

Chapter 2

Analysis of Mercury Species in the Environmental Samples

Abstract Accurate analysis of mercury (Hg) species is the basis of understanding fate and behavior of Hg in aquatic environment. In this chapter, different Hg speciation systems were introduced according to analytical theory or research aim. In this chapter, we elucidate the detailed procedures, including the sample collection, preparation, and analytical methods in water, sediment, plankton, and sediment employed in this study.

Keywords Mercury · Species · Measurement · Water · Sediment · Plankton · Fish

2.1 Mercury Speciation in Water

2.1.1 Definition of Mercury Species

Mercury (Hg) widely exists in nature, including the lithosphere, pedosphere, hydrosphere, biosphere, and atmosphere. Hg has three chemical states (0, +1, +2) and exists as elemental, inorganic, and organic Hg in the environment. Concentrations of Hg usually represent much lower levels than other heavy metals in the natural environment, like Cu, Cd, Pb. etc. It is a great challenge to accurately analyze the trace level Hg species in samples, such as uncontaminated water samples, which hampered the understanding of the Hg biogeochemical cycle and the estimation of the potential Hg exposure in aquatic food chain. With the improvement of sensitive, specific, and precise Hg species analysis method, it appears that the data of Hg in natural waters before the 1980s are not reliable. High concentration of Hg in water samples was caused by contamination during the sample collection or pretreatment. Furthermore, trace level Hg species in natural water sample could not be measured since the Lowest Detection Limit of Method for Hg is not good enough (Bloom and Fitzgerald 1988; Yan et al. 2005a).

Biogeochemical cycling of Hg in aquatic ecosystems involves the distribution, transportation, and transformation of Hg in sediment, water, sediment/water

interface, water/air interface, phytoplankton, zooplankton, shellfish, fish, etc. Elemental Hg (Hg^0), divalent Hg (Hg^{2+}), and methylmercury (MeHg) are the main concerned Hg species in aquatic ecosystem. Hg^0 mainly exists in the atmosphere due to its high volatility, and accounts for more than 95% of Hg in the atmosphere. The Hg^0 exchange between water/air interface is a key transportation process between the two large Hg pools, including both emission and deposition process. The deposition of Hg from the atmosphere to the water includes wet deposition and dry deposition. The deposition can directly input into water surface or input from the watershed to the water body as runoff after it deposited on the land. Divalent Hg (Hg^{2+}) is the main fraction of Hg in water, which is regarded as high activity, named as reactive Hg (RHg). MeHg is the most concerned species due to its high toxicity, bioaccumulation, and biomagnification through the food chains. Generally, MeHg is the predominant form of Hg in fish tissues. MeHg is formed from inorganic Hg (such as Hg^0 , Hg^{2+} , etc.) via methylation usually involving with bacteria. Sediment is the pool of Hg in aquatic system and its anaerobic environment favors the methylation of Hg. The diffusion of Hg^{2+} and MeHg between sediment/water interface is the key transportation process from sediment to water body.

In chemistry, speciation analysis refers to the analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample (IUPAC 1997). For Hg, the speciation analysis includes both chemical defined species, like MeHg, ethylmercury (EtHg), etc., and operationally defined species, like dissolved Hg (DHg), reactive Hg (RHg), etc. In general, the analytic species are first extracted from the sample matrix, then following separation of Hg species, and detected by an appropriate detector. With regard to different sample media, acid or alkaline digestion technique will be processed before organic solvent extraction. Chromatographic technique is often applied for the separation of different chemical forms, such as high-performance liquid chromatography (HPLC), gas chromatography (GC), supercritical Fluid chromatography (SFE), or capillary zone electrophoresis (CZE). A number of detection methods are available, but its sensitivity, multielemental capability, and the possibility of isotopic information make inductively coupled plasma mass spectrometry (ICP-MS) the detector of first choice. Hg detectors usually include cold vapor atomic absorption spectroscopy (CVAAS), ICP-MS, cold vapor atomic fluorescence spectrometry (CVAFS), and atomic emission spectroscopy (AES), in which CVAFS is most popular due to its high sensitivity and low cost.

