Chemosphere 225 (2019) 803–809

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

New insights into the chemical forms of extremely high methylmercury in songbird feathers from a contaminated site

Zhidong Xu [a, b], Kasun S. Abeysinghe [a, b], Xiaohang Xu [a, b], Chunhao Gu [c], Longchao Liang [d], Qinhui Lu [a, b], Yubo Zhang [e], Lirong Zheng [f], Wen-Xiong Wang [g], Guangle Qiu [a, *]

[a] State Key laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang, 550081, China
[b] University of Chinese Academy of Sciences, Beijing, 100049, China
[c] Department of Ecosystems Science and Management, University of Wyoming, 82071, United States
[d] College of Resource and Environmental Engineering, Guizhou University, Guiyang, 550025, China
[e] Department of Agricultural Sciences, Anshun College, Anshun, 561000, China
[f] Institute of High Energy Physics, Chinese Academy of Sciences, Beijing, 100049, China
[g] Department of Ocean Science, Hong Kong University of Science and Technology, Clearwater Bay, Kowloon, Hong Kong

HIGHLIGHTS

- MeHg in songbird feathers comprised of 74.1–95.9% of THg.
- MeHg in feathers was mainly bound to cysteine and reduced glutathione, while IHg was bound to reduced glutathione.
- Hg chemical forms in feathers was highly dependent on the exposure sources.

ARTICLE INFO

Article history:
Received 14 December 2018
Received in revised form
21 February 2019
Accepted 10 March 2019
Available online 14 March 2019

Handling Editor: Martine Leermakers

Keywords:
Songbird feathers
MeHg
Chemical forms
Speciation
XANES

ABSTRACT

The chemical forms of mercury (Hg), particularly methylmercury (MeHg), in songbird feathers from an abandoned mining region were analyzed via X-ray absorption near-edge structure analysis (XANES). In feathers, proportions of MeHg as total mercury (75.6–100%) quantified by the XANES were directly comparable to the chemical extraction values (74.1–95.9%). Most of MeHg were bound with cysteine (Cys) and reduced glutathione (GSH), whereas inorganic mercury (IHg) was mainly bound with GSH. These results were consistent with those found in fish muscles and human hairs of both fish consumers and occupational Hg exposure populations. Our study suggested that chemical forms and speciation of Hg were highly dependent on the exposure sources and food consumption, respectively. Bird feathers were able to selectively accumulate MeHg due to their special binding ways. However, detailed mechanisms of Hg accumulation in bird feathers remain to be further elucidated.

1. Introduction

Mercury (Hg) is a global pollutant and one of the most hazardous substances in the world (ATSDR, 2017). Methylmercury (MeHg), an organic Hg form with high neurotoxicity and biomagnification potential, can be produced under anoxic conditions by bacteria, and transported and biomagnified along the food chain in aquatic systems (Morel et al., 1998; Chen et al., 2005). In terrestrial systems, invertebrates such as spiders can also be involved in the transfers of Hg between avian and aquatic food chains, exacerbating the biomagnification of Hg (Cristol et al., 2008). Terrestrial birds may suffer from high Hg exposure risks through invertebrates' consumption (Cristol et al., 2008; Rimmer et al., 2010).

Bird feather is a noninvasive bioindicator of environmental Hg pollution and also can reflect the body Hg burden or exposure risks. (Furness et al., 1986; Becker et al., 1993; Agusa et al., 2005). Reproductive success reduction, reduced survival and immune

* Corresponding author. State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences No. 99, Lincheng Western Road, Guanshanhu District, Guiyang, China.
E-mail address: qiuguangle@vip.skleg.cn (G. Qiu).

https://doi.org/10.1016/j.chemosphere.2019.03.060

competences in songbirds such as Tree Swallows (*Tachycineta bicolor*), Zabra Finches (*Taeniopygia guttata*) and Carolina Wrens (*Thryothorus ludovicianus*) can occur under elevated Hg exposure both in feeding experiment and field study (Hawley et al., 2009; Hallinger et al., 2011; Jackson et al., 2011; Paris et al., 2018). In seabird feathers, Hg speciation was mainly dominated by MeHg, which accounted for 90–100% of total mercury (THg) (Thompson and Furness, 1989; Bond and Diamond, 2009). Over the last decade, X-ray absorption near-edge structure analysis (XANES) has been used to identify the Hg chemical forms in biological samples, such as plants, fish muscles, seabird's liver, human brain and hair (Wang et al., 2012; Harris et al., 2003; Arai et al., 2004; Korbas et al., 2010; Li et al., 2008b). For birds, it reported that HgS and HgSe were the dominant Hg forms in seabird's liver (Arai et al., 2004). However, little is known about the Hg chemical forms and its proportions in bird feathers.

Recently, we found that Hg in feathers (Hg_f) of insectivorous songbirds (Spot-breasted Scimitar Babbler *Pomatorhinus mcclellandi*) from an Hg-contaminated terrestrial ecosystem reached as high as 123.3 ± 34.2 mg kg^{-1} (Abeysinghe et al., 2017), which was higher than the previously reported highest values of 91.6 mg kg^{-1} in feathers of seabird (Wandering albatross *Diomedea exulans*) (Renedo et al., 2017), suggesting extremely high exposure risks of songbirds in contaminated sites. Meanwhile, such elevated Hg levels in feathers provided an ideal material to investigate the Hg chemical forms via XANES. The objectives of this study were 1) *in-situ* analyze the Hg chemical forms and its proportions in feathers using XANES technique and 2) discuss the potential factors affecting the proportions and chemical binding of such extremely high MeHg in songbird feathers.

2. Materials and methods

2.1. Sampling and preparation

The sampling site, situated in the Wanshan Hg mine (WSMM) area, was adjacent to abandoned artisanal Hg retorting sites (Gouxi, GX) and historically intensive Hg mining sites (Wukeng and Meizixi, WK and MZX) with an area of approximately 4×4 km. In WSMM, large scale Hg mining and smelting activities lasted about 3000 years and finally ceased after 2004, and artificial smelting was subsequently prohibited several years ago.

