

Final report:

**Assessing Mercury Contamination and Bioavailability in Great Smoky Mountains
National Park Aquatic Habitats**

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**Prepared for: Great Smoky Mountains Conservation Association
James Tanner Fellowship Committee**

and

The Great Smokey Mountains National Park / National Parks Service

Summary

This study represents an initial exploration of Hg contamination in sediments, soils and aquatic macroinvertebrates collected from different locations in the Great Smoky Mountains National Park. Mercury was readily measurable in all soil, sediment and macroinvertebrate samples, though the magnitude of Hg concentrations varied widely across locations.

The range of all sediment/soil total Hg measurements ranged from 14-378 ng/g on a dry weight basis, with mean and median values of 101.6 and 93.3 ng/g, respectively. A limited number of sediment samples (5) analyzed for methyl Hg had concentrations ranging from 0.02 – 3.83 ng/g on a dry weight basis. These values represent a range of contribution (0.2 to 8.2 %) to the total Hg pool present at the sites. Macroinvertebrate samples were taken from each of three locations – Kerr Branch, Chilogatee Creek and Mossy Branch. These sites differed considerable in their physical habitats, sediment geochemistry, and Hg loading. For example, Mossy Branch (47 ng/g), Kerr Branch (78 ng/g) and Chilogatee Creek (117.6 ng/g) differed almost 3-fold in total Hg concentrations, yet the methyl Hg profiles did not reflect the total Hg profiles. Mossy Branch, despite having the lower total Hg among the 3 sites, had methyl Hg concentrations (3.8 ng/g) that were comparable to Kerr Branch (3.7 ng/g). Chilogatee Creek had the lower methyl Hg concentration (1.6 ng/g) of the three sites despite its higher total Hg concentrations.

The texture of soils and sediments are sandy loam and loamy sand, respectively. Quartz, biotite, kaolinite, and potassium feldspar are major mineral components. Most of sediments and soils are moderately acidic (4.2) to near neutral pH (6.8). While soils exhibit cation exchange capacity (CEC) of 13-23 cmol/kg, CEC of sediments are CEC 7-12 cmol/kg, suggesting that lack of cation exchangeability in sediments. Mercury desorption in sediments with high C (e.g., Kerr Branch) was much lower than that from low C sediments (Laffete and Mossy Branch sediments). These results indirectly suggest that organic carbon sources might strongly retain the labile Hg fractions in sediments (1-7%).

Comparisons of invertebrate tissue Hg concentrations among sites are complicated by the fact that the same taxa did not occur at each site. Nevertheless there are some interesting observations. First, Hg (total and methyl) concentrations in sediment are likely not good predictors of food web Hg incorporation. For example, Hg burdens in the caddis fly *Diplectrona modesta* were more than two fold higher in Mossy Branch than in Kerr Branch, despite the fact that Kerr Branch had higher sediment total Hg and comparable methyl Hg. Second, trophic position did not appear to be a major driver of Hg body burdens among species at a given site. However, based on limited data, it appears that the proportion of methyl to total Hg increases with trophic status.

Introduction:

Our limited understanding of the extent of mercury (Hg) contamination in the Great Smoky Mountains National Park (GSMNP) prompted the investigations described in this report. Our activities, supported by the Tanner Fellowship fell under three specific aims:

- 1). Characterize mercury concentrations in sediments/soils
- 2). Measure mercury concentrations in select aquatic biota (invertebrates)
- 3). Assess physiochemical factors affecting the bioavailability of Hg from sediments and soils

Another major goal of these studies was to identify locations and species that might be suitable for incorporation into a long term monitoring effort should the Park Service elect to establish long term trends in Hg deposition.

Materials and methods:

Objective 1: Characterize mercury concentrations in sediments/soils

Site Selection: In March, 2008, we met with Park staff to discuss potential sampling locations for this study. Park Service personnel selected 11 sampling sites, and through volunteer efforts, three sediment samples were collected from each of 11 locations throughout the Park for analysis of total Hg by Cold Vapor Atomic Fluorescence Spectroscopy at North Carolina State University. Based on the results of these analyses, we visited several of these sites in June, 2008 to assess their suitability for further studies. Due to drought conditions, some of these sites were dry, and others did not provide suitable habitat for aquatic invertebrates. In some cases, dry soil samples were taken for Hg analysis. Ultimately, two of the initial 11 sites were deemed suitable for analysis of invertebrate tissues – Kerr Branch and Chilogatee Creek, though the latter was not sampled during this June sampling event. A third, “new” site was selected during the June 08 trip – Mossy Branch in the Cataloachie basin.

