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Use of mercury isotopes to quantify sources of human inorganic mercury exposure and metabolic processes in the human body

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ABSTRACT

The pathways of human mercury (Hg) exposure are complex and accurate understanding of relative contributions from different pathways are crucial for risk assessment and risk control. In this study, we determined total Hg concentration and Hg isotopic composition of human urine, dietary components, and inhaled air in the Wanshan Hg mining area (MA), Guiyang urban area (UA), and Changshun background area (BA) to understand Hg exposure sources and metabolic processes in human body. At the three studied sites, total gaseous mercury (TGM) showed negative δ^{202}Hg (−3.11‰ to + 1.12‰) and near-zero Δ^{199}Hg (−0.16‰ to + 0.13‰), which were isotopically distinguishable from Hg isotope values of urine (δ^{202}Hg: −4.02‰ to − 0.84‰; Δ^{199}Hg: −0.14‰ to 0.64‰). We observed an offset of −1.01‰ to −1.6‰ in δ^{202}Hg between TGM and urine samples, and an offset of −1.01‰ to 0.80‰ in δ^{202}Hg between rice and urine samples, suggesting that lighter isotopes are more easily accumulated in the kidneys and excreted by urine. We proposed that the high positive Δ^{199}Hg in urine samples of UA was derived from fish consumption. The results of a binary mixing model based on Δ^{199}Hg were compared with those from a classic dietary model. The results from the MIF binary model showed that fish consumption accounted for 22% of urine Hg in the families at UA, whereas fish consumption contributed limited Hg to MA and BA. This study highlighted that Hg isotopes can be a useful tracer in understanding the sources and fates of Hg in human bodies.

1. Introduction

Mercury (Hg) is a non-essential and toxic element in the human body, and the toxicity of Hg largely depends on its chemical form. Methylmercury (MeHg) is one of the most toxic form of Hg and can bioaccumulate in aquatic food webs. However, inorganic mercury (IHg) is also believed to be a toxic form of Hg produced in tissues after inhalation of gaseous elemental Hg (Driscoll et al., 2013). Gaseous elemental Hg is highly diffusible, lipid-soluble, and can be absorbed rapidly and efficiently from inhaled air via the alveolar membranes in the lungs (Iranmanesh et al., 2013). The primary target organs of gaseous elemental Hg are the brain and kidneys. Inhaled gaseous elemental Hg is readily absorbed in the lungs at a rate of ~ 80%, and is quickly diffused into the blood and distributed to all body organs (Iranmanesh et al., 2013; Solis et al., 2000). In the brain, elemental Hg^0 can be oxidized to inorganic Hg^{2+} which induces toxic effects to the central nervous system such as tremors, delusions, memory loss, and neuro-cognitive disorders (Clarkson et al., 2002; 2010; Crea et al., 2014; Roger et al., 2018). In the kidneys, the absorbed gaseous elemental Hg can led to tubular necrosis and glomerulonephritis (Chan, 2011; Dhanapriya et al., 2016).

MeHg is readily absorbed via consumption of food and nearly 100% of consumed MeHg is retained in the human body after ingestion (Blough et al., 2014; Li et al., 2015). Absorbed MeHg primarily binds to protein sulfhydryl groups in blood and tissue and then may be transported through cell membranes, mainly bound to cysteine by large neutral amino acid transporters (Yin et al., 2008; Wei et al., 2018). Approximately 10% of the body burden of MeHg is found in the head region, while the liver and the kidneys are also MeHg accumulation

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areas (Clarkson et al., 2010; Ma et al., 2017; NRC, 2000). Occupational exposure (e.g., workers in chlor-alkali plants, Hg mines, gold mines, Hg processing factories, and dental clinics) remains a predominant route of human exposure to Hg vapor and IHg (WHO, 1990, 1991; Anglen et al., 2015; Black et al., 2017), while consumption of fish and rice are the major MeHg exposure pathways (WHO, 2007; Cerveny et al., 2016; Feng et al., 2008; Zhang et al., 2010; Du et al., 2016). Hair total mercury (THg) is often used as a proxy of MeHg exposure, since the majority of THg in hair is presented as MeHg (>80%) among populations that consume fish (WHO, 2007; Cerveny et al., 2016; Manceau et al., 2016). Urine is the main pathway of excretion of IHg in human body (Beasley et al., 2014; Wang et al., 2017); therefore, urine THg is commonly used as the biomarker of IHg exposure (Solis et al., 2000; Black et al., 2017). However, some studies also argued THg concentrations in human hair and urine could not accurately reflect human exposure to MeHg and/or IHg (Ohno et al., 2007; Sherman et al., 2013, 2015; Riazet al., 2016; Kwaansa et al., 2019). Ohno et al (2007) reported about 30% of urine Hg resulted from degradation of MeHg in the consumed fish. Sherman et al. (2013) reported that urine Hg in dental professionals reflected a mixture of IHg demethylated from fish-derived MeHg and amalgam-derived IHg. Laffont et al. (2009) demonstrated that both inhalation of Hg vapor and consumption of fish contributed directly to hair THg concentrations in gold miners.

