Eben Goodale ORCID iD: 0000-0003-3403-2847

Guangle Qiu ORCID iD: 0000-0002-1599-4795

*19-00342*

*Environmental Toxicology*

C. He et al.

Heavy metals in bird feathers from southern China

# Heavy Metal, Arsenic and Selenium Concentrations in Bird Feathers from a Region in Southern China Impacted by Intensive Mining of Non-ferrous Metals

Chao He[1], Tongping Su[1,2], Shilong Liu[1], Aiwu Jiang[1]**, Eben Goodale[1]*, Guangle Qiu[3]

[1] Guangxi Key Laboratory of Forest Ecology and Conservation, College of Forestry, Guangxi University, Nanning, Guangxi, China.

[2] Key Laboratory of Beibu Gulf Environment Change and Resources Use, Ministry of Education, Nanning Normal University, Nanning, China

[3] State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang, China

*(Submitted 22 May 2019; Returned for Revision 17 June 2019; Accepted 17 October 2019)*

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/etc.4622.

---

**Abstract:** Heavy metal pollution is widespread in China, particularly in its mining regions. Mercury [Hg] concentrations in birds from Guizhou Province were recently reported to be above adverse effect levels, even in non-mining areas. We sampled birds to investigate whether Hg might be a threat near lead [Pb], zinc [Zn] and tin [Sn] mines in Guangxi Zhuang Autonomous Region. We measured concentrations of eight metals/metalloids in feathers of 627 resident birds representing 60 species sampled across 14 sites on five rivers. We found Hg concentrations (mean ± SD, 1.27 ± 2.02 ppm) were lower than in the recent Guizhou study. Mercury had the fewest correlations with other metals/metalloids; the mined metals (Pb, Sn, Zn) had the most such correlations. Levels of cadmium (Cd, 1.12 ± 2.64 ppm), As (4.78 ± 17.11 ppm), and Pb (17.18 ± 24.49 ppm) were closer to thresholds of adverse effects, or relatively high compared to other studies. With the exception of a few hotspots for Hg, Cd, and As near mines, metal/metalloid levels were fairly evenly distributed among sites, consistent with the regional occurrence of mineable ore deposits. Hg appears not to be threatening to all avian species in China, although it may be problematic for some species near Hg mines. In addition to Hg, however, other metals/metalloids may pose wildlife health hazards.

**Keywords**: biomonitoring, ecotoxicology, heavy metals, mining pollution, wildlife toxicology

This article includes online-only Supplemental Data.

*Address correspondence to ebengoodale@gxu.edu.cn, eben.goodale@outlook.com; aiwuu@163.com

DOI: 10.1002/etc.xxxx

## INTRODUCTION

Heavy metals, whether they be naturally or anthropogenically released, may constitute an environmental threat in the "planetary boundaries" concept, and crossing these boundaries could trigger abrupt environmental change (Rockström et al. 2009). Heavy metals are known to be a major source of disease for humans (Järup 2003), and several of these metals, such as mercury (Hg, Scheuhammer et al. 2007), are also known to be toxic to fish and wildlife. These metals may be mobilized by a variety of human activities, and also vary in their manner of distribution and contamination. Some metals such as Hg are emitted into the atmosphere by industrial processes and are then deposited back to earth, even in areas that never received direct discharges (e.g., polar regions, Pacyna et al. 2010). In addition, they can also be concentrated at local pollution point sources (e.g., Kocman et al. 2013 for Hg), particularly in mining regions (Dudka and Adriano 1997). Heavy metals also vary in their chemical properties and the degree to which they are accumulated by living matter and biomagnify across trophic levels, and so different metals pose particular problems for specific ecosystems and species (e.g., Hg methylates in anoxic conditions, Ullrich et al. 2001, and biomagnifies, Lavoie et al. 2013). Although Hg has been the focus of particular concern, other metals/metalloids such as cadmium (Cd, Burger 2008), arsenic (As, Sánchez-Virosta et al. 2015), and lead (Pb, Williams et al. 2018) may also threaten wildlife, and particularly birds.

Pollution is a particularly important issue for China because of the scale and rapidity of its economic development and urbanization (Chan and Yao 2008). Due to the large amount of coal-burning powerplants and other industrial activity, some heavy metals that are spread through deposition, such as Hg, have particularly high emissions in

China (Pacyna et al. 2010). As China is also a major mining center, heavy metal pollution is a particular problem in mining regions (Li et al. 2014). However, much of the research activity on heavy metals in China has been confined to soil, water, the atmosphere and humans. Less research has been devoted to wildlife as indicators for biomonitoring studies (but see Hsu et al. 2006, Abeysinghe et al. 2017). Birds in particular are good bioindicators because they are common, vary in their trophic levels, and can be sampled non-destructively (e.g., Abdullah et al. 2015).

The present study focused on the Guangxi Zhuang Autonomous Region (**Figure 1**), one of the mining regions highlighted to be of special concern (Li et al. 2014). We studied areas in the northwest and southwest of Guangxi. In these areas mining tends to be focused on Pb-zinc (Zn), Zn-copper (Cu), or tin (Sn), with some published reports of human health concerns arising from this mining (see Supplemental Methods). We sampled four rivers with known point sources of pollution tied to the mining industry, and measured eight heavy metals/metalloids. Sampling was designed to target locations near the known point source, and then at two locations further downstream (~10 km and 50 km) to understand the extent of the contamination. We also chose one river with clean drinking water as a reference site (see Supplemental Methods). We hypothesized that the biomagnifying metal Hg, and to a lesser extent Cd, might be present at high concentrations in insectivorous or carnivorous species, even at river sites that had Pb/Zn (and not Hg) mining. We were concerned about the levels of Hg, given that they were previously shown (Abeysinghe et al. 2017) to be above levels of adverse effects in the feathers of insectivorous passerine birds in mining and non-mining areas of Guizhou Province, the province to the north of Guangxi, perhaps due to aerial deposition. Further, Hg requires its own kind of testing and thus is sometimes not measured along with other heavy metals/metalloids, so it could be a hidden threat in mining regions.

