Determination of Methylmercury Levels in Mercury-contaminated Soils from Oak Ridge TN by Cold Vapor Generation

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New Orleans, LA
- Released into the atmosphere from natural sources, such as volcanic eruptions and ocean emissions, as well as from anthropogenic sources.

- Distributed throughout the environment in various inorganic and organic forms, including elemental (Hg\(^0\)), mercurous (Hg\(_2^+\)), mercuric (Hg\(_2^{+}\)) and alkylated compounds (methylmercury and ethylmercury).

- Strong neurotoxin impacting the central and peripheral nervous system. Organomercury species bioaccumulate in many different environments.

- Mineral cinnabar (HgS) is the most insoluble (4.65\times10^{-25} \text{ g/L at 25 °C}) form. HgO and HgCl\(_2\) are relatively soluble Hg species in water, with solubilities of 0.051 g/L at 25 °C and 69 g/L at 20 °C, respectively.
Methylmercury (CH$_3$Hg) is the most toxic form of mercury that bioaccumulates compared to other forms of mercury.

- Impairs the central nervous system, as well as genetic and enzymatic information.

- CH$_3$Hg represents the predominant form of mercury in the aquatic food chain.
Methods for Mercury Determination

- **Gas Chromatography (GC)**
  - Volatilization of analyte and detection via GC.
  - Westoo’s method is popular for sample prep to determine organic mercury species.

- **HPLC**
  - Preferred over GC because of no volatilization step.
  - Column preconcentration is still necessary.
  - Reversed-phase HPLC is preferred for organomercury detection due to hydrophobic stationary phase, ideal for organic compound detection.

- **Cold vapor generation (CVG)**
  - Ideal for trace Hg analysis
  - Allows speciation of Hg.
  - Popular for determination of Hg in blood, hair and urine.

- **ICP-MS**
  - Favored for isotopic Hg determinations
  - Low LOD and multi-sample analysis in short amount of time.
Coupled Methods for Mercury Determination

- Cold Vapor Generation Atomic Fluorescence Spectroscopy (CVG-AFS)
- Cold Vapor Generation Atomic Absorption Spectroscopy (CVG-AAS)
- Gas Chromatography - Atomic Fluorescence Spectroscopy (GC-AFS)
- HPLC-Cold vapor generation Atomic Absorption Spectroscopy (HPLC-CVG-AAS)
- Cold Vapor Generation Electrothermal Atomic Absorption Spectroscopy (CV-ETAAS)
- Cold Vapor generation - Optical Emission Spectrometry (CVG-ICP-OES)
- Microwave Induced Plasma Atomic Emission Spectroscopy (MIP-AES)
- HPLC Inductively Coupled Plasma Mass Spectrometry (HPLC-ICP-MS)
- Gas chromatography Inductively Coupled Plasma Mass Spectrometry (GC-ICP-IDMS)
Legacy Hg Contamination in Oak Ridge, TN

- In 1960’s, elemental Hg was used at Y-12 National Security Facility for manufacturing components of nuclear weapons.

- It is estimated that 350 tons of Hg was released to environment.

- Hg distributed through run-off by the East Fork Poplar Creek (EFPC) which ran through the Y-12 complex, flowing into the surrounding community and terminating at the Clinch River.
Through remedial actions over the last 25 years, Hg concentrations and fluxes have been reduced, but Hg levels in water at the Y-12 Complex boundary continue to exceed both the regulatory limit (51 ng/L) and the remediation goal (200 µg/L) (Brooks and Southworth 2011). Commensurate reductions in the fish tissue concentrations (to achieve the EPA criteria of 0.3 µg/g) have not been observed (Peterson et al. 2011).
Hypothesis

Methylmercury is water-soluble and thus could directly affect contamination in water and wildlife.

It is hypothesized that Hg-contaminated soils in Oak Ridge TN contain significant levels of methylmercury that is responsible for elevated Hg contamination in soil and water.

Mercury contaminated soil from the Y-12 Complex. Adopted from the Knoxville News Sentinel.
Objectives

• To develop a procedure for selective separation and determination of trace amounts of methylmercury in Hg-contaminated soils.