Mercury analysis: Mercury analyses of sediment/soil samples were conducted in 3 different laboratories. Initially 33 samples (3 each from 11 locations taken for site selection purposes) were conducted at North Carolina State University (see A below for description of methods and QA/QC procedures). Sediment and soil samples collected in June were analyzed by the Pacific Northwest National Laboratory, Sequim, WA. Finally, sediment from Chilogatee Creek collected in October, 2008 was analyzed by US Geological Survey, Menlo Park, CA.

A. Sediment samples for pre-screening/site selection.

Total mercury concentrations in sediment and macroinvertebrates were analyzed using USEPA method 1631 revision E with a modified digestion procedure. Samples were freeze-dried (weights of freeze-dried samples ranged from 0.0157 to 0.3152 g for macroinvertebrates, and 0.22 to 0.56 g for sediment samples) and then digested in 4.5 ml

of ultrapure 16M HNO₃ (OmniTrace Ultra, Merck, Darmstadt, Germany) and 1.5 ml of 18M H₂SO₄ (Leeman Labs Inc, West Chester, Pennsylvania, USA) in 50 ml Teflon digestion vessels using a microwave heating system (MarsXpress, CEM Inc, Mathews, North Carolina, USA). Samples were then subjected to BrCl oxidation (overnight) and SnCl₂ reduction. Samples were diluted with 0.2 % HCl for total mercury analysis by flow-injection cold-vapor atomic fluorescence spectrophotometry (CVAFS) with a Leeman Laboratories Hydro AF Gold plus analyzer (Leeman Labs Inc, USA). The accuracy of total mercury determination was evaluated by analysis of certified standard reference material from the National Institute of Standards and Technology (NIST Mussel 2976), method blanks (acid), and calibration standards. In the analysis of Hg in the sediment samples, our measured concentration (mean ± SD) of total Hg in NIST Mussel 2976 was 75.3 ± 5.9 ng/g dry wt (n = 4) (certified value: 61 ± 3.6, total recovery 123 ± 0.2%). Total Hg in method blanks were 1.18 ± 0.2 ng/L, which is slightly higher than the lowest concentration to generate the standard curve (0.5 to 100 ng/L) but much lower than measured values of the sediment samples. In the analysis of Hg in the macroinvertebrate samples, our measured concentration (mean ± SD) of total Hg in NIST Mussel 2976 was 68.0 ± 4.2 ng/g dry wt (n = 2) (recovery 111 ± 0.1%). Total Hg in method blanks were 0.94 ± 0.26 ng/L. Mercury levels in all the samples exceeded our method detection limit of 1.3 ng/g dry weight, calculated as 3 times the standard deviation of the method blank mass divided by the average sample mass.

B. Sediment/soil samples (June, 2009) – Pacific Northwest National Laboratory

All sediments and soil samples were stored frozen at -80°C until analysis. All desorption samples were acidified and were kept at 4 ± 2°C prior to the total Hg analysis. All samples were analyzed in duplicates (except for desorption samples) for total mercury by EPA Method 7473 (Thermal Decomposition, Amalgamation, and Cold Vapor Atomic Spectrophotometry). The range of recovery, ongoing precision and recovery (OPR), achieved detection limit for the total Hg analysis is 8-120%, ≤ 20%, and 0.00199 µg/g, respectively.

Samples were analyzed for methylated Hg by a modification of EPA Method 1630. Methylmercury in the extracted sample was ethylated and then purged onto carbon traps as a means of preconcentration and interference removal. The ethylated methylmercury was thermally desorbed into a fluorescence cell. Fluorescence (peak area) is proportional to the quantity of methylmercury collected, which is quantified using an average response factor as a function of the quantity of sample purged. The range of recovery, ongoing precision and recovery (OPR), achieved detection limit for the total Hg analysis is 65-135%, ≤ 33%, and 0.00275 ng/g, respectively.

C. Sediment sample from Chilogatee Creek (October, 2009) – US Geological Survey

Sediment total Hg (THg) sub-samples were extracted with aqua regia (6 ml concentrated HCl and 2 ml concentrated HNO₃) overnight at room temperature, then diluted with 5% BrCl and heated for 24 hrs at 50 °C (Olund and others, 2004). The digestate was then quantified for THg with the tin-chloride reduction, dual gold trap cold vapor atomic fluorescence Spectrophotometry (CVAFS) quantification approach (USEPA, 2002) using an automated Tekran 2600 Total Mercury Analyzer.

Sediment MeHg sub-samples were extracted at 60 °C for 4 hours in an organic / alkaline solution (25% KOH in methanol) and quantified using a Brooks-Rand automated methylmercury analyzer (FDEP, 2008).