Mercury isotope serves as a new tool to understand the fate of Hg in human bodies and ecosystems. Mercury has seven natural stable isotopes (^{196}Hg, ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg, ^{204}Hg). Almost all physical, chemical, and biological processes could lead to mass dependent fractionation (MDF, mainly reported as δ^{202}Hg) of both odd and even Hg isotopes (Reviewed by Blum et al. (2014)). However, only a few processes, mainly photochemical (Hg^{2+} photo-reduction and MeHg photo-degradation) can cause mass independent fractionation of odd Hg isotopes (MIF, mainly reported as Δ^{199}Hg) (Bergquist and Blum, 2007, 2010). Combined δ^{202}Hg and Δ^{199}Hg signals, Hg isotopes can provide multi-dimensional insights into the sources and processes of Hg in the human body (Sherman et al., 2013, 2015; Laffont et al., 2009, 2011; Du et al., 2018).

Offsets of about + 1.7 to + 2.8‰ in δ^{202}Hg have been observed between consumed food and human hair, suggesting Hg-MDF readily occur during Hg metabolism in the human body (Bonsignore et al., 2015; Laffont et al., 2009, 2011; Li et al., 2014, 2016; Rothenberg et al., 2017; Du et al., 2018). However, studies reported no differences in Δ^{199}Hg between consumed food and human hair, suggesting limited Hg-MIF occurs during metabolic processes and therefore Hg-MIF can be a direct tracer of consumed Hg. Hg MIF signals of human hair have been be used to distinguish MeHg intake from rice versus fish; as the proportion of MeHg intake from rice increases, hair Δ^{199}Hg decreases. For rice consumers who seldom eat fish, hair Δ^{199}Hg is close to zero, whereas fish consumers have significantly positive hair Δ^{199}Hg (Du et al., 2018; Rothenberg et al., 2017). When exposed to high Hg vapor environment, hair Δ^{199}Hg in miners indicated a mixture of ingested fish MeHg and absorbed IHg (Sherman et al., 2015). In dental professionals, MeHg can be demethylated within the human body, causing IHg excretion in urine (Sherman et al., 2013). Demethylation of fish-derived MeHg can contribute significantly to urine THg concentrations and may result in the overestimation of Hg^0 exposure from dental amalgams (Sherman et al., 2013).

It should be noted that although hair Hg isotopes have been extensively studied, the isotopic composition of urine remains poorly studied. To fill this knowledge gap, this study was designed to investigate the Hg isotopic composition of human urine samples from residents in mining (MA), background (BA) and urban areas (UA) in Guizhou Province, SW China. It aimed to (i) trace the source of Hg in urine, and (ii) understand Hg metabolic processes in the human body.

2. Experimental section

2.1. Sample collection

Three primary schools in the Guiyang urban area (UA), Wanshan mining area (MA), and Changshun background area (BA) were selected for sampling (Figure S1). UA is located in Guiyang, the capital of Guizhou Province. Coal combustion is the major source of Hg emission in Guiyang (Wang et al., 2013). The average of gaseous elemental Hg in ambient air of Guiyang in 2009 was 9.72 ng m^{-3}, which was slightly higher than these in other cities in China, such as Beijing, Shanghai, Chongqing, and Nanjing (Fu et al., 2011). MA is located in a small village of the Wanshan Hg mine. Long-term Hg mining and retorting activities in Wanshan area have resulted in serious Hg contamination to the surrounding environment (water, soil, and atmosphere). BA is located in a small residential community, 70 km away from Guiyang city. This primary school was previously chosen as a control site due to the lack of significant local industrial activities (Feng et al., 2008).

