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# TOTAL MERCURY AND METHYLMERCURY CONCENTRATIONS OVER A GRADIENT OF CONTAMINATION IN EARTHWORMS LIVING IN RICE PADDY SOIL

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**Abstract:** Mercury (Hg) deposited from emissions or from local contamination, can have serious health effects on humans and wildlife. Traditionally, Hg has been seen as a threat to aquatic wildlife, because of its conversion in suboxic conditions into bioavailable methylmercury (MeHg), but it can also threaten contaminated terrestrial ecosystems. In Asia, rice paddies in particular may be sensitive ecosystems. Earthworms are soil-dwelling organisms that have been used as indicators of Hg bioavailability; however, the MeHg concentrations they accumulate in rice paddy environments are not well known. Earthworm and soil samples were collected from rice paddies at progressive distances from abandoned mercury mines in Guizhou, China, and at control sites without a history of Hg mining. Total Hg (THg) and MeHg concentrations declined in soil and earthworms as distance increased from the mines, but the percentage of THg that was MeHg, and the bioaccumulation factors in earthworms, increased over this gradient. This escalation in methylation and the incursion of MeHg into earthworms may be influenced by more acidic soil conditions and higher organic content further from the mines. In areas where the source of Hg is deposition, especially in water-logged and acidic rice paddy soil, earthworms may biomagnify MeHg more than was previously reported. It is emphasized that rice paddy environments affected by acidifying deposition may be widely dispersed throughout Asia. *Environ Toxicol Chem* 2017;36:1202–1210. © 2016 SETAC

**Keywords:** Biomagnification Earthworms Ecotoxicology Mercury biomonitoring Soil contamination

## INTRODUCTION

Mercury (Hg) is toxic to humans and animals, even at low concentrations [1–3]. Mercury is released through natural and anthropogenic processes [4], with anthropogenically released Hg having a global distribution. Emissions from fossil-fuel combustion travel long distances in air, and Hg also can be locally distributed through contamination of water by industrial processes [5,6]. In aquatic ecosystems, inorganic forms of Hg will change into methylmercury (MeHg), by the methylating activity of some microorganisms [7]. Methylmercury is more readily accumulated by biological processes and is more toxic than inorganic Hg, affecting neurological, physiological, and reproductive processes [2,3]. Mercury bioaccumulation through food webs has traditionally been perceived as a problem for aquatic piscivores [3]. However, recent evidence demonstrates that species at the top of terrestrial food chains can also be affected, especially in contaminated areas [8–10].

The recent rapid industrialization of China has led it to be 1 of the largest Hg emitters in the world [6,11]. The burning of fossil fuels is the largest source of emissions in the country, and has a strong impact on the regional level of atmospheric Hg [12]. At the same time, mining and industrial processes can also lead to high levels of Hg at more local scales [13,14]. In the latter case, contamination in some areas—such as the Wanshan Mercury Mine District in the province of Guizhou, China—can be quite high, with levels of Hg far above the national limits for environmental quality [15]. Apart from high levels of Hg, Asia may be different from other regions of the world because of differences in the ways that Hg flows through ecosystems. For example, in some parts of China, human exposure comes more from the ingestion of rice than from fish [16], because rice is grown in wet, seasonally flooded soils that facilitate methylation [17]. Some research has shown that animals in rice paddy environments, such as fish and aquatic invertebrates, may accumulate Hg [18,19], but this topic has not yet been well studied in the main rice-growing region of Asia.

Earthworms play an important role in the decomposition of organic matter, the mixing of soil layers, and nutrient cycling, and therefore provide crucial ecosystem services, as well as being good indicators of ecosystem health [20]. Earthworms are consumed by a variety of animals, especially vertebrates such as reptiles, birds, and mammals [21–23], and thus may serve as a vector for Hg transfer upward in the food web. Because of their direct contact with the soil, earthworms are good research organisms to understand how soil properties affect Hg methylation and thus Hg bioavailability to animals. For

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example, research has shown that soil characteristics such as low pH and high organic matter content can lead to high methylation [7,24,25]. Earthworms offer a good model to ask whether such properties influence methylation in rice-paddy soils, which can be both acidic and organic matter-rich, and how this process varies between contaminated sites, and those in which the source of Hg is deposition. We know that earthworms accumulate MeHg already in the soil, and in addition, recent research has suggested that further methylation may occur in the earthworm alimentary canal [26,27], potentially making them even more important in understanding Hg bioaccumulation.

We measured concentrations of total mercury (THg) and MeHg in earthworms at progressive distances away from abandoned Hg mines in the Wanshan Mercury Mining District as well as in another region of Guizhou Province, China without a history of mining. The present study describes the pattern of Hg and MeHg across distances and in locations with different soil properties, examines what percentage of Hg in earthworms is methylated, and quantifies bioaccumulation factors. The objective was to determine whether earthworms are important to the bioaccumulation of Hg in terrestrial, rice-based ecosystems.