Feathers were collected in Wanshan mercury mining areas (WSMM) with permission from the Forest Department of Wanshan District and the Institute of Geochemistry Chinese Academy of Sciences from 2014 to 2015. Detailed information of study areas and sampling procedures can be found in our previous study of Abeysinghe et al. (2017). A total of thirty two flight feather samples were collected from residential adult songbirds and washed by deionized water, acetone and deionized water with ultrasonic cleaners, air dried until of constant weight, then cut into pieces about 1–2 mm length for analysis. Among these feathers, twenty eight were measured for their proportions of MeHg as THg (MeHg %), and four feather samples containing high THg concentrations were selected for XANES analysis. Detailed bird information is listed in Table S1 in supporting information (SI). Based on the main diet composition of birds, the birds were roughly categorized as primary insectivorous (PI) including Chinese Hwamei (*Garrulax canorus*), Chestnut-headed Tesia (*Cettia castaneocoronata*) and Spot-breasted Scimitar Babbler (*Pomatorhinus mcclellandi*), primary omnivorous (PO) including Grey-headed Parrotbill (*Psittiparus gularis*) and Collared Finchbil (*Spizixos semitorques*), primary frugivorous (PF) including Mountain Bulbul (*Ixos mcclellandii*), and primary granivorous (PG) including Tree Sparrow (*Passer montanus*).

2.2. Hg and XANES analysis

2.2.1. Hg analysis

THg concentrations were measured by the USEPA Method 1631E (USEPA, 2002). Approximately 0.05 g of feathers were digested at 95 °C in a mixed acid (HNO_3: $H_2SO_4 = 4{:}1$, v/v), oxidized by BrCl, reduced by NH_4OH HCl and $SnCl_2$ and measured by cold vapor atomic fluorescence spectrometry (F-732VJ) (Shanghai Huaguang Company, China). For MeHg analysis, approximately 0.01 g feathers were digested in 25% (m/m) KOH methanol solvent for 3 h at 75 °C, diluted to 50 ml with ultrapure water and measured via aqueous ethylation, purge, trap and gas chromatography cold vapor atomic fluorescence spectrometry (GC-CVAFS) (Brook Rand Model III, Brooks Rand Company, United States) following USEPA Method 1630 (USEPA, 1998).

For THg and MeHg, certificated reference materials (CRMs) including human hair (GBW 0910b) (Shanghai Institute of Applied Physic, China) and lobster hepatopancreas (TORT-2) (NRDC, Canada) and duplicated samples (10%) were used to ensure data quality. Method blanks and sample blanks were also applied. The recoveries of CRMs were $98 \pm 1.5\%$ and $102 \pm 8\%$ for THg and MeHg, respectively. Each sample was measured by three times and relative standard deviation (RSD) of duplicated samples was less than 5%.

2.2.2. XANES analysis

Hg L_{III} X-ray absorption spectra were collected at XAFS beamline 1W1B at the Beijing Synchrotron Radiation Facility (BSRF). Four feather samples including Chinese Hwamei (S1-hw), Chestnut-headed Tesia (S2-cht), Spot-breasted Scimitar Babbler (S3-sbsb) and Tree Sparrow (S7-ts) were selected for the XANES analysis. THg concentrations in these samples are listed in Table 1. To investigate the Hg chemical forms in feathers, thirteen Hg standard reference materials (SRMs) including mercuric acetate ($Hg(AC)_2$), mercuric sulfate ($HgSO_4$), cinnabar (α-HgS), metacinnabar (β-HgS), mercuric oxide (HgO, yellow and red), mercuric chloride ($HgCl_2$), mercuric L-cysteinate (HgCys), mercuric homocysteine (HgDicys), mercuric selenide (HgSe), methylmercury L-cysteinate (MeHgCys), methylmercury glutathionate (MeHgSG) and mercury glutathionate ($Hg(SG)_2$) were employed. Most of these compounds were purchased. However, HgDicys and HgCys were synthesized according to Andrews and Wyman (1930) and Neville and Drakenberg (1974), respectively. MeHg-Cys, MeHgSG and $Hg(SG)_2$ were synthesized according to Lemes and Wang (2009) and Mah and Jalilehvand (2010). All XANES data were processed and linearly fitted by Athena (version 0.9.24) in the IFEFFIT package developed by Ravel and Newville (2010).

3. Results and discussion

3.1. THg and MeHg

Feathers of four dietary birds (n = 28) were quantified for their Hg concentrations and the proportions of MeHg as THg (MeHg%). The concentrations of THg and paired MeHg are listed in Table 1. THg concentrations in PI, PO, PG and PF bird feathers displayed a decreasing trend that 7.4–177 (average 126.7–161.0) mg kg^{-1}, 7.4–33.3 (average 19.2–29.1) mg kg^{-1}, 15.8–24.9 (average 20.7 ± 4.1) mg kg^{-1}, and 0.6–1.7 (average 1.2 ± 0.4) mg kg^{-1}, respectively. In addition, differences were observed in MeHg% for feathers of four dietary type birds. Except for feathers of PF birds which had the lowest percentage ($74.1 \pm 4.6\%$), MeHg% of most bird feathers was elevated and in a similar variation (89.0–95.9%).

Such difference of MeHg concentration and proportions may be related to their dietary types. Rice is an anomalous plant for MeHg accumulation and has high MeHg% reaching up to 93% than other

Table 1
THg and paired MeHg concentrations in bird feathers from WSMM and the literatures (mg kg^{-1}).