Sampling was conducted from November 2013 to October 2014. A total of 15, 15, and 13 pupils and their respective guardians (1:1 comparison) from MA, UA, and BA, respectively were recruited in this study. Age, gender, weight, height, dental Hg amalgam use, and dietary information data for each participant were summarized in Table S1. Every participant signed a consent agreement before participation. Ethical approval for this study was obtained from the Institute of Geochemistry, Chinese Academy of Sciences.

Urine samples were collected in disposable paper cups and then transferred into 50 mL pre-cleaned plastic centrifugal tubes. Urine samples were preserved by adding trace-metal grade HNO_3 (to 10% of the total volume) and then hermetically sealed and transported to the laboratory (4 °C). Total gaseous mercury (TGM) was collected by iodinated carbon traps and was measured according to a previous method (Fu et al., 2014). Air sampling systems were installed 1.5 m above the ground floor, outside the classrooms of the three schools. A Hg vapor analyzer (Tekran 2537B, Tekran Instruments, Canada) was installed to continuously monitor the TGM concentrations at both the inlet and outlet of the iodinated carbon trap. No breakthrough of Hg vapor was detected during the continuous sampling for 30 days.

THg concentrations and Hg isotope in fish from UA, vegetable from MA, and, rice and hair from the three sites were adopted from our previous study (Du et al., 2018). All the food samples were obtained from the students' kitchens. Rice and vegetables samples collected from MA and BA were grown and harvested locally, while food samples collected from UA were bought from the market. Both herbivorous and carnivorous fish were included.

2.2. THg analysis

Approximately 0.2 to 3 mL of each urine sample was digested with 5 mL of acid mixture (HNO_3:H_2SO_4 = 4:1, v:v) at 95 °C for 3 h. The THg concentrations of the digests solutions were determined by cold vapor atomic fluorescence spectroscopy (CVAFS; model 2500, Tekran Instruments, Canada) following the USEPA Method 1631E (USEPA, 2002). The limit of detection of THg in urine was 0.1 ng mL^{-1}. Method blanks, Certified Reference Materials (CRMs; ZK020-1, ZK020-2, human urine, China CDC), and duplicates were used for quality control. The mean recoveries of the CRMs were 94 ± 9% (n = 15) and 95 ± 7% (n = 15) for ZK020-1 and ZK020-2, respectively. The relative percentages of difference were < 10% for duplicate samples.

The collected TGM samples were processed using a double-stage offline combustion-trapping technique (Yu et al., 2016). A catalyst tube (LECO, USA) was utilized to remove iodine and iodine-containing oxidation products from the iodinated carbon traps, which may cause negative interference in the subsequent Hg isotopic measurements. Ten mL of 40% reverse *aqua regia* (1:3 HNO_3:HCl, v/v) was utilized to capture the decomposed Hg. The Hg mass in each trapping solution was

measured by CVAFS. A series of tests with iodinated carbon traps being spiked with a specified amount of Hg^0 from an external Hg vapor calibration unit (Tekran model 2505) were conducted. The average recovery after catalyst unit was 95 ± 14% (2SD; n = 6). The procedural blanks from the iodinated carbon traps averaged at 49 ± 48 pg (2SD; n = 3), which was < 1% of the Hg in the air sample. CRM BCR482 (lichen, IRMM) was prepared using the same process to test the accuracy of the Hg isotopic values.

2.3. Hg isotope measurement

Approximately 2 to 12 mL of each urine sample was digested in a water bath (95 °C, 3 h) using 5 to 30 mL of fresh HNO_3/H_2SO_4 mixture (v/v, 4:1). Each digestion was diluted to 25–150 mL and reduced by $SnCl_2$, and the Hg was purged into 5 mL 40% reverse *aqua regia* by Hg-free N_2 gas (200 to 300 mL min^{-1}). Then, the trapping solutions were diluted to a THg concentration of 1 ng mL^{-1} and an acid concentration of 10% to 20%. CRM BCR482 (lichen) and urine samples were prepared for duplicate analysis. For TGM samples, the trapping solutions were also diluted to a THg concentration of 1 ng mL^{-1}, with an acid concentration of 10% to 20%. Milli-Q water (18.2 ΩM, Millipore, Bedford, USA) was used for dilution. Hg isotopes were measured by multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS; Nu-Plasma II) according to our previous method (Yin et al., 2010). Hg MDF is expressed in δ^{202}Hg notation in units of permil (‰) referenced to the neighboring NIST-3133 Hg standard:

$$\delta^{xxx}\text{Hg} = [(^{xxx}\text{Hg}/^{198}\text{Hg}_{sample})/(^{xxx}\text{Hg}/^{198}\text{Hg}_{NIST\ SRM\ 3133}) - 1] \times 1000 \quad (1)$$

where xxx refers to the mass of each isotope between 199 and 202 amu.