## MATERIALS AND METHODS

### *Site selection, sample collection, and preparation*

All sampling was done in accordance with agreements between the Institute of Geochemistry, Chinese Academy of Sciences, and the districts of Wanshan and Leishan, Guizhou Province, and followed the laws and regulations of the People's Republic of China. In the Wanshan Mercury Mine District, we worked at 3 river catchments (the Xia Xi, Gao Louping, and Ao Zhai rivers), which have large Hg tailings, now covered by concrete (Figure 1). For each catchment, we selected 3 distances from the tailing (0–1 km, 2–3 km, and 7–8 km). We selected the 0-km to 1-km distance to be as close as possible to the mines, and the 7-km to 8-km distance because it is thought that the water in rivers of these areas is safe for human consumption at a distance of 8 km from the tailings [28]. Because contamination in the area likely decreases exponentially away from the mines, we selected 2 km to 3 km as the intermediate or medium distance [28]. At each distance, 3 sampling sites were established, 1 per river catchment. Each site constituted 4 10-m × 10-m plots, at least 100 m from each other and within a paddy field that was less than 500 m from the catchment river.

Sampling was also conducted in a control region, the Leishan District of Guizhou, approximately 200 km from Wanshan, where there is no history of Hg mining. Here we selected 3 sites on separate rivers of approximately the same size as those in Wanshan. Two of these 3 sites were 500 m from each other, both upstream of a confluence of the rivers on which they were located. They were 5 km from the other site (Figure 1).

Sample collection was the same for all plots (Wanshan and control). In each plot, 15 to 20 adult (i.e., clitellate) earthworms were collected within 10 cm of the surface, with earthworms being between 9 cm and 11 cm in length. Representative samples of all earthworms were put into a 4% formaldehyde solution for later species identification [29]. For identification, a Nikon SMZ800 anatomical lens was used to observe both external characteristics (body length, body width, segmentation, pigmentation, first dorsal pore, setae, prostomium, male pore, and spermathecal pore) and internal structures (gizzard, septa, spermathecae, testis-sacs, seminal vesicles, prostate gland, accessory gland, and internal ceca) [29]. From their pigmentation (heavily pigmented on the dorsal surface and lightly pigmented ventrally), we believe the majority of earthworms in this area were endogeic, living in the upper layers of the soil, consuming mineral soil with a preference for material rich in organic matter, and rarely coming to the surface [30]. These species have also been classified as endogenic in some previous research [31–33].

Earthworms were brought back to the laboratory in soil. They were washed 8 times with deionized water, and then depurated over wet filter papers for 48 h to purge their gut contents [34]. The earthworms were washed again a further 8 times, placed in separate polythene bags (1 earthworm to a bag), and placed in a freezer. Later the whole earthworm samples were freeze-dried (EYELA model FDU-1100) and then ground into homogenous powder using an agate mortar and pestle that was cleaned between samples. All samples were then again preserved in sealed polythene bags.

For soil sampling, each plot was further divided into 10 replicate 1-$m^2$ subplots. Twelve samples of approximately 50 g of soil from the 0-cm to 20-cm soil depth were collected from within each subplot using a corer. The 12 samples from each subplot were then mixed into a single composite soil sample; there were 10 soil composite samples per plot. All soil samples were stored in sealed polythene bags, placed in a cooler, and then carried to the laboratory within 24 h. When collecting, storing, and transporting the soil samples, we followed the procedure recommended by US Environmental Protection Agency (USEPA) method 1630 [34]. In the laboratory, all soil samples were freeze-dried, ground in a ceramic disc mill, and sieved to 150 mesh [35]. During the grinding process for both earthworms and soil, precautions were taken to avoid any cross-contamination.

Fifteen earthworm samples (randomly selected from the 15–20 per subplot) and 10 soil samples were analyzed per plot for THg, and 5 earthworm samples and 5 soil samples for MeHg (with the specific samples randomly chosen). Results are expressed on a dry weight basis. The 10 soil samples per plot were also analyzed for soil physical–chemical properties.

### *Laboratory analytical methods*

To determine THg concentrations, 0.2 g of the dry soil sample was digested in a water bath (95 °C) for 5 min, using a fresh mixture of concentrated HCl and $HNO_3$ (3:1,v/v), then BrCl was added, and the mixture was heated to 95 °C for another 30 min [15,36]. A subsample of ground earthworm (0.3–1 g) was digested using 10 mL $HNO_3/H_2SO_4$ (8:2, v/v) heated to 95 °C using a water bath for 3 h [37–39]. The Hg concentration in all digested solutions was determined using BrCl oxidation and $SnCl_2$ reduction, coupled with cold-vapor atomic absorption spectrometry, using a F732-S spectrophotometer (Shanghai Huaguang Machinery and Instrument) [15,40].

