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Tracing aquatic bioavailable Hg in three different regions of China using fish Hg isotopes

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ABSTRACT

To trace the most concerned bioavailable mercury (Hg) in aquatic environment, fish samples were collected from three typical regions in China, including 3 rivers and 1 lake in the Tibetan Plateau (TP, a high altitude background region with strong solar radiation), the Three Gorges Reservoir (TGR, the largest artificial freshwater reservoir in China), and the Chinese Bohai Sea (CBS, a heavily human-impacted semi-enclosed sea). The Hg isotopic compositions in fish muscles were analyzed. The results showed that anthropogenic emissions were the main sources of Hg in fish from TGR and CBS because of the observed negative $\delta^{202}Hg$ and positive $\Delta^{199}Hg$ in these two regions (TGR, $\delta^{202}Hg$: − 0.72 to − 0.29‰, $\Delta^{199}Hg$: 0.15 – 0.52‰; CBS, $\delta^{202}Hg$: − 2.09 to − 0.86‰, $\Delta^{199}Hg$: 0.07 – 0.52‰). The relatively higher $\delta^{202}Hg$ and $\Delta^{199}Hg$ ($\delta^{202}Hg$: − 0.37 – 0.08‰, $\Delta^{199}Hg$: 0.50 – 1.89‰) in fish from TP suggested the insignificant disturbance from local anthropogenic activities. The larger slopes of $\Delta^{199}Hg/\Delta^{201}Hg$ in fish from TGR (1.29 ± 0.14, 1 SD) and TP (1.25 ± 0.06, 1 SD) indicated methylmercury (MeHg) was produced and photo-reduced in the water column before incorporation into the fish. In contrast, the photoreduction of Hg^{2+} was the main process in CBS (slope of $\Delta^{199}Hg/\Delta^{201}Hg$: 1.06 ± 0.06, 1 SD). According to the fingerprint data of Hg isotopes, the most important source for aquatic bioavailable Hg in TP should be the long-range transported Hg, contrasting to the anthropogenic originated MeHg from surface sediments and runoffs in TGR and inorganic Hg from continental inputs in CBS. Therefore, the isotopic signatures of Hg in fish can provide novel clues in tracing sources and behaviors of bioavailable Hg in aquatic systems, which are critical for further understanding the biogeochemical cycling of Hg.

1. Introduction

As a global pollutant, mercury (Hg) can transport long distances in the atmosphere posing risks to human health and wildlife (Bergquist and Blum, 2007; Jiang et al., 2006; Kalinchuk et al., 2017). In aquatic systems, inorganic Hg can be biologically and chemically transformed into methylmercury (MeHg), a more toxic and bioavailable Hg species (Hsu-Kim et al., 2013; Lin et al., 2015; Wang et al., 2013; Yin et al., 2014). Worse, MeHg can be easily accumulated and magnified by fish (Harris et al., 2007; Pickhardt and Fisher, 2007; Zhang et al., 2014; Pouilly et al., 2012). In the case of human beings, the consumption of fish becomes the main exposure pathway to MeHg (Harris et al., 2003; Gochfeld, 2003; Li et al., 2011). Therefore, tracing the sources and

behaviors of bioavailable Hg is of great value to assess the risks of Hg in aquatic environment.

In the last decade, Hg isotopic tracing method has been conducted on various environmental compartments. Both mass-dependent fractionation (MDF) and mass-independent fractionation (MIF) of Hg isotopes have been observed during the environmental processes of Hg (Bergquist and Blum, 2007; Sun et al., 2014; Lepak et al., 2015; Feng et al., 2010; Yin et al., 2013; Chen et al., 2016). The combination of MDF and MIF signatures inherited from the Hg sources and the shifts following environmental processes can provide multi-dimension information for source apportionments. In the particular case of MIF in odd Hg isotopes, it only shifts during specific photochemical processes (i.e., the photoreduction of inorganic Hg(II) and the

photodemethylation of MeHg) in natural environment, making the isotopic method suitable for evaluating the sources and unique biogeochemical processes of Hg (Bergquist and Blum, 2007; Rose et al., 2015; Blum et al., 2014; Lepak et al., 2015).