MIF is expressed as the difference between the measured δ^{xxx}Hg value and the predicted value based on the δ^{202} Hg value (Equations 2, 3, and 4).

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

$$\Delta^{200}\text{Hg} = \delta^{200}\text{Hg} - 0.502 \times \delta^{202}\text{Hg} \quad (3)$$

$$\Delta^{201}\text{Hg} = \delta^{201}\text{Hg} - 0.752 \times \delta^{202}\text{Hg} \quad (4)$$

The Hg concentrations and acid matrices were matched between samples and NIST-3133. UM-Almadeń secondary standard solutions (diluted to 1 ng mL^{-1} Hg in 10% acid) were analyzed as a secondary standard using the same analytical treatment (Blum and Bergquist, 2007). Our results of UM-Almadeń (δ^{202}Hg = − 0.56 ± 0.10‰; Δ^{199}Hg = 0.04 ± 0.04‰; Δ^{200}Hg = 0.02 ± 0.06‰; Δ^{201}Hg = 0.01 ± 0.06‰; Mean ± 2SD, n = 12) and BCR482 (δ^{202}Hg = − 1.53 ± 0.18‰; Δ^{199}Hg = − 0.61 ± 0.14‰; Δ^{200}Hg = 0.04 ± 0.04‰; Δ^{201}Hg = − 0.56 ± 0.12‰; Mean ± 2SD; n = 8) agreed well with previous results (Bergquist and Blum, 2007, 2010; Estrade et al., 2010). The external precision of the replicate UM-Almadeń secondary standard solution was used to represent the analytical uncertainty (2SD).

2.4. Statistical analysis

Statistical analyses were performed using SPSS 19 for Windows. Extreme values based on statistical outlier analysis for each class of measurements were removed. We characterized the outliers as urine THg that were 1.5 times higher than the third quartile or lower than the first quartile. This procedure removed 7% of all data from the database.

The data were tested for normal distribution by the Kolmogorov-Smirnov test. If they were not normally distributed, the data were log transformed for further statistical analysis. Descriptive statistics are presented as Mean ± Standard Deviation (SD). Mean values of each variable across each site were compared using independent-samples t-tests and one-way analysis of variance (ANOVA). Correlation coefficients were determined by Pearson correlation analysis. The results of statistical tests were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. TGM and Hg isotope

TGM concentrations and isotopic compositions at the three sites are shown in Fig. 1 and Table S2. The highest TGM values were observed at MA (111 ± 165 ng m^{-3}, 2SD, n = 10), compared to UA (11.2 ± 8.89 ng m^{-3}, 2SD, n = 9) and BA (8.28 ± 10.6 ng m^{-3}, 2SD, n = 10). The TGM showed distinguishable Hg isotopes among MA (δ^{202}Hg = − 0.52 ± 1.18‰; Δ^{199}Hg = 0.07 ± 0.07‰; 2SD, n = 10), UA (δ^{202}Hg = − 1.35 ± 2.06‰; Δ^{199}Hg = 0.01 ± 0.10‰; 2SD; n = 9) and BA (δ^{202}Hg = − 0.77 ± 1.72‰; Δ^{199}Hg = 0.01 ± 0.19‰; 2SD, n = 10).

In this study, the means of TGM δ^{202}Hg and Δ^{199}Hg values from MA were significantly higher than previous results also conducted in the Wanshan Hg mine (δ^{202}Hg = − 2.14 ± 0.42‰; Δ^{199}Hg = − 0.29 ± 0.08‰; 2SD, n = 4; Yin et al., 2013). The much lower δ^{202}Hg and Δ^{199}Hg values in TGM samples collected over rice paddies in the Wanshan Hg mine has been explained by preferential photo-reduction of isotopically lighter Hg isotopes in the paddy water (Yin et al., 2013a). The Hg isotopic compositions of TGM at the three studied sites were comparable with previous results from other Chinese cities (Beijing and Xian), but different with the results from remote sites in China (eg. Mt. Ailao and Mt. Changbai, Fig. 1). This indicates that the main source of TGM in UA and MA is local and anthropogenic, since previous studies demonstrated negative δ^{202}Hg and near-zero Δ^{199}Hg for TGM influenced by anthropogenic Hg emissions (Yu et al., 2016; Xu et al., 2017; Fu et al. 2019). Interestingly, Hg isotopic compositions of TGM of BA were quite different from these of remote areas (Mt. Ailao and Mt. Changbai). The main reason for the difference between BA and Mt. Ailao is that BA is also affected by anthropogenic activities. Although this site was chosen as the control site, but it is only 70 km away from the industrial areas in Guiyang City. In comparison, Mt. Ailao is about 500 km from the known cities.

