**Introduction**

In the US, seafood consumed by the population account for more than >90% of the exposure to neurotoxic mercury. To regulate mercury consumption it is essential to understand the bioaccumulation of mercury in fish and shellfish. Methylmercury is the most toxic and highly bioaccumulative form of mercury. We studied the molecular speciation of Hg (i.e., inorganic mercury (InHg) and methylmercury (MeHg)) as well as the Mass Dependent Fractionation (MDF) and Mass Independent Fractionation (MIF) of Hg isotopes in fish skeletal muscle and liver tissue. MDF has been found to occur in kinetic processes such as volatilization, evaporation, and biotic reduction, however significant MIF has been observed mostly in photochemical reactions. Thus, comparing both tissues of the same fish in terms of molecular speciation (InHg, MeHg) and Hg isotopic composition (MDF, MIF) might help to unravel the bioaccumulation of Hg within fish, which may provide better understanding of the mercury cycle in aquatic ecosystems, something that hasn’t been done before.

**Procedure**

- **Specimen Preparation**
  - Livers and muscles of the grouper were freeze-dried, then isolated from each other and subsequently ground using an agate mortar.
  - **Digestion for Hg Speciation Analysis (MeHg, InHg)**
    - Sample was digested in 8 M HNO₃ and baked for 8 hours in a 60°C oven.
    - Each sample was diluted with DI water and filtered.
  - **Tekran2700 Mercury Analyzer**
    - Tekran vials were filled with 25 mL of DI water.
    - Sodium acetate (NaAc-HAc) 2 M buffer solution was added to each vial.
    - Species extract was added to each vial and diluted with DI water.
    - The pH of the solution in the vial was measured to ensure it ranged between 4.3-4.7.
  - **Sodium tetrahydroborate (NaBH₄) was added to each vial (derivationization that volatilize Hg species by adding an ethyl group).**
  - Samples were analyzed for mercury speciation using the Tekran2700 mercury analyzer.

- **Second Digestion for Total Mineralization**
  - Samples were digested in reverse Aqua Regia (1:4 HCl:HNO₃, v/v) and left overnight.
  - Samples were heated to 80°C for 4 hours on a hot plate after being diluted with DI water.
  - Once cooled BRCI was added to each vial to ensure conversion of all Hg to Hg(II).

- **Neptune MC-ICP-MS**
  - Samples and standard solutions were prepared in the same matrix (12% AR + 5% BRCI).
  - Before analysis Hydroxylamine was added to each sample to neutralize excess BRCI.
  - Samples were bracketed with international standard (NIST1131) to report mercury isotopic composition allowing comparison with literature.
  - A secondary standard (YM-ALMADEN) was measured periodically to check accuracy and precision of the measurements.

**Data and Results**

**FIGURE 1. North East region of the Gulf of Mexico, region where specimens were caught**

**FIGURE 2. HGA-200 Advance Membrane Cold Vapor and Hydride Generation System, sample test tubes, wash process, and peristaltic pumps**

**FIGURE 3(a). Percent of MeHg and InHg in grouper fish liver tissue**

**FIGURE 3(b). Percent of MeHg and InHg in grouper fish skeletal muscle tissue**

**FIGURE 4(a). δ²⁰⁹Hg vs. δ²⁰⁷Hg for muscle and liver tissue of grouper showing MDF for these isotopes; line is calculated isotopic compositions based on exponential mass fractionation law.**

**FIGURE 4(b). δ²⁰⁹Hg vs. δ²⁰⁷Hg for muscle and liver tissue of grouper; line is calculated isotopic compositions based on MDF only. Deviation from the line is magnitude of MIF (δ²⁰⁷Hg).**

**FIGURE 5(a). δ²⁰⁹Hg, which represents MIF, versus %MeHg in grouper fish skeletal muscle and liver tissue**

**FIGURE 5(b). δ²⁰⁷Hg, which represents MDF, versus %MeHg in grouper fish skeletal muscle and liver tissue**

**REFERENCES**


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