

SUPPORTING INFORMATION

Localization and speciation of mercury in brown rice with implications for pan-Asian public health

BO MENG[†], XINBIN FENG^{†, *}, GUANGLE QIU[†], CHRISTOPHER W. N. ANDERSON[‡],
JIANXU WANG[†], LEI ZHAO^{§, †}

[†]State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550002, P. R. China

[‡]Soil and Earth Sciences, Institute of Agriculture and Environment, Massey University, Palmerston North, New Zealand

[§]College of Resources Environment, Southwest University, Chongqing 400716, P. R. China

*Corresponding Author:

Xinbin Feng

Phone: 86-851-5891356

Fax: 86-851-5891609

Email: fengxinbin@vip.skleg.cn

12pages in total, 4 Figures, 4 Tables

Description of sampling locations

Each of these locations experiences conditions of similar climate and is subject to subtropical monsoon-type weather. Mining activity in the Wanshan district generated an estimated cumulative 2.0×10^4 tons of Hg and 1.0×10^8 tons of calcine (roasted ore) and waste rock that is today spread throughout the environment. Formal mining ceased in 2001, but illegal artisanal and small-scale mining activities continue today. Numerous environmental studies have shown serious Hg contamination of air, stream water, soil, and organisms throughout the Wanshan district (1-6). In contrast to the other sites, there is no significant source for Hg emission in proximity to the Huaxi control site, and the environment at this site is characteristic of the regional background for southwestern China (7). Concentrations of Hg in ambient air, surface soil samples (upper 0~20 cm), and stream water samples reported for the artisanal Hg mining site (Gouxi), abandoned Hg mining sites (Wukeng and Dashuixi), and the control site (Huaxi) are summarized in Table S1. More detail on these locations has been reported by Meng et al. (4).

Detailed information concerning sample collection and preparation

Eighty nine ripe rice grain samples were collected by hand from the control site (n=10), the abandoned Hg mining sites (n=39), and the artisanal Hg mining site (n=40) during the harvest seasons of 2006-2009 (Table S2). Each sample constituted a composite of at least five subsamples within an area of about 5-10 m² in the same paddy field. All samples were rinsed in the field using drinking water, and then further cleaned with de-ionized water in an ultrasonic bath in the laboratory. Clean samples were air dried and stored in polyethylene bags.

The edible parts of the rice samples (brown rice) were shelled from their hulls using a laboratory huller (Taizhou Foodstuff Instrument Inc. China). The brown rice was then milled to remove the bran layers (including the germ) using a laboratory polisher (Taizhou Foodstuff Instrument Inc. China). The mass of hull, bran, and white rice for each sample was recorded, and proportionally constituted 21 ± 3.3 %, 8.0 ± 1.5 %, and 71 ± 3.1 % of the raw rice weight, respectively. These figures are comparable with proportions reported in the literature (8,9). All samples were ground to less than 150 mesh (IKA-A11 basic, IKA®, Germany). The grinder was thoroughly cleaned after each sample as a precaution to avoid cross-contamination during sample processing. Powdered samples were packed into plastic dishes, sealed in polyethylene bags and stored in

desiccators kept dark and cold prior to analysis.

Synchrotron radiation microscopic X-ray fluorescence analysis (SR- μ XRF) analysis required the preparation of thin sections. In order to avoid any redistribution of Hg during preparation, thin sections were prepared without resin embedding of the grains. In detail, a grain was first fractured using a scalpel, both longitudinally and transversely, in order to obtain a flat surface that could be attached to a plastic support with adhesive tape. Mounted samples were further sliced with a freezing microtome (CM3050S, Leica[®], Germany). In this way, approximately 150 μ m thick longitudinal and latitudinal sections were placed on Kapton[®] tape for SR- μ XRF analysis.

