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Mercury flow through an Asian rice-based food web☆

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ABSTRACT

Mercury (Hg) is a globally-distributed pollutant, toxic to humans and animals. Emissions are particularly high in Asia, and the source of exposure for humans there may also be different from other regions, including rice as well as fish consumption, particularly in contaminated areas. Yet the threats Asian wildlife face in rice-based ecosystems are as yet unclear. We sought to understand how Hg flows through rice-based food webs in historic mining and non-mining regions of Guizhou, China. We measured total Hg (THg) and methylmercury (MeHg) in soil, rice, 38 animal species (27 for MeHg) spanning multiple trophic levels, and examined the relationship between stable isotopes and Hg concentrations. Our results confirm biomagnification of THg/MeHg, with a high trophic magnification slope. Invertivorous songbirds had concentrations of THg in their feathers that were 15x and 3x the concentration reported to significantly impair reproduction, at mining and non-mining sites, respectively. High concentrations in specialist rice consumers and in granivorous birds, the later as high as in piscivorous birds, suggest rice is a primary source of exposure. Spiders had the highest THg concentrations among invertebrates and may represent a vector through which Hg is passed to vertebrates, especially songbirds. Our findings suggest there could be significant population level health effects and consequent biodiversity loss in sensitive ecosystems, like agricultural wetlands, across Asia, and invertivorous songbirds would be good subjects for further studies investigating this possibility.

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1. Introduction

Mercury is a well-known threat to both human and wildlife health, even at low concentrations (Mergler et al., 2007; Scheuhammer et al., 2007). The majority of global anthropogenic emissions are to the atmosphere and originate from Asia, particularly China and India (Pacyna et al., 2010) and the proportion of global output contributed by Asia is expected to increase (Streets

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et al., 2009). The number of locally contaminated sites in Asia is also substantial (Kocman et al., 2013). Yet biomonitoring studies in Asia have concentrated mostly on animals consumed by humans, such as fish (Liu et al., 2012, 2014; Pan et al., 2014), with a general lack of attention to full food webs which can be used to assess ecosystem health. Wildlife studies have focused on invertebrates (Zhang et al., 2010c), and herons and cranes (Burger and Gochfeld, 1993; Zhang et al., 2006; Luo et al., 2014), with few studies on top predators (Guo et al., 2000) or community-wide investigations (Hsu et al., 2006). For example, there have been no studies on passerine birds (hereafter "songbirds") in Asia. Songbirds have recently been found to be especially strong accumulators of Hg in terrestrial contaminated areas (Evers et al., 2005; Cristol et al., 2008; Jackson et al., 2015), and are prone to reproductive declines due to Hg (Heinz et al., 2009; Jackson et al., 2011).

Apart from high exposure, Asia may also have different patterns of ecosystem Hg flow compared to other parts of the world. In inland regions of China, human exposure to Hg comes more from rice than fish (Zhang et al., 2010a). Mercury concentrations in fish in China are relatively low, perhaps due to the prevalence of farmed fish of short lifespan and low trophic level (Cheng and Hu, 2012), and high fishing intensity on wild fish (Feng et al., 2008; Pan et al., 2014). In contrast, rice paddies are ephemeral wetlands that are regularly flooded, facilitating the methylation process that makes Hg more biologically available (Ackerman and Eagles-Smith, 2010; Rothenberg et al., 2014) and rice grains in particular accumulate MeHg (Qiu et al., 2008; Zhang et al., 2010b). The global distribution of rice cultivation, like that of Hg emissions, is concentrated in Asia (Rothenberg et al., 2014). Rice fields provide habitat for a variety of arthropods (Chen et al., 2013; Dalzochio et al., 2016), and their vertebrate predators (Fasola and Ruiz, 1996; Elphick, 2000). Therefore, there is substantial, but undocumented, potential risk of Hg exposure for wildlife in rice-based ecosystems throughout Asia, particularly in areas of known Hg contamination.

The objectives of the Minamata Convention (International Negotiating Committee, 2013) include protecting the environment, especially when the environment directly affects public health. In different parts of Asia, hunting of wildlife such as mammals and birds is common (Yiming and Wilcove, 2005; Liang et al., 2013; Sreekar et al., 2015), and therefore high levels of Hg in wildlife can directly expose people to Hg. Where animals are confined to specific areas and consume all their diet from that area, they can be used as bioindicators of the environmental risks to people in contaminated areas, even highly contaminated areas from which people have been evacuated, but in which the efficacy of restoration needs to be evaluated. Here we report on Hg levels in mining and non-mining regions of Guizhou Province, China, in soil, rice, and a range of resident (non-migratory) animals spanning the entire food web.

2. Methods

2.1. Ethical guidelines

All samples were obtained following an agreement between the Wanshan Mercury Mine District, Leigongshan District, and the Institute of Geochemistry, Chinese Academy of Sciences. Birds were mist netted with the permission of the Guizhou Forestry Department; no species in this study was on the protected species list of China.

2.2. Sampling sites

The mining region was the Wanshan Mercury Mining District, one of Asia's largest Hg mines, with a history of Hg production that spanned 3000 years until its closure in 2001 (Dai et al., 2012; Fig. S1). The non-mining region was the Leigongshan area, about 180 km away, with a similar geological and ecological setting, but no record of mining activities. At Wanshan, Hg mine-waste is today capped under concrete, but Hg concentrations in soil samples near these caps remain high (~80 μg/g, or parts per million [ppm] THg DW) (Feng et al., 2013), and Hg concentrations in the atmosphere are elevated due to *in situ* emissions from contaminated soil (Dai et al., 2012). Human habitations have been removed from areas near the most contaminated large-scale Hg tailings at Wukeng, although people continue to come to that area to conduct agriculture. In Leigongshan, the source for Hg is deposition from the atmosphere (Fu et al., 2009; Zhang et al., 2013).