To determine the concentration of MeHg in soil samples, 0.5 g to 1.0 g of dry soil was weighed into 50-mL centrifuge tubes and leached in 1.5 mL 2 M copper sulfate, 7.5 mL 25% $HNO_3$, and 10 mL $CH_2Cl_2$ [41]. The MeHg concentration in soil extracts was quantified using cold vapor atomic fluorescence spectroscopy (CVAFS), following USEPA method 1630 [34], which requires a progressive sequence of distillation, addition of 2 M acetate buffer, ethylation with 1% sodium tetraethylborate, purge and trap of MeHg onto Tenax traps, thermal desorption, separation by gas chromatography, and detection by CVAFS (Brooks Rand Model III). The determination of MeHg concentrations in earthworms followed the same methodology as described above, after extraction using a potassium hydroxide methanol/solvent [42,43]. The pH of soil samples

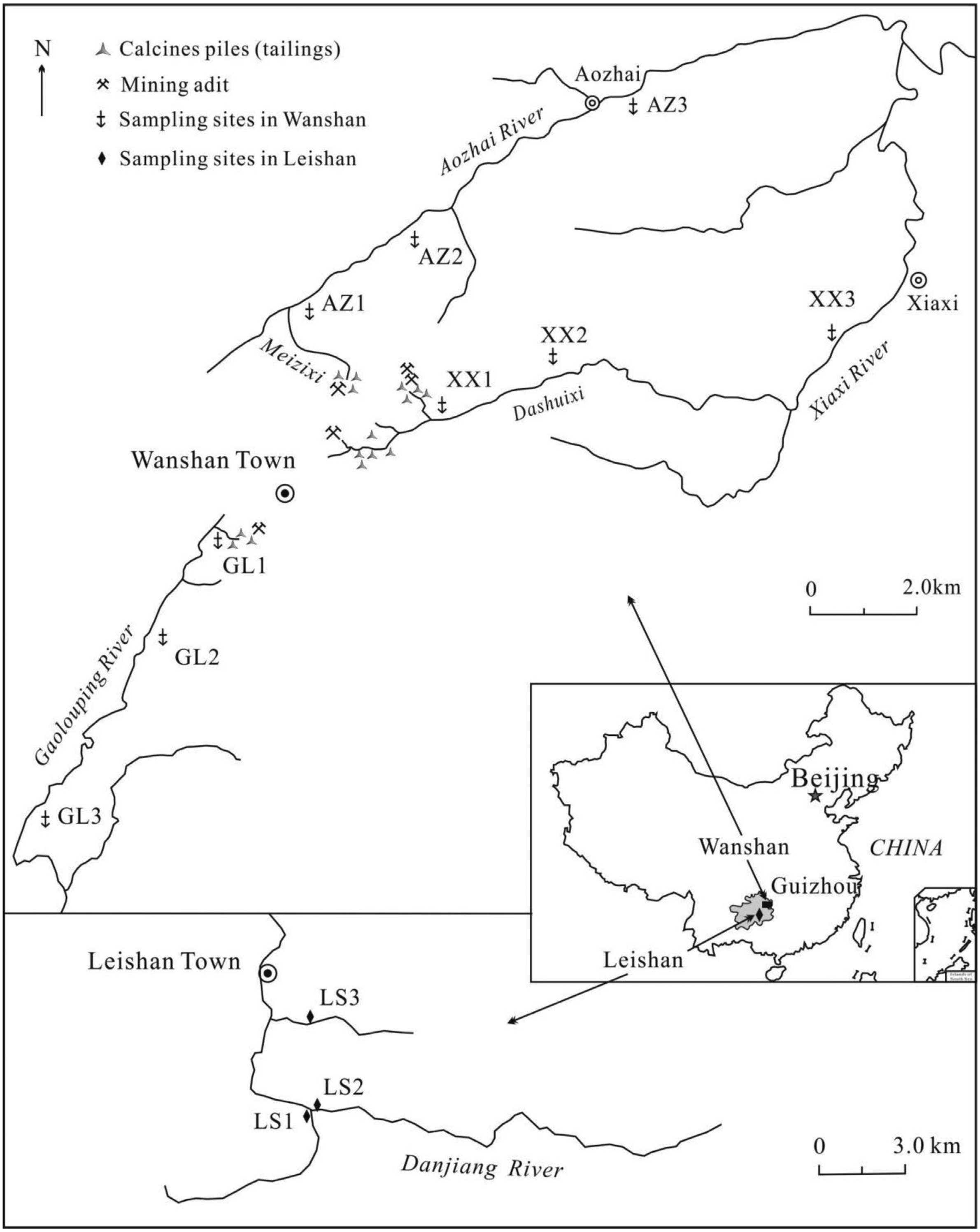


Figure 1. The present study sites in Guizhou Province, China, with sites of varying distance to abandoned mines in Wanshan district (upper inset), and control sites in Leishan district (lower inset). The scale bar in the insets is 6 km; white dots in Guizhou on the larger map are not to scale. We sampled 3 distance categories, with 3 sites at each distance (close, 0–1 km to the mines [XX1, AZ1, GL1]; middle, 2–3 km to the mines [XX2, AZ2, GL2]; far, 7–8 km to the mines [XX3, AZ3, GL3]) as well as Leishan (LS1–3), which is approximately 200 km away from Wanshan. At each site, 4 plots were laid in rice paddy soil, spaced at least 200 m from each other and within 500 m of the river. Abbreviations of river catchments in Wanshan: XX = Xia Xi river, AZ = Ao Zhai, GL = Gao Louping; LS = Leishan control sites.

was quantified using an electrode with a solid:water ratio of 1:2.5. A 5-mg sample was measured for the percentage of organic matter, using potassium dichromate ($K_2Cr_2O_7$) oxidation coupled with volumetric analysis [36,44]. The dichloromethane ($CH_2Cl_2$) was high-performance liquid chromatography/American Chemical Society quality (99.9% purity), and all other reagents were ultra pure quality (>95% purity), purchased from Shanghai Chemicals.