3.2. Urine THg and Hg isotope

Each single urine sample was tested for THg concentration, and, a mixture of all the urine samples for each participant were analyzed for the Hg isotope composition. The urine THg concentrations in MA averaged at 22.1 ± 27.6 ng mL^{-1} (2SD, n = 19), which were significantly higher than these of UA (0.61 ± 0.85 ng mL^{-1}, 2SD, n = 20) and BA (0.98 ± 1.53 ng mL^{-1}, 2SD, n = 20). At each site, no significant correlation was found between urine THg concentrations and age, weight, height, and gender ($p > 0.05$; Figure S2 and Table S1). However, urine THg showed distinguishable Hg isotopes among the three sites (Fig. 2). Urine δ^{202}Hg and Δ^{199}Hg values were − 1.65 ± 0.78‰ and 0.08 ±

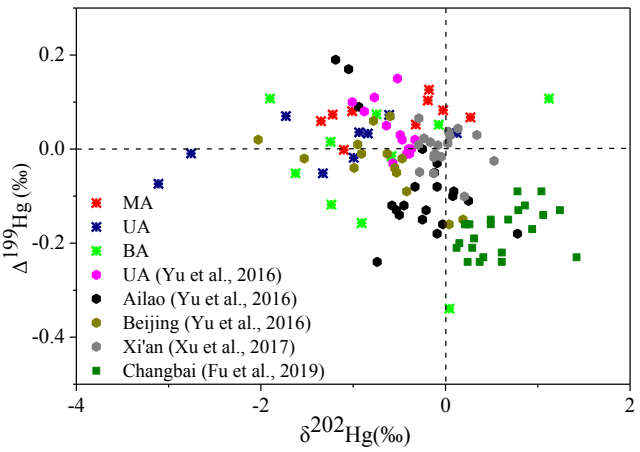

Fig. 1. Hg isotopic compositions of TGM in the mining area (MA), urban area (UA), background area (BA) in this study and previous studies.

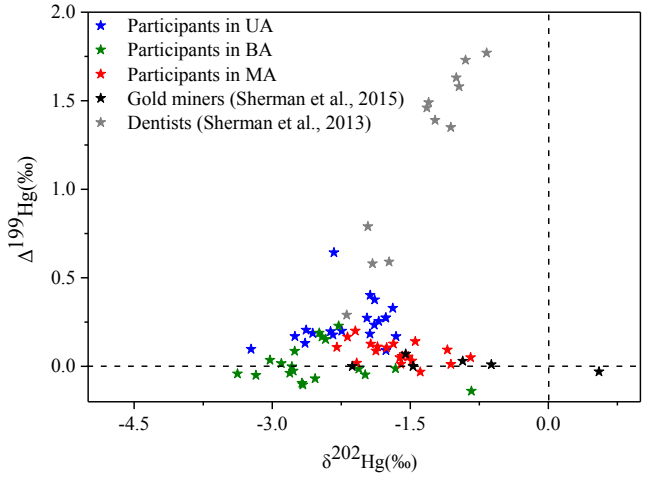

Fig. 2. Urine Hg isotope in the mining area (MA), urban area (UA), background area (BA) in this study and previous studies.

0.12‰ (2SD, n = 19), −2.16 ± 0.86‰ and 0.24 ± 0.26‰ (2SD, n = 20), and − 2.59 ± 1.26‰ and 0.02 ± 0.20‰ (2SD, n = 20) in MA, UA, and BA, respectively. As shown in Fig. 2, urine Hg isotopic data in this study were distinguished from the U.S. dentists, but overlapped with gold miners (Sherman et al., 2013, 2015). For dentists who frequently ingest fish, urine THg exhibited negative δ^{202}Hg values (−1.35 ± 0.96‰, 2SD, n = 20) and large positive Δ^{199}Hg (1.22 ± 1.02‰, 2SD, n = 20), and for gold miners who experienced high occupational exposure to gaseous elemental Hg and limited fish consumption, urine THg was characterized by negative δ^{202}Hg (−1.03 ± 1.86‰, 2SD, n = 6) and near-zero Δ^{199}Hg (0.01 ± 0.06‰, 2SD, n = 6) (Sherman et al., 2013, 2015).