Objective 2: Measure mercury concentrations in select aquatic biota (invertebrates)

In June, 2008, Insects were sampled from 2 locations – Kerr Branch and Mossy Branch. In October, 2008, Kerr Branch and Mossy Branch were re-sampled, and invertebrates were collected from a third site - Chilogatee Creek. Each of these sites differed considerably in character. Kerr Branch is in a wooded watershed with mixed sediment and cobble substrate. Mossy Branch flows through an open meadow habitat with mixed fine and coarse sediment. No cobble substrate occurs in Mossy Branch. Chilogatee Creek has been reduced to a limited flow through a mudflat environment (Table 1).

Invertebrates were sampled with a D-frame kick net and sorted in the field. Representative samples were taken for each taxon, and preserved in ethanol for taxonomic identification. All taxonomic identifications were provided by the North Carolina Department of Environment and Natural Resources (NCDENR). We were unable to get species identifications for juvenile crayfish, a notoriously difficult group for identifying immatures. The remaining animals were rinsed with deionized water and immediately frozen on dry ice and transported to NCSU for Hg analysis by CVAFS as described above. A subset of samples was sent to Frontier Geosciences for methylmercury analysis.

Site	Description	Dates	# of taxa
Kerr Branch	Wooded creek, cobble/sediment substrate	June '08	4
		October '08	1
Chilogatee Creek	Small channel through mudflat	October '08	4
Mossy Branch	Meadow, fine sediment substrate	June '08	4
		October '08	4

Table 1. Invertebrate sampling locations and characteristics.

Objective 3: Assess factors affecting the bioavailability of Hg from sediments

Soil and Sediment Sampling:

Grab samples (surface soils and sediment samples) were collected in acid washed vials. Samples for methylmercury analysis were immediately frozen, and were kept at ~0°C until further analysis.

Physicochemical Characterization and Mineralogical Analysis of Soils.

All soils and sediments were air-dried, and were passed through a 2mm sieve. Texture of soils was analyzed using the hydrometer method (Klute 1986). Soil pH_{water} was

determined in deionized water using a soil:solution ratio of 1: 1. Loss-on-ignition and hydrometer methods were used to measure % organic matter (OM) and particle size, respectively (Sims and Heckendorn, 1991). Cation exchange capacity (CEC) was measured using an unbuffered salt extraction method (Grove et al., 1982). Total Ca, Al, Mg, Na were analyzed using inductively coupled plasma-atomic emission spectroscopy (ICP-AES). Organic matter and metal oxides were removed from the soils using sodium hypochlorite and citrate-bicarbonate-dithionite method, and than sand, silt and clay fractions were obtained using centrifugation-sedimentation methods described by Jackson (Jackson, 1956). Freeze-dried samples were analyzed for bulk mineralogy using a polarized microscopy method.

Desorption Experiments.

A batch replenishment method was used to investigate total Hg desorption for 15 d. Fresh moist sediments (approximately 8g) were placed in 50 ml Teflon tubes. 50 ml of Hg free desorption solutions (0.01 M NaCl solutions with 1mM of MES buffer that were adjusted to the sediment pH_{water} values) were added to sediments. The soil suspensions were shaken on an end-over-end shaker at 50 rpm. At sampling times (1-15d), the tubes were centrifuged at 11,950 g for 5 min, and the supernatants were replaced with the same Hg-free 0.1M NaCl solutions. This process was repeated when samples were taken.

Results:

Objective 1: Characterize mercury concentrations in sediments/soils

A. Mercury in sediment samples chosen for site selection/pre-screening

Total mercury concentrations varied widely among sites chosen for initial screening (Figure 1). These sites, chosen by Park Service personnel represent a variety of habitat types and elevations. Not all of these sites were suitable for subsequent invertebrate sampling due to seasonal dryness, lack of sufficient biomass, or difficulty finding the exact location of the original sample. These data do provide some baseline

data for moist habitats in the Park.

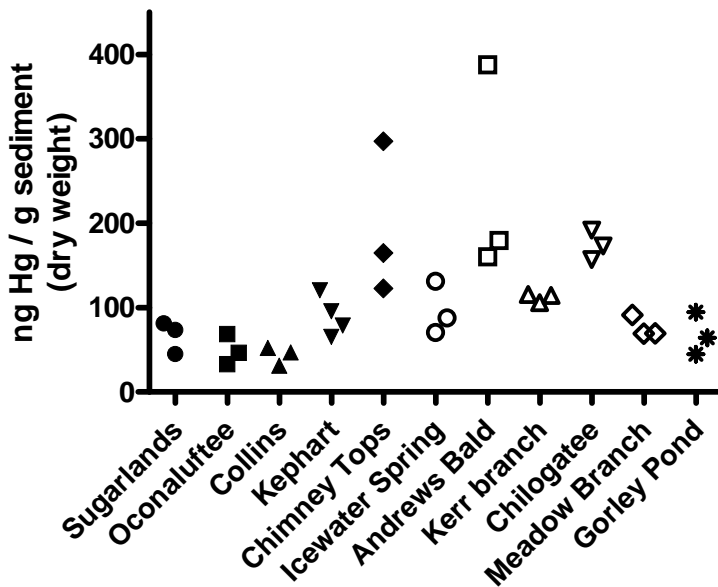


Figure 1. Mercury concentrations in GSMNP sediment samples collected in the Spring of 2008. Each data point represents a single run of freeze dried sediment analyzed by CVAFS.