(1) Selective extraction of methylmercury from top soil samples.
(2) Eliminate inorganic mercury from soil extracts.
(3) Quantify trace methylmercury in soils using cold vapor generation.
Results: Total Mercury Levels

- Total Hg analysis were made by acid digestion of the soils. About 0.1 g soil samples (n=3) was digested on a Graphite Digestion Block at 140 °C in 5 mL HNO₃ and then treated with H₂O₂ to oxidize organic material.

- Montana soil (SRM 2710) and Domestic Sludge (SRM 2781) from National Institute of Standards and Technology (NIST, Gaithersburg, MD) were used for verification of total mercury analysis.

ICP-MS analysis showed that contaminated top soil contained about 57 to 95 mg/kg Hg.
Selective extraction: Sample Preparation

- Extraction of CH$_3$Hg from soil matrix is carried with template soils collected from Biloxi, MS. They were ground and sieved through 0.25 mm apertures. Samples were analyzed by ICP-MS for Hg content before utilizing in any experimental work.

- Samples of template soils (0.5 g) were contaminated with 0.2 mL of 100 $\mu$g/mL Hg(II) or CH$_3$HgCl. Dried at room temperature for at least 48h to simulate the soil matrix from Oak Ridge, TN.

- 20 $\mu$g Hg or CH$_3$HgCl spiked into 0.5 g soil (40 mg/kg)
Extraction

- Extraction with HCl and HNO$_3$ was attempted for selective extraction of MeHg from the soils.
- Extractions were performed via shaking of soil suspensions up to 24 h in HCl or HNO$_3$.
- Similar suspensions were subjected to ultrasounds using a sonic dismembrator equipped with Titanium probe for time intervals up to 6 minutes.
Effect of shaking

HCl extraction
- In-Hg was extracted substantially in dilute HCl with shaking. However, it was not extracted fully within 24-h of shaking in HNO₃.
- MeHg was extracted from soils in 5% HCl within 3-h shaking.

HNO₃ extraction
- Spiked MeHg was extracted in HNO₃ in 1 h of shaking into solution.
- In-Hg extraction was minimal in HNO₃.
Effect of ultrasounds

**HCl extraction**
- Ultrasound sonication in HCl and HNO₃ yielded similar results with shaking.

**HNO₃ extraction**
- Extraction was much faster. In 3 min sonication, all MeHg extracted into solution with 5% HNO₃.
More optimization needed for removal of extracted Hg(II)

Coprecipitation was attempted to remove residual Hg(II) from soil extracts

- Coprecipitating agents: bismuth (III), lanthanum (III), iron (III) and ammonium sulfide
- Complexing agents: L-cysteine and thiourea
- Precipitation of 100 ppb Hg(II) or CH$_3$HgCl was made in 1000 ppm La(III), Bi(III) or Fe(III) with or without L-cysteine and thiourea. Metal hydroxides were precipitated with triethylamine (TEA)
- Separate coprecipitations were made with ammonium sulfide, (NH$_4$)$_2$S.

100 µL TEA
100 ppb Hg(II) or CH$_3$HgCl + 1000 ppm La(III), Bi(III) or Fe(III)

Hg or MeHg by ICP-MS
### Coprecipitation trials without ligands

<table>
<thead>
<tr>
<th>Matrix/Precipitant</th>
<th>Recovery (%)</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MeHg</td>
<td>Hg(II)</td>
<td></td>
</tr>
<tr>
<td>1000 µg/mL La(III)</td>
<td>2.7 ± 1.2</td>
<td>7.6 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>1000 µg/mL Bi(III)</td>
<td>6.3 ± 1.9</td>
<td>5.8 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>1000 µg/m Fe(III)</td>
<td>8.8 ± 1.0</td>
<td>22.8 ± 5.2</td>
<td></td>
</tr>
</tbody>
</table>

Fe(III) removed the most Hg(II) from solution without impacting CH$_3$HgCl concentration. Coprecipitation of Hg(II) with Bi(OH)$_3$ and La(OH)$_3$ provided very little Hg(II) recovery.
Thiourea and L-cysteine were made to 0.1% (m/v) in 2 mL solution of 100 μg/L CH₃HgCl or Hg(II) and 1000 μg/mL La(III), Bi(III) or Fe(III). The contents were precipitated with 0.2 mL TEA.