Sites	Species/Latin name	No.	Primary diets	N	THg		MeHg		MeHg%	Sources
					Range	Mean	Range	Mean		
Wanshan, China	Chinese Hwamei/*Leucodioptron canorum*	S1-hw	Insectivorous	4	92.2–114.0	94.8 ± 24.5[a]	90.1–104.4	84.3 ± 22.2	89.0 ± 7.4	This Study
	Chestnut-headed Tesia/*Cettia castaneocoronata*	S2-cht	Insectivorous	4	127.0–177.0	161.0 ± 23.0	101.6–166.5	147.2 ± 30.6	90.7 ± 7.2	
	Spot-breasted Scimitar Babbler/*Pomatorhinus mcclellandi*	S3-sbsb	Insectivorous	4	79.6–153.0	126.7 ± 32.8	69.7–140.4	113.3 ± 31.4	89.1 ± 2.8	
	Grey-headed Parrotbill/*Psittiparus gularis*	S4-ghp	Omnivorous	4	25.6–33.3	29.1 ± 3.3	24.3–32.3	27.9 ± 3.4	95.9 ± 1.0	
	Collared Finchbill/*Spizixos semitorques*	S5-cf	Omnivorous	4	7.4–32.8	19.2 ± 10.5	5.9–31.9	17.7 ± 10.8	89.7 ± 7.5	
	Mountain Bulbul/*Ixos mcclellandii*	S6-mb	Frugivorous	4	0.6–1.7	1.2 ± 0.4	0.5–1.1	0.9 ± 0.3	74.1 ± 4.6	
	Tree Sparrows/*Passer domesticus*	S7-ts	Granivorous	4	15.8–24.9	20.7 ± 4.1	14.3–23.3	19.3 ± 4.3	93.0 ± 2.6	
Wanshan and Leishan, China	Oriental Scops Owl/*Otus sunia*		Predatory	21	–	7.71–27.1[b]	–	–	94.2 ± 3.1	Abeysinghe et al. (2017)
	Common Kingfisher/*Alcedo atthis*		Fish eating	20	–	5.89–22.48	–	–	93.5 ± 4.9	
	Songbirds		Insectivorous	44	–	1.54–123.28	–	–	94.3	
	Songbirds		Frugivorous	20	–	0.11–5.09	–	–	74.1	
	Songbirds		Granivorous	37	–	0.30–19.34	–	–	94.5	
Foula, England	Seabirds		Fish eating	109	–	1.5–30.7	–	1.7–29.2	77–118	Thompson et al. (1990)
Gough, England	Seabirds		Fish eating	7	–	9.4 ± 3.9	–	9.1 ± 4.0	97	
Chaun, Russia	Seabirds		Fish eating	15	–	0.7–6.1	–	0.9–6.5	106–128	Kim et al. (2004)
New Brunswick, Canada	Seabirds		Fish eating	25	–	0.9–4.9	–	0.8–5.3	82–133	Bond and Diamond (2009)
North Canada	Seabirds		Fish eating	51	0.24–43.66	0.59–15.79	0.21–27.88	0.52–11.28	67–91	Mallory et al. (2015)
Southern Ocean	Seabirds		Fish eating	175	0.3–91.6	0.4–39.9	–	–	81–97	Renedo et al. (2017)

[a] Mean is mean concentration with standard deviation.
[b] Range of mean value.

plants in grain (Horvat et al., 2003; Qiu et al., 2008; Zhang et al., 2010), and its consumption is the dominant MeHg exposure pathway for local residents in mining areas (Feng et al., 2008; Zhang et al., 2010). Rice consumption was likely an important Hg exposure pathway for granivorous birds and specialized rice pests, and elevated concentrations of THg (0.35–21.8 mg kg^{-1}) and MeHg % (25.8–65.9%) in insects at WSMM were also documented in earlier study (Abeysinghe et al., 2017). Data on Hg concentrations in fruits were not available, but the exposure dosage via fruit consumption may account for only a small proportion based on studies mentioned above. Consumption of such high MeHg% food increased the potential exposure of MeHg, finally resulting in elevated MeHg burden and MeHg% in songbird feathers. Table 1 also summarizes the results of MeHg% in bird feathers from the literature. Due to the consumption of high MeHg containing fish, Hg in seabird feathers reached up to 91.6 mg kg^{-1} and MeHg was the main speciation (67–133%) in aquatic bird feathers (Renedo et al., 2017; Thompson and Furness, 1989; Bond and Diamond, 2009). In the present study, we also found that MeHg% in the feathers of songbirds with different dietary types was close to the proportions observed in seabird feathers, suggesting similarly high MeHg% also exits in terrestrial bird feathers.

3.2. Hg chemical forms in bird feathers

3.2.1. Hg L_{III} XANES spectra process

Normalized Hg-L_{III} edge XANES spectra of SRMs and the four samples are shown in Fig. 1. The similarity in spectra of the samples suggested that the Hg forms in songbirds were similar despite the large variations of Hg concentrations. By principal component analysis (PCA), the three principal compounds represented almost all (>99%) Hg in the samples and the SRMs were restricted to only five compounds including $Hg(SG)_2$, MeHgCys, MeHgSG, HgCys and HgDicys. The linear combination fitting (LCF) analysis was conducted by two methods, including normalized $\mu(E)$ and the first derivative $n\mu(E)$ used in mine wastes and human hair (Kim et al., 2004; Elgazali et al., 2018). However, due to large difference in MeHg% by normalized $\mu(E)$ in fitting results (Table S3), we found that the MeHg% in LCF results by the first derivative $\mu(E)$ was much closer to the chemical extraction analysis (90.6–98.0% and 89.1–96.5%), exhibiting a higher resolution for complex biological materials and providing more accurate results.

3.2.2. Hg chemical forms

The LCF fitting results and the Hg-L_{III} spectra of the four samples are shown in Table 2 and Fig. 2. Based on the LCF analysis, the percentage of MeHgCys was 71.5–100%, while it ranged from undetectable (Nd) to 27.7% for MeHgSG, suggesting that MeHgCys was the dominant form. In this study, MeHg was predominated by MeHgCys and MeHgSG, and both two forms accounted for 90.6–98.0% of total MeHg. Because LCF analysis usually had an approximately 10% error (Kim et al., 2004), our result was representative of the main Hg chemical forms.