### *Quality assurance and quality control considerations*

Quality control measures consisted of method blanks, field blanks, triplicates, matrix spikes, and parallel analysis of several certified reference materials and blind duplicates, as described in the Supplemental Data, Table S1. For THg, the method detection limit (3 × standard deviation of the blank) was 0.01 μg/kg. Each sample was determined 3 times, and we calculated the mean concentration. The method was validated using reference materials TORT-2 (National Research Council of Canada) and GBW07405 (China Standard Material Research Center, Beijing, China). Determined THg concentrations were in good agreement with the certified values, as described in the Supplemental Data, Table S1. Recoveries on matrix spikes of THg in soil and earthworm samples ranged from 96% to 113% and 98% to 103%, respectively.

For MeHg, the method detection limit was 0.05 ng/g. The method was validated using reference materials TORT-2 and ERMCC580 (Institute for Reference Materials and Measurements, Belgium). Determined MeHg concentrations were in good agreement with the certified values, as described in the Supplemental Data, Table S1. Recoveries on matrix spikes of MeHg in soil and earthworm samples ranged from of 94% to 118% and 97% to 109%, respectively. Careful attention was paid to the blank controls, and blanks were introduced in each digestion to ensure the purity of chemicals used.

*Statistical analyses*

The bioaccumulation factor (BAF) for earthworms was calculated as the concentration of THg or MeHg in earthworms (mg/kg) divided by the concentration in soil (mg/kg) for a plot. The BAF values for THg and MeHg were calculated separately. Because we are only analyzing 1 taxon in the present study (earthworms, as opposed to multiple taxa at different trophic levels), this bioaccumulation factor is equivalent to a bioconcentration factor.

For statistical analyses, we used mixed linear models, with plot and river catchment as random factors, and distance (close, middle, far, and very far, the last being the control site) as the fixed factor. Although the river catchments in Leishan were different from those in Wanshan, they were randomly assigned to be similar to Wanshan, to run the mixed models (we confirmed that different assignments made very little difference to the results). Variables were log-transformed when appropriate to better fit parametric assumptions. Mixed model analysis was conducted with the lme4 mixed modeling program [45] and the multcomp program for multiple comparisons [46] in R Ver 3.1. To understand relationships between soil characteristics (organic matter %, pH) and the percentage of THg that was methylated in earthworms, we performed simple linear regressions, using arcsine transformations when appropriate. We also developed multiple regression models, including soil concentrations of THg as well as soil properties, to explain the percentage of Hg methylated in earthworms. Regression models were simplified by removing interactions when they were not significant. Results in which $p$ values were $< 0.05$ were considered significant.

## RESULTS

There was a sharp decline in soil THg concentration with increasing distance from the mines (Figure 2A and see Supplemental Data, Table S2 for a full summary of the data). The THg concentration for all Wanshan sampling sites at all distances from the mine was above the Chinese National