## MATERIAL AND METHODS

### *Study Sites*

All 14 sites used in this study are in two prefectures of Guangxi Zhuang Autonomous Region, southern China (see Figure 1). Eleven sites were in Hechi Prefecture, northwest Guangxi, known as a center for non-ferrous metals in China, and has a subtropical monsoonal climate. Three sites were in Chongzuo Prefecture, southwest Guangxi, in the northern border of the tropics. Both counties include large portions of limestone karst, and all sites were between 150 and 500 m above sea level. These sites were located on five rivers, including four rivers affected by mining and smelting operations (Qingshui, Diaojiang, Dahuanjiang and Dongling Reservoir Rivers), and one apparently uncontaminated river (Chengjiang River; the reference site). In general, our sampling scheme for each river was to place the first sampling site slightly downstream of a known point source of pollution (or, for the reference river, the point at which it arose from underground), a second one 10 km downstream and the last one a further 30-50 km downstream, with the intent to examine the spatial extent of the contamination. We systematically selected sites that were in agricultural areas with some disturbed natural vegetation. Specifically, the sites were laid out as follows (for more details see Supplemental Methods and Table S1):

1) Qingshui River (Hechi Prefecture), the smallest river we sampled in northwest Guangxi. The first sampling site was at Yulan village, where two Hg mines operated for approximately 50 years before becoming inactive quite recently

(about 2015). A second site was located 10 km downstream. As the Qingshui then joins the larger Hongshui River a few km from the second sampling site, we did not have a third sampling site for this river.

2) Diaojiang River (Hechi Prefecture), of intermediate size between the Qingshui and the Dahuanjiang, described below, and the subject of earlier pollution studies (see Supplemental Methods). Many Pb, Zn and pyrite mines are located in its upper reaches. The first sampling site was about 3 km south of the town of Chehe, which is a center for the smelting of many nonferrous metals, and close to the famous Dachang Sn/polymetallic ore field. The second sampling site was 10 km downstream, and a third sampling site 50 km further downstream.
3) Dahuanjiang River (Hechi Prefecture), the largest river we sampled. Recent reports suggest that levels of metals currently in the river are high for some metals (see Supplemental Methods).

   The first sampling site was near Shangchao Town, which is < 5 km downstream from some Pb and Zn mines; there are more mines further upstream. A second sampling site was 10 km downstream from the first, and a third sampling site 50 further km downstream.
4) Chengjiang River (Hechi Prefecture), chosen as a reference river for the project because it has no mining operations. It emerges from an underground source and is considered by local communities to be clean and has also been determined to have potable water. The first sampling site was near the town of Daxing, 1 km downstream from the emergence of the river from underground. Two other sampling sites were located 10 km downstream and 30 km further downstream.

5) Dongling Reservoir River (Chongzuo Prefecture), is a smallest river sampled, smaller than the Qingshui, and in places it goes underground. It is the outlet for the Dongling Reservoir, which is located inside the mining area of the Daxin Pb/Zn mine. This mine started operating in 1955 and was a large-scale operation for 40 years until activity stopped in 2001, due to resource exhaustion and environmental pollution. The first sampling site was at the reservoir itself. The second sampling site was located 10 km and the third a further 50 km downstream.

*Feather Sampling*

Permission for the field study was obtained from the Hechi Forestry Bureau and Chongzuo Forestry Bureau, and procedures followed the laws of the People's Republic of China. Bird feather samples were collected in the months of December 2017 to March 2018. We placed ten mistnets, 9-m long and 3-m high, on the periphery of farmlands within 100 m of the rivers. We used passive methods (no birdsong playback; sampling all species captured), placing nets from dawn to dusk, and patrolling them at least once per hour, placing captured birds in cotton bags until they were processed. Birds were identified using MacKinnon and Phillipps (2000), and in consultation with experienced field ornithologists (TS, AJ). We took standard measurements (tarsus, mandible, wing, tail, weight) and plucked the second secondary feather from both wings, before we color-banded and released each bird. Feathers were stored in polythene bags at -20°C after returning to the laboratory. Before analysis, all feathers were washed vigorously, alternating acetone and deionized water, and then air-dried, weighed, and cut up into pieces with stainless steel scissors (Burger and Gochfeld 2000b, Abdullah et al. 2015).

Concentrations may be considered wet weight because intensive heating was not used; nevertheless, the differences between dry weight and wet weight are likely to be minimal for feathers.

*Laboratory Procedures*

We measured total Hg concentration in feathers with a DMA-80 Direct Hg analyzer (Milestone Italy) at Guangxi University. We tested a human hair reference (IAEA-086) after every 15 samples in order to determine accuracy and precision. We considered the data met acceptability criteria if we obtained between 0.53~0.61 mg/kg (hereafter, ppm), the 95% confidence interval for this standard (Bleise et al. 2000). Mercury recovery was 101.02 ± 6.62%.