2.3. Sample collection

In Wanshan, soil, rice, herbivorous and predatory invertebrates, and frogs (hereafter referred to as "taxa with multiple sites in Wanshan"), were sampled at five sites that had different soil contamination concentrations, including sites very close to large-scale Hg tailings, a site near a small-scale artisanal mine, and downstream sites as far as 20 km away from contamination point sources (Table 1, map in Fig. S1), between August and December, 2014. At the same time, we sampled a sixth site in Leigongshan. Within each site we concentrated sampling in a rice paddy area of approximately 500 m × 500 m. For soil and rice, we set out 7 to 13 replicate 1 m^2 sampling plots, spaced at least 50 m from each other. Within each sampling plot we took 12 subsamples of approximately 50 g of soil using a corer from the 0–20 cm soil depth and

Table 1
Sample species, collection sites and tissue type.

Taxa	Sampling scheme	Tissue[a]
Soil, rice, herbivorous and predatory insects, frogs	5 sites, 0–20 km, from mines in Wanshan[b] 1 site in Leigongshan	Frogs: leg muscles (DW) Other taxa: whole sample (DW)
Fish, rats, snakes	Within 500 m of most contaminated mine in Wanshan 1 site in Leigongshan	Fish: axial muscles (DW) Rats: hair (FW) Snakes: tail tissue (DW)
Kingfishers and passerine birds	Within 4 km of large-scale mines in Wanshan 1 site in Leigongshan	Feathers (FW)
Owls	Recently captured birds from Wanshan District (within 20 km from mines) recently captured birds, Leishan District	Feathers (FW)

[a] FW = freshweight; DW = dry weight.
[b] The five sites were: 1) within 500 m of Wukeng tailing, the most contaminated site; 2) within 500 m of Meizixi, another large mercury tailing; 3) Gouxi, a small-scale artisanal mining site, now abandoned, approximately 12 km from Wukeng, but not downstream; 4) Baiguoshu, a village 8–10 km downstream from Meizixi; 5) Shenchongkou, a village 20 km downstream from both Wukeng and Meizixi.

then mixed these subsamples together. Four panicles of rice were collected from each sampling plot in October, 2014 before harvest. Sweep nets (38 cm diameter) were used to catch insects in rice fields, walking with a 'zig-zag' sampling pattern. Light traps were placed to catch moths, and long handled aquatic nets were used to catch frogs in the rice fields. Invertebrates and frogs were euthanized by placing them with 95% ethyl alcohol in a centrifuge tube and storing them at −14 °C.

For other taxa, including fish, rats, snakes, kingfishers, owls, and songbirds, one group of samples in Wanshan was sampled as close to the large-scale Hg tailings as possible (although the exact distances to the tailings differed between taxa, see Table 1), as well as in Leigongshan, over an area there of 5 km^2. We mist-netted adult passerine birds and kingfishers between August and December, 2014, and between August and October, 2015, using 10–13, 12-m, 36-mm mesh nets. At Wanshan, birds were captured near rice fields 0–4 km from tailings areas near the abandoned mines now covered by concrete. Nets were situated mainly at the edge of shrub forests near rice paddies, with the exact placement chosen according to vegetation structure. We used passive capture techniques for most species, attracting selected species by playback, and checking the nets every 30 min. We plucked the second secondary feather from each side from birds with adult plumage, banded the bird with a plastic colored band, and then released it. Owls were captive birds that we found being sold in the markets in Wanshan City in August, 2015 and Zhang Au, a city near Leigongshan, in September, 2015. The sellers told us that these birds had been collected in the respective districts within the past several weeks. We released them after taking the second secondary feathers.

Snakes, rats and fish were all collected between August and October 2015 in a rice paddy near the most contaminated area of Wanshan district (Wukeng, see Table 1), and in Leigongshan. From adult snakes we cut a tail tissue sample of about 1 cm, following the protocol described by Drewett et al. (2013), and then released the animals. Rats were considered pests by the local farmers, and we sampled them destructively by using standard small mammal traps that were locally available, and euthanizing with CO_2; from rats we sampled hair clippings. Farmers in the region use pools in rice paddies to farm Crucian Carp (*Carassius carassius*); we sampled fish collected by the farmers, of about 10–15 cm in length. Farmers also helped us catch the wild Small Snakehead (*Channa asiatica*), of about 20–30 cm in length, in a pool that collects runoff from the tailing areas within 500 m of Wukeng. Fish were placed on ice and transferred into a portable freezer.

2.4. Storage, processing and selection of samples

All samples were stored following collection in sealed polyethylene bags to avoid cross contamination. At the lab, samples were washed six times with deionized water. Feathers were washed with acetone, followed by further washing with deionized water. Hair samples were washed with nonionic detergent and then with acetone and deionized water (Feng et al., 2008).

Rice was set out to dry in the lab without direct sunlight; feather and hair samples were similarly dried for 48 h. For fish, we dissected and sampled axial muscles, which have been used as biomarkers for Hg (Scheuhammer et al., 2016). For frogs, we dissected and sampled leg muscles (Hothem et al., 2010). Whole insect and muscle samples were freeze dried (using EYELA model FDU-1100, Tokyo, Japan). For the smaller invertebrates, multiple individuals were used together to make one sample. Feathers were cut into small fragments to homogenize them, and all insect and muscle samples were ground into homogenous powder using an agate mortar and pestle, cleaning between samples. All samples were then again preserved in sealed polythene bags.

While THg was conducted on samples from all sites, MeHg and stable isotope measurements were only made on samples collected at Wanshan, or, for taxa with multiple sites in Wanshan, at Wukeng. The sample that was tested for MeHg was the same as that tested for THg, except in the case of four insect species (see Appendix 2; in these exceptional cases, the percent MeHg was calculated by dividing the average MeHg concentration of all samples of that species by the average THg concentration of all samples of that species).

2.5. Laboratory analyse

The person conducting analysis was blind to the source of the samples. The concentration of Hg in feather and hair is presented on a fresh weight (FW) basis, whereas the concentration of Hg in all other samples types is presented as a dry weight concentration (DW).