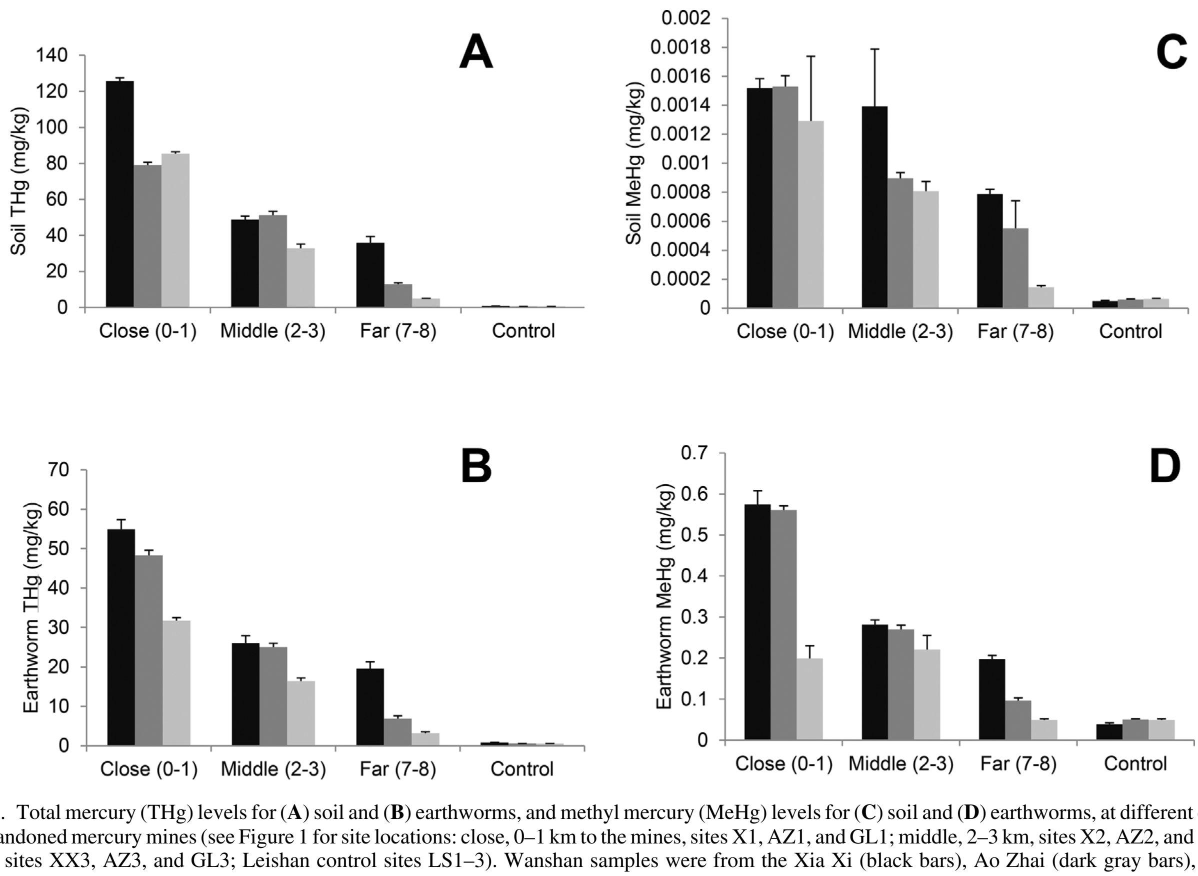


Figure 2. Total mercury (THg) levels for (**A**) soil and (**B**) earthworms, and methyl mercury (MeHg) levels for (**C**) soil and (**D**) earthworms, at different distances from abandoned mercury mines (see Figure 1 for site locations: close, 0–1 km to the mines, sites X1, AZ1, and GL1; middle, 2–3 km, sites X2, AZ2, and GL2; far, 7–8 km, sites XX3, AZ3, and GL3; Leishan control sites LS1–3). Wanshan samples were from the Xia Xi (black bars), Ao Zhai (dark gray bars), and Gao Louping (light gray bars) rivers. Data are expressed as mean ± standard error. For THg, each site represents 40 data points (10 from each of the 4 plots); for MeHg, there are 20 data points (5 from each of the 4 plots).

Standards maximum allowable concentration of Hg in soil for agricultural or residential use (1.5 mg/kg) [15]. The THg concentration in soil for each of the 4 distance categories was significantly different from the other categories ($z$ values $> 12.3$, $p < 0.0001$). Soil THg levels at the farthest distances in Wanshan were still 29 times the background levels at the Leishan control sites, and particularly high contamination was recorded for the Xia Xi River catchment of Wanshan (Figure 2).

The species of earthworms identified in the paddy soils were similar at all sites (*Drawida japonica japonica*, *Metaphire californica*, and *Amyntha* spp; Table 1), and the THg concentration of these earthworms was correlated with the soil concentration of THg ($r^2 = 0.96$, $p < 0.0001$). At Wanshan, the concentration of THg in earthworms was approximately half that of soil (mean BAF = 0.55; Figure 2B). However, the BAF for THg was almost twice as high in the Leishan control sites (mean 1.06 in Leishan; $z$ values for comparison between Leishan and other 3 distance categories in Wanshan were $> 3.5$, $p < 0.001$; Figure 3A).

The MeHg concentration in soil and earthworms also decreased with increasing distance from the mines (Figure 2C and D). Differences between the Leishan control sites and the 3 Wanshan distance categories for soil MeHg were significant (all $z$ values $> 2.8$, $p < 0.030$). However, the concentration of MeHg for earthworms from the Wanshan site most distant from the mines was not significantly different compared with the Leishan control sites ($z = 1.72$, $p = 0.31$; all other $z$ values $> 3.6$, $p < 0.002$).

The results indicate that the concentration of THg decreased with distance from the mines more dramatically (i.e., with a higher level of statistical significance) than the concentration of MeHg. This can be explained by an increasing proportion of soil THg present as MeHg (as a percentage) when further from the mines, and greatest for the control sites (for soil, $z$ values for comparison in methylation percentage between Leishan and the other 3 distance categories in Wanshan were $> 3.0$, $p < 0.014$; Figure 3C; for earthworms, $z$ values for comparison between Leishan and other 3 distance categories were $> 16.9$, $p < 0.0001$; Figure 3D).

The MeHg concentration in earthworms was related to the concentration of MeHg in soil ($r^2 = 0.63$, $p < 0.0001$), but not as closely as to the concentration of THg in soil. Similar to that for THg, the earthworm BAF for MeHg was higher in Leishan than Wanshan ($z$ values for comparison between Leishan and other 3 distance categories were $> 3.5$, $p < 0.003$).