In this chapter, the analysis of Hg species in water, sediment, and biota is reviewed, including the cleaning procedure of sampling vessels or equipment, sample collection, storage, pretreatment, analytical methods, etc.

There are various Hg species (Fig. 2.1) in natural water in river, lake, reservoir, etc. In chemistry, Hg can be categorized into inorganic Hg and organic Hg. Inorganic Hg includes elemental Hg, divalent Hg (Hg^{2+}), monovalent Hg, which is not stable and easily transforms into elemental Hg and divalent Hg through disproportionation reaction. Organic Hg includes methylmercury (MeHg), dimethylmercury (DMeHg), ethylmercury (EtHg), phenylmercury (PhHg), etc.

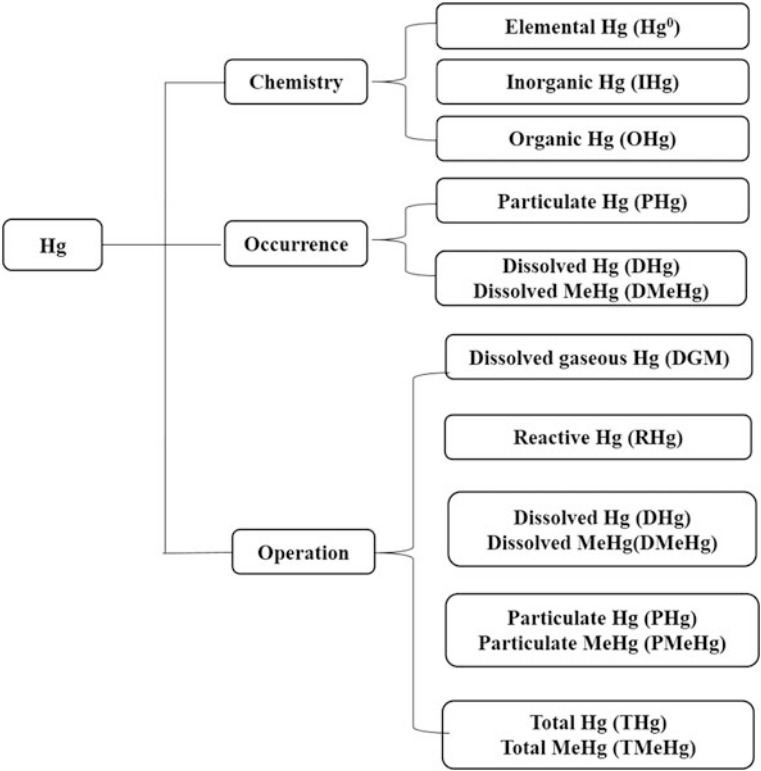


Fig. 2.1 Hg speciation in natural water

In water, the dissolved Hg^0 is defined as the Hg trapped by N_2 bubbling, named as dissolved gaseous mercury (DGM). Hg^{2+} is operationally defined as SnCl_2 reduced Hg, named reactive mercury (RHg) (Lindqvist et al. 1991). MeHg is the most concerned species in water, which is easily bioaccumulated in food chain. The concentration of DMeHg, EtHg, and PhHg in water is much lower than MeHg, and they are not measured so popular and only reported in a few references in deep sea and wetland systems (Cai et al. 1996; Cossa et al. 1994; Wallschlager et al. 1995).

According to the operation definition of filtration, Hg in water was operationally defined as total mercury (THg), soluble Hg (dissolved Hg, DHg), and particulate Hg (PHg). Accordingly, MeHg in water is categorized into total methylmercury (TMeHg), dissolved methylmercury (DMeHg), and particulate methylmercury (PMeHg).

2.1.2 Sample Collection Program in River and Reservoirs

1. Cleaning procedure of vessels and equipment

Due to the extremely low mercury concentration (ppt and sub-ppt level) in natural water, quality control of every step including blank of sampling vessels, sampling equipment, sample collection, storage, and determination is vital to get accurate measurement of Hg. It is quite essential to do ultra-clean treatment during the preparation of sampling bottle and sampling equipment.