For MeHg, the proportions of MeHgCys (MeHgCys%) and MeHgSG (MeHgSG%) varied within the four feathers of different dietary type birds. In all samples, MeHgCys% ranged from 69.5% to 90.6%, while MeHgSG% ranged from Nd to 27.7%. In addition, except for feather of S2-cht, there was no large difference of MeHg forms between feathers of the two diet (PI and PG) birds. Both the elevated MeHg in rice grains and insects in contaminated sites and the high diet consumption may result in similar proportions of MeHg in various dieted bird feathers. In addition, the dietary habitats may affect the MeHg chemical forms in feathers. For example, S2-cht, which mainly preyed on spiders with elevated

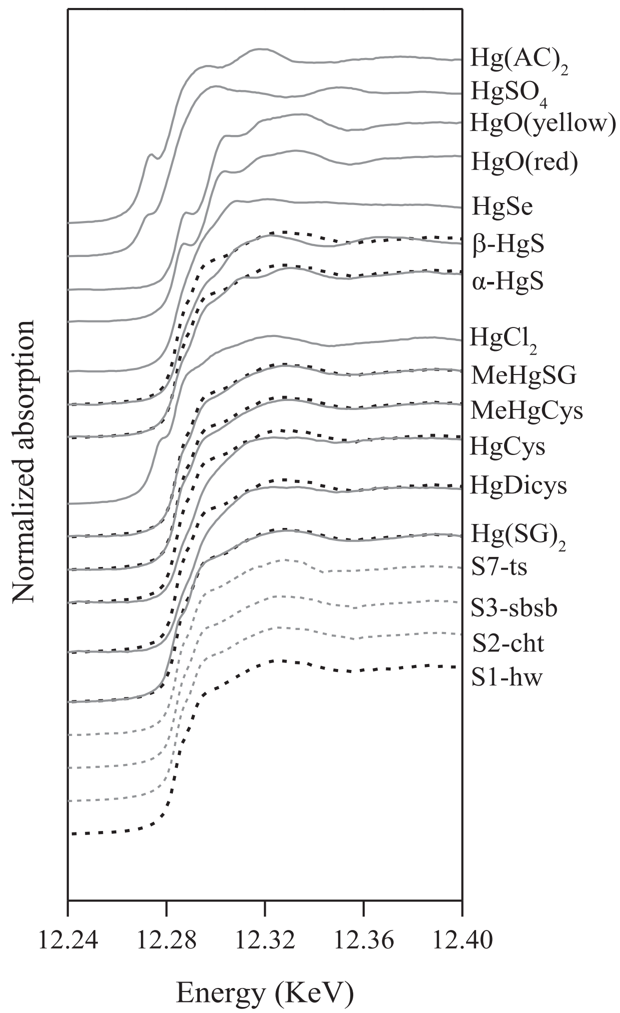

Fig. 1. Hg L_{III} XANES spectra of bird feathers and standard reference compounds. Solid lines in grey color refer to spectra of SRMs, dotted lines in grey color refer to spectra of samples, and black dotted lines in black color refer to the spectrum of S1-hw.

MeHg% (65.9%) (Abeysinghe et al., 2017) had the highest proportion of MeHgCys and the lowest proportion of MeHgSG among the four samples. Thus, consumption of such elevated MeHg food like spiders may affect the percentages of MeHgCys and MeHgSG in feathers. In addition, other factors such as gender, molting, life stage and individual difference in birds may also affect the proportions.

IHg was mainly bound to glutathione compounds and existed as $Hg(SG)_2$ complex. In four samples, the proportions of $Hg(SG)_2$ were within a small variation (Nd to 9.4%). Our present results were different from IHg forms in seabirds' liver which were dominated by HgS and HgSe (Arai et al., 2004). Unlike MeHg which was mainly accumulated through fish or rice consumption, sources of IHg in feathers included direct accumulation or MeHg demethylation like in human hair (George et al., 2010). In addition, bird feathers mainly consisted of keratin (or α-keratin) enriched in cysteine residue (Gregg and Rogers, 1986), which had high affinity for MeHg (Rabenstein et al., 1982). However, because liver and kidney can store Hg from ingested food and demethylated MeHg to other tissues (Thompson et al., 1990; Ek et al., 2004; Kim et al., 1996). Thus, the chemical compositions and function differences may relate to such difference of Hg chemical forms in feathers and liver.

3.2.3. *Potential affect factors*

In biological tissues, MeHg can form water-soluble complexes via the attachment of thiol groups (-SH) in proteins, certain peptides like GSH and amino acids (Clarkson and Magos, 2006). MeHgCys has a close structure as methionine and can enter into cells by neutral amino acids carriers such as L-type amino transporter (LAT). In liver cells, MeHg can be transported into bile as a MeHgSG complex (Ballatori and Clarkson, 1985), which is then broken down to constituent amino acids such as MeHgCys complex by extracellular enzymes (Clarkson and Magos, 2006), and MeHgCys can be re-absorbed into bloodstream in the gallbladder and finally deposited into feathers (Dutczak et al., 1991). Meanwhile, thiol groups in the reduced glutathione molecules can combine with Hg^{2+} and form $Hg(SG)_2$, which was exported from liver cells by glutathione carriers such as multidrug resistance-associated proteins (MRPs) (Ballatori et al., 2002; Bosnjak et al., 2009). Although there was no direct studies on the transport of various Hg forms (e.g. MeHgCys and MeHgSG) in human hair and feather follicle, the neutral amino acids carriers may likely be involved.

Recent reports on the Hg chemical forms in various non-biota and biota samples are listed in Table 3. Among these samples, only Hg in rice, fish and hair exists as HgCys, MeHgCys and MeHgSG. Meanwhile, feather and human hair had similar compositions of keratin (or α-keratin) enriched in cysteine residues (Gregg and Rogers, 1986; Powell and Rogers, 1986). Thus, Hg in feather and human hair should have certain similar chemical forms. However, it has been reported that IHg in hair of occupational or extremely high IHg (Hg vapor) exposure populations (i.e., workers from Hg mining sites or chemical plants) was mainly $Hg(SG)_2$ rather than MeHgCys, which is dominat form in hair of fish consumption populations (Li et al., 2008b; Elgazali et al., 2018; George et al., 2010). These results strongly indicated that exposure routes highly affected the Hg chemical forms in hair.