Detailed information concerning analytical methods

Total mercury and Methylmercury Analysis. For THg analysis, each fraction of rice grain was digested in a freshly prepared mixture of HNO₃/H₂SO₄ (4:1, v/v) at 95°C (10,11). The THg concentration in the digest solution was determined using cold vapor atomic fluorescence spectrometry (CVAFS, Tekran 2500, Tekran Inc., Canada) preceded by BrCl oxidation, SnCl₂ reduction, pre-concentration and finally thermo-reduction to Hg⁰ (11). MeHg was extracted from samples using a KOH-methanol/solvent extraction technique (12). The MeHg concentration in the extract was quantified using GC-CVAFS (Brooks Rand Model III, Brooks Rand Labs, Seattle, WA, USA) following analytical method 1630 (13). The concentration of IHg in each rice grain fraction was calculated as the concentration difference between THg and MeHg in the sample (4).

X-ray Absorption Near-Edge Structure Analysis. Due to the relatively low concentration of Hg (IHg and MeHg) in white rice, Hg speciation analysis by XANES was restricted to rice bran samples which contained a concentration of Hg that was appropriate for analysis using this technique. Specifically, two rice bran samples with high concentrations of THg were chosen. This was done to ensure the quality of the XANES spectra (rice bran samples and reference compounds) obtained using beamline 1W1B of the Beijing Synchrotron Radiation Facility (BSRF). An energy range of -200 to 1000 eV relative to the edge absorption of the L_{III}-electron of Hg (lowest energy inflection at 12.28 keV) was used to acquire each spectra. Hg L_{III}-edge spectra were collected using a 19 element Ge array solid state detector tuned to the Hg L α line fluorescence. In order to investigate different Hg oxidation states and chemical structures, ten IHg standards, including

cinnabar (α -HgS), metacinnabar (β -HgS), mercuric oxide (yellow as well as red HgO), mercuric chloride (HgCl₂), mercuric sulphate (HgSO₄), mercuric acetate (CH₃COO)₂Hg, mercuric L-cysteinate (hereafter Hg-cysteine), mercuric dicysteinate (hereafter Hg-dicysteine or Hg-cystine), and mercuric selenide (HgSe), and one organic Hg standard, methylmercury L-cysteinate (MeHg-cysteine), were employed in this study. Most of these compounds were purchased (Dongxin Chemical Reagent Corp., China). However, Hg-dicysteine and Hg-cysteine were synthesized according to Andrews and Wyman (14) and Neville and Drakenberg (15), respectively. MeHg-cysteine was kindly provided by Professor Feiyue Wang (University of Manitoba, Winnipeg, Canada) and was synthesized according to the method of Lemes and Wang (16). XANES spectra of the IHg reference compounds attached to Kapton[®] tape were acquired in transmission mode, while pressed thin tablets of the rice bran samples and the MeHg reference compounds (diluted in starch) were collected in fluorescence mode under ambient conditions. The XANES data (average of two scans) were normalized (baseline and background corrections) and analysed by linear combination fitting (LCF) employing the IFEFFIT software package (CARS, University of Chicago). The minimal R-factor, Chi-square, and Reduced Chi-square were used in the quality control of the fitting. The correctness of the LCF is controlled by many factors (17) and therefore the results we report are not free of uncertainties and limitations.

Synchrotron radiation microscopic X-ray fluorescence analysis. SR- μ XRF analysis of thin sections (longitudinal as well as latitudinal) was conducted using beamline 4W1B of the BSRF. The detection limit for analysis was in the order of mg kg⁻¹. The incident X-ray beam (15 keV) was generated using a Si (111) double crystal monochromator and was focused by toroidal mirror and poly capillary lens to resolve a spot size 50 μ m in diameter. Mapping data (SR- μ XRF) were collected using a stepsize of 50~100 μ m depending on the size of the investigated sample, and with 60 s per point. SR- μ XRF spectra were processed using the PyMCA software package (18). Elemental maps of the analyzed sections of rice grain were generated using Origin 8.0 software (OriginLab Corp., USA). Parts of the rice grain that have higher density or thickness appear with a stronger SR- μ XRF signal due to the compression of 3D-spatial information into 2D during the imaging process (19).