For Hg measurement, collection of water samples should be bottled with fluoropolymer or borosilicate glass. For water sample filtering, borosilicate glass filter device was employed. Great caution is needed during the cleaning of fluoropolymer bottle or glass bottle to avoid any kind of contamination.

All of the borosilicate glass sampling bottle or filtration device are subjected to a strict ultra-clean treatment before sampling. The processes are as follows:

- (1) Cleaning: cleaning the bottle with tap water with detergent.
- (2) Acid soaking: soaking the bottle in 10% HNO_3 (V/V) at least 24 h.
- (3) Rinsing: first rinsing the bottle or device from the acid with tap water, and then rinsing with double-distilled water (DDW).
- (4) Baking: Putting the bottle or device into muffle furnace, keeping the temperature at 500 °C at least 1 h.
- (5) Storage: Sealing the bottle or device with double-layer polyethylene bags.

If fluoropolymer bottle is used, the cleaning procedure should follow EPA Method 1631E.

- (1) Keep the bottle in hot 4 N HCl for more than 48 h.
- (2) After cooling and rinsed with DDW, the bottle was filled with 1% HCl in an oven at 60–70 °C overnight.
- (3) After cooling and rinsed with DDW, the bottle was filled with 0.4% (V/V) HCl.

After cleaning, the bottle is tightly capped (with a wrench), double bagged in new polyethylene zip bags and stored in wooden or plastic boxes.

2. Sampling method for atmospheric deposition

A bulk precipitation sampler was designed based on the version of the collector used by European countries (Commission OaP 1997). It is demonstrated that there is no significant difference (at the 10% level) between co-located bulk and wet deposition samples for THg (Landing et al. 1998).

To reduce the Hg volatilization in the precipitation samples, the precipitation sample should avoid the exposure to air and sunlight as less as possible. The sampling train consisted of three borosilicate glass components (Fig. 2.2), a funnel (~15 cm diameter), a connecting tube (~2 cm diameter), and a sampling bottle (~1 L volume). The connecting tube plays a role of capillary to prevent the volatilization of Hg in the precipitation sample. The connecting tube and

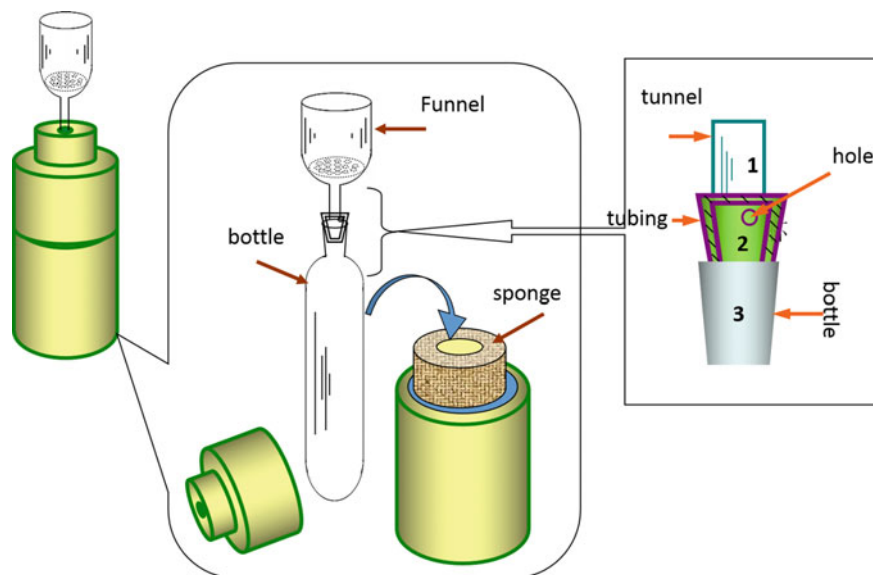


Fig. 2.2 Sampling equipment for wet deposition

the sampling bottle were placed inside a PVC column which was filled with sponge to be shielded from sunlight.

The cleaning of the sampling train components follows the cleaning procedure for borosilicate glass bottles. The sample collector was mounted on the roof of houses approximately 1.5 m above the ground to avoid contamination from soil particles by splashing during heavy rainfall.