The urine Δ^{199}Hg/Δ^{201}Hg ratios in UA and BA were 1.18 ± 0.24 (2SD, r^2 = 0.85, p < 0.01) and 1.15 ± 0.36 (2SD, r^2 = 0.66, p < 0.01), respectively (Fig. 3). These ratios fall between the values reported for Hg^{2+} photo-reduction (Δ^{199}Hg/Δ^{201}Hg ~ 1) and MeHg photo-degradation (Δ^{199}Hg/Δ^{201}Hg ~ 1.3) (Bergquist and Blum, 2007), suggesting urine Hg reflected a mixture of signal from IHg photo-reduction and MeHg photo-degradation. However, a Δ^{199}Hg/Δ^{201}Hg ratio of 0.94 ± 0.18 (2SD, r^2 = 0.85, p < 0.01) was observed in urine from MA, which is consistent with previous results on Hg^{2+} photo-reduction. The main form of Hg in fish tissue is MeHg. According to our previous study, δ^{202}Hg and Δ^{199}Hg in fillet fish samples collected from Guizhou Province were − 0.48 ± 1.04‰ and 0.96 ± 1.30‰ (2SD, n = 18), respectively (Du et al., 2018). Therefore, we hypothesized that the high Δ^{199}Hg/Δ^{201}Hg ratios at UA and BA resulted from the consumption of fish. As well, IHg is the dominant Hg species in vegetables and rice. The

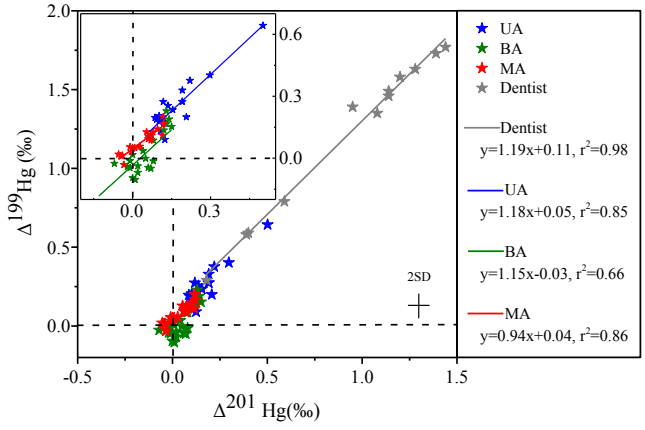

Fig. 3. Urine Δ^{199}Hg versus Δ^{201}Hg in the mining area (MA), urban area (UA) and background area (BA).

observation of Δ^{199}Hg/Δ^{201}Hg ~ 1 in the urine samples of MA was consistent with our previous results on vegetables and rice (Du et al., 2018). For instance, the mean δ^{202}Hg and Δ^{199}Hg values in rice samples were − 2.59 ± 0.98‰ and 0.03 ± 0.06‰ (2SD, n = 15), −1.35 ± 0.38‰ and 0.02 ± 0.08‰ (2SD; n = 11), and − 1.83 ± 0.24‰ and − 0.07 ± 0.08‰ (2SD, n = 14) in MA, UA, and BA, respectively. Vegetables collected from MA showed δ^{202}Hg of − 2.34 ± 1.12‰ and Δ^{199}Hg of − 0.05 ± 0.14‰ (2SD; n = 8). Therefore, vegetables and rice, rather than fish, is the major source of urine Hg in MA.

3.3. MDF

Previous studies have demonstrated that Hg metabolic processes in the human body may cause significant MDF. A large offset of 1.7‰ to 2.2‰ in δ^{202}Hg was observed between fish and the hair of fish consumers (Sherman et al., 2013; Li et al., 2008, 2018; Bonsignore et al., 2015; Laffont et al., 2009; Li et al., 2014), and this offset can be even larger (2.5‰ to 2.7‰) between rice and the hair of rice consumers (Du et al., 2018). The offset between consumed food and hair may be caused by demethylation of MeHg in human body (Sherman et al., 2013; Du et al., 2018). In this study, we observed an offset of − 1.01‰ to − 1.6‰ in δ^{202}Hg between TGM and urine samples, and an offset of − 1.01‰ to 0.80‰ in δ^{202}Hg between rice and urine samples in UA and BA (Fig. 4A and 4C). It demonstrated that lighter isotopes are more easily accumulated in the kidneys and excreted by urine, while heavier isotopes are more likely to remain in the human body.