Detailed information concerning the QC/QA procedure employed

Quantification of the THg and MeHg concentration in rice tissues was performed using daily calibration curves with a coefficient of variation (r^2) ≥ 0.99 . Quality control measures consisted of method blanks, triplicates, matrix spikes, and certified reference materials. The method detection limits (3σ) were calculated as $0.01 \mu\text{g kg}^{-1}$ for THg and to $0.002 \mu\text{g kg}^{-1}$ for MeHg. The precision and bias for triplicate samples were estimated less than 7.2% for THg and MeHg analysis. The recoveries for matrix spikes ranged from 96 to 110% for THg analysis, and from 86 to 105% for MeHg. The certified reference materials employed in this study were as follows: rice (GBW08508 and GBW10010, National Research Centre for Certified Reference Materials, China), poplar leaf (GBW07604, National Research Centre for Certified Reference Materials, China), and shellfish (TORT-2, National Research Council of Canada). The results of the analysis of the certified reference materials are described in Table S3.

Statistical analysis was performed using SPSS 13.0 software (International Business Machines Corp.). Relationships between covariant sets of data were subjected to regression analysis. Correlation coefficients (r) and significance probabilities (p) were computed for the linear regression fits. Significant differences were determined using the Kruskal-Wallis test and declared at $p < 0.05$. The Hg content of each fraction of rice grain was calculated by multiplying the Hg concentration by the corresponding biomass.

Factors controlling mercury levels in rice grain

Previous work has defined atmospheric Hg as the principal source of IHg to the aboveground parts of rice plant (4,20). However, MeHg in paddy soil is believed to be the dominant source of MeHg in rice (4,21). The rice root surface acts as a potential Hg barrier, which sequesters IHg and consequently reduces the translocation of Hg through the root system to aboveground parts of rice plant such as the leaf, stalk, seed, and hull (4,20). As shown in Table S1, the concentration of Hg^0 in ambient air at the artisanal Hg mining site (Gouxu) was much higher than that at the abandoned Hg mining sites (Wukeng and Dashuixi) and the control site (Huaxi). Although the concentration of THg in soil at the abandoned Hg mining sites was significantly higher than that at the artisanal Hg mining site and the control site, the highest concentration of IHg in white rice ($89 \pm 7.3 \mu\text{g kg}^{-1}$) was observed at Gouxu, followed by the artisanal Hg mining site ($7.9 \pm 3.6 \mu\text{g kg}^{-1}$) and Huaxi ($2.7 \pm 0.25 \mu\text{g kg}^{-1}$). Therefore, the bioaccumulation factors of IHg in the Hg

contaminated area seem to be lower than in the control site, an observation which is in good agreement with previous studies (4,22).

Meng et al. (21) clearly demonstrated that newly deposited Hg to paddy agricultural fields is more readily transformed into MeHg and accumulated by rice plants than Hg forms that have resided in paddy sediment for an extended time. Therefore, atmospheric mercury can also be inferred as the principle source of MeHg in plants, albeit via the soil-plant vector. Environmental quality data show that ambient air at the Gouxì artisanal mining site is intermittently polluted by Hg⁰ emissions from adjacent artisanal Hg mining operations to a level that is orders of magnitude above the background (control) level for China (Table S1). Furthermore, available data indicates that the atmospheric Hg concentration at Gouxì is, on average, more than one order of magnitude higher than at Wukeng/Dashuixi. Dai et al. (23) demonstrated that atmospheric Hg dry deposition fluxes in the Wanshan mining area exhibit a high degree of variability, both temporarily and spatially. They reported a Wanshan mercury flux as high as 6000 $\mu\text{g m}^{-2} \text{yr}^{-1}$, which is about 2 orders of magnitude higher than in the background flux in southwest China (24). We therefore propose that elevated IHg and MeHg concentrations in rice cultivated in the Wanshan Hg mining area (artisanal and abandoned sites) can be attributed to the atmospheric flux of mercury at this location, although the pathway for uptake differs between the two species (MeHg via soil; IHg via atmosphere). The current study, considered jointly with previous observations, supports the hypothesis that Hg in ambient air is the key factor that controls the concentration of both IHg and MeHg in rice grain (4,20,21). However, further work needs to be done to elucidate the mechanisms underpinning uptake.