3. Water sample sampling method

Water samples of river reservoirs system were collected using an acid-washed, Teflon-coated, 10 L Niskin sampler (Model 1010X series, General Oceanics Inc. U.S.A.). The Niskin bottle was positioned in the upstream direction relative to the operator. All samples were collected following ultra-clean sample handling protocols. Water samples were transferred from the sampler into pre-cleaned bottles. Each bottle was rinsed three times with reservoir/river water before sample collection.

Unfiltered samples were packed into double layer of zip bags for the analysis of THg, RHg, TMeHg, and total suspended solid (TSS) in lab. Water samples were filtered on site using a 0.45 μm filter (Millipore) to get dissolved water samples for analysis of DHg and DMeHg. All the water samples were acidified on site to 0.4% (v/v) with ultra-pure concentrated hydrochloric acid (HCl). The sample bottles were then capped, sealed with Parafilm, and stored in a refrigerator at 3–4 °C in the dark. The analysis of concentrations of Hg species in water samples was conducted within 28 d after sampling.

2.1.3 Analysis Total Mercury in Water Samples

Basically, the analysis of THg and inorganic mercury in water samples followed US EPA Method 1631 (US EPA 2001b). For RHg analysis, take an aliquot of unfiltered water samples into bubbler with 50 mL DDW, 0.1 mL HNO_3 and 0.1 mL of 20% SnCl_2 . Then purge with $300 \text{ mL} \cdot \text{min}^{-1} \text{ N}_2$ for 20 min to collect RHg by gold trap. Finally, connect the gold trap with analysis system, RHg will be analyzed by due amalgamation combined with CVAFS.

Analysis of DHg and THg in water followed a same method. In 100 mL of water samples, add 0.5 mL BrCl at room temperature for 24 h. Just prior to analysis, 0.2 mL 20% NH_2OHHCl were added into the sample bottle to remove the excess amount of BrCl . The sample was further reduced with 0.1 mL 20% SnCl_2 , purged with N_2 , the formed elemental Hg vapor (Hg^0) was trapped by gold amalgamation, and finally thermally desorbed and analyzed by dual amalgamation combined with CVAFS. PHg was calculated as the difference between the concentration of THg and DHg in the sample.

2.1.4 Analysis of Methylmercury in Water Samples

The analysis of MeHg in water followed the distillation- NaBeT_4 ethylation-Tenax trap-GC-CVAFS as EPA Method 1630 (US EPA 2001a). MeHg analysis in water samples based on distillation of CH_3HgCl , which is formed by adding 0.4% HCl into water samples during the sample collection. For distillation, 45 mL of water sample and 200 μL of 1% APDC was placed into a fluoropolymer distillation vial. For the distillate receiving vial, 5.0 mL of reagent water and 200 μL of acetate buffer was added. The distillation vial was in an Al-heating block at 125 with purging with $60 \text{ mL} \cdot \text{min}^{-1} \text{ N}_2$. The receiving vial was kept at about 4 °C in a water-ice bath.

Distilled water samples were added into bubblers for ethylation by adding 50 μL 1% NaBeT_4 . MeHg were collected onto Tenax trap by purging the solution with $300 \text{ mL} \cdot \text{min}^{-1} \text{ N}_2$. Then Tenax trap was heated to release MeHg into the carrier gas and MeHg finally was analyzed by GC-CVAFS. Analysis of TMeHg and DMeHg in water samples was following the same method. PMeHg was calculated as the difference between the concentrations of TMeHg and DMeHg in the sample.

2.2 Analysis of Mercury Species in Sediment Samples

2.2.1 Sample Collection

Sediment samples were collected by a gravity corer with a 6.3 cm diameter 64 cm long plexiglass tube. The overlying water in the core tube was collected into acid-cleaned borosilicate glass bottle by siphoning down to about 10–20 cm above the sediment surface. Sediment cores were sectioned every 1 or 2 cm under N₂ atmosphere in a glove bag. Sediment samples were placed in centrifuge tubes, capped and sealed with parafilm.