Since the abandon of Hg mining activities in WSMM in 2004, the total gaseous mercury (TGM) concentration in atmosphere was $28 \pm 13\ ng\ m^{-3}$ (abandoned mining site) and $403 \pm 399\ ng\ m^{-3}$ (artificial Hg smelting site) (Zhao et al., 2016), which was much lower than a previously reported value of $718–40{,}000\ ng\ m^{-3}$ in a typical artificial Hg smelting sites in Wuchuan (Li et al., 2008a). In addition, studies reported that Hg in workers' hair was mainly

Table 2
Hg- L_{III} LCF analysis results of four feathers by the first derivative of $n\mu(E)$.

Samples	THg[a]	MeHg[a]	$Hg(SG)_2$	MeHgCys	MeHgSG	MeHg[b]	R-factor
	mg kg^{-1}	%	%	%	%	%	
S1-hw	114	89.0 ± 7.4	2.0 ± 5.1	71.5 ± 8.7	26.5 ± 5.0	98.0	0.0202
S2-cht	190	90.7 ± 7.2	9.4 ± 4.2	90.6 ± 7.2	Nd±4.2[c]	90.6	0.0137
S3-sbsb	100	96.5 ± 2.7	2.8 ± 3.8	69.5 ± 6.2	27.7 ± 3.5	97.2	0.0100
S7-ts	35	93.1 ± 2.6	Nd±4.6[c]	82.2 ± 20.2	17.8 ± 21.1	100	0.0236

[a] Analyzed by GC/CVAFS.
[b] Sum of MeHgSG and MeHgCys.
[c] Nd means undected data.

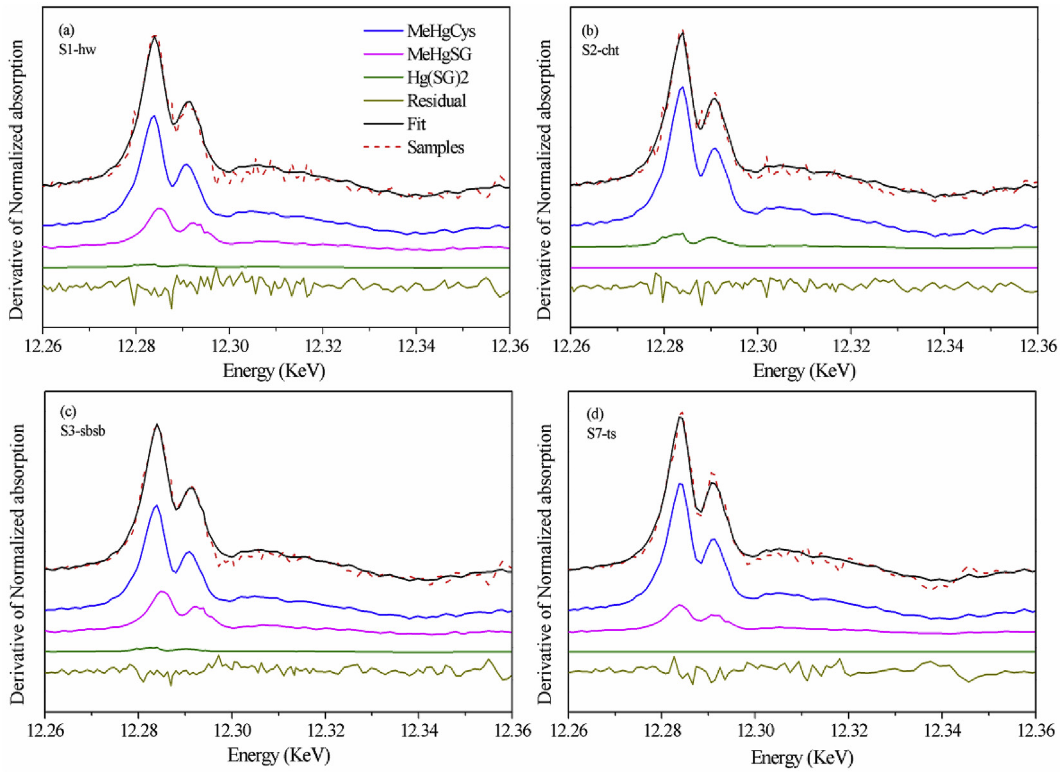

Fig. 2. Hg-L_{III} spectra of the four feathers based on LCF analysis. Dotted lines refer to spetra of samples, colorful soild lines refer to spetra of SRMs while sold line in black color refers to fitting spectra. a) LCF result of S1-hw, b) LCF result of S2-cht, c) LCF result of S3-sbsb and d) LCF result of S7-ts. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Hg forms and its proportions reported in biological samples based on XAFS methods.

Sample	Pollution	IHg (%)	MeHg (%)[a]	MeHg (%)[b]	Sources
Soil, Wanshan, China	Hg mining	α-HgS (1.6–16.4), β-HgS (67.4–79.1), $HgCl_2$ (1.4–12.7), HgO (4.4–16.7)	–[c]	<1	Yin et al. (2016a); Zhao et al. (2016)
Soil, Mt. Amiata District, Italy	Hg mining	β-HgS (100)	–	–	Rimondi et al. (2014)
Calcine, Wanshan, China	Hg mining	α-HgS (8.3–45.9), β-HgS (35.6–85.0), $HgCl_2$ (<5.5), HgO (12–25.8)	–	<1	Yin et al. (2016b)
Calcine, Mt. Amiata District, Italy	Hg mining	α-HgS (21–40), β-HgS (37–60), Mosesite (42)	–	–	Rimondi et al. (2014)
Rice, Wanshan, China	Hg mining	Hg-Cys (76–77)	MeHg-Cys (24–25)	3.7–8.3	Meng et al. (2014)
Fish muscles	–	–	MeHg-Cys (~100)	~100	Harris et al. (2003)
Human brain	–	HgSe (35)	MeHg-Cys (65)	–	Korbas et al. (2010)
Human hair, Seychelles	–	$[Hg(SR)_3]^-$ (19–23)	MeHg-Cys (77–81)	–	George et al. (2010)
Human hair, Wanshan, China	Hg mining	Like $Hg(SG)_2$	–	7.08 ± 0.78	Li et al. (2008b)
Human hair, Pakistan	Chemical industry	$Hg(SG)_2$	–	1.4 ± 2.3	Elgazali et al. (2018)
Seabirds' liver, North Pacific	–	HgSe and β-HgS	–	–	Arai et al. (2004)
Songbird feathers, Wanshan, China	Hg mining	$Hg(SG)_2$ (Nd-24.4)	MeHg-Cys (71.5–90.6) and MeHg-SG (Nd-28.5)[d]	89.0–96.5	This study

[a] Result of XANES fitting.
[b] Analyzed by GC/CVAFS or other chemical extraction methods.
[c] means no data.
[d] Nd means not detected.