Site	Total Hg (ng/g)	Methyl Hg (ng/g)	% Methyl Hg
Kerr Branch sediments	78	3.71	4.7
Mossy Branch sediments	47	3.83	8.2
*Chilogatee Creek	117.6	1.56	1.3
Oconoluftee sediments	14	0.667	4.8
Chimney tops sediments	14	0.025	0.2
Chimney tops soil	99	-	
Andrews bald soil	177	-	
Kerr Branch soil	97	-	
Purchase Knob soil	173	-	

Table 2. Mercury in sediments/soils from June 2008 and *October 2008 sampling. Sites in bold were chosen for subsequent invertebrate sampling on the basis of Hg concentrations in sediments and availability of invertebrates.

Note that there are cases where sediment/soil samples are consistent with previous measures (Fig.1), and other cases where values are quite different. It is important to note that 1). Samples were taken at different times, 2). Analyses were performed by different labs, and 3). Precise sampling locations within a site were likely different as sampling was performed by different people. Nevertheless, there is reasonably good agreement among sampling events/laboratories.

Consistencies: Chilogatee (high Hg), Oconoluftee (low Hg), Andrews Bald (high Hg) and Kerr Branch (moderate Hg) samples were reasonably consistent between sampling events.

Inconsistencies: Chimney Tops sediments were quite different between sampling events, likely as a result of different sampling locations within the site.

Objective 2: Measure mercury concentrations in select aquatic biota (invertebrates)

The second major objective was to measure Hg concentrations in selected invertebrate species from each of three sites. Sites were chosen with very different physical habitats (Table 1), and where possible, species common to the different sites were sampled for Hg analysis. Additionally, we selected taxa representing different taxonomic groups and feeding strategies (e.g. predatory and non-predatory species).

Of the three sites, Mossy Branch contained the highest diversity of taxa and is recommended as a potential location for long term monitoring. Chilogatee Creek had been essentially drained, leaving only a very small channel flowing through a broad mudflat. Kerr Branch had very low flow and was difficult to sample in both June and October sampling trips.

<i>Site</i>	<i>Season</i>	<i>Taxon</i>	<i>Sample Mass (g dry weight)</i>	<i>Total Hg (ng/g dry weight)</i>
Chilogatee Cr.	Fall	<i>Basiaeschna janata</i> ¹	0.080	332.4
Chilogatee Cr.	Fall	<i>Basiaeschna janata</i> ¹	0.178	171.5
Chilogatee Cr.	Fall	Crayfish ²	0.254	82.7
Chilogatee Cr.	Fall	Crayfish ²	0.315	103.6
Chilogatee Cr.	Fall	Crayfish ²	0.098	100.5
Chilogatee Cr.	Fall	<i>Lanthus</i> sp ¹	0.070	99.9
Chilogatee Cr.	Fall	<i>Lanthus</i> sp ¹	0.111	107.7
Chilogatee Cr.	Fall	<i>Tipula</i> sp ⁴	0.077	160.7
Kerr Branch	Fall	<i>Acroneuria abnormis</i> ¹	0.109	80.6
Kerr Branch	Summer	<i>Acroneuria abnormis</i> ¹	0.186	99.2
Kerr Branch	Summer	<i>Acroneuria abnormis</i> ¹	0.120	71.6
Kerr Branch	Summer	Crayfish ²	0.071	85.7
Kerr Branch	Summer	<i>Diplectrona modesta</i> ²	0.108	64.0
Kerr Branch	Summer	<i>Maccaffertium piducum</i> ³	0.013	166.3
Mossy Branch	Fall	<i>Cordulegaster</i> sp ¹	0.118	142.6
Mossy Branch	Fall	Crayfish ²	0.088	148.0
Mossy Branch	Fall	Crayfish ²	0.055	142.9
Mossy Branch	Fall	<i>Diplectrona modesta</i> ²	0.034	145.5
Mossy Branch	Fall	<i>Ephemera</i> sp ⁴	0.100	285.6
Mossy Branch	Summer	Crayfish ²	0.280	191.2
Mossy Branch	Summer	Crayfish ²	0.038	261.0
Mossy Branch	Summer	Crayfish ²	0.090	217.9
Mossy Branch	Summer	Crayfish ²	0.103	182.2
Mossy Branch	Summer	<i>Diplectrona modesta</i> ²	0.101	145.0
Mossy Branch	Summer	<i>Ephemerella subvaria</i> ²	0.082	81.9
Mossy Branch	Summer	<i>Lanthus</i> sp ¹	0.057	148.4

Table 3. Total Hg in aquatic invertebrates collected from 3 locations. Each value represents single sample run by CVAFS at NSCU as described above. ¹Represents predatory taxa, ²represents omnivores/collectors, ³represents scrapers, and ⁴represents taxa who likely process organic material in ingested sediments.