In natural water, the concentrations of Hg, especially the MeHg with higher bioavailability, are extremely low (Zhang and Wong, 2007; Wang et al., 2009; Guo et al., 2008), which makes it difficult to preconcentrate enough Hg for isotopic analysis. To date, only a few studies have reported the Hg isotopes in water samples (Chen et al., 2010, 2016, 2012; Foucher et al., 2013; Yuan et al., 2015). Although the Hg isotopic signatures in sediments have been successfully used for tracing the sources and behaviors of Hg (Feng et al., 2010; Yin et al., 2013; Chen et al., 2016; Lepak et al., 2015), most Hg in sediment is not bioavailable (Gochfeld, 2003; Shi et al., 2005). After methylation in aquatic systems, the produced MeHg could enter food chains by multiple pathways (*i.e.* uptake by benthos or plankton) more easily and be biomagnified in the habitant fish. Thus, the fish Hg isotopes may provide indicative information for tracing the bioavailable Hg in aquatic systems and, further understanding the biogeochemical cycling of Hg.

Hg isotopic signatures in different fish from certain sites have been reported to vary depending on the living conditions (*e.g.*, the habitat depth, the latitude and the diet differences) (Jackson et al., 2008; Gantner et al., 2009; Perrot et al., 2012). Kwon et al. (2015) used fish Hg isotopes to explore the potential MeHg sources and Hg transfer pathways between Douglas Lake and adjacent terrestrial systems. They concluded that ~ 20% MeHg was photo-demethylated prior to bioaccumulation by comparing the isotopic compositions in fish and sediments (Kwon et al., 2015). These studies implied the possibility of fish Hg isotopes in tracing the bioavailable Hg in aquatic systems. However, the environmental behaviors of Hg in different aquatic systems are complicated due to the various physicochemical conditions and anthropogenic influences (Balogh et al., 2015; Tsui et al., 2014), which may perplex the efficiency of tracing Hg sources and processes in aquatic environment using Hg isotopes in fish.

In this work, we selected the aquatic systems in 3 distinct regions of China, including a high altitude plateau, the largest artificial freshwater reservoir, and a semi-enclosed sea with heavy anthropogenic impacts. The Hg isotopic compositions in the collected fish samples were measured and compared categorically to evaluate the application of fish isotopic method in investigating the sources and potential environmental processes of bioavailable Hg in these aquatic systems.

2. Materials and methods

2.1. Study area and sample collection

As shown in Fig. 1, different types of aquatic systems in three typical regions over large spatial scales in China were selected.

The Tibetan Plateau (TP), located in the southwest of China, is the highest plateau in the world with an average altitude of *ca.* 4000 m above sea level. It is also called the "Third Pole" or "Roof of the World" (Zhang et al., 2014; Shao et al., 2016a, 2016b; Xu et al., 2016; Ren et al., 1999; You et al., 2013). Despite being a remote and mostly non-industrialized region, very high concentrations of Hg have been observed in fish as a result of the long lifecycle of fish and the long-range atmospheric Hg transport recently (Zhang et al., 2014; Shao et al., 2016b). Furthermore, TP is the birthplace of the ten largest rivers in Asia, which provide drinking water for numerous people (Shao et al., 2016a; Huang et al., 2009).

The Three Gorges Reservoir (TGR), newly built in 2009 and covering more than 1080 km², is the largest man-made freshwater reservoir in China. The maximum water depth can reach 175 m in flood season (Li et al., 2015). TGR is situated at a region with Hg contamination via natural enrichment and anthropogenic emissions (Li et al., 2015; Feng and Qiu, 2008). Further, flooded conditions may promote the Hg methylation and mobilization, thereby potentially increasing the Hg levels in aquatic organisms (Bloom and Lasorsa, 1999; Zhao et al., 2016; Eggleton and Thomas, 2004; Blum et al., 2013).