$Hg(SG)_2$ and exogenous Hg^0 or gaseous mercury was also absorbed in the cuticle (Li et al., 2008b; Elgazali et al., 2018). However, because birds in the present study dwelled in a heavily Hg contaminated environment, the possibility that feathers received IHg such as elemental Hg (Hg^0) from the ambient atmosphere cannot be excluded. Considering rice and fish consumption were the two major MeHg exposure pathways for human beings in mining areas and coastal areas without occupational exposure (Feng et al., 2008; Zhang et al., 2010), consumption of food such as plant grains and insects were the dominant Hg exposure pathways for birds. The large difference among Hg forms in hair of both fish-eating and occupational exposure population and songbird feathers in contaminated sites suggested that Hg exposure routes also highly affected the Hg chemical forms in bird feathers.

4. Conclusions

Overall, this study is the first time to examine the chemical forms of Hg in songbird feathers. We demonstrated that MeHg is the main Hg speciation in songbird feathers from a contaminated site, which was similar to seabird feathers. We also found that MeHgCys and MeHgSG were the dominant chemical forms of MeHg, while $Hg(SG)_2$ was the main form of IHg. Food consumption affected the proportions of MeHgCys and MeHgSG, while the Hg exposure routes can highly affect the chemical forms. Considering Hg in feather mainly come from inner organs with a dynamic process of body burden, it is worthy to measure the Hg concentrations during the feather formation. In addition, because Hg toxicity was highly dependent on its speciation or chemical forms, a better understanding of its speciation is critical for elucidating the Hg exposure risks to birds. However, further studies such as synchrotron microscopic X-ray fluorescence analysis (μ-XRF), high-resolution (HR) XANES and Hg stable isotopes are needed to reveal the detailed accumulation processes and sources of Hg in bird feathers.

Acknowledgements

We greatly thank the assistances from Dr. Jianxu Wang from Institute of Geochemistry and Dr. Tao Chen from Institute of High Energy Physics, Chinese Academy of Sciences in data analyses and samples' measurements of XANES, and anyone who involved in the present study. This work was financial supported by the National Natural Science Foundation of China (NSFC, 41573135) and the Sino-Norwegian Cooperative Project on Mercury (SINOMER III).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2019.03.060.

References

Abeysinghe, K.S., Qiu, G.L., Goodale, E., Anderson, C.W.N., Bishop, K., Evers, D.C., Goodale, M.W., Hintelmann, H., Liu, S.J., Mammides, C., Quan, R.C., Wang, J., Wu, P.P., Xu, X.H., Yang, X.D., Feng, X.B., 2017. Mercury flow through an Asian rice-based food web. Environ. Pollut. 229, 219–228. https://doi.org/10.1016/j.envpol.2017.05.067.

Agency for Toxic Substances and Disease Registry (ATSDR), 2017. Priority List of Hazardous Substances. http://www.atsdr.cdc.gov/SPL/index.html.

Agusa, T., Matsumoto, T., Ikemoto, T., Anan, Y., Kubota, R., Yasunaga, G., Kunito, T., Tanabe, S., Ogi, H., Shibata, Y., 2005. Body distribution of trace elements in black-tailed gulls from rishiri island, Japan: age-dependent accumulation and transfer to feathers and eggs. Environ. Toxicol. Chem. 24, 2107–2120. https://doi.org/10.1897/04-617r.1.

Andrews, J.C., Wyman, P.D., 1930. Hg derivatives of cysteine. J. Biol. Chem. 87, 427–433.

Arai, T., Ikemoto, T., Hokura, H., Terada, Y., Kunito, T., Tanabe, S., Nakai, L., 2004. Chemical forms of mercury and cadmium accumulated in marine mammals and seabirds as determined by XAFS analysis. Environ. Sci. Technol. 38, 6468–6474. https://doi.org/10.1021/es040367u.

Ballatori, N., 2002. Transport of toxic metals by molecular mimicry. Environ. Health Perspect. 110, 689–694. https://doi.org/10.1289/ehp.02110s5689.

Ballatori, N., Clarkson, T.W., 1985. Biliary secretion of glutathione and of glutathione-metal complexes. Fundam. Appl. Toxicol. 5, 816–831. https://doi.org/10.1016/0272-0590(85)90165-4.

Becker, P.H., Furness, R.W., Henning, D., 1993. The value of chick feathers to assess spatial and interspecific variation in the mercury contamination of seabirds. Environ. Monit. Assess. 28, 255. https://doi.org/10.1007/BF00545769.

Bond, A.L., Diamond, A.W., 2009. Total and methyl mercury concentrations in seabird feathers and eggs. Arch. Environ. Contam. Toxicol. 56, 286–291. https://doi.org/10.1007/s00244-008-9185-7.

Bosnjak, I., Uhlinger, K.R., Heim, W., 2009. Multidrug efflux transporters limit accumulation of inorganic, but not organic, mercury in sea urchin embryos. Environ. Sci. Technol. 43, 8374. https://doi.org/10.1021/es901677r.

Chen, C.Y., Stemberger, R.S., Kamman, N.C., Mayes, B.M., Folt, C.L., 2005. Patterns of Hg Bboaccumulation and transfer in aquatic food webs across multi-lake studies in the northeast US. Ecotoxicology 14, 135–147. https://doi.org/10.1007/s10646-004-6265-y.

Clarkson, T.W., Magos, L., 2006. The toxicology of mercury and its chemical compounds. Crit. Rev. Toxicol. 36, 1–54. https://doi.org/10.1080/10408440600845619.