<i>Site</i>	<i>Taxon</i>	<i>Season</i>	<i>Methyl Hg (ng/g) wet weight</i>	<i>*Methyl Hg (ng/g) dry weight</i>	<i>Total Hg (ng/g) dry weight</i>	<i>* % methyl Hg</i>
Chilogatee Creek	<i>Tipula</i>	Summer	5.0	23.0	160.67	14.3
Chilogatee Creek	<i>Basiaeschna</i>	Summer	31.55	145.13	171.53	84.6
Mossy Branch	<i>Diplectrona</i>	Summer	14.9	68.54	145.51	47.1
Mossy Branch	<i>Crayfish</i>	Fall	12.7	58.42	145.43	40.7
Mossy Branch	<i>Ephemera</i>	Fall	19.45	89.47	192.51	46.4
Kerr Branch	<i>Lanthus</i>	Summer	28.4	130.64	148.42	88.0
Kerr Branch	<i>Crayfish</i>	Summer	9.6	44.16	85.71	51.5

Table 4. Comparisons of methyl and total Hg in select invertebrate taxa. *Note that methyl mercury analyses were performed by Frontier Geosciences, and results were reported on a wet weight basis. We assumed samples to be 78% water weight for estimates of methyl Hg on a dry weight basis, and % methyl Hg.

Objective 3: Assess factors affecting the bioavailability of Hg from sediments

Physicochemical and Mineralogical Characterization.

Table 1 shows the particle size and mineralogical properties of soils and sediments. The texture of soils and sediments was sandy loam and loamy sand. Major mineral components, confirmed by micropetrographic analyses, were quartz, biotite and potassium feldspar. Kaolinite was also present in soils. The soil organic carbon (OC) content was highest in soils from Andrew bald, followed by soils from Purchase Knob, and Chimney tops. All sediments have relatively low OC contents (< 6.6 %) comparing to the OC content in soils. Strong Hg adsorption on humic substances and dissolved OC has been previously reported (Meili, 1991; Krabbenhoft and Babiarez, 1992; Khwaja et al., 2006), and these soil organic carbon components might be important in controlling inorganic- and organic-Hg retention in the soils. Soil pH ranges from 4.2 to 7.2. Most of sediments had moderately acidic to near neutral pH. However, pH of some surface soils (e.g., Purchase Knob and Andrew Bald) is strongly acidic, suggesting the low cation retention capacity. A large amount of exchangeable Ca was measured in all soils/sediments except for the soils from Andrew Bald. It is likely that the soils from Andrew Bald have been exposed to acidic rain, resulting in the depletion of exchangeable base cations. The low % base saturation suggests that the soil from Andrew Bald is the most sensitive to Hg cation leaching of all soils. On the contrarily, the highest CEC value (23 cmol/kg) was observed in Chimney top soils. The retention of base cations is probably due to relatively high pH (7.2).

Sample	pH _{water}	Acidity (cmol/kg)	CEC (cmol/kg)	%C	% base saturation	% Base Saturation			
						Ca	Mg	K	Na
Purchase Knob soil	4.2	9.2	13.3	19.0	31	21	6	3	0
Andrews bald soil	4.9	8.4	9.1	21.3	8	4	2	1	0
Kerr Branch soil	5.1	7.2	15.8	16.2	54	10	2	0	0
Chimney tops soil	7.2	2.8	23.6	8.7	88	77	11	0	0
Chimney tops sediments	5.65	2.3	8.7	5.1	53	45	7	1	0
Kerr Branch sediments	6.15	3.6	7.4	6.6	48	44	3	1	0
Oconoluftee sediments	5.81	3.1	6.4	1.1	42	38	4	0	0
Mossy Branch sediments	5.55	4	6.5	1.1	38	32	5	1	1
Chilogatee Creek sediments	6.82	1.6	10.5	5.5	85	76	8	1	0