In summary, the concentration of Hg in air is not directly reflected in the bioaccumulation factor, and therefore for Hg at sites with high atmospheric mercury flux, bioaccumulation factors need to be interpreted with caution.

Figure S1. Map of the study area and sampling locations.

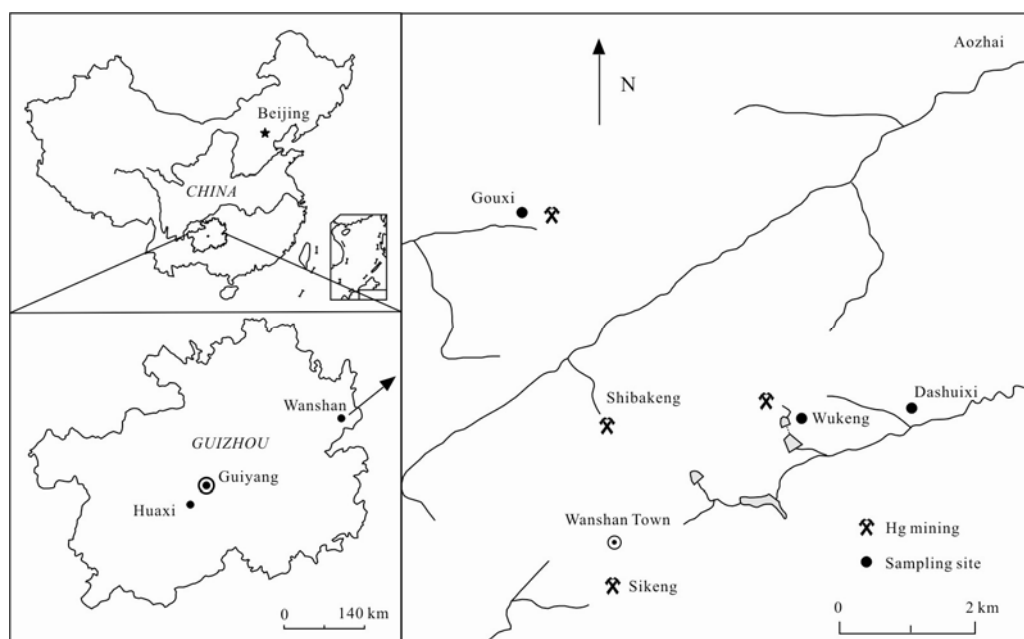


Figure S2. IHg and MeHg concentration in fractions of rice grain collected from the control site, abandoned Hg mining sites, and artisanal Hg mining site (with standard deviations; dry weight).