For THg measurements, a 0.01–0.07 g sample was measured using thermal decomposition, amalgamation, and atomic absorption spectrophotometry, following method 7473 of the U.S. E.P.A (2007), using a portable Hg vapor analyzer (Lumex, Model RA-915 +/Pyro-915+, St. Petersburg, Russia).

For MeHg, measurements were on 0.1–0.2 g of sample, after extraction with KOH–methanol/solvent, when appropriate (for whole invertebrate samples, vertebrate muscles, mammal hair, bird feathers, vertebrate tissue and muscle; Liang et al., 1994; Liang et al., 1996), following method 1630 of the U.S. E.P.A (2001). The method requires a progressive sequence of distillation for soil samples, addition of 2M acetate buffer, ethylation with 1% sodium tetraethylborate, purge and trap of methylethylmercury onto Tenax traps, thermal desorption, separation by gas chromatography, and detection by CVAFS (Brooks Rand Model III, Seattle, USA).

For stable isotope analysis, 400–500 μg of each sample was packed into a tin container. The relative abundance of carbon and nitrogen isotopes was determined with a continuous-flow mass spectrometer (Thermo Scientific MAT 253) coupled to an elemental analyzer (Thermo Scientific Flash EA 2000 HT). All isotopic data were expressed in the conventional delta notation (‰): $\delta^{13}C_{sample}$ or $\delta^{15}N_{sample} = (R_{sample}/R_{reference} - 1) \times 1000$ with $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$.

2.6. Quality control

Quality-control measures consisted of method blanks, field blanks, triplicates, matrix spikes, and parallel analysis of several certified reference materials, as further described in Table S1. THg detection limits were 0.5 ng/g for soil, 2 ng/g for rice grains, 20 ng/g for hair samples, and 5 ng/g for all other biological samples. The method was validated using two reference materials (TORT-2, GBW07405), and the found concentrations were in good agreement with the certified values. For MeHg, the method detection limit was 0.05 ng/g. The method was validated using two reference materials (TORT-2, ERMCC580), and similar to THg, the found MeHg concentrations agreed well with the certified values. Recoveries on matrix spikes of MeHg in soil and biological samples were in the range of 97–107% and 95–115%, respectively. Careful attention was paid to the blank controls and blanks were introduced in each digestion to ensure the purity of chemicals used.

For stable isotopes, the reference used was PDB for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$. The $\delta^{13}C$ mean percent recoveries were in range of 99.1–100.7%. The $\delta^{15}N$ mean percent recoveries were in range of 99.8–100.2%. Analysis uncertainty was less than 0.2‰.

2.7. Statistical analysis

We used the Handbook of the Birds of the World on-line data (www.hbw.com) to categorize birds into four categories (majority insectivorous, majority granivorous [grain-eating], majority frugivorous [fruit-eating], and omnivorous [no clear majority]) according to their diet in the non-breeding season, when they were caught; bird taxonomy follows this electronic database. We used MacKinnon and Phillipps (2000) to exclude any bird species that had migratory populations in China.

To understand whether soil contamination influences the THg concentration in soil and taxa with multiple sites in Wanshan, we coded Wanshan sites 1–5 in order of the severity of contamination based on the analysed soil samples (Wukeng was 1), and coded Leigongshan as 6. We then conducted restricted likelihood ratio tests to assess the influence of contamination on THg levels, using the function "ordAOV" in the R package "ordPens", which uses a maximum likelihood approach, running 10,000 simulations (Gertheiss, 2015). Because the THg concentration was heteroschedastic and not normal, and even a log transformation of the data did not correct these problems, we took the rank of the data as the response variable. We ran these tests separately for each species except passerine birds, for which we had multiple diet classes. For these birds, we built general linear mixed models for the different diet classes separately, with treatment – Wanshan vs. Leigongshan – as the fixed factor and species as a random factor, using the function "lmer" in the package "lme4" (Bates et al., 2014). Again, we first rank-transformed the THg concentration data. To assess whether each of the 10 bird species found at both sites (Wanshan and Leigongshan) had higher levels of THg in Wanshan we used Wilcoxon nonparametric tests. The average THg concentration in granivorous species at Wanshan was compared to the average concentration in frugivorous species with a Student's t-test (the data met parametric assumptions).

Assessment of the relationship between $\delta^{15}N$ and log THg, and between $\delta^{15}N$ and log MeHg, was conducted using simple linear regression (Lavoie et al., 2013). In this analysis, the THg data, like the data for MeHg and stable isotopes, was taken from Wanshan, and specifically from Wukeng for taxa with multiple sites in Wanshan. We conducted two analyses, one for whole samples or tissues, and one for feathers. We excluded aquatic organisms or those species that have a diet of aquatic organisms (dragonflies and frogs for whole samples and tissues; kingfishers for feathers), as they appeared to be a different food chain, as evidenced by their high $\delta^{13}C$ readings. All analyses were run in R version 3.3.1 (R Core Team 2017).

3. Results

3.1. Relationship between THg concentrations in living organisms and contamination

Mercury concentrations in rice, and in all invertebrate and frog samples increased with increasing severity of soil contamination (all restricted likelihood ratios > 108, $P < 0.0001$ for all 15 species). For all passerines considered together, and for each diet class of passerines, THg concentrations were higher in Wanshan than Leigongshan (all $X^2_1 > 72$, $P < 0.0001$ for all five comparisons). For all single vertebrate species that had one sampling group in Wanshan and a comparison group in Leigongshan (rats, fish, snakes, kingfishers, owls), THg concentrations were higher in Wanshan than in Leigongshan (all Mann-Whitney U < 5, $P < 0.0015$ for all five comparisons). Of the 10 species of year-round resident passerines sampled in both Wanshan and Leigongshan, all had significantly higher THg concentrations at Wanshan (all Mann-Whitney U < 14, $P < 0.0013$ for all 10 comparisons).