The offsets of δ^{202}Hg values of TGM relative to urine in MA were − 1.01‰, while the difference between rice and urine δ^{202}Hg were + 0.94‰ (Fig. 4). These offsets were much higher than those in UA and BA. This may be as a result of human IHg exposure from soil and vegetable ingestion. Soil ingestion constituted 18% of the total IHg exposure in MA on average, which were significantly higher than these in UA and BA (2%; Table S3). Since the soil THg concentrations in UA and BA were relative low, we did not measure soil Hg isotopes in UA and BA. The mean of soil δ^{202}Hg in MA was − 0.02 ± 0.16‰ (Yin et al., 2013b), which is higher than that in TGM, rice, and vegetable (Fig. 4). This may result in more positive urine δ^{202}Hg values in MA. Meanwhile, vegetable ingestion is an important source of IHg exposure and contributed to 18% to 38% of urine THg. The urine Hg isotope in MA reflected the mixture from inhalation of TGM and ingestion of rice, vegetable, and soil.

3.4. MIF and quantifying Hg exposure source

Due to the absence of MIF during metabolic processes in human body, MIF in human urine may be used as an indicator to differentiate Hg exposure sources and pathways. A binary mixing model based on MIF data was used to quantify Hg exposure via consumption of fish versus non-fish sources (Equations 3–4):

$$\Delta^{199}Hg_{urine} = F_{non\text{-}fish} * \Delta^{199}Hg_{non\text{-}fish} + F_{fish} * \Delta^{199}Hg_{fish} \quad (3)$$

$$F_{fish} = 1 - F_{non\text{-}fish} \quad (4)$$

where $\Delta^{199}Hg_{urine}$, $\Delta^{199}Hg_{fish}$, and $\Delta^{199}Hg_{non\text{-}fish}$ are the Δ^{199}Hg values in urine, fish, and non-fish (i.e. rice, vegetables, air via TGM) sources at each site, respectively. F_{fish} and $F_{non\text{-}fish}$ represent the fraction of Hg exposure from fish and non-fish sources, respectively. The mean Δ^{199}Hg values in UA fish were used as the end-member of the fish source. The mean of the Δ^{199}Hg values in rice and TGM (as well as vegetables in MA) were used as the end-member for the non-fish dietary source at each site.

According to the model output, fish consumption contributed 22 ± 26% (2SD, n = 20) of urine Hg at UA, but only contribute 8.3 ± 12% (2SD, n = 19) and 4.1 ± 5% (2SD, n = 20) of urine Hg at MA and BA, respectively. The urine Δ^{199}Hg inherited the positive signal from fish-derived MeHg, and the MIF binary mixing model can trace this process.

We calculated the contribution of IHg intake from the diet by the dietary model (SI) and the relative contributions of different IHg exposure sources were shown Fig. S3. Significant differences of the contributions from fish intake were observed between the dietary model and