The Chinese Bohai Sea (CBS) is a shallow, semi-enclosed and human-impacted coastal ecosystem in the northwestern Pacific. CBS covers an area of *ca.* 77,100 km², with a coastline length of *ca.* 3800 km, and a mean water depth of 18 m (Zou et al., 2011; Meng et al., 2015; Liu et al., 2016a; Bian et al., 2016). It is surrounded by 17 highly industrialized and urbanized cities and receives large amounts of freshwater from over 40 rivers (Liu et al., 2016a; Bian et al., 2016). The presence of ship-traffic and developed industry in the CBS region resulted in relatively high concentrations of heavy metals such as Hg and persistent organic pollutants (Meng et al., 2015; Liu et al., 2016a). In addition, this region is a major source of fish for consumers and the overfishing phenomenon has been reported previously, which can pose latent exposure risks after the fish consumption by human (Jin, 2004).

A total of 39 fish samples were collected from 2012 to 2015, including 23 samples from TP (9 from Lhasa River, 3 from Niyang River, 6 from Yarlung Zangbo River and 5 from Nam Co Lake), 6 samples from TGR, and 10 samples from CBS. After collection, fish samples were ice preserved in boxes and transported to the laboratory as soon as possible. The weight and length of all fish samples were measured (Table 1). Subsequently, the fish muscle samples were separated, freeze-dried, and homogenized with an agate mortar.

2.2. Analysis of total Hg

The total Hg (THg) concentrations were determined following the USEPA method 1631 after microwave digestion (Shao et al., 2016b; USEPA, 2002). Briefly, 0.35 g fish muscle samples were weighed into 50 mL Teflon® vials. Then, 3 mL concentrated HNO_3 (65%, v/v) and 3 mL H_2O_2 (30%, v/v) were added. All samples were pre-digested at 50 °C on a heating plate for 0.5 h. The vials were moved to a microwave digestion system and digested by programmed temperature (MARSX, CEM, USA). THg concentrations of all digestion solutions were determined by MERX Automatic Total Mercury Analytical System (Brooks Rand Lab, USA). All the THg concentrations mentioned in this work were shown as ng/g (dry weight).

2.3. Analysis of Hg stable isotope ratios by MC-ICP-MS

Hg stable isotope ratios were analyzed by MC-ICP-MS (Nu Plasma II, Nu Instruments, UK) following a previous publication (Yin et al., 2010). The digested solutions were diluted with suitable volume Milli-Q water aiming to match the isotope standard (NIST SRM-3133) concentration (1.0 ng/mL Hg in 3% (v/v) HNO_3) for stable isotope measurement. The sample solution was introduced by an peristaltic pump (Gilson, USA) and continuously mixed with 3% (v/v) $SnCl_2$ solution in a HGX-200 Hydride Generation (Teledyne CETAC Technologies, USA) to convert Hg(II) to Hg(0). The produced Hg(0) was separated from the bulk solution on a frosted glass phase separator and then introduced with Tl aerosol into the MC-ICP-MS subsequently. The internal Tl standard (NIST SRM-997) and sample-standard bracketing (SSB) method were used for instrumental mass bias corrections during the isotope measurement. Both MDF signatures ($\delta^{202}Hg$) and MIF signatures (including $\Delta^{199}Hg$, $\Delta^{200}Hg$ and $\Delta^{201}Hg$) were calculated following publication (Bergquist and Blum, 2007). Isotopic ratios are shown as δ in permil (‰) referred to NIST 3133 according to the following equation:

$$\delta^{xxx}Hg = (((^{xxx}Hg/^{198}Hg)_{sample}/(^{xxx}Hg/^{198}Hg)_{NIST3133}) - 1) \times 1000 \quad (1)$$

MIF represents the difference between the measured $\delta^{xxx}Hg$ values and the values predicted based on MDF (the $\delta^{202}Hg$ value). MIF is reported as Δ in permil (‰) in this work according to publication (Bergquist and Blum, 2007). For δ < 10‰, a valid approximation is:

$$\Delta^{199}Hg = \delta^{199}Hg - (\delta^{202}Hg \times 0.252) \quad (2)$$