Sample	Texture	Horizon /Color Description	Bulk Mineralogy	Total Hg (µg/g) dry	Mono methyl Hg (ng/g) dry	Drainage	% texture		
							Sand	Silt	Clay
Purchase Knob soil	Sandy loam	A ₁ (10YR 3/2)	Q, B, K, F	0.173	NA	excessively well drained	61	25	14
Andrews bald soil	Sandy loam	A ₁ (10YR 3/2)	Q, B, K, F	0.177	NA	excessively well drained	59	25	16
Kerr Branch soil	Sandy loam	A ₁ (10YR 3/3)	Q, B, K, F	0.097	NA	excessively well drained	63	25	12
Chimney tops soil	Sandy loam	A ₁ (10YR 3/2)	Q, B, K, F	0.099	NA	excessively well drained	61	30	9
Chimney tops sediments	Loamy sand	NA	Q, K, K-F	0.014	0.025	NA	82	17	1
Kerr Branch sediments	Loamy sand	NA	Q, F	0.078	3.71	NA	79	15	5
Oconoluftee sediments	Loamy sand	NA	Q, F	0.014	0.667	NA	80	19	1
Mossy Branch sediments	Loamy sand	NA	Q, F	0.047	3.83	NA	80	15	5
Chilogatee Creek sediments	Loamy sand	NA	Q, F	0.118	1.56	NA	83	10	7

Mineralogy determined by polarized microscope analysis. Mineral abbreviations: Q: quartz, F: potassium feldspar, K: kaolinite, B: biotite

Table-1: Physicochemical characterization, total Hg and monomethyl Hg levels in GSM surface soils and sediments.

Mercury Desorption Experiments.

Results of the Hg desorption are shown in Figure-1. During the desorption experiments, total Hg concentrations for all samples were fluctuated between 4.58 and 9.95 ppt (ng/L). Samples from Mossy Branch, released the most Hg after 15 days, and followed by samples from Kerr Branch, Laffete, and Chimney tops. The degree of Hg desorbability is not correlated with differences in the amount of total Hg, suggesting a complex desorption mechanism in these sediments. It seems that the extent of Hg desorption in these sediments have two different trends in different sediment types. Mercury desorption in sediments with high C (i.e., Chimney Tops and Kerr Branch) was low (less than 7% of total Hg release after 15 days), and nearly reached plateau after 10 days, followed by a steady continuous desorption reactions. On the contrary, sediments with low C content (1.1% in Laffete and Mossy Branch) showed biphasic reactions; initial fast desorption followed by a slow desorption which increases with the number of replenishments. These results indirectly suggest that organic carbon sources might strongly retain the labile Hg fractions in sediments.

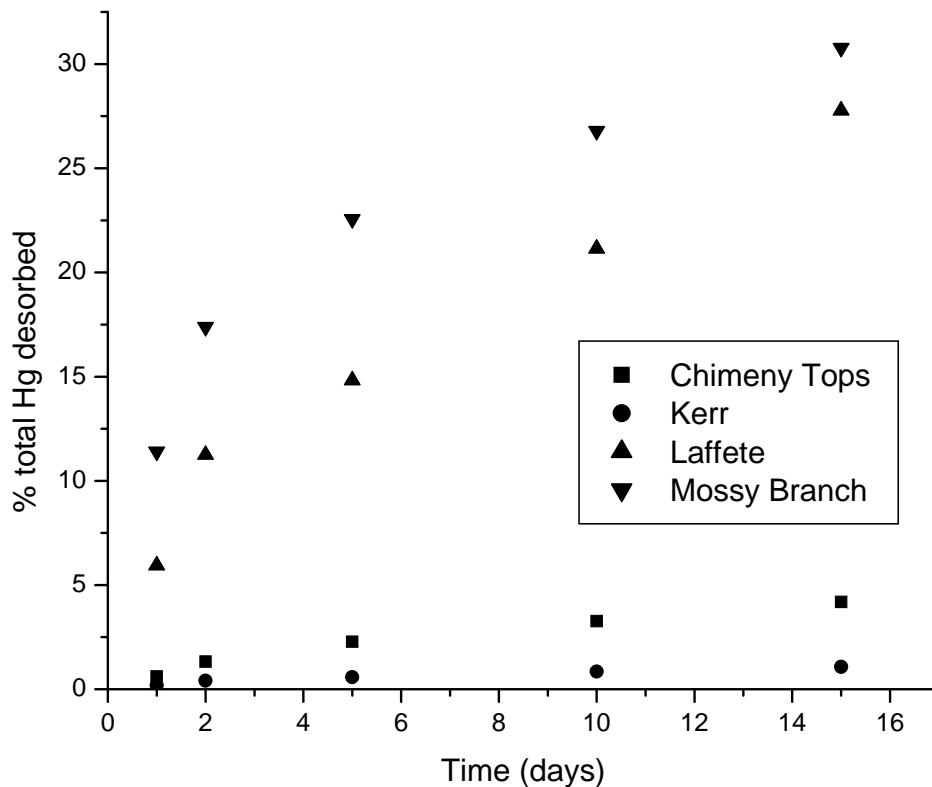


Figure-1: Total Hg desorption from GSM sediments. % RSD of total Hg analysis is approximately 6% for each analysis. Error bars are hidden within each symbol.