For the other metals/metalloids, we digested feather samples with nitric acid ($HNO_3$, GR) following standard methods (State Health and Family Planning Commission and State Food and Drug Administration 2017) and then ran analyses on the Inductively Coupled Plasma Mass Spectrometer (Perkin Elmer, USA) at Guangxi University. A mixed elements standard solution (for As, Cd, Cu, Pb, Se, Sn, Zn) was used to create standard curves ($R^2 > 0.99$). Feather samples were analyzed and the mixed standard solution (As = 10μg/L, Cd = 10μg/L, Cu = 50μg/L, Pb = 10μg/L, Se = 30μg/L, Sn = 5μg/L, Zn = 200μg/L) was tested every 15 samples to confirm that the instrument was not drifting out of range. The acceptability criteria was between 80-120% recovery; recovery rates were: 95.73 ± 13.27% for As, 97.66 ± 5.01% for Cd, 98.77 ± 8.83% for Cu, 98.34 ± 6.31% for Pb, 99.56 ± 6.07% for Se, 98.89 ± 4.56% for Sn, and 113.41 ± 8.31% for Zn. The sample size for Se was smaller than the other metals/metalloids (n = 236 individuals of 44 species at seven sites), as it was added towards the end of the study as a comparison to other metals/metalloids such as Hg with which it is antagonistic, and only quantified in those samples that had additional material. The instrument and method detection levels are shown in Supplemental Table 2.

### *Bird Species Data*

To decide which bird samples to use in the statistical analysis, we first categorized birds as resident or migrant species (see Supplemental Methods for information on data sources). For all resident species (migratory birds were not analyzed), we classified their diet into four categories (carnivores [eating primarily vertebrate prey], insectivores [eating primarily invertebrate prey], frugivores and granivores), judging the category for a species by the majority of its diet (Supplemental Table 3). For this work we used three sources (see Supplemental Methods), and found a consensus category for each species, giving some weight to the genera if the data on a species was contradictory. Finally, we hypothesized that the proximity of birds to the ground might influence feather concentrations of metals/metalloids. Therefore, we classified species by vertical strata, using three categories: ground, understory or shrub or grass, and subcanopy or canopy.

### *Data Analysis*

We first investigated how the traits of birds affected heavy metal/metalloid concentrations in feathers, using general linear mixed models (GLMMs), R software version 3.5+, and the package ‘lme4’ (Bates et al. 2017). The concentrations of the eight types of heavy metals/metalloids were the response variables. For this and the subsequent modeling, we transformed these concentrations separately for the different metals/metalloids using either a log or a square root transformation, guided by Box/Cox analysis and visual assessment of residual graphs. Fixed explanatory factors included:

diet, vertical strata, and the interaction between these two factors. Random explanatory factors included the sampling site, nested in river, and the species of bird. We progressively simplified models, removing first non-significant interactions, and then non-significant variables.

Our second set of analyses focused on the location and extent of contamination, again with the transformed concentrations of the eight types of heavy metals/metalloids as separate response variables. First, we compared the five rivers in their heavy metal/metalloid concentrations in bird feathers. These GLMMs had the fixed factors of river and diet, and random factors of site and species. Second, we compared all 14 sites to each other, with a model that had site as the fixed factor and species as a random factor. Third, we ran a model that compared the sites in their proximity to the known sources of contamination. Fixed factors included the position of the site on the river ('proximity to pollution': close [upstream], intermediate [midstream] and far [downstream]) and diet, and the random factor was species.

Our last analysis explored the correlations between the different kind of metals/metalloids, with the question being how much information does the concentration of one metal/metalloid infer about the concentrations of other metals/metalloids, accounting for differences related to both species and site. Given that some metals/metalloids varied by diet category, for this analysis we used only insectivorous birds. We first averaged the concentrations of metals/metalloids for different individuals of a species captured at the same site (with the total sample size being 139 mean values for a species at a site), and then determined the relationship between all pairs of metals/metalloids. For all these analyses, we ran Pearson correlations on transformed concentrations, and likewise found the P-value associated with each correlation. We calculated the correlations with Se and the other metals/metalloids separately, because of the smaller sample size for Se (65 mean values for a species at a site).

A significant problem was that we had a substantial number of non-detections for some elements (especially As, Cd, and Cu). For the main analysis, we coded a sample with an undetectable concentration as 0. However, to understand the effect on non-detections on the results, we also tried a second approach, coding any sample with an undetectable concentration, or a sample that had a detected concentration below the method detection limit, as having half of the method detection limit (following Nardiello et al. 2019). The results of these analyses were similar and are summarized in Supplemental Results.

## RESULTS

### *Summary Statistics on the Concentration of Heavy Metals/Metalloids*

We sampled 625 individuals of 60 species (606 individuals of 54 passerine species; see Supplemental Table 3 for species list). The average concentrations of the eight different metals/metalloids are shown in Table 1. Several metals/metalloids had very high standard deviations because many samples had undetectable concentrations (coded as 0). For example, As was undetectable in 377 of 622 samples, and hence had a coefficient of variance (CV) of 3.58; Cd was undetectable in 139 of 622 samples, and it had the second highest CV of 2.35. To describe the extreme values in the dataset, we also show in Table 1 summary statistics for the site with the greatest average concentrations for each element. Further, for elements that showed differences among diet categories (see

Results: Bird Traits), summary statistics are presented for the diet category with the greatest average concentrations, at the site with the greatest average concentrations.

*Bird Traits.* The interaction between vertical strata and diet, and vertical strata itself were never significant factors, so all models were simplified to diet as the single fixed factor. Diet was strongly associated with concentrations of Hg, in the direction of biomagnification: carnivores had higher concentrations than insectivores (Z value = 4.41, $P < 0.001$), and insectivores had higher concentrations than both frugivores and granivores (Z values > 3.10, $P < 0.0094$; **Figure 2**). Selenium had a similar pattern, with carnivores and insectivores higher than frugivores and granivores (Z values > 2.73, $P < 0.029$). Diet was also significantly associated with concentrations of Zn and Sn, but not in direction of biomagnification. Specifically, for Zn, granivores and insectivores had higher levels than carnivores (Z values > 2.74, $P < 0.030$), and the same pattern held for Sn (Z values > 2.87, $P < 0.021$).