In the lab, sediment samples were centrifuged at 3500 rpm for 25 min at 4 °C. After being filtered through 0.45 µm membrane filter (Millipore), pore water samples were preserved with 0.4% (V/V) HCl in acid clean borosilicate glass tubes and stored in a refrigerator at 3–4 °C in the dark until DMeHg and DHg analysis. Field blanks were also prepared by adding Milli-Q water in sampling tubes. Subsequently, the freeze-dried sediment samples were ground and homogenized to a size of 150 meshes per inch with a mortar for solid-phase THg, MeHg, and organic matter (OM) concentration analysis.

2.2.2 Total Mercury Analysis in Sediment Samples

THg measurement in sediments was performed by reduction with SnCl₂ following oxidation by acid digestion (Li et al. 2005). Sediment samples were freeze-dried and ground before analysis. The digestion procedure required approximately a 0.20 g dry weight sample. The sample was placed inside a 25 mL glass tube covered with a glass ball, and 5 mL DDW and 5 mL aqua regia were added in turn, digested at 95 °C for 5 min. After that, 1 mL BrCl solution was added continuously to digest at 95 °C for 30 min. After cooling, the sample reacted for an additional 24 h and 0.2 mL NH₂OHHCl solution (25 g of reagent grade NH₂OHHCl is dissolved in 100 mL DDW) was added in the sample. Then, THg was determined by CVAFS. The lowest limit of detection was 0.01 ng g⁻¹ for THg analysis, which was calculated based on 3 times the standard deviation of blank measurements. Quality control for the THg determinations was addressed with method blanks, blank spikes, matrix spikes, certified reference materials, and blind duplicates.

2.2.3 Methylmercury Analysis in Sediment Samples

MeHg in sediment samples were analyzed following the method of He et al. (2004). Approximately 0.3 g of sediment was placed into a 50 mL centrifuge tube. 1.5 mL of 1 M CuSO₄, 7.5 mL of 3 M HNO₃, and 10 mL of CH₂Cl₂ were added. The tube

was closed and shaken for 30 min. 5 mL of the CH_2Cl_2 layer was pipetted into another 50 mL centrifuge tube after the tube was centrifuged at 3000 rpm for 30 min. About 40 mL of double-deionized water was added to the tube. The tube was heated at 45 °C in a water bath until no visible solvent was left in the tube and the remaining liquid was then purged with nitrogen for 8 min in a water bath at 80 °C to remove solvent residue. The sample was brought to 50 mL with double-deionized water before an appropriate volume (generally 15 mL) of the sample was transferred to a borosilicate bubbler for MeHg analysis following the procedure described previously. Quality control for the MeHg determinations was addressed with method blanks, blank spikes, matrix spikes, certified reference materials, and blind duplicates.

2.3 Analysis of Mercury Species in Plankton and Fish

Hg transport and accumulation in aquatic ecosystems is mainly through the food chain. Due to the biomagnification effect of Hg in the food chain, Hg levels are low at the bottom of the food chain and in aquatic organisms of relatively small age. As the trophic level increases, aquatic organisms absorb Hg into the body through ingestion, mainly MeHg, and progressively with trophic levels. In order to study the enrichment characteristics of Hg in foodstuffs in the reservoirs of different evolution stages, the dominant aquatic species of different trophic levels were collected from upper to lower reaches of the Wujiang River for Hg and MeHg analysis.

2.3.1 Sample Collection

1. Plankton

Phytoplankton is an ecological concept that refers to the life of small plants floating in the water, usually refers to the phytoplankton, mainly including Cyanophyta, Bacillariophyta, Chrysophyta, Xanthophyta, Pyrrophyta, Cryptophyta, Euglenophyta, and Chlorophyta. Phytoplankton is in the first trophic level in the aquatic food chain.

The phytoplankton was collected by plankton net with different pore size, and finally classified according to their size. Phytoplankton was fixed with formalin 3–5% in the field and identified and enumerated (random fields) under the microscope using the settling technique in the laboratory. In addition, the cells colonies and filaments were enumerated to at least 300 specimens of the combined specie (Li et al. 2014).