Discussion:

1). Total Hg in sediments

Total Hg in sediment varied greatly among sites and between seasons. From Spring, 2008 sediment samples (3 samples from each site) from 11 sites, site average total Hg ranged from the lowest of 43.5 ± 10.8 in Collins Boglet to 242.6 ± 126.2 ng/g in Andrews Bald Bog. Generally the within site variation in total Hg levels was small, with coefficient of variation (CV = standard deviation/mean) close to 30% for most sites, and approximately 50% for Chimney Tops and Andrews Bald Bog due to the large deviation of one sample from the other two samples from the same sites (Figure 1). Of the June 2008 and October 2008 sediment and soil samples from 9 sites, total Hg ranged from 14 (Oconoluftee and Chimney Tops sediments) to approximately 180 ng/g (Andrew Bald soil). The summer and fall sediments from same sample sites were generally lower in total Hg than those of the Spring samples but showing similar pattern (i.e., high, moderate, low total Hg in the sediments in the Spring remain high, moderate, low in the Summer and Fall sediments). For examples, Kerr Branch (summer) and Chilogatee Creek (Fall) had 78 and 118 ng/g, while they had 120 and 174 ng/g in the Spring 2008 respectively. This may reflect the temporal changes in total Hg in the sediments. Surprisingly, Chimney Tops sediment had 14 ng/g total Hg in the Fall, which is only about 7% of value in the Spring, but it is unclear whether sediments were collected from the same location within this area.

To put these sediment concentrations in perspective, we compare our results to those of other studies in different geographical regions. Total mercury in surface sediment ranged from < 10 to 3700 ng/g with an overall average concentration of 190 ng/g from 579 sites across northeastern North American (Kamman et al., 2005). Shallow littoral sediments from St. Lawrence River averaged 82 ng/g dw Hg (Filion and Morin, 2000). A median value of 120 ng/g Hg (range = 0.1 to 8.50 ug/g) of Hg was reported in sediment from the Great Lakes (Jaagumagi and Persaud 1992). A Canadian study compared sediments from a natural lake (40 to 106 ng/g) and a hydroelectric reservoir (47 to 229 ng/g) (Tremblay and Lucotte, 1997). The highest reported total sediment Hg values we found in the literature were from a historically mercury contaminated reservoir, which ranged from 260 to 39000 ng/g dw (Yan et al. 2008).

The U. S. National Oceanic and Atmospheric Administration (1999) provide sediment quality guidelines expressed as threshold, probable and upper effects levels at which toxicity is likely to occur to benthic organisms. For total Hg, these guideline values are 0.174, 0.486, and 0.560 ug/g, respectively. In our study, 7 out of 44 (16%) of the sediment samples we measured were at or above this lower threshold value. None exceeded the upper threshold.

Difference in total Hg in GSMNP sediments may reflect both differences in deposition rates as well as different sediment characteristics. For example, sediment from Mossy Branch with relatively lower C content had faster Hg desorption and lower total Hg compared to those from Kerr Branch and Chilogatee Creek. Several studies indicate that total Hg in sediment is positively related to total C contents in sediment (Tremblay and Lucotte, 1997, Lucotte et al., 1995).

2). Methyl Hg in sediments

As methyl mercury analyses are costly, we were only able to measure MeHg in a limited number of samples. In our study, MeHg ranged from 0.025 – 3.83 ng/g. Kamman et al (2005) report a range (0.15 to 21 ng/g) of MeHg in sediments from different freshwater ecosystems. The mean MeHg value reported from that study is identical to the highest value found in our study (3.83 ng/g).

In our study, the % MeHg ranged from 0.2-8.2%. To put this range in perspective, average values from other systems include $4.4 \pm 0.40\%$ for rivers, $3.31 \pm 0.44\%$ for lakes, and $4.44 \pm 0.42\%$ for reservoirs (Bell and Scudder, 2007). Interestingly, these averages are also very similar to the percentages found in periphyton from different habitats. It is thought that fluctuating water levels can stimulate mercury methylation in sediments. Thus, meadow habitats such as the Mossy Branch drainage may provide conditions conducive to Hg methylation, with seasonal changes in moisture. This could explain the relatively high methyl Hg concentrations at this site despite the relatively low total Hg present there. Additionally, it is likely that methyl mercury concentrations may be highly variable over time in Chilogatee Creek now that the hydrology has been radically altered. We may expect to find more Hg methylation in this new mudflat habitat.