*Comparing Rivers, Sites and the Extent of the Contamination*

Birds from the largest river, Dahuanjiang, exhibited relatively high levels of Cu and As. Specifically, Dahuanjiang birds had higher Cu concentrations than those on all other rivers (Z values > 2.88, $P < 0.033$), and higher As concentrations than other rivers (Z values > 2.74, $P < 0.048$) except the Dongling Reservoir River. Birds from the Qingshui River had higher Hg concentrations than those from the Dahuanjiang and those from the Diaojiang (Z values > 2.77, $P < 0.044$). No other contrasts between any pair of rivers for any metal/metalloid were significant.

Differences in concentrations among sites were apparent. For Hg, the most upstream site on the Qingshui near the Yulan Mine (QS1) had higher levels in feathers than all other sites (Z values > 12.56, P < 0.001). For Cd, the most upstream site on the Dongling Reservoir River (DLR1), inside the Daxin Pb/Zn mine area, had higher levels than most other sites (Z values > 4.10, P < 0.01). For As, the midstream site on the Dahuanjiang (DHJ2) was higher than most other sites (Z values > 4.33, P < 0.001).

The proximity to pollution models (all rivers combined except for the reference site) followed our predictions for Hg, Cd and Pb, but did not for As, Cu, Sn, Se and Zn. Mercury in bird feathers was significantly greater at sites close to the pollution source than intermediate sites (Z value = 3.15, P = 0.0046), and intermediate sites were higher than far sites (Z value = 6.05, P < 0.001). For Cd and Pb, concentrations at the sites close to the source were higher than both intermediate and far sites (Z values > 8.10, P < 0.001). However, for As, Cu, Sn and Zn, close sites were lower than intermediate or far sites (Z values > 2.80, P < 0.014), and for Se far sites were higher than intermediate or close sites (Z values > 4.17, P < 0.001; although in this model there were only four locations). Models of the proximity to pollution source, conducted on the individual rivers separately (Supplemental Table 4), showed that the Dongling Reservoir River in particular had levels of many metals/metalloids (other than Cd) that were higher at intermediate and far sites, driving the results of the overall models wherein all rivers were combined.

### *Correlations between Metals/Metalloids*

For the correlations, 11 of 28 possible pairwise interactions were significant, and all significant correlations were positive. The metals that are targets for mining in the region (Pb, Sn and Zn) had the most significant correlations with other metals/metalloids (four significant correlations each). Cadmium, Se and Hg had the lowest correlations with other metals/metalloids (one significant correlation each). Mercury, in particular, had four non-significantly negative correlations with other metals/metalloids, more than any other metal/metalloid.

## DISCUSSION

Feathers are a useful matrix for biomonitoring because they can be collected nondestructively and can address exposure to a broad range of metals (e.g., Abdullah et al. 2015). One limitation is that feather concentrations represent the body burden at the time of feather growth, so that migration or dispersal of birds can be a source of variability. For this reason, we analyzed only Guangxi resident birds. Other limitations are that toxicity thresholds are better understood for other tissue types (e.g., internal organs for Cd, Burger 2008, blood or eggs for Hg, Ackerman et al. 2016). Finally, a recent analysis documented substantial variation within each feather (although we used a whole feather sample), within feather types, and between feather types (Peterson et al. 2019), leading to the recommendation that many feathers should be sampled for each bird. Removing multiple feathers might mean, however, that the technique might be destructive. This potential variation should be kept in mind when comparing our results to other studies.

Overall, metal/metalloid levels in bird feathers were fairly similar across sites. In fact, five of the eight metals/metalloids studied had results in the proximity to pollution source models in the opposite direction of what would be expected, with levels higher away from what we had identified as the pollution source. This may represent a lack of knowledge about all the sources of pollution in these areas, and/or non-consideration of other tributaries running into the rivers. The river chosen as the reference unexpectedly did not have significantly lower concentrations of any metal/metalloid than any other river. This suggests that the background level of the metals/metalloids in this limestone karst area is high, consistent with the regional occurrence of minable ore deposits. On the other hand, concentrations of three of the most toxic metals/metalloids (As, Cd, and Hg) were high near known mining areas (Table 1; concentrations at the highest site were 4.3 X, 3.0 X, and 5.0 X mean levels for the three elements, respectively). Further, some of the correlations – Cd with Pb (r=0.58), As as a common waste of Cu smelting (r=0.51) – are likely a result of these ores being associated in the mining and smelting process (Dudka and Adriano 1997).

### *Mercury*

Mercury is a well-known neurotoxin that can be a threat to wildlife health (Scheuhammer et al. 2007, Ackerman et al. 2016) as well as human health. Our study was consistent with many in showing that Hg biomagnifies across trophic levels, and thus was highest in carnivores (Lavoie et al. 2013). Mercury is most bioavailable and toxic when it is methylated, which usually is facilitated by bacteria in anoxic conditions (Ullrich et al. 2001). A rough level of adverse effects is given as 5 ppm in feathers (Burger and Gochfeld 2000b), although it can be much higher for large species (e.g. 40 ppm for loons; Evers et al. 2008). More recently it has been suggested that adverse effects could be encountered even at 3 ppm in tail feathers for small passerines (Jackson et al. 2011), although this level may be lower than warranted (Fuchsman et al. 2017).