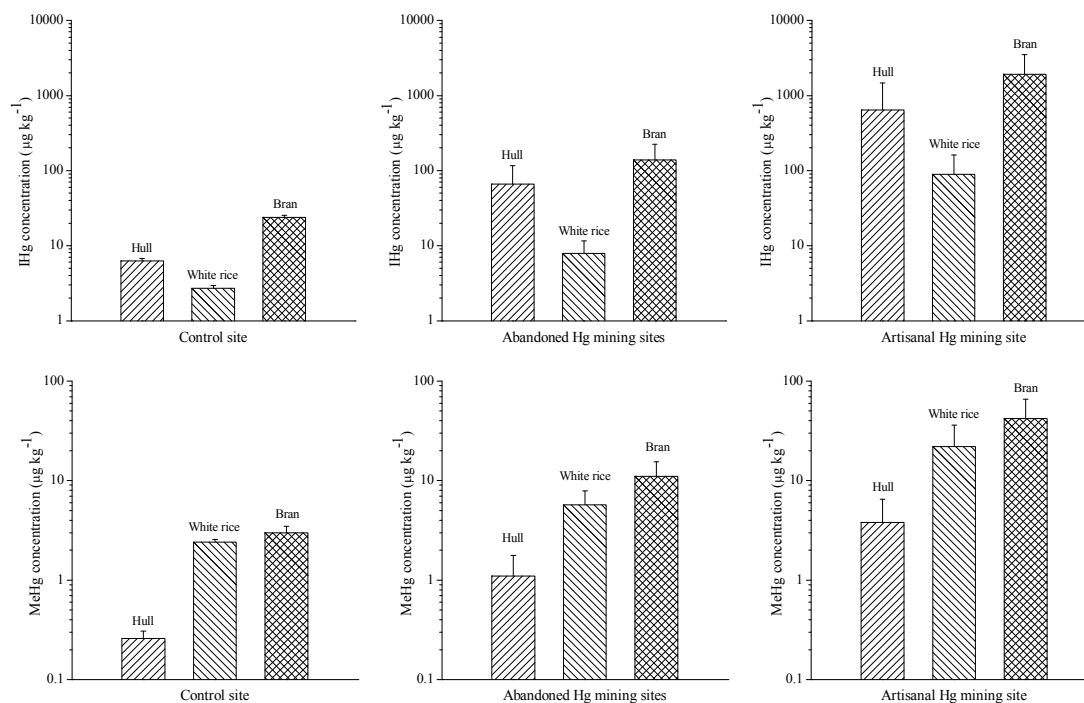


Figure S3. Comparison of normalized mercury L_{III} XANES spectra of rice bran samples with selected mercury species.

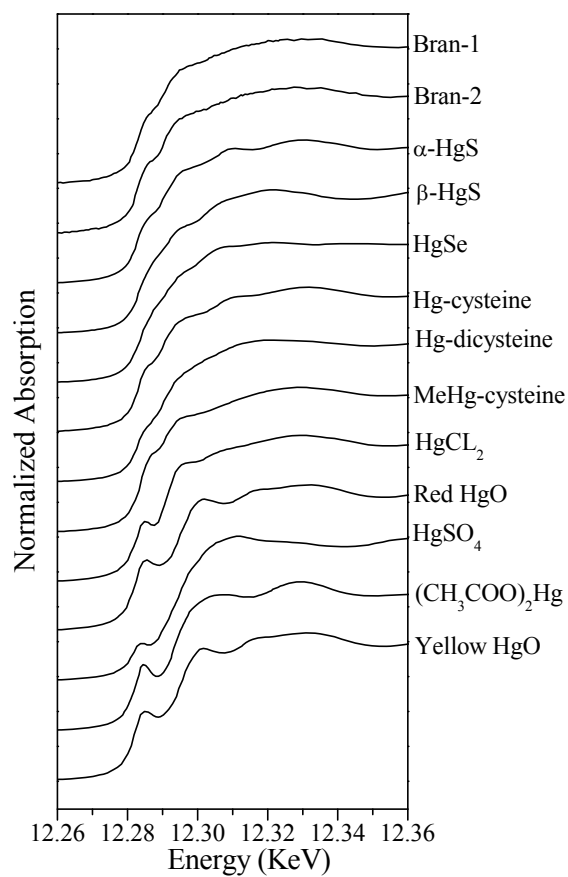


Figure S4. Comparison of mercury L_{III} XANES spectra of rice bran samples from two individuals. The essential identity of the two spectra is illustrated by the difference spectrum.

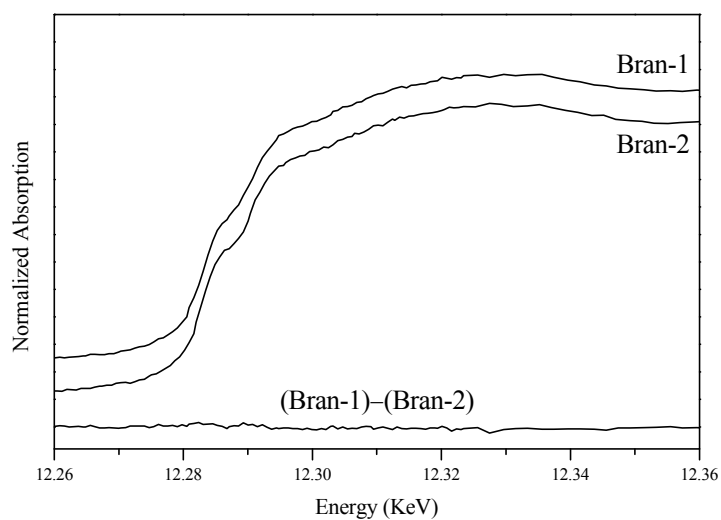


Table S1 Concentrations of Hg in ambient air, soil, and stream water at the artisanal Hg mining site (Gouxi), abandoned Hg mining sites (Wukeng and Dashuixi), and the control site.