3.2. Trophic structure of the food web

The diet information from the literature (Appendix S1) and the results from staple isotopes gathered at the most contaminated sites, gave a similar picture of the food web of the animals for which

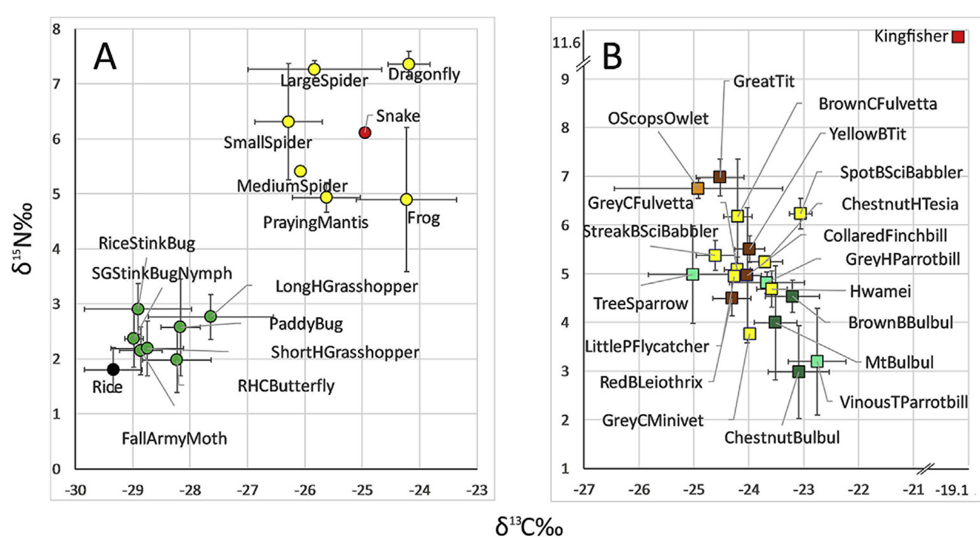

Fig. 1. Biplot of stable isotope ($\delta^{13}C$ versus $\delta^{15}N$) values for (A) organisms with whole samples or tissues, and (B) bird feathers. Data points are mean values with error bars representing standard deviation, and are color coordinated by trophic level, as judged by literature on the species' diets (Appendix 1): rice is black, herbivorous insects (in A only) are green, frugivorous birds (in B only) are dark green, granivorous birds (in B only) are blue green, omnivores (consuming plant tissue and insects) are brown, invertebrate-eating animals are yellow, species that eat both invertebrates and vertebrates are orange, and vertebrate carnivores are red. Notice that the axes have different scales in the two panels and that the axis of panel B is cut off to show the outlying point of Common Kingfisher (−19.1 ± 0.3, 11.6 ± 0.3). All species had at least six samples, except for the Small Spider and Large Spider species (four samples each), and four species shown in the graph without error bars (two samples each). Names are abbreviations of the English in Tables 2 and 3 (see those tables for scientific names). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

we had tissues or whole samples (Fig. 1A). Rice had the lowest $\delta^{15}N$ value (1.80‰ ± 0.50), followed by a group of seven species of herbivorous insects, all averaging $\delta^{15}N$ values below 3‰. Carnivorous species had $\delta^{15}N$ values above 4.89‰, with large spiders 7.26‰ (±1.66) and dragonflies 7.36‰ (±0.37) the highest values. The large spiders appear to be more than one trophic level higher than the herbivorous insects, given trophic fractionation of 3.4‰ (Post, 2002; (7.26–2.41 [average of 7 species]/3.4 = 1.42)). Aquatic species – dragonflies and frogs – had the highest values for $\delta^{13}C$.

The stable isotope values for bird feathers correlated loosely with their trophic categorizations made from the literature (Fig. 1B). Frugivores had $\delta^{15}N$ values below 4.5‰; six of seven insectivores were above this value. However, omnivores and granivores showed high amounts of variation. Kingfishers had by far the highest $\delta^{15}N$ values (11.56‰ ± 0.30), but also had anomalous $\delta^{13}C$ values, indicating an aquatic carbon source.

3.3. THg and MeHg concentrations across trophic levels in the rice-based food web

Due to the differences among tissue types, we present the values of THg and MeHg separately for taxa that were collected as whole samples or tissue (Table 2), and taxa for which feathers or hair was collected (Table 3). Graphically, the food web and the levels of THg are presented in Fig. 2. The figure is simplified from all the species listed in Tables 2 and 3, by removing species not found at both sites (e.g., wild fish, omnivorous passerines), those of intermediate trophic level (e.g., small and medium sized spiders), those with different kinds of tissues (e.g., mammal hair), and those for which it was unclear what taxa was most consumed (e.g., small owls).

Some patterns in this data suggest rice as a source of THg. Two insect species (Paddy Bug *Leptocorisa acuta* and Rice Stink Bug *Oebalus pugnax*) that are known to specialize on eating rice grains had the highest THg levels of herbivorous insects (other species were crop generalists; Appendix S1). Granivorous bird species had as high THg levels in Wanshan as piscivorous kingfishers (see Fig. 2A). The average THg concentrations for three species of granivorous passerines collected from Wanshan were significantly higher than that for three species of frugivorous passerines ($t_{3.08} = 8.21$, $P < 0.004$).

The data also demonstrate high THg levels at the highest trophic levels, which were occupied by spiders and in passerines. All three size classes of spiders (representing three differently sized species) had higher THg levels than carnivorous insects (Table 2). The bird species that was found with the greatest individual THg concentration (190 ppm), Chestnut-headed Tesia (*Oligura castaneocoronata*) is a spider specialist (Appendix S1). Invertivorous passerine species had the highest THg concentrations both in the most contaminated mining site and at the non-mining site. Comparison of the THg concentrations reported in the current work to 60 studies conducted globally on feather concentrations highlights the high levels recorded for both Wanshan and Leishan (Table 4; see Appendix S3 for full data). In Wanshan, 14/18 passerine species had higher THg levels than previously recorded in passerine feathers for point source locations. In Leigongshan, 3/10 species (all invertivores) had higher levels than previously recorded for passerine feathers from non-point source locations, not including one outlying measurement from a population of Rusty Blackbird (Edmonds et al., 2010).