<table>
<thead>
<tr>
<th>Matrix/precipitant</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MeHg</td>
</tr>
<tr>
<td>0.1% L-cysteine</td>
<td>2.5 ± 0.7</td>
</tr>
<tr>
<td>1000 μg/mL La(III) + 0.1% L-cysteine</td>
<td>11.6 ± 3.8</td>
</tr>
<tr>
<td>1000 μg/mL Bi(III) + 0.1% L-cysteine</td>
<td>1.8 ± 0.7</td>
</tr>
<tr>
<td>1000 μg/mL Fe(III) + 0.1% L-cysteine</td>
<td>7.6 ± 3.5</td>
</tr>
<tr>
<td>0.1% Thiourea</td>
<td>12.8 ± 1.8</td>
</tr>
<tr>
<td>1000 μg/mL La(III) + 0.1% Thiourea</td>
<td>41.1 ± 2.8</td>
</tr>
<tr>
<td>1000 μg/mL Bi(III) + 0.1% Thiourea</td>
<td>67.2 ± 1.0</td>
</tr>
<tr>
<td>1000 μg/mL Fe(III) + 0.1% Thiourea</td>
<td>24.8 ± 9.1</td>
</tr>
<tr>
<td>0.005 M Ammonium sulfide*</td>
<td>102 ± 3</td>
</tr>
</tbody>
</table>
La(III) + L-cysteine yielded the most effective for separation of CH$_3$Hg from Hg(II). However, CH$_3$Hg concentration in solution was reduced by about 12%.

Thiourea gave very sporadic recoveries with Fe(III), Bi(III) and La(III). Highest and most selective precipitation enhancement being with that of Bi(III).
Coprecipitation with Ammonium Sulfide

- Attempts were made to selectively remove Hg(II) from soil with ammonium sulfide, (NH₄)_2S.
- 25 to 30 μL of 0.35 M (NH₄)_2S was added to 2 mL test solutions that contained 100 μg/L CH₃Hg or Hg(II).
- After centrifugation, supernatant was analyzed by ICP-MS because HgS is very insoluble, even in concentrated acids.
- Recovery of MeHg was quantitative. Hg(II) was effectively eliminated.
Optimization of cold vapor generation

Cold vapor generation manifold and ICP-MS instrument for determination of CH₃Hg
Examining Acid Concentration in CVG System

- For both CH$_3$HgCl and Hg(II), CVG signals increased with HNO$_3$ concentration, and gained consistency at around 4% HNO$_3$.

- This is an indication that no modifications need to be done to mercury suspensions prior to determination with CVG-ICP-MS.

- SnCl$_2$ did not affect CH$_3$Hg at all, but performed similarly to NaBH$_4$ for vapor generation from Hg(II).
Examining reducing agent in CVG System

- NaBH$_4$ and SnCl$_2$ were examined on Hg(II) and CH$_3$HgCl in 5% (v/v) HNO$_3$.
- There was no notable vapor generation of CH$_3$HgCl, and none at all proceeding 3% of SnCl$_2$, whereas Hg(II) signals showed a steady pattern at 0.5% (m/v) SnCl$_2$.
- 0.5 and 1% (m/v) NaBH$_4$ gave max readings for both CH$_3$HgCl and Hg(II).
- Signals decreased with increasing NaBH$_4$ levels which generates excessive H$_2$ that changed sampling position in the plasma.
When compared to CH$_3$HgCl in 5% HNO$_3$ with 1% NaBH$_4$:

La(III) + L-cysteine showed no significant interference on MeHg CVG system.

Ammonium sulfide showed some signal depression, but it was not significant.
Calibration curves constructed with CH$_3$HgCl solutions prepared in 5% HNO$_3$ with La(III) + L-cysteine and 0.005 M (NH$_4$)$_2$S additives. La(III) + L-cysteine and (NH$_4$)$_2$S media showed similar calibration slopes to that of MeHg solutions in 5% HNO$_3$.
The highest LODs were from La(III) + L-cysteine system due to the high background blanks.