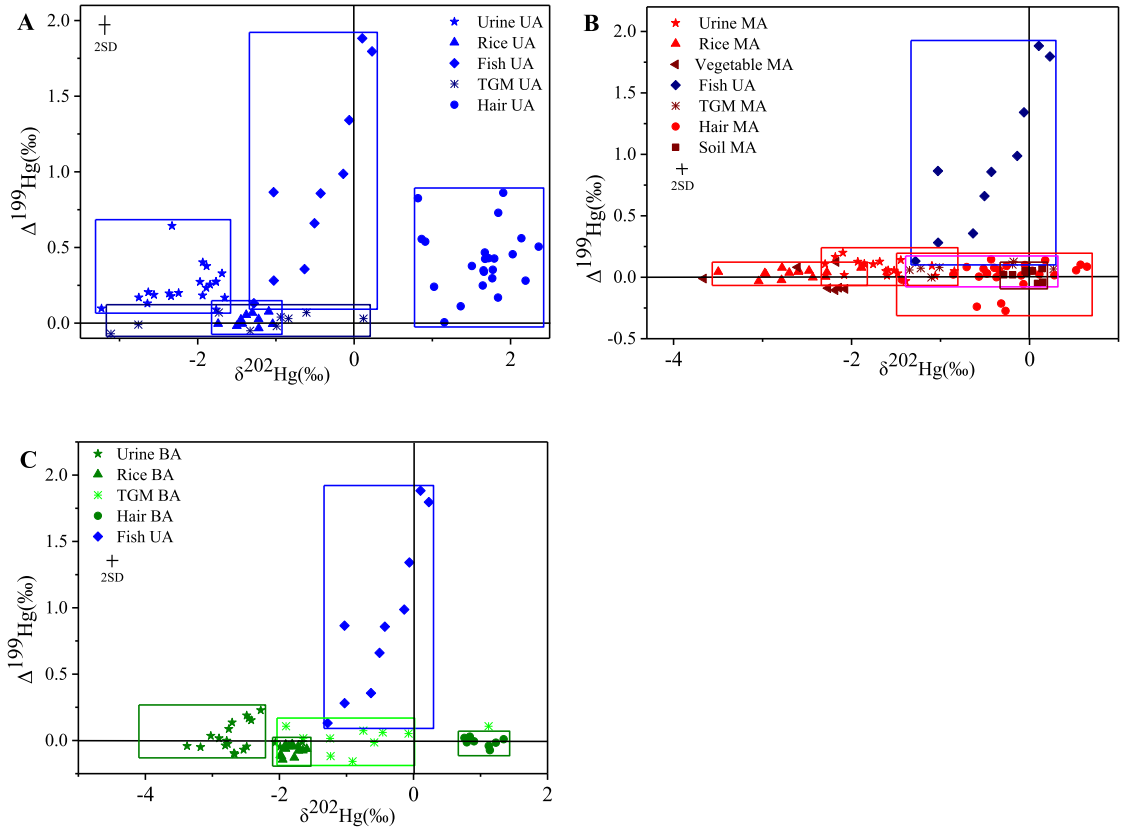

Fig. 4. Urine, hair, TGM, soil, and diet Hg isotope the urban area (UA), the mining area (MA), and the background area (BA).

MIF binary mixing model in UA. According to the dietary model, fish intake contributed only 2.8 ± 0.63% (2SD) of urine THg in UA (Fig. S3), while 22 ± 26% (2SD, n = 18) according to the MIF binary mixing model. Significant variations in amount of daily diet intake may cause significant uncertainty of the contribution from different exposure sources. However, no MIF was observed during the Hg metabolic processes in the human body. Therefore, the MIF in human urine could be used as a more effective indicator to quantify Hg exposure sources. More importantly, MeHg demethylation can also occur in the human intestine (Wang et al., 2017). We proposed that fish-derived MeHg can be demethylated into IHg in the human intestine and finally excreted in the urine. The urine Δ^{199}Hg inherited the positive signal from fish-derived MeHg, and the MIF binary mixing model can trace this process. However, the dietary model neglected this process and underestimated the contribution of fish consumption to IHg exposure in human urine.

4. Conclusions

Fish and rice consumption are important dietary sources of Hg to humans. In this study, we demonstrated that Hg isotopes can be effective tracer for "fingerprinting" sources and metabolic processes of IHg in the human body. We observed a negative shift of δ^{202}Hg between TGM and urine THg and between diet sources and urine THg, indicating greater excretion of lighter Hg isotope than heavier Hg isotope. Using a MIF mixing model, we confirmed that fish consumption can be an important source of Hg in urine. Fish mainly contain MeHg, however, this MeHg can be demethylated within the human body and excreted via urine. Soil ingestion contributed 18% of IHg exposure in MA, which influenced Hg isotopic signatures in human urine. Our study thus demonstrated that Hg isotopes can be useful tracers to understand the sources and fates of Hg in human bodies.

CRediT authorship contribution statement

Buyun Du: Conceptualization, Methodology, Investigation, Data curation, Writing - review & editing. **Runsheng Yin:** Methodology, Data curation. **Xuewu Fu:** Methodology. **Ping Li:** Conceptualization, Methodology, Investigation, Data curation, Writing - review & editing. **Xinbin Feng:** Conceptualization, Methodology, Data curation, Writing - review & editing, Funding acquisition. **Laurence Maurice:** Conceptualization, Methodology, Data curation, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.106336.

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