The percentage of THg present as MeHg was very low in soil, but increased through the food web (see Tables 2 and 3), as MeHg was biomagnified from 13.9 ± 13.7% of THg in rice to 38.1 ± 13.9% in herbivorous invertebrates, to 48.8 ± 11.7% in predatory invertebrates. In vertebrates (remembering differences in the tissues

Table 2
Mean concentrations of THg, their variability, and the percentage of THg that is methylated for taxa collected as whole samples or tissues. Values are given for the most contaminated sites (Wukeng for taxa that had multiple sites in Wanshan; otherwise, the one Wanshan sample), and the reference site (Leigongshan). For groups of species, the standard deviation represents the variability among species, whereas for species it represents the variability among individual samples. For data broken down by single species, and including multiple sites within Wanshan for those species sampled in this way, see Appendix S2.

Species or taxonomic group (Figs. 1 and 2 Exclusion and Abbreviation)	Wukeng or Wanshan			Leigongshan			% of THg that is MeHg		
	Mean	SD	N	Mean	SD	N	Mean % MeHg	SD % MeHg	N
Soil	71.68	6.88	11	0.07	0.01	9	0.00	0.00	19
Rice *Oryza sativa*	0.07	0.01	14	0	0	11	13.88	13.75	35
Herbivorous Insects (Separate points in Fig. 1; "Herb Insect" in Fig. 2)	1.60	1.33	318 (9 sp)	0.03	0.01	288 (9 sp)	38.13	13.87	49 (6 sp)
Short-horned Grasshopper *Oxya* sp.	0.35	0.097	34	0.037	0.026	40	59.20	16.98	5
S. Green Stink Bug Nymph *Nezara viridula*	0.45	0.25	39	0.002	0.004	42	NA	NA	0
S. Green Stink Bug Adult *Nezara viridula*	0.76	0.3	37	0.004	0.004	25	25.84	NA[a]	7
Long-horned Grasshopper *Phaneroptera falcata*	0.49	0.06	30	0.026	0.019	35	51.46	8.72	10
Leafhopper *Graminella* sp.	1.25	0.53	40	0.013	0.011	27	NA	NA	0
Rice Horned Caterpillar Butterfly *Melanitis leda*	1.77	0.55	39	0.036	0.025	33	31.10	NA[a]	10
Fall Armyworm Moth *Spodoptera frugiperda*	2.15	0.65	33	0.028	0.026	31	26.95	8.78	10
Paddy Bug *Leptocorisa acuta*	2.89	1.03	33	0.046	0.041	33	NA	NA	0
Rice Stink Bug *Oebalus pugnax*	4.27	1.99	33	0.0097	0.01	22	34.20	NA[a]	7
Carnivorous Insects (Separate points in Fig. 1; "Carn Insect" in Fig. 2)	2.68	NA	78 (2 sp)	0.58	NA	70 (2 sp)	56.47	7.82	20 (2 sp)
Dragonfly *Sympetrum flaveolum*	2.66	0.84	43	0.68	0.26	35	65.90	NA[a]	10
Praying Mantis *Tenodera sinensis*	2.72	0.44	35	0.48	0.18	35	47.03	7.82	10
Small Spider *Leucauge* sp. (Not in Fig. 2)	3.55	1.9	46	0.12	0.08	33	NA	NA	0
Medium Spider *Tetragnatha nitens* (Not in Fig. 2)	14.41	7.11	10	0.3	0.17	10	41.25	10.30	10
Large Spider *Nephila pilipes*	21.77	3.63	12	1.56	0.77	10	41.13	16.35	10
Crucian Carp *Carassius carassius* (no data for Fig. 1; "Farm Fish" in Fig. 2)	1.41	0.38	38	0.51	0.1	28	31.75	9.35	10
Small Snakehead *Channa asiatica* (Not in Fig. 1 or Fig. 2)	3.26	1	10	0	0	0	42.55	13.29	10
Frog *Fejervarya limnocharis*	5.35	2.27	46	0.26	0.09	37	58.38	7.68	10
Big-eye Rat Snake *Ptyas dhumnades* ("Snake")	9.77	2.25	11	2.36	0.35	4	87.02	7.91	11

[a] Percent MeHg calculated as calculated by dividing the average MeHg concentration of all samples of that species by the average THg concentration of all samples of that species.

Table 3
Mean concentrations of THg, their variability, and the percentage of THg that was methylated for taxa collected as feathers (birds) or hair (mammals). Values presented as in Table 2.