3). Hg in invertebrates

Total Hg was determined in invertebrates collected from three sites: Chilogatee Creek, Kerr Branch, and Mossy Branch. Total Hg varied among sites, changed temporarily, and differed according to the trophic levels. Total Hg ranged from 64 ng/g dw in *Diplectrona modesta* in the summer from Kerr Branch to approximately 332 ng/g in *Basiaeschna janata* in the Fall from Chilogatee Creek (Table 3). Crayfish species collected in the Summer from Mossy Branch accumulated more total Hg than those in the Fall. Interestingly, Crayfish species had significantly higher total Hg in the Summer and Fall seasons from Mossy Branch than those in the Fall from Chilogatee Creek, though Mossy Branch sediment had a relatively low total Hg compared to Chilogatee Creek sediment. Similarly, caddisfly species *D. modesta* in the Summer contained high total Hg relative to this species in the Fall from Chilogatee Creek. Due to insufficient data, no clear trend was observed between total Hg and the trophic levels, though the total Hg body burden differed among the species among these categories (Table 3)

Total Hg in the invertebrates from our study is comparable from the results in the literature. Reported values for mayflies collected from lakes ranged from 50 to over 200 ng/g dry wt (Tremblay and Lucotte, 1997). Total Hg in insects sampled from a hydroelectric reservoir ranged from 186 to 1215 ng/g dw (Tremblay and Lucotte, 1997). In North Carolina, total Hg concentrations varied among 4 collector/gatherer insects from the Eno River, with two caddisfly species (~ 60 ng/g) were slightly higher in total Hg than two mayfly species (~40 ng/g) (Xie et al., 2009).

In our study, the relative contribution of MeHg to total Hg varied widely among taxa. The shredder/collector species (i.e. *Tipula*, *Ephemera*, *Diplectrona*) had less than 50% MeHg, while predatory species from the order Odonates (i.e., *Basiaeschna* and *Lanthus*) had more than 80%. This pattern is similar to that found in aquatic insects from natural lakes and hydroelectric reservoirs (Tremblay and Lucotte, 1997). These results suggest that MeHg content in macroinvertebrates is closely related to trophic level in the benthic food web.

Orihel et al. (2006, 2007) suggest that total both total Hg and MeHg in water, particles, periphyton, sediments and aquatic biota are proportional to inorganic Hg loading. This raises fundamental questions regarding the relative importance of exposure routes for both MeHg and total Hg bioaccumulation. The extent to which Hg bioaccumulation is driven by dissolved vs. dietary exposure pathways remains unclear. Work by Wang et al. (2004) and Xie et al. (2009) demonstrate that inorganic (Hg II) uptake from solution can be significant in freshwater aquatic insects and in a marine mussel. Without data on wet deposition rates, and the relative importance of exposure routes, this question will remain unanswered. This also complicates the interpretation of some of our data. It is not feasible to explain the observed discrepancy (for instance, crayfish from Mossy Branch which has a lower Hg levels in sediment have higher total Hg than those from the other two sample sites). Apparently more research and monitoring is needed to better understand total mercury in invertebrates from this region.

Our understanding of mercury bioaccumulation in benthic macroinvertebrates remains limited. Here we show that total Hg and/or methyl Hg concentrations in sediment were not necessarily good predictors of invertebrate tissue Hg with our limited data. Previous research has not resulted in definitive conclusions on the relationship between total Hg concentrations in sediment and total Hg tissue burdens in invertebrates. For examples, Filion and Morin (2000) found that there is no correlation between sediment Hg concentration and Hg in invertebrates from a river in Canada. In contrast, Naimo et al. (2000) showed that there is a significant relationship between sediment Hg concentration and Hg in *Hexagenia* mayflies in a contaminated floodplain river. This discrepancy may reflect the difference in the Hg bioaccumulation and regulation mechanisms, functional feeding (trophic position) in the food web in different invertebrate species. In fish, Kamman et al. showed that fishes that feed primarily or exclusively on benthic substrates have typically low and invariant Hg concentrations. Therefore, the lack of a clear relationship between sediment total Hg concentrations and insect tissue Hg concentrations implies the importance sediment characteristics in determining Hg bioavailability. Additionally, food webs clearly influence tissue Hg bioaccumulation. It also implies that monitoring of sediment total Hg may not be reliable as an inexpensive surrogate to evaluate whether tissue total Hg (probably including fish due to their diet consisting mainly invertebrates) is likely to be elevated in a particular freshwater ecosystem.

Acknowledgements:

This work was supported by the Great Smoky Mountains Conservation Association/James Tanner Fellowship. We acknowledge the help and support of National Park Service Personnel and volunteer sediment collectors. Dr. Robin Stewart, USGS Menlo Park provided total and methyl Hg analysis of Chilogatee Creek sediments. Jennifer Flippin (NCSU) provided field collecting support.

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