Comparing the concentrations of Hg in this study to the levels of adverse effects, our highest levels were found at the QS1 site, near the Yulan Hg mine, where insectivores averaged 6.4 ppm. Of the 625 total birds sampled in this study, 55 were over the 3 ppm level, and in particular 31/41 of the Yulan birds exceeded this level; 27/625 of all birds and 21/41 of Yulan birds were over the 5 ppm threshold. Hence, some birds close to the Yulan mine, especially carnivorous and insectivorous species, may experience adverse effects from Hg exposure. Yet the site 10 km away from Yulan (QS2) did not have very high levels of Hg, with the insectivores having 0.78 ± 0.53 ppm (n=30 of 10 species), so it seems the contamination in Hg is not very spread out.

The Hg levels in the present study are substantially lower than recently reported from nearby Guizhou Province. In a recent study of a non-mining location in Guizhou, concentrations in the feathers of insectivorous passerines averaged 11.3 ± 9.9 ppm (44 individuals, 5 insectivorous species; Abeysinghe et al. 2017; in a mining area they were even higher). In contrast, our Guangxi study insectivores averaged 1.58 ± 2.10 ppm (392 individuals, 38 species); carnivores averaged 4.35 ± 4.83 ppm (18 individuals, 5 species). It is unclear why there is such a difference between our results and those of Abeysinghe et al. 2017. The habitats sampled in the two studies – paddy fields near rivers – were quite similar. The methods of sampling feathers (one secondary feather) and processing those feathers before Hg determination were identical. We believe the differences between the studies is more due to the scale and source of pollution rather than from methodological issues. Guizhou Province is known for particularly high levels of Hg emissions and return deposition (Li et al. 2013). Indeed, the scale of contamination of the Wanshan Hg mining region in Guizhou, once one of the largest Hg mines in the world, and one that combined mining and processing, might even influence the reference site for that study. In contrast, the Yulan mine is small and did not include processing. Overall, the Abeysinghe et al. (2017) study raised the potential that passerine birds throughout

China could be at risk for the adverse effects of Hg due to high aerial deposition, and our study is thus useful in showing that these risks are not universal.

*Cadmium*

Cadmium is of special concern to Guangxi, due to potential biomagnification and the reports of human illness in the area, especially tied to pollution from Pb/Zn mining (see Supplemental Methods). Cadmium concentrates in the kidneys, which leads to kidney damage (Järup and Åkesson 2009); at low concentrations its most adverse effect may be on bone structure (Åkesson et al. 2014), and it can also cause reproductive problems, specifically interfering with spermatogenesis (Marettová et al. 2015). Many of these effects can be observed in animals under experimental conditions (Burger 2008). However, there is less evidence that Cd has toxic effects from field studies on wild populations (Beyer 2000), and its effects may be noticeable most in areas of low calcium availability (e.g., Larison et al. 2000). Cadmium bioaccumulates, and, under some conditions may biomagnify (Burger 2008). A rough estimate of the level of adverse effects in feathers was estimated by Burger and Gochfeld (2000b) at 2 ppm.

We did not find evidence of biomagnification in this study for Cd. Perhaps this is because Cd can also reach high levels in certain plants (Larison et al. 2000), so that the contrast between herbivorous and carnivorous species may not be evident. The levels of Cd that we found in bird feathers were somewhat closer to the threshold for adverse effects than for Hg. Of the 622 total birds sampled, 89 were above 2 ppm, and in particular 25/71 of birds at DLR1 were above this threshold. Our mean levels in bird feathers of 1.1 ppm is above most earlier feather records from unpolluted areas (see further details in Supplemental Discussion). Nevertheless, our levels are far below the feather concentrations of dosed wood ducks (*Aix sponsa*) that died due to renal problems due to levels of 100 µg/g in their diet (resulting in 29.6 µg/g in feathers; Mayack et al. 1981), or of starlings (*Sturnus vulgaris*) that were given half that dietary concentration (50 µg/g in the diet resulting in 7.4 µg/g in feathers; Pilastro et al. 1993), and some exceptional field measurements (Abdullah et al. 2015, mean 41 ppm at highest site, and references within).

### *Arsenic*

Arsenic can be naturally abundant in regions with certain geological characteristics and thus can be a problem contaminating drinking water (Smedley and Kinniburgh 2002). Arsenic is also a problem specifically for mining activities, where it is often associated with smelting, especially Cu smelting (Matschullat 2000), and can be particularly concentrated in mine tailings and wastewater (Williams 2001). Seventy percent of globally explored As reserves are in China, and of this 95% is in the southwestern limestone karst region (Guangxi, Guizhou and Yunnan; Zhang et al. 2017). Guangxi has the greatest As pollution of all provinces in China (Li et al. 2014), and contamination of groundwater has already been reported in Nandan County inside Hechi Prefecture (see greater detail on the study sites in the Supplemental Methods).

Humans can be exposed to a variety of forms of inorganic and organic As that may vary in their bioavailability and toxicity, although all forms seem hazardous. For example, some forms of methylated As can be genotoxic (Mandal and Suzuki 2002). Overall, As acts as a toxin inactivating enzymes (Mandal and Suzuki 2002), and it can deplete the major antioxidants of cells (Koivula and Eeva 2010), and be carcinogenic (Sánchez-Virosta et al. 2015). In birds, high doses (e.g., those given diets dosed with > 24 µg/g monomethylarsonic acid), are fatal, and sublethal effects range across reproductive, behavioral and physiological categories (Sánchez-Virosta et al. 2015).