There were also differences between the physical–chemical properties of the soil in Leishan control and Wanshan sites. Soil pH at Leishan was lower than at the Wanshan sites (the extreme alkaline values of Wanshan are because of mercury tailings; $z$ values for comparison between Leishan and other 3 distance categories were $> 8.6$, $p < 0.001$). Soil organic matter was greater at Leishan relative to Wanshan ($z$ values for comparison between Leishan and other 3 distance categories were $> 3.2$, $p < 0.007$). These 2 soil properties were correlated with the percentage of Hg that was methylated (Figure 4). Including data at all sites, increased methylation was significantly related to decreased pH (Figure 4A; $p < 0.0001$, $r^2 = 0.60$), and increased organic matter (Figure 4B; $p < 0.0002$, $r^2 = 0.26$). The multivariate model ($r^2 = 0.64$) showed that pH had a strong influence on the percentage MeHg in earthworm tissues ($t = -5.34$, $p < 0.0001$), and organic matter a marginal 1 ($t = 1.74$, $p = 0.089$), without an influence of soil THg concentrations ($t = 0.39$, $p = 0.70$).

Because the differences between the Leishan and Wanshan sites were so dramatic (and these sites are very far apart), we further investigated whether the correlations between increased methylation and pH and organic matter would hold just for the Wanshan data. The relationship between increased methylation and organic matter continued to be highly significant when only the Wanshan data were used ($p < 0.004$, $r^2 = 0.23$). The relationship between increased methylation and pH tended to be significant in Wanshan ($p = 0.063$, $r^2 = 0.10$). The multivariate model ($r^2 = 0.34$) demonstrated that organic matter was the most important predictor of the percentage methylation of earthworms within Wanshan ($t = 2.60$, $p = 0.014$), and neither pH ($t = -1.35$, $p = 0.19$) nor THg ($t = -1.15$, $p = 0.26$) were strongly influential.

Table 1. The species composition of earthworms at the sampling sites[a]

| Species | Close (0–1 km) | Middle (2–3) | Far (7–8) | Control |
|---|---|---|---|---|
| *Drawida japonica japonica* | 3/3 | 2/3 | 3/3 | 3/3 |
| *Metaphire californica* | 3/3 | 3/3 | 3/3 | 3/3 |
| *Amynthas* - species 1 | 3/3 | 3/3 | 2/3 | 2/3 |
| *Amynthas* - species 2 | 3/3 | 3/3 | 3/3 | 2/3 |
| *Amynthas* - species 3 | 1/3 | 2/3 | 3/3 | 1/3 |
| *Amynthas* - species 4 | 1/3 | 2/3 | 1/3 | 1/3 |

[a]The number of sites in which the earthworm species was found is shown for each distance category out of the 3 sites sampled. The *Amynthus* earthworms are part of a species complex, for which there may be several undescribed species.

## DISCUSSION

We show 2 major patterns in the distribution of Hg within soil and earthworms in rice paddies of Guizhou Province, China. First, THg and MeHg concentrations decrease with increasing distance away from the mercury mines (Figure 2), as would be expected. Our results show high Hg concentrations even at 7 km to 8 km from the mines, where drinking water is considered to be safe [28]. The Xia Xi rivershed was particularly contaminated, and this information may be useful in the re-evaluation of this contaminated area. Second, the percentage of Hg that is methylated, and the earthworm BAF values for THg and especially for MeHg, are higher with increasing distance from the mines, and are particularly high in the control region (Figure 3). We propose below that this pattern of higher methylation and higher intrusion of Hg into organisms and food webs may be related to differences in soil conditions. The acidic, high-organic matter soils of the control region are commonly found throughout rice paddy fields in Asia [17,47], and hence earthworms may be playing more important roles in the amplification of Hg in the food web than previously recognized.

A comparison of the percentage of methylation and BAF values of the present study with other recent earthworm Hg studies [48–51] shows that levels found in the present study are relatively high, especially for the sites at greater distances from the mines in Wanshan and in Leishan (Table 2 and Figure 4). We propose that the described methylation and BAF patterns may be influenced by differences between the soil conditions at different distances from the mines, and particularly pH and organic matter. As to pH, the sites closest to the Hg mines have very high pH values because of the contamination of the limestone tailings (for the most part, Guizhou soils are naturally acidic [52]). High pH values are known to limit the methylation process [7,24,53,54]. However, we need to acknowledge that

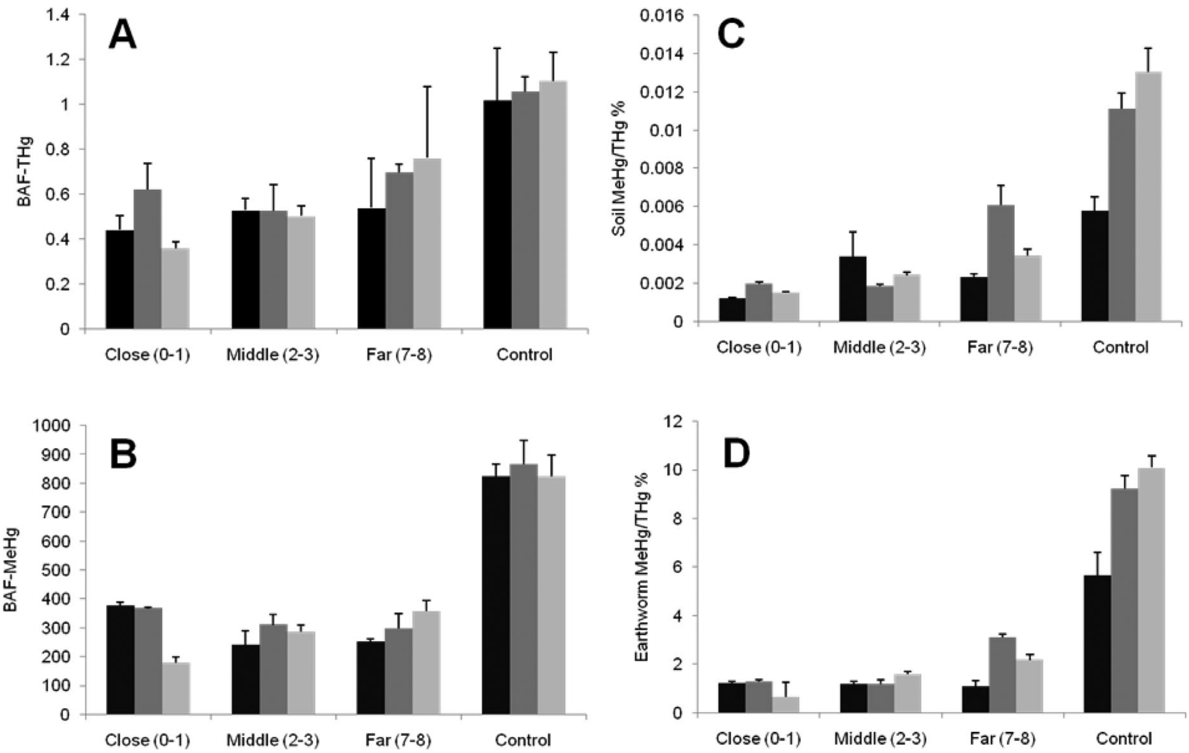


Figure 3. Bioaccumulation factors (BAF = concentration in earthworms/concentration in soil) for (**A**) total mercury (THg) and (**B**) methylmercury (MeHg), and the amount of MeHg expressed as % of THg in (**C**) soil and (**D**) earthworms, at different distances from abandoned mercury mines (see Figure 1 for site locations: close, 0–1 km to the mines, sites X1, AZ1, and GL1; middle, 2–3 km, sites X2, AZ2, and GL2; far, 7–8 km, sites XX3, AZ3, and GL3; Leishan control sites LS1–3). Wanshan samples were from the Xia Xi (black bars), Ao Zhai (dark gray bars), and Gao Louping (light gray bars) rivers. Data are expressed as mean ± standard error. Each site was represent by 40 data points, 10 from each of the 4 plots, for THg and 20 data points, 5 from each of the 4 plots, for MeHg.

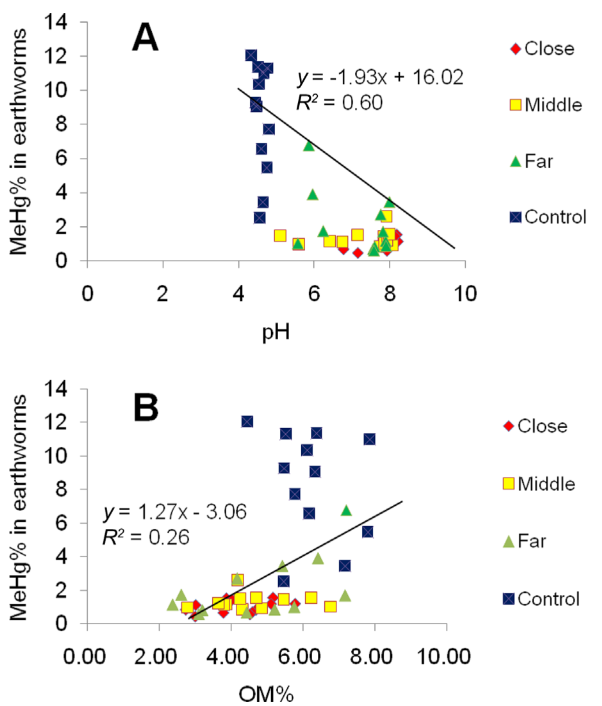


Figure 4. The relationship between the % of methylmercury (MeHg) in earthworms and (**A**) pH and (**B**) organic matter (OM), with data points corresponding to their distance category (see Figure 1 for site locations: close, 0–1 km to the abandoned mines, sites X1, AZ1, and GL1; middle, 2–3 km, sites XX2, AZ2, and GL2; far, 7–8 km, sites XX3, AZ3, and GL3; Leishan control sites LS1–3). Each data point represents a study plot, and its values were calculated from a mean of 10 THg or 5 MeHg data points, and 10 soil measurements.

our analyses showing that pH is strongly correlated with MeHg are driven by very low pH readings from the control site, much lower than those in Wanshan. Further research should be conducted in sites that have pH values ranging from 4 to 6 throughout Guizhou Province to confirm the relationship of pH to the percentage of Hg that is methylated in earthworm tissues.

Low concentrations of organic matter have also been reported to limit methylation [7,17,24,36], and organic matter may be related to pH, because high pH values like those in Wanshan may limit plant growth [7,55–58]. Indeed, organic matter was the strongest predictor of the percentage of MeHg in earthworms within the contaminated area, although it should be noted that our model explained all but 34% of the variation in the data. Other soil properties, such as texture, or redox conditions, may also be important factors. Furthermore, earthworms may vary within and between species in their capacity to methylate inorganic Hg in their digestive systems [26,27], and these differences may be producing the high levels of variation that were observed (although we note that the earthworm communities were similar at different sites). The present study is unable to answer questions about in vivo methylation processes in earthworms, and this issue should be a priority research direction in the future.

In contrast to the mine-contaminated soils, rice-paddy environments in which atmospheric deposition is the main source for Hg, like our control sites, may have higher rates of methylation. A number of studies have found that Hg derived from deposition is more easily methylated [24,59–61]. Several features of rice paddies make them conducive sites for methylation, including flooded conditions, anoxic soils, and high primary productivity, which leads to high amounts of organic matter [17,58]. Rice paddy cultivation in this part of China involves rice straw amendment, whereby the stems and

Table 2. Comparison of the present study to other related earthworm studies, in the percentage of total mercury (THg) that was methylated (MeHg) in earthworms, and their bioaccumulation (BAF) values for THg and MeHg

| Study | Species | %MeHg[a] | BAF THg | BAF MeHg |
|---|---|---|---|---|
| Burton et al. [46] | *Eisenia fetida* | – | 0.6 to 0.33 | 175 to 249 |
| Zhang et al. [47] | *Drawida sp.* | 3.0 | 0.040 to 0.539 | 10.163 to 31.387 |
| | *Alolobophora sp.* | 12.0 | | |
| Rieder et al. [48] | *Aporrectodea longa* | 5.7 | 1 to 15 | 15 to 191 |
| | *Lumbricus terrestris* | 10.1 | | |
| Álvarez et al. [49] | *Lumbricus terrestris* | 0.01 to 0.2 | 0.02 to 0.62 | 0.8 to 17.3 |
| Present study | *Drawida japonica japonica*<br>*Metaphire californica*<br>*Amynthas sp* | 0.4 to 12.0 for combined species | 0.55 | 177 to 865 |

[a]Multiple values on different lines represent measurements for separate species.

roots from the older plants are allowed to decay into the soil; such practices ensure high organic content and may also acidify soils [25].

These soil conditions in rice paddies may make the earthworms important biomagnifiers of MeHg. As mentioned above, besides simply accumulating MeHg through the assimilation of methylated species from soils, earthworms are capable of methylating Hg in their own digestive systems [26,27]. Acidic soils in particular may allow earthworm lipids to bind and accumulate Hg [48]. Some species of earthworms may live up to 8 yr, and such a long lifespan can allow them to accumulate Hg [62]. Indeed, compared with other invertebrates at low trophic levels, earthworms may have relatively high Hg accumulation. For example, at sites close to the mines in the present study, earthworms averaged nearly twice the MeHg concentrations (0.44 mg/kg) of grasshoppers (*Oxya* sp., 0.22 mg/kg; a significant difference, Welch's $t$ test, $t_{72.7} = 7.33$, $p < 0.0001$; K.S. Abeysinghe, unpublished data). Earthworms are in turn consumed by a wide variety of animals, especially vertebrates, that biomagnify Hg further [21–23]. Indeed, the hog badger (*Arctonyx collaris*) is a mammal in our region that feeds predominantly on earthworms [63], and many Turdidae bird family species common to the region consume earthworms as a large proportion of their diet (E. Goodale, personal observation).

## CONCLUSIONS

We have shown that the percentage of Hg that is methylated in earthworms and their BAF values were relatively high in rice-paddy soils, compared with studies in other environments. These rates were particularly elevated in control areas where soil conditions were acidic and soils had high organic matter content. The origin of Hg is mainly from atmospheric deposition in these control areas, and, given the deposition patterns of Hg in China [64,65], the MeHg concentrations we report for the control site might be representative of concentrations found over wide regions of China, as well as other parts of Asia. Emissions from industrial activities also acidify environments [47,66], and acidification is particularly problematic in China, where the use of nitrogen fertilizer is very high [67]. Our data add to the evidence that Hg can flow through earthworms into rice-based ecosystems [18], and further research should focus on these ecosystems as part of international efforts to control the emissions and mitigate the adverse effects of Hg.

*Supplemental Data*—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3643.

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*Data Availability*—Average values for THg, MeHg, organic matter, and pH are given in the Supplemental Data, Table S2, and all measurements are available on request from the corresponding authors (qiuguangle@vip.skleg.cn. or yangxd@xtbg.ac.cn).

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