	Huaxi	Wukeng and Dashuixi	Gouxi	References
Ambient air Hg ⁰ (ng m ⁻³)	2.0-12	6.0-290	12-1652	(4,25)
Soil THg (mg kg ⁻¹)	0.24-0.38	31-527	13-36	(4)
Stream water THg (ng L ⁻¹)	3.5-8.7	20-10580	92-14000	(1,2,4,26)

Table S2 Description of sampling method

Sampling periods	Control site	Abandoned Hg mining sites	Artisanal Hg mining site
September 2006	3	9	3
September 2008	4	21	12
September 2009	3	9	25
Total amount	10	39	40

Table S3. List of certified reference materials used in the present study and the results obtained (mean \pm S.D, $\mu\text{g kg}^{-1}$).

Producer	CRM	n	Hg speciation	Obtained value	Certified value
NRC CRM*	GBW10014	15	THg	10.2 \pm 0.10	10.9 \pm 1.6
NRC CRM*	GBW10020	15	THg	149 \pm 6.9	150 \pm 20
NRCC**	TORT-2	15	THg	270 \pm 36	270 \pm 60
NRCC**	TORT-2	30	MeHg	144 \pm 12	152 \pm 13

*NRC CRM: National Research Centre for Certified Reference Materials;

**NRCC: National Research Council Canada.

Table S4 Hg concentration ratio for adjacent fractions of rice grain (mean \pm S.D)

sampling sites	IHg concentration ratio		MeHg concentration ratio	
	bran/hull	bran/white rice	bran/hull	bran/white rice
control site(n=10)	3.9 \pm 0.33	9.1 \pm 0.84	12 \pm 2.8	1.2 \pm 0.15
abandoned Hg mining sites(n=39)	2.4 \pm 1.2	18 \pm 6.3	13 \pm 6.1	1.9 \pm 0.6
artisanal Hg mining site(n=40)	4.1 \pm 2.9	22 \pm 6.6	14 \pm 9.9	2.1 \pm 0.7

Literature cited

1. Horvat M, Nolde N, Fajon V, Jereb V, Logar M, Lojen S, Jacimovic R, Falnoga I, Qu LY, Faganeli J, Drobne D. (2003) Total mercury, methylmercury and selenium in mercury polluted areas in the province Guizhou, China. *Sci Total Environ* 304(1-3):231-256.
2. Li P, Feng XB, Shang LH, Qiu GL, Meng B, Liang P, Zhang H. (2008) Mercury pollution from artisanal mercury mining in Tongren, Guizhou, China. *Appl Geochem* 23(8):2055-2064.
3. Li P, Feng XB, Qiu GL, Shang LH, Wang SF, Meng B. (2009) Atmospheric mercury emission from artisanal mercury mining in Guizhou Province, Southwestern China. *Atmos Environ* 43(14):2247-2251.
4. Meng B, Feng XB, Qiu GL, Wang DY, Li P, Shang LH, Sommar J. (2010) Distribution patterns of inorganic mercury and methylmercury in tissues of rice (*Oryza sativa* L.) plants and possible bioaccumulation pathways. *J Agric Food Chem* 58(8):4951-4958.
5. Qiu GL, Feng XB, Li P, Wang SF, Li GH, Shang LH, Fu XW. (2008) Methylmercury accumulation in rice (*Oryza sativa* L) grown at abandoned mercury mines in Guizhou, China. *J Agric Food Chem* 56(7):2465-2468.
6. Qiu GL, Feng XB, Wang SF, Shang LH. (2005) Mercury and methylmercury in riparian soil, sediments, mine-waste calcines, and moss from abandoned Hg mines in east Guizhou province, southwestern China. *Appl Geochem* 20(3):627-638.
7. Fu XW, Qiu GL, Shang LH, Zhang H. (2011) Speciated atmospheric mercury and its potential source in Guiyang, China. *Atmos Environ* 45(25):4205-4212.
8. Juliano BO. (1972) The rice caryopsis and its composition, In: Houston DF (ed); Rice chemistry and technology. Minnesota: AACC.
9. Pedersen B, Eggum BO. (1983) The influence of milling on the nutritive value of flour from cereal grains. IV. Rice. *Qual Plant Plant Foods Hum Nutr* 33(4):267-278.
10. Horvat M, Lupsina V, Pihlar B. (1991) Determination of Total Mercury in Coal Fly-Ash by Gold Amalgamation Cold Vapor Atomic-Absorption Spectrometry. *Anal Chim Acta* 243(1):71-79.
11. U.S. EPA (2002) Method 1631: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry (Revision E), EPA-821-R-02-019. Washington,