Species or taxonomic group (Figs. 1 and 2 Exclusion and Abbreviation)	Wukeng or Wanshan			Leigongshan			% of THg that is MeHg		
	Mean	SD	N	Mean	SD	N	Mean % MeHg	SD % MeHg	N
Common Kingfisher *Alcedo atthis*	22.48	5.52	11	5.89	1.49	9	93.45	4.94	11
Oriental Scops Owl *Otus sunia* (Not in Fig. 2)	27.10	6.13	13	7.71	3.01	8	94.23	3.05	10
Passerines: Frugivores (Separate points in Fig. 1; "Frug Bird" in Fig. 2)	3.31	1.99	54 (3 sp)	0.21	0.09	20 (3 sp)	74.11	4.65	4 (1 sp)
Mountain Bulbul *Ixos mcclellandii*	1.16	0.37	14	0.11	0.038	9	74.11	4.65	4
Chestnut Bulbul *Hemixos castanonotus*	3.67	2.00	26	0.25	0.19	6	NA	NA	0
Brown-breasted Bulbul *Pycnonotus xanthorrhous*	5.09	4.17	14	0.27	0.12	5	NA	NA	0
Passerines: Granivores (Separate points in Fig. 1; "Gran Bird" in Fig. 2)	23.12	3.67	124 (3 sp)	0.29	NA	37 (2 sp)	94.46	1.81	8 (2 sp)
Tree Sparrow *Passer montanus*	19.34	13.08	69	0.30	0.12	22	93.05	2.64	4
Vinous-throated Parrotbill *Paradoxornis webbianus*	23.32	9.95	4	NA	NA	0	NA	NA	0
Grey-headed Parrotbill *Paradoxornis gularis*	26.68	14.66	51	0.28	0.085	15	95.86	0.98	4
Passerines: Invertivores (Separate points in Fig. 1; "Invt Bird" in Fig. 2)	59.82	35.11	125 (8 sp)	11.3	9.90	44 (5 sp)	94.32	2.95	54 (6 sp)
Little Pied Flycatcher *Ficedula westermanni*	22.06	12.88	5	NA	NA	0	NA	NA	0
Grey-chinned Minivet *Pericrocotus solaris*	29.50	14.87	3	NA	NA	0	NA	NA	0
Grey-cheeked Fulvetta *Alcippe morrisonia*	42.67	11.40	14	NA	NA	0	94.78	1.67	10
Brown-cheeked Fulvetta *Alcippe poioicephala*	45.09	14.14	12	1.54	0.56	13	96.73	1.54	10
Hwamei *Leucodioptron canorum*	52.57	33.28	21	2.12	0.98	12	88.98	7.41	4
Streak-breasted Scimitar Babbler *Pomatorhinus ruficollis*	62.52	34.77	38	13.38	4.91	10	96.46	2.66	10
Chestnut-headed Tesia *Oligura castaneocoronata*	100.9	45.66	19	25.47	2.2	3	95.14	1.81	10
Spot-breasted Scimitar Babbler *Pomatorhinus mcclellandi*	123.28	34.21	13	14	6.56	6	93.82	2.61	10
Passerines: Omnivores (Separate points in Fig. 1; not in Fig. 2)	21.76	14.46	97 (4 sp)	NA	NA	0 (0 sp)	91.64	7.45	4 (1 sp)
Red-billed Leiothrix *Leiothrix lutea*	5.71	1.88	15	NA	NA	0	NA	NA	0
Collared Finchbill *Spizixos semitorques*	17.07	18.65	38	NA	NA	0	91.64	7.45	4
Yellow-browed Tit *Sylviparus modestus*	23.99	34.47	18	NA	NA	0	NA	NA	0
Great Tit *Parus major*	40.27	29.62	26	NA	NA	0	NA	NA	0
Rice Field Rat *Rattus argentiventer* (Not in Fig. 1 or Fig. 2)	3.70	6.88	42	0.33	0.07	8	39.50	10.83	10

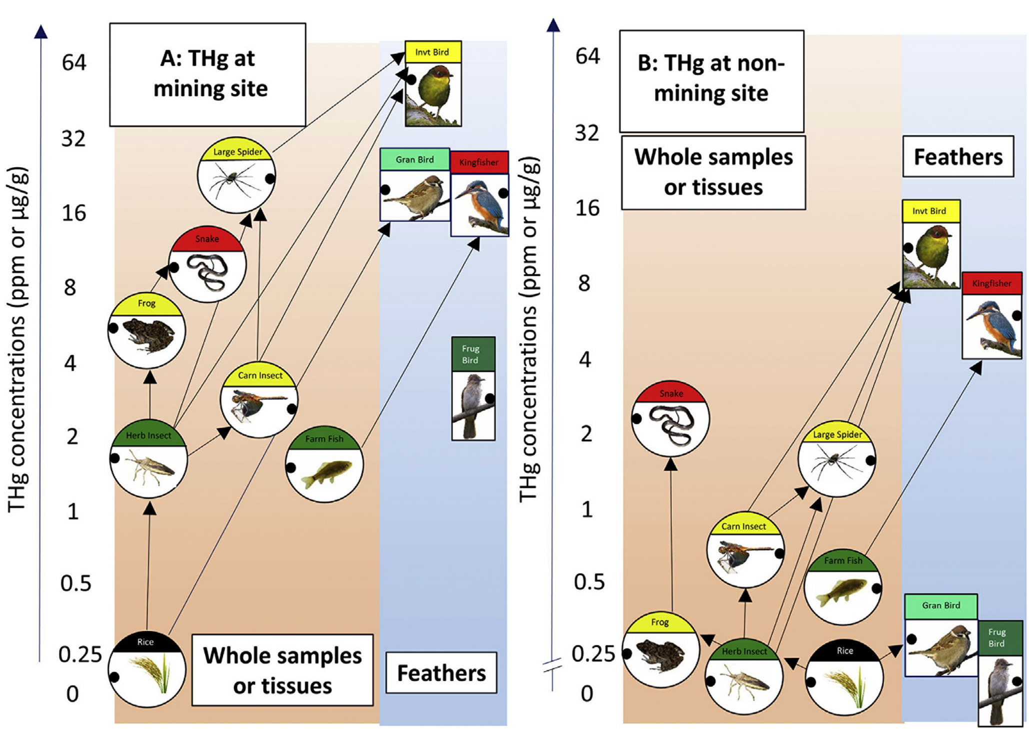

Fig. 2. The concentrations of THg in rice-based ecosystems at (A) the most contaminated mining and the (B) non-mining site. Mean THg concentrations (placement of black dots) are shown on a 2^n scale (for exact concentration, error and the proportion of THg that was MeHg, see Tables 2 and 3). The concentrations in feathers (right side of each panel in blue) are not directly comparable to concentrations measured in tissue or whole samples (left side in beige). Arrows indicate trophic relationships that are supported by published literature or web resources on the respective species (see Appendix S1). Color coding of species by trophic level, as in Fig. 1. Abbreviations follow Tables 2 and 3. All photographs modified from images on Wikimedia Commons; Invt Bird (*Oligura castaneocoronata* pictured) by Umesh Srinivasan, Gran Bird (*Passer montanus*) and Kingfish by Andreas Trepte, Frug Bird (*Ixos mcclellandii*) by Jason Thompson, Snake (*Pytas* sp.) by Bernard Dupont, Lrg Spdr by David Monniaux, Frog by Wibowo Djatmiko, Carn Insect (*Sympetrum flaveolum*) by André Karwath, Farm Fish by Piet Spaans, Herb Insect (*Oebalus pugnax*) by Ryan Kaldari, Rice by IRRI Images. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Average measurements of THg in bird feathers from 60 studies conducted globally, inside or outside Hg locally contaminated areas ("point source"), for comparison with this study. The unit of replication is a group of birds of one species, of the same age or sex and sampling location ($n = 238$). Maximum values are the highest THg reported from one such unit among all published papers. For the full data, see Appendix S3.