According to size difference, zooplankton can be divided into macrozooplankton, mesozooplankton, and microzooplankton. Zooplankton has a wide range of feeding behavior: filter feeding, predation, and symbiosis with autotrophic

phytoplankton as seen in corals. Zooplankton feed on bacterioplankton, phytoplankton, other zooplankton, detritus and even nektonic organisms.

Zooplankton samples were collected using a conical net of 64 μm mesh. The organisms were rinsed with filtered (0.45 μm porosity) reservoir water to remove adhered particulate matter and filtered through Nitex sieves of 610, 216, 108, and 38 μm mesh size to obtain four size fractions: 38–108, 108–216, 216–610, and >610 μm . Samples collected for zooplankton identification as well as for Hg and MeHg determination were separated into four fractions as described above. Samples for zooplankton identification were stored in vials and fixed in 4% formalin solution, while samples for THg and MeHg analyses were kept frozen until lyophilization, then again stored frozen for analysis (Wang et al. 2011).

They were taken back to the laboratory for freeze-drying and used to determine THg, MeHg, carbon (C) and nitrogen (N) isotopic composition. Samples of C and N isotopes were freeze-dried and ground through a 60 mesh nylon sieve. The samples were wrapped in aluminum foil and packed in a centrifuge tube and sealed in a dry dish.

2. Fish

The fish was collected from the fisherman in each reservoir. The number of fish species and the number of specimens sampled for each species were limited by availability of fish during the sampling campaign. All fish were visually inspected for fin and body deformations to avoid farmed fish in the samples. The collected fish were stored alive in barrels with water and air purge, or dead on ice in freeze boxes until sampling in the lab. The fish collected may not represent all species in the reservoirs, but the most abundant ones should be included.

The weight and length of the fish were measured before a sample of the dorsal muscle was removed and stored frozen for Hg analysis. The length and weight of the fish were recorded before collecting the fish muscle. About 20 g of muscle in the dorsal muscle of the fish was collected and wrapped in a tin foil and stored in a zip bag at $-18\text{ }^{\circ}\text{C}$. Fish scales are used to identify their age, fish without scales, or partial scales, and their otoliths are age-matched.

2.3.2 Total Mercury Analysis in Plankton and Fish Samples

All samples were analyzed for THg following the method of acid digestion, SnCl_2 reduction, gold trap collection, and CVAFS. About 0.5–1.0 g of fresh muscle tissue was added into a 25 mL glass tube covered with a glass ball, and 10 mL of HNO_3 : H_2SO_4 (8:2, V/V) were added in turn, digested at $95\text{ }^{\circ}\text{C}$ for 3 h. After the samples cooled down, add appropriate volume of DDW to 25 mL. Then 0.5 mL BrCl were added for 24 h. Before analysis, 0.2 mL 25% $\text{NH}_2\text{OH}\cdot\text{HCl}$ were added to remove excess of BrCl . An aliquot of the digestate were taken for Hg analysis by SnCl_2 , gold trap collection, and CVAFS following EPA Method 1631 (US EPA 2001b).

Quality control consisted of duplicates, method blanks, and standard reference material. Blank spikes and duplicates were taken regularly (>10% of samples) throughout each sampling process. The analysis of THg in plankton is following the similar method with fish THg analysis, but 0.10–0.20 g of dry samples was taken.

2.3.3 Methylmercury Analysis in Plankton and Fish Samples

About 0.5–1.0 g of fresh fish samples and 5 mL of 20% KOH were added into 25 mL fluoropolymer vials. Then the fluoropolymer bottles were kept at 75 °C in a water bath for 3 h. An aliquot of fish sample digestate were taken for MeHg analysis by aqueous ethylation, Tenax trap, GC-CVAFS following US EPA (2001a) and Yan et al. (2005b). Analysis of MeHg in plankton is similar with fish samples, but only about 5–10 mg of dry sample was taken for digestion. Quality control consisted of duplicates, method blanks, and standard reference material. Blank spikes and duplicates were taken regularly (>10% of samples) throughout each sampling process.

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