DC.

12. Liang L, Horvat M, Cernichiari E, Gelein B, Balogh S. (1996) Simple solvent extraction technique for elimination of matrix interferences in the determination of methylmercury in environmental and biological samples by ethylation gas chromatography cold vapor atomic fluorescence spectrometry. *Talanta* 43(11):1883-1888.
13. U.S. EPA (2001) Method 1630: Methylmercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and CVAFS, EPA-821-R-01-020. Washington, DC.
14. Andrews JC, Wyman PD. (1930) Hg derivatives of cysteine. *J. Biol. Chem.* 87, 427-433.
15. Neville GA, Drakenberg T. (1974) Mercury(II) complexation of cysteine, methyl cysteineate, and S-methyl cysteine in acidic media. *Can J Chem* 52(4):616-622.
16. Lemes M, Wang FY. (2009) Methylmercury speciation in fish muscle by HPLC-ICP-MS following enzymatic hydrolysis. *J Anal At Spectrom* 24(5):663-668.
17. Beauchemin S, Hesterberg D, Beauchemin M. (2002) Principal component analysis approach for modeling sulfur K-XANES spectra of humicacids. *Soil Sci Soc Am J* 66(1):83-91.
18. Solé VA, Papillon M, Cotte M, Walter Ph, Susini J. (2007) A multiplatform code for the analysis of energy-dispersive X-ray fluorescence spectra. *Spectrochim Acta B* 62(1):63-68.
19. McNear DH, Peltier E, Everhart J, Chaney RL, Sutton S, Newville M, Rivers M, Sparks DL. (2005) Application of quantitative fluorescence and absorption-edge computed microtomography to image metal compartmentalization in *Alyssum murale*. *Environ Sci Technol* 39(7):2210-2218.
20. Meng B, Feng XB, Qiu GL, Wang DY, Liang P, Li P, Shang LH. (2012) Inorganic mercury accumulation in rice (*Oryza sativa L.*). *Environ Toxicol Chem* 31(9):2093-2098.
21. Meng B, Feng XB, Qiu GL, Liang P, Li P, Chen CX, Shang LH. (2011) The process of methylmercury accumulation in rice (*Oryza sativa L.*). *Environ Sci Technol* 45(7):2711-2717.
22. Zhang H, Feng XB, Larssen T, Shang LH, Li P. (2010). Bioaccumulation of methylmercury versus inorganic mercury in rice (*Oryza sativa L.*) grain. *Environ Sci Technol* 44(12):4499-4504.
23. Dai ZH, Feng XB, Sommar J, Li P, Fu XW. (2012) Spatial distribution of mercury deposition fluxes in Wanshan Hg mining area, Guizhou province, China. *Atmos Chem Phys*

12(14):6207-6218.

24. Fu XW, Feng XB, Sommar J, Wang SF. (2012) A review of studies on atmospheric mercury in China. *Sci Total Environ* 421-422:73-81.
25. Zheng W. (2007) Mercury Species in the urban air of Guiyang. Ph.D. Thesis, The Graduate School of the Chinese Academy of Sciences (in Chinese with English abstract).
26. Feng XB, Qiu GL, Wang SF, Shang LH. (2003) Distribution and speciation of mercury in surface waters in mercury mining *areas in Wanshan*, Southwestern China. *J Phys IV France* 107:455-458.