	Point source locations	Non-point source locations
Waterbirds[a]		
Mean	5.55	5.31
SD	8.48	6.21
Maximum	66.31	26.68
n species	70	23
n studies	14	13
Passeriformes		
Mean	2.66	1.44
SD	3.11	3.15
Minimum	0.07	0.10
Maximum	8.76	19.57
n species	6	25
n studies	6	5
Raptors[b]		
Mean	8.09	4.24
SD	9.62	2.75
Minimum	0.32	1.07
Maximum	40.00	6.08
n species	17	1
n studies	13	1

[a] From orders Anseriformes, Charadriiformes, Gaviiformes, Gruiformes, Pelecaniformes, Podicipediformes, Procellariiformes, Sphenisciformes, Suliformes, following www.worldbirdnames.org.
[b] From orders Accipitriformes, Falconiformes, Strigiformes, following www.worldbirdnames.org.

sampled), MeHg rose from 31.8 ± 9.4% for farmed fish, 39.5 ± 10.8% for rats, 42.6 ± 13.3% for wild fish, 58.4 ± 7.7% for frogs, 87.0 ± 7.9% for snakes, 93.5 ± 4.9% for kingfishers, 94.2 ± 3.1% for owls, and 92.1 ± 6.7% in passerines (10 species).

3.4. Relationship between THg and MeHg and stable isotopes among all taxa

We conducted separate biomagnification analyses for taxa collected as whole samples or tissues, and birds. For terrestrial species for which we collected whole samples or tissues, and including rice as the bottom of the food web, there was a significant correlation between $\delta^{15}N$ and THg ($n = 13$, $P = 0.0003$, $R^2 = 0.68$), with a trophic magnification slope of 0.40 (Fig. 3A). The regression between $\delta^{15}N$ and MeHg explained more of the variance ($n = 10$, $P < 0.0001$, $R^2 = 0.87$; Fig. 3C), although the magnification slope was actually lower (0.36).

Analogous analyses for bird feathers (only terrestrial, resident avian species) showed strong correlations between $\delta^{15}N$ and THg ($n = 18$, $R^2 = 0.51$, $P < 0.0001$, Fig. 3B) and between $\delta^{15}N$ and MeHg ($n = 10$, $R^2 = 0.49$, $P < 0.0001$, Fig. 3D).

4. Discussion

4.1. Bioaccumulation of Hg across the food web and implications for animal health

Our results suggest that rice is an exposure source for animals, as rice grain consumers had the highest concentrations of THg and MeHg among herbivorous insects, and granivorous birds had higher concentrations than frugivorous ones. Contrary to the usual findings for a bioaccumulating toxin, we found in Wanshan that the THg and MeHg concentrations of granivorous songbirds were as high as those of kingfishers. Wild fish are quite rare and highly harvested in this part of China, with most fish found in human-dominated landscapes being farmed, and of low trophic level. A good example is the carp sampled in our study. Hence, the 'rice, not fish' Hg exposure route appears to hold for animals, as it does for humans in this part of China (Zhang et al., 2010a).

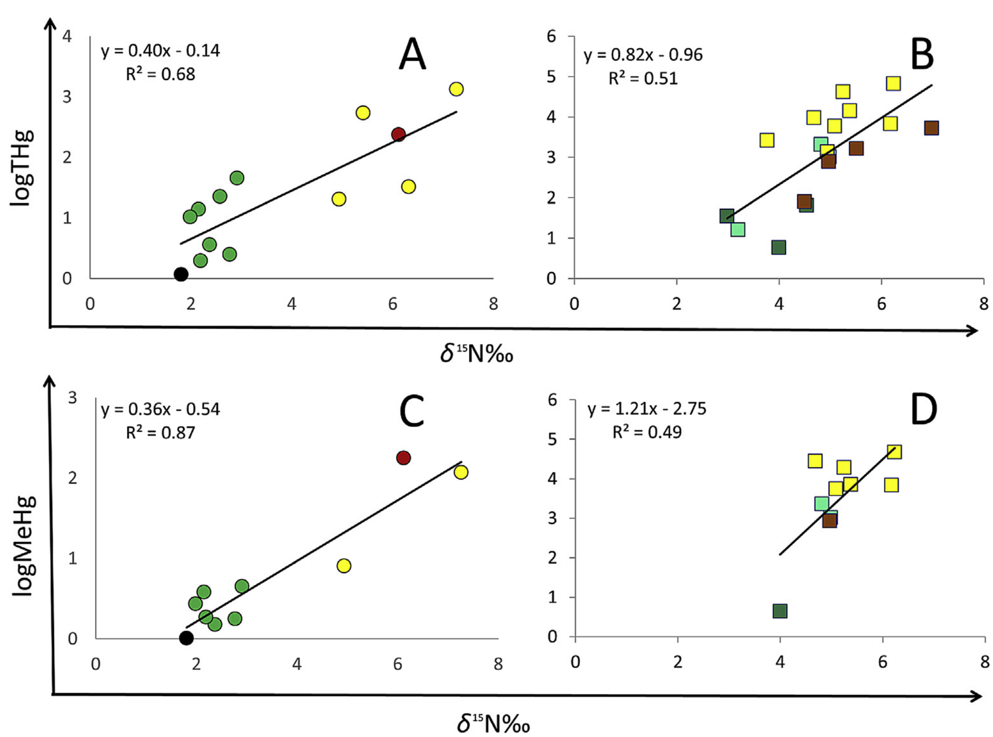

Fig. 3. The relationship between trophic position, as measured by $\delta^{15}N$, and (A) THg levels for those taxa collected as whole samples or tissues, (B) THg levels for avian taxa that had feather samples, (C) MeHg for whole samples or tissues, (D) MeHg for bird feathers. Color coding by trophic level, as in Fig. 1. Notice that the Y-axes scales vary for the different panels.