LODs for ammonium sulfide were similar to those with 5% HNO₃.

<table>
<thead>
<tr>
<th>Medium</th>
<th>200Hg</th>
<th>201Hg</th>
<th>202Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% HNO₃</td>
<td>0.082</td>
<td>0.09</td>
<td>0.085</td>
</tr>
<tr>
<td>La (III) + L-cysteine</td>
<td>0.41</td>
<td>0.38</td>
<td>0.42</td>
</tr>
<tr>
<td>Ammonium sulfide</td>
<td>0.12</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>
Method validation

<table>
<thead>
<tr>
<th>Sample</th>
<th>CH$_3$Hg concentration (ng/g)</th>
<th>Certified value (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^{200}$Hg</td>
<td>$^{201}$Hg</td>
</tr>
<tr>
<td>SQC1238</td>
<td>13.0 ± 3</td>
<td>13.2 ± 3</td>
</tr>
<tr>
<td>ERM – CC580</td>
<td>81 ± 7</td>
<td>79 ± 8</td>
</tr>
</tbody>
</table>

- **Reference materials SQC1238 (methylmercury sediment)**
  \[\text{CH}_3\text{Hg} = 0.01 \pm 0.00291 \ \mu g/g\]
- **ERM – CC580 (estuarine sediment)**
  \[\text{Total Hg} = 132 \pm 4 \ \mu g/g \ \text{and} \ \text{CH}_3\text{Hg} = 0.075 \pm 0.004 \ \mu g/g\]

- Samples (0.2 g for SQC1238 and 0.1 g for ERM-C580) were agitated via ultrasound for 3 min in 5% HNO$_3$ (5 mL).
- 2 mL of the extract was then taken and treated with 30 µL of 0.35 M (NH$_4$)$_2$S for removal of Hg(II).
- Contents were centrifuged at 12,000 rpm for 20 min, then transferred to 2-mL micro-centrifuge tubes and analyzed by CVG-ICP-MS.
Soils from Oak Ridge TN were processed with optimized method, and analyzed along with SRMs.

CH$_3$Hg levels ranged from 0.030 to 0.051 µg/g. CH$_3$Hg distribution in the floodplain soils were much lower compared with Hg(II) or total Hg levels.

CH$_3$Hg concentrations are within the proximity of regulatory limit (0.051 µg/g).

<table>
<thead>
<tr>
<th>Sample</th>
<th>CH$_3$Hg concentration (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^{200}$Hg</td>
</tr>
<tr>
<td>Soil 1</td>
<td>0.043 ± 0.006</td>
</tr>
<tr>
<td>Soil 2</td>
<td>0.040 ±0.008</td>
</tr>
<tr>
<td>Soil 3</td>
<td>0.040 ± 0.012</td>
</tr>
<tr>
<td>Soil 4</td>
<td>0.032 ± 0.003</td>
</tr>
<tr>
<td>Soil 5</td>
<td>0.032 ± 0.009</td>
</tr>
<tr>
<td>Soil 6</td>
<td>0.050 ± 0.01</td>
</tr>
<tr>
<td>Soil 7</td>
<td>0.046 ± 0.005</td>
</tr>
<tr>
<td>Soil 8</td>
<td>0.029 ± 0.002</td>
</tr>
<tr>
<td>Soil 9</td>
<td>0.033 ± 0.009</td>
</tr>
<tr>
<td>Soil 10</td>
<td>0.042 ± 0.011</td>
</tr>
</tbody>
</table>
Conclusions

• The method developed for selective extraction of MeHg from contaminated sediments provides rapid extraction and high selectivity with effective removal of Hg(II) prior to determination.

• Ultrasounds extraction in HNO₃ and further sample treatment with ammonium sulfide precipitation allowed the highest selectivity for accurate determination of CH₃Hg with cold vapor generation.

• Results show that there is MeHg in the contaminated soil from the Y-12 National security complex.

• The distribution of MeHg in the topsoil in the contaminated regions are at the threshold of regulatory limits of 0.051 µg/g.
Acknowledgements

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