The mean level of As we report here for all birds, 4.78 ppm, rank fifth highest of 13 published data for feathers from polluted areas, and are above 14 of 15 records from unpolluted areas (Sánchez-Virosta et al. 2015). If one compared only the levels found in the large river Dahuanjiang (DHJ; 14.66 ± 31.21 ppm; n = 159), or the highest site levels at DHJ1 (mean 20.89, Table 1), they would rank third for pollution records and be above all published values from unpolluted sources. Most studies of the effect of As in birds have been correlative and also have trouble distinguishing the effect of As from other heavy metals (Sánchez-Virosta et al. 2015). However, a recent paper that dosed Great Tits (*Parus major*) in the field (using nest boxes) showed that nestlings with feather concentrations averaging 1.3 ppm exhibited some declines in wing growth, whereas nestlings with feather concentrations that averaged 13.6 ppm showed increased mortality in the nest (Sánchez-Virosta et al. 2018). Hence, the levels that we report here for adult birds may have health consequences, particularly at specific sites on the largest river (Dahuanjiang).

### *Lead*

Lead exposure may come through many sources (e.g., car emissions, paint), but in our region it is probably directly associated with mining. Lead exposure has long been known to cause neurobehavioral, physiological and reproductive effects in humans (Järup 2003), with cognitive effects in children even at low doses (Lanphear et al. 2005). Lead toxic effects on birds includes stresses on the organ system and reproductive toxicity (Burger and Gochfeld 2000a, Williams et al. 2018). An approximate level of adverse effects for feathers was placed by Burger and Gochfeld (2000b) at 4 ppm.

Lead had the highest levels of the non-essential metals tested in the present study in absolute terms and also relative to the threshold of adverse effects. Of the 622 birds sampled, 469 were above 4 ppm, and 38/41 birds at site QS1, which had the highest Pb levels, were above this threshold. Our mean levels (17.1 ppm) were above all but one of 29 seabird studies reviewed by Vizuete et al. (2018), and above all four species of raptor studied by Dauwe et al. (2003). We hope that future studies will sample bird blood in our region, as toxicity thresholds are better understood in blood than in feathers (Williams et al. 2018).

### *Essential metals/metalloids (Cu, Se, Zn) and Sn*

Three other metals/metalloids that we measured (Cu, Se and Zn) are considered essential trace elements for human health (WHO 1996), while Sn has no known human function, and documentation of adverse effects is scarce (Thomas and McGill 2008). Like all essential elements, there can be situations in which extreme levels of Cu, Se and Zn (and perhaps Sn) can lead to adverse effects. For example, extreme levels of Zn (e.g. ~ 1000 ppm in liver) has been suggested to have caused mortality in waterfowl in a polluted mining region (Beyer et al. 2004). However, we are not aware of data that would help interpret Zn concentrations in feathers. In our study, Zn had the highest levels of the essential elements, perhaps directly linked to it being a target metal in this mining region. Indeed, of all our metals/metalloids, the levels of Zn (mean 506 ppm) were closest to the extreme levels in Pakistan (Abdullah et al. 2015; mean 529 ppm at highest site), and thus further work should investigate the effects these levels might have on wildlife in our region.

Interestingly, we detected a pattern in Zn and Sn in herbivorous species, and particularly granivores, had higher concentrations. We included the vertical strata of the birds' foraging as a variable in the modeling process, and did not find this significant, so it does not seem this result is due to granivores having more direct contact with the soil. A pattern of biodilution of Zn over trophic levels has also recently be shown in a freshwater environment (Montañez et al. 2018).

We added Se to our original list of metals/metalloids because of its relationship as an antagonist to Hg, due to it forming an inert and stable compound with Hg that then reduces Hg toxicity (Koeman et al. 1973, Yang et al. 2008). The idea that Se forms the compound with Hg is consistent with our result that Se was elevated in higher trophic species (and the results of Qiu et al. 2019 when Hg was at levels below 10 ppm). Although Hg was not correlated to Se in our analysis, it is important to note that our analysis used only insectivores. If all species are used, there is a significant correlation between the two elements (r=0.26, P = 0.013, n = 94). Selenium is also known to reduce the toxicity of Cd (Lin et al. 2012), so its presence might be critical to how birds cope with high levels of the most toxic metals/metalloids in our region.

## CONCLUSIONS

The hypothesis that Hg might be a hidden risk in this mining region was not supported. Mercury was much lower than in another study focusing on feathers of passerines in a neighboring province in China (Abeysinghe et al. 2017), and only high in one small-scale Hg mining area. Our study is therefore useful to show that not all passerine birds in China are at risk from Hg. Further, the levels of Hg exhibited weakest correlation with levels of other metals/metalloids, with only one significant correlation with Pb. However, the potential for metals/metalloids other than Hg to pose adverse effects on wildlife is suggested by: 1) elevated Cd close to Pb/Zn mines; 2) high levels of As in one river that also had high Cu pollution, 3) Pb concentration that approached the threshold for adverse effects, and 4) Zn concentrations in feathers that approached levels reported in a very contaminated part of Pakistan (Abdullah et al. 2015). Other than a few hotspots (Hg at Hg mine, Cd at Pb/Zn mines, As in large river), concentrations of most heavy metals/metalloids were evenly dispersed among sites, even in a river without mining, suggesting wide spread contamination of heavy metals/metalloids in this region with mineable deposits.

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

*Acknowledgment*—We are grateful to the Hechi Forestry Bureau and Chongzuo Forestry Bureau for permission to do fieldwork, and R. He and G. Mo for much help it. We thank the Agricultural College and the College of Light Industry and Food Engineering of Guangxi University for providing access to the laboratory equipment, and X. Fan, R. Wang, X. Xu, and X. Zhou for technical assistance with and advice about the laboratory procedures. We appreciate the input of three anonymous reviewers who helped to improve the manuscript. This research was supported by the Special Talents Recruitment Grant from Guangxi University to EG and a Key Grant of Guangxi Nature and Science Foundation (2018GXNSFDA281016) to AJ

*Data accessibility*—Data, associated metadata, and calculation tools are available from the corresponding author (ebengoodale@gxu.edu.cn, eben.goodale@outlook.com; aiwuu@163.com).