Cristol, D.A., Brasso, R.L., Condon, A.M., Fivargue, R.E., Friedman, S.L., Monroe, A.P., White, A.E., 2008. The movement of aquatic mercury through terrestrial food webs. Science 320, 335. https://doi.org/10.1126/science.1154082.

Dutczak, W.J., Clarkson, T.W., Ballatori, N., 1991. Biliary-hepatic recycling of a xenobiotic: gallbladder absorption of methyl mercury. Am. J. Physiol. 260, 73–80. https://doi.org/10.1152/ajpgi.1991.260.6.G873.

Ek, K.H., Morrison, G.M., Lindberg, P., Rauch, S., 2004. Comparative tissue distribution of metals in birds in Sweden using ICP-MS and laser ablation ICP-MS. Arch. Environ. Contam. Toxicol. 47, 259–269. https://doi.org/10.1007/s00244-004-3138-6.

Elgazali, A.A.S., Gajdosechova, Z., Abbas, Z., Lombi, E., Scheckel, K.G., Donner, E., Fiedler, H., Feldmann, J., Krupp, E.M., 2018. Reactive gaseous mercury is generated from chloralkali factories resulting in extreme concentrations of mercury in hair of workers. Sci. Rep. 8, 1–5. https://doi.org/10.1038/s41598-018-20544-5.

Feng, X.B., Li, P., Qiu, G.L., Wang, S.F., Li, G.H., Shang, L.H., Meng, B., Jiang, H.M., Bai, W.Y., Li, Z.G., Fu, X.W., 2008. Human exposure to methylmercury through rice intake in mercury mining areas, Guizhou province, China. Environ. Sci. Technol. 42, 326–332. https://doi.org/10.1021/es071948x.

Furness, R.W., Muirhead, S.J., Woodburn, M., 1986. Using bird feathers to measure mercury in the environment: relationships between mercury content and moult. Mar. Pollut. Bull. 17, 27–30. https://doi.org/10.1016/0025-326X(86)90801-5.

George, G.N., Singh, S.P., Myers, G.J., Watson, G.E., Pickering, I.J., 2010. The chemical forms of mercury in human hair: a study using X-ray absorption spectroscopy. J. Biol. Inorg. Chem. 15, 709–715. https://doi.org/10.1007/s00775-010-0638-x.

Gregg, K., Rogers, G.E., 1986. Feather keratin: composition, structure and biogenesis. In: Biology of the Integument, Vertebrates, vol. 33. Springer, Berlin Heidelberg. https://doi.org/10.1007/978-3-662-00989-5_33, 667-604.

Hallinger, K.K., Cornell, K.L., Brasso, R.L., Cristol, D.A., 2011. Mercury exposure and survival in free-living tree swallows (*Tachycineta bicolor*). Ecotoxicology 20, 39–46. https://doi.org/10.1007/s10646-010-0554-4.

Harris, H.H., Pickering, I.J., George, G.N., 2003. The chemical form of mercury in fish. Science 301, 1203. https://doi.org/10.1126/science.1085941.

Hawley, D.M., Hallinger, K.K., Cristol, D.A., 2009. Compromised immune competence in free-living tree swallows exposed to mercury. Ecotoxicology 18, 499–503. https://doi.org/10.1007/s10646-009-0307-4.

Horvat, M., Nolde, N., Fajon, V., Logar, M., Lojen, S., Jacimovic, R., Falnoga, I., Qu, L.Y., Faganeli, J., Drobne, D., 2003. Total mercury, methylmercury and selenium in mercury polluted areas in the province Guizhou, China. Sci. Total Environ. 304, 231–256. https://doi.org/10.1016/S0048-9697(02)00572-7.

Jackson, A.K., Evers, D.C., Etterson, M.A., Condon, A.M., Folsom, S.B., Detweiler, J., Schmerfeld, J., Cristol, D.A., 2011. Mercury exposure affects the reproductive success of a free-living terrestrial songbird, the carolina wren (*thryothorus ludovicianus*). Auk 128, 759–769. https://doi.org/10.1525/auk.2011.11106.

Kim, C.S., Rytuba, J.J., Brown, G.E., 2004. Geological and anthropogenic factors influencing mercury speciation in mine wastes: an EXAFS spectroscopy study. Appl. Geochem. 19, 379–393. https://doi.org/10.1016/S0883-2927(03)00147-1.

Kim, E.Y., Murakami, T., Saeki, K., Tatsukawa, R., 1996. Mercury levels and its chemical form in tissues and organs of seabirds. Arch. Environ. Contam. Toxicol. 30, 259–266. https://doi.org/10.1007/BF00215806.

Korbas, M., O'Donoghue, J.L., Watson, G.E., Pickering, I.J., Singh, S.P., Myers, G.J., Clarkson, T.W., George, G.N., 2010. The chemical nature of mercury in human brain following poisoning or environmental exposure. ACS Chem. Neurosci. 1, 810–818. https://doi.org/10.1021/cn1000765.

Lemes, M., Wang, F., 2009. Methylmercury speciation in fish muscle by HPLC-ICP-MS following enzymatic hydrolysis. J. Anal. At. Spectrom. 24, 663–668. https://doi.org/10.1039/b819957b.

Li, P., Feng, X.B., Qiu, G.L., Shang, L.H., Wang, S.F., 2008a. Mercury exposure in the population from Wuchuan mercury mining area, Guizhou. China. Sci. Total Environ. 395, 72–79. https://doi.org/10.1016/j.scitotenv.2008.02.006.

Li, Y.F., Chen, C.Y., Li, B., Li, W., Dong, Z.Q., Nomura, M., Gao, Y.X., Zhao, Z.X., Hu, W., Zhao, Y.L., Chai, Z.F., 2008b. Mercury in human hair and blood samples from people living in Wanshan mercury mine area, Guizhou province, China: a XAS study. J. Inorg. Biochem. 102, 500–506. https://doi.org/10.1016/j.jinorgbio.2007.11.005.