The trophic magnification slopes for THg (0.40) and MeHg (0.38) were much above the average values of 0.16 ± 0.11 [SD] for THg (range −0.19 to 0.48), and 0.24 ± 0.08 for MeHg (range 0.08–0.53) in the review of Lavoie et al. (2013). Another related way of measuring biomagnification are trophic transfer factors (TTF), which are the ratio of concentrations of a metal at one trophic level divided by the concentrations at the level beneath that. TTFs of this study again are high. For example, the concentration of THg in rice at Wukeng was measured at 74 ppb, whereas the two rice grain eating species, Paddy Bug and Rice Stink Bug, had concentrations of 2890 ppb and 4270 ppb, for TTFs of 39–51 times. Similar values were found at the reference site: rice at 1 ppb and TTFs of 10–46 times. TTFs for MeHg in aquatic studies are usually between 1 and 7 (and TTFs of MeHg are usually greater than those for THg; DeForest et al., 2007). But comparing our values to those of Lavoie et al. (2013) and DeForest et al. (2007) is complicated by the fact that our food web is terrestrial, and in a highly contaminated area (stable isotopes and MeHg were only collected from the mining site).

Biomagnification of Hg along the invertivorous songbird food-chain, from herbivorous insects to predatory insects to predatory spiders, makes these animals particularly vulnerable to the adverse effects of Hg. Other studies have also noted the importance of spiders in biomagnifying MeHg in the food web (Cristol et al., 2008; Gann et al., 2015). The large spiders in this study had the highest trophic position, as measured by $\delta^{15}N$, of all the terrestrial organisms for which we sampled whole bodies or tissues. They appear more than one (precisely 1.4) trophic levels above herbivorous insects, suggesting that these orb-weaving spiders are eating some percentage of carnivorous insects (Fig. 2). Indeed, this genus of spiders, *Nephila*, creates webs large enough to capture even small birds (although the spiders are unlikely to eat birds; Walther, 2016), and thus could capture some carnivorous species like dragonflies.

The concentrations reported here in invertivore feathers are among the highest ever recorded for passerines (Table 4). Such concentrations may impact the health of the animals involved, even at the reference non-mining site (Leigongshan). The critical THg level for adverse effects on piscivorous birds based on feathers is estimated to be 40 ppm FW (Evers et al., 2008). For passerines, reproduction impairment can be observed at feather THg concentrations as low as 4 ppm FW (Jackson et al., 2011), although there is limited knowledge about Hg toxicity in natural conditions for a wide range of passerine species. Average concentrations for invertivorous songbirds at Leigongshan were 3 times this 4 ppm level, and 3 of 5 species reported an average concentration above this threshold. At the contaminated sites 8 of 8 species reported an average THg concentration above this established reproductive threshold. The effects of Hg on passerines are pervasive, and include lowered immunity (Hawley et al., 2009), increased stress hormones (Franceschini et al., 2009), diminished muscular performance that controls flight (Carlson et al., 2014), and poorer performance on cognitive tasks such as song (McKay and Maher, 2012) and risk evasion (Kobiela et al., 2015).

The source of Hg at Leigongshan is atmospheric deposition (Fu et al., 2009; Zhang et al., 2013) and the concentration in soil (0.07 ppm THg) is below the average concentration across China (Zhang and Wong, 2007; Shi et al., 2013). This freshly deposited Hg is readily methylated and has been shown in previous work to be accumulated by rice (Zhao et al., 2016). Despite the low concentration in soil, Hg at Leigongshan was biomagnified to a substantial concentration in invertivorous songbirds, kingfishers, snakes, and spiders (Fig. 2). Given that rice cultivation in Asia is highest in China and India, where Hg emissions are also highest (Rothenberg et al., 2014), research is needed to determine if reproductive impairment from MeHg is leading to lowered population health of avian invertivores in the region. Loss of biological diversity in terrestrial ecosystems of Asia may be an unknown consequence of Hg pollution. We recommended a multi-national biomonitoring effort across Asia using wetland invertivorous songbirds as particularly important bioindicators for evaluating the effectiveness of the Minamata Convention on Mercury to improve environmental health.

4.2. Implications of Hg biomagnification through the terrestrial food chain for public health

The animal data collected in this study can be used to guide human health policy in the mining region. Residents of the area frequently collect fish and frogs, and although hunting for birds and mammals is illegal, this remains a common practice. Education strategies should target this audience and inform the population of the risk of consuming wildlife from the area. With respect to agricultural policies, farmers could be encouraged to grow crops that bioaccumulate less MeHg than rice, such as maize (Qiu et al., 2008). If farmers continue rice cultivation, research on how agricultural practices affect mercury accumulation suggest that stopping rice straw amendment can lower MeHg bioaccumulation, and recycling of field water can limit export of Hg from the systems (Windham-Myers et al., 2014). In terms of food safety, rice from small-scale fields in contaminated areas might be mixed from the large amount of rice produced in non-contaminated areas in Guizhou, which would reduce exposure of the local population to Hg and reduce the health risk associated with consuming rice only from the Wanshan region. Finally, we hope these measurements of Hg concentrations in animals can be repeated over time, to assess the success of restoration measures in the mining region. Biomonitoring with animals would seem an effective way to evaluate human safety in the many locally contaminated areas in the world (Kocman et al., 2013), even in the most contaminated areas from which people have been evacuated.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2017.05.067.

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