## REFERENCES

Abdullah M, Fasola M, Muhammad A, Malik SA, Bostan N, Bokhari H, Kamran MA, Shafqat MN, Alamdar A, and Khan M. 2015. Avian feathers as a non-destructive bio-monitoring tool of trace metals signatures: A case study from severely contaminated areas. *Chemosphere* 119:553-561.

Abeysinghe KS, Qiu G, Goodale E, Anderson CWN, Bishop K, Evers DC, Goodale MW, Hintelmann H, Liu S, Mammides C, Quan R-C, Wang J, Wu P, Xu X-H, Yang X-D, and Feng X. 2017. Mercury flow through an Asian rice-based food web. *Environ Pollut* 229:219-228.

Ackerman JT, Eagles-Smith CA, Herzog MP, Hartman CA, Peterson SH, Evers DC, Jackson AK, Elliott JE, Vander Pol SS, and Bryan CE. 2016. Avian mercury exposure and toxicological risk across western North America: A synthesis. *Sci Total Environ* 568:749-769.

Åkesson A, Barregard L, Bergdahl IA, Nordberg GF, Nordberg M, and Skerfving S. 2014. Non-renal effects and the risk assessment of environmental cadmium exposure. *Environ Health Persp* 122:431-438.

Bates D, Maechler M, Bolker B, and Walker S. 2017. Linear mixed-effects models using S4 classes. Accessible at: http://cran.r-project.org/web/packages/lme4/index.html. Date accessed: December 2017.

Beyer WN. 2000. Hazards to wildlife from soil-bourne cadmium reconsidered. *J Environ Qual* 29:1380-1384.

Beyer WN, Dalgarn J, Dudding S, French JB, Mateo R, Miesner J, Sileo L, and Spann J. 2004. Zinc and lead poisoning in wild birds in the Tri-State Mining District (Oklahoma, Kansas, and Missouri). *Arch Environ Con Tox* 48:108-117.

Bleise AR, Heller-Zeisler SF, and Parr RM. 2000. Reference sheet IAEA-086: Methylmercury, total mercury and other trace elements in human hair. International Atomic Energy Agency, Vienna, Austria.

Burger J. 2008. Assessment and management of risk to wildlife from cadmium. *Sci Total Environ* 389:37-45.

Burger J, and Gochfeld M. 2000a. Effects of lead on birds (Laridae): A review of laboratory and field studies. *J Tox Environ Heal B* 3:59-78.

Burger J, and Gochfeld M. 2000b. Total metal levels in feathers of twelve species of seabirds from Midway Atoll in the Northern Pacific Ocean. *Sci Total Environ* 257:37-52.

Chan CK, and Yao X. 2008. Air pollution in mega cities in China. *Atmos Environ* 42:1-42.

Dauwe T, Bervoets L, Pinxten R, Blust R, and Eens M. 2003. Variation of heavy metals within and among feathers of birds of prey: Effects of molt and external contamination. *Environ Pollut* 124:429-436.

Dudka S, and Adriano DC. 1997. Environmental impacts of metal ore mining and processing: A review. *J Environ Qual* 26:590-602.

Evers DC, Savoy LJ, DeSorbo CR, Yates DE, Hanson W, Taylor KM, Siegel LS, Cooley Jr JH, Bank MS, Major A, Munney K, Mower BF, Vogel HS, Schoch N, Pokras M, Goodale MW, and Fair J. 2008. Adverse effects from environmental mercury loads on breeding common loons. *Ecotoxicology* 17:69-81.

Fuchsman PC, Brown LE, Henning MH, Bock MJ, and Magar VS. 2017. Toxicity reference values for methylmercury effects on avian reproduction: Critical review and analysis. *Environ Toxicol Chem* 36:294-319.

Hsu MJ, Selvaraj K, and Agoramoorthy G. 2006. Taiwan's industrial heavy metal pollution threatens terrestrial biota. *Environ Pollut* 143:327-334.

Jackson AK, Evers DC, Etterson MA, Condon AM, Folsom SB, Detweiler J, Schmerfeld J, and Cristol DA. 2011. Mercury exposure affects the reproductive success of a free-living terrestrial songbird, the Carolina wren (*Thryothorus ludovicianus*). *Auk* 128:759-769.

Järup L. 2003. Hazards of heavy metal contamination. *Brit Med Bull* 68:167-182.

---

Järup L, and Åkesson A. 2009. Current status of cadmium as an environmental health problem. *Toxicology and Applied Pharmacology* 238:201-208.

Kocman D, Horvat M, Pirrone N, and Cinnirella S. 2013. Contribution of contaminated sites to the global mercury budget. *Environ Res* 125:160-170.

Koeman JH, Peeters WH, Koudstaal-Hol CH, Tjioe PS, and de Goeij JJ. 1973. Mercury-selenium correlations in marine mammals. *Nature* 245:385-386.

Koivula MJ, and Eeva T. 2010. Metal-related oxidative stress in birds. *Environ Pollut* 158:2359-2370.

Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurstl P, Bellinger DC, Canfield RL, Dietrich KN, Bornschein R, Greene T, Rothenberg SJ, Needleman HL, Schnaas L, Wasserman G, Graziano J, and Roberts R. 2005. Low-level environmental lead exposure and children's intellectual function: An international pooled analysis. *Environ Health Persp* 113:894-899.

Larison JR, Likens GE, Fitzpatrick JW, and Crock JG. 2000. Cadmium toxicity among wildlife in the Colorado Rocky Mountains. *Nature* 406:181-183.

Lavoie RA, Jardine TD, Chumchal MM, Kidd KA, and Campbell LM. 2013. Biomagnification of mercury in aquatic food webs: A worldwide meta-analysis. *Environ Sci Technol* 47:13385-13394.

Li Z, Ma Z, van der Kuijp TJ, Yuan Z, and Huang L. 2014. A review of soil heavy metal pollution from mines in China: pollution and health risk assessment. *Sci Total Environ* 468:843-853.

Lin L, Zhou WH, Dai HX, Cao FB, Zhang GP, and Wu FB. 2012. Selenium reduces cadmium uptake and mitigates cadmium toxicity in rice. *J Hazard Mater* 235:343-351.

MacKinnon J, and Phillipps K. 2000. A field guide to the birds of China. Oxford University Press, Oxford, UK.

Mandal BK, and Suzuki KT. 2002. Arsenic round the world: A review. *Talanta* 58:201-235.

Marettová E, Maretta M, and Legáth J. 2015. Toxic effects of cadmium on testis of birds and mammals: A review. *Anim Reprod Sci* 155:1-10.

Matschullat J. 2000. Arsenic in the geosphere - A review. *Sci Total Environ* 249:297-312.

Mayack LA, Bush PB, Fletcher OJ, Page RK, and Fendley TT. 1981. Tissue residues of dietary cadmium in wood ducks. *Arch Environ Con Tox* 10:637-645.

Montañez JC, Arribere MA, Rizzo A, Arcagni M, Campbell L, and Ribeiro Guevara S. 2018. Zinc in an ultraoligotrophic lake food web. *Environ Sci Pollut R* 25:15422-15435.

Nardiello V, Fidalgo LE, Lopez-Beceiro A, Bertero A, Martinez-Morcillo S, Miguez MP, Soler F, Caloni F, and Perez-Lopez M. 2019. Metal content in the liver, kidney, and feathers of Northern gannets, *Morus bassanus*, sampled on the Spanish coast. *Environ Sci Pollut R* 26:19646-19654.

Pacyna EG, Pacyna JM, Sundseth K, Munthe J, Kindbom K, Wilson S, Steenhuisen F, and Maxson P. 2010. Global emission of mercury to the atmosphere from anthropogenic sources in 2005 and projections to 2020. *Atmos Environ* 44:2487-2499.

Peterson SH, Ackerman JT, Toney M, and Herzog MP. 2019. Mercury concentrations vary within and among individual bird feathers: A critical evaluation and guidelines for feather use in mercury monitoring programs. *Environ Toxicol Chem* 38:1164-1187.

Pilastro A, Congiu L, Tallandini L, and Turchetto M. 1993. The use of bird feathers for the monitoring of cadmium pollution. *Arch Environ Con Tox* 24:355-358.

Qiu G, Abeysinghe KS, Yang X-D, Xu Z, Xu X, Luo K, and Goodale E. 2019. Effects of selenium on mercury bioaccumulation in a terrestrial food chain from an abandoned mercury mining region. *B Environ Contam Tox* 102:329-334.

Rockström J, Steffen WL, Noone K, Persson Å, Chapin III FS, Lambin E, Lenton TM, Scheffer M, Folke C, Schellnhuber HJ, Nykvist B, de Wit CA, Hughes T, van der Leeuw S, Rodhe H, Sörlin S, Snyder PK, Costanza R, Svedin U, Falkenmark M, Karlburg L, Corell RW, Fabry VJ, Hansen J, Walker B, Liverman D, Richardson K, Crutzen P, and Foley J. 2009. Planetary boundaries: Exploring the safe operating space for humanity. *Ecol Soc* 14:32.

Sánchez-Virosta P, Espín S, García-Fernández AJ, and Eeva T. 2015. A review on exposure and effects of arsenic in passerine birds. *Sci Total Environ* 512-513:506-525.

Sánchez-Virosta P, Espín S, Ruiz S, Salminen J-P, García-Fernández AJ, and Eeva T. 2018. Experimental manipulation of dietary arsenic levels in great tit nestlings: Accumulation pattern and effects on growth, survival and plasma biochemistry. *Environ Pollut* 233:764-773.

Scheuhammer AM, Meyer MW, Sandheinrich MB, and Murray MW. 2007. Effects of environmental methylmercury on the health of wild birds, mammals, and fish. *AMBIO* 36:12-18.

Smedley PL, and Kinniburgh DG. 2002. A review of the source, behaviour and distribution of arsenic in natural waters. *Appl Geochem* 17:517-568.

State Health and Family Planning Commission and State Food and Drug Administration. 2017. National food safety standard determination of multicomponents in food. Pages 1-14, China.

Thomas VG, and McGill IR. 2008. Dissolution of copper, tin, and iron from sintered tungsten-bronze spheres in a simulated avian gizzard, and an assessment of their potential toxicity to birds. *Sci Total Environ* 394:283-289.

Ullrich SM, Tanton TW, and Abdrashitova SA. 2001. Mercury in the aquatic environment: A review of factors affecting methylation. *Crit Rev Environ Sci Technol* 31:241-293.

Vizuete J, Pérez-López M, Míguez-Santiyán MP, and Hernández-Moreno D. 2018. Mercury (Hg), lead (Pb), cadmium (Cd), selenium (Se), and arsenic (As) in liver, kidney, and feathers of gulls: A review. *Rev Environ Con Tox* 247:85-146.

WHO. 