Mah, V., Jalilehvand, F., 2010. Glutathione complex formation with mercury(II) in aqueous solution at physiological pH. Chem. Res. Toxicol. 23, 1815–1823. https://doi.org/10.1021/tx100260e.

Mallory, M.L., Braune, B.M., Provencher, J.F., Callaghan, D.B., Gilchrist, H.G., Edmonds, S.T., Allard, K., O'Driscoll, N.J., 2015. Mercury concentrations in feathers of marine birds in Arctic Canada. Mar. Pollut. Bull. 98, 308–313. https://doi.org/10.1016/j.marpolbul.2015.06.043.

Meng, B., Feng, X.B., Qiu, G.L., Anderson, C.W.N., Wang, J.X., Zhao, L., 2014.

Localization and speciation of mercury in brown rice with implications for pan-Asian public health. Environ. Sci. Technol. 48, 7974–7981. https://doi.org/10.1021/es502000d.
Morel, F.M.M., Kraepiel, A.M.L., Amyot, M., 1998. The chemical cycle and bioaccumulation of mercury. Annu. Rev. Ecol. Systemat. 29, 543–566. https://doi.org/10.2307/221718.
Neville, G.A., Drakenberg, T., 1974. Mercury (II) complexation of cysteine, methyl cysteineate, and S-methyl cysteine in acidic media. Can. J. Chem. 52, 616–622. https://doi.org/10.1139/v74-095.
Paris, O.J., Swaddle, J.P., Cristol, D.A., 2018. Exposure to dietary methyl-mercury solely during embryonic and juvenile development halves subsequent reproductive success in adult zebra finches. Environ. Sci. Technol. 52, 3117–3124. https://doi.org/10.1021/acs.est.7b04752.
Powell, B.C., Rogers, G.E., 1986. Hair Keratin: composition, structure and biogenesis. In: Biology of the Integument, Vertebrates. Springer, Berlin Heidelberg, pp. 695–751. https://doi.org/10.1007/978-3-662-00989-5_34.
Qiu, G.L., Feng, X.B., Li, P., Wang, S.F., Li, G.H., Shang, L.H., Fu, X.W., 2008. Methylmercury accumulation in rice (*Oryza sativa L.*) grown at abandoned mercury mines in Guizhou, China. J. Agric. Food Chem. 56, 2465–2468. https://doi.org/10.1021/jf073391a.
Rabenstein, D.L., Isab, A.A., Reid, R.S., 1982. A proton nuclear magnetic resonance study of the binding of methylmercury in human erythrocytes. Biochim. Biophys. Acta Mol. Cell Res. 720, 53–64. https://doi.org/10.1016/0167-4889(82)90038-6.
Ravel, B., Newville, M., 2010. ATHENA, ARTEMIS, HEPHAESTUS: data analysis for X-ray absorption spectroscopy using IFEFFIT. J. Synchrotron Radiat. 12, 537–541. https://doi.org/10.1107/S0909049505012719.
Renedo, M., Bustamante, P., Tessier, E., 2017. Assessment of mercury speciation in feathers using species-specific isotope dilution analysis. Talanta 174, 100–110. https://doi.org/10.1016/j.talanta.2017.05.081.
Rimmer, C.C., Miller, E.K., Mcfarland, K.P., 2010. Mercury bioaccumulation and trophic transfer in the terrestrial food web of a montane forest. Ecotoxicology 19, 697–709. https://doi.org/10.1007/s10646-009-0443-x.
Rimondi, V., Bardelli, F., Benvenuti, M., Costagliola, P., Grey, J.E., Lattanzi, P., 2014. Mercury speciation in the Mt. Amiata mining district (Italy): interplay between urban activities and mercury contamination. Chem. Geol. 380, 110–118. https://doi.org/10.1016/j.chemgeo.2014.04.023.
Thompson, D.R., Furness, R.W., 1989. Comparison of levels of total and organic mercury in seabird feathers. Mar. Pollut. Bull. 20, 577–579. https://doi.org/10.1016/0025-326X(89)90361-5.
Thompson, D.R., Stewart, F.M., Furness, R.W., 1990. Using seabirds to monitor mercury in marine environments: the validity of conversion ratios for tissue comparisons. Mar. Pollut. Bull. 21, 0–342. https://doi.org/10.1016/0025-326x(90)90795-a.
United States Environmental Protection Agency (USEPA), 1998. Method 1630: Methyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry. USEPA, Washington, DC, USA, pp. 1–55.
United States Environmental Protection Agency (USEPA), 2002. Method 1631: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry. USEPA, Washington, DC, USA, pp. 1–33.
Wang, J.X., Feng, X.B., Anderson, C.W.N., Wang, H., Zheng, L.R., Hu, T.D., 2012. Implications of mercury speciation in thiosulfate treated plants. Environ. Sci. Technol. 46, 5361. https://doi.org/10.1021/es204331a.
Yin, R.S., Gu, C.H., Feng, X.B., Hurley, J.P., Krabbenhoft, D.P., Lepak, R.F., Zhu, W., Zheng, L.R., Hu, T.D., 2016a. Distribution and geochemical speciation of soil mercury in Wanshan Hg mine: effects of cultivation. Geoderma 272, 32–38. https://doi.org/10.1016/j.geoderma.2016.03.003.
Yin, R.S., Gu, C.H., Feng, X.B., Zheng, L.R., Hu, N.N., 2016b. Transportation and transformation of mercury in a calcine profile in the Wanshan Mercury Mine, SW China. Environ. Pollut. 219, 976–981. https://doi.org/10.1016/j.envpol.2016.10.034.
Zhang, H., Feng, X.B., Larssen, T., Qiu, G.L., Vogt, R.D., 2010. In inland China, rice, rather than fish, is the major pathway for methylmercury exposure. Environ. Health Perspect. 118, 1183. https://doi.org/10.1289/ehp.1001915.
Zhao, L., Anderson, C.W.N., Qiu, G.L., Meng, B., Wang, D.Y., Feng, X.B., 2016. Mercury methylation in paddy soil: source and distribution of mercury species at a Hg mining area, Guizhou province, China. Biogeosciences 13, 2429–2440. https://doi.org/10.5194/bg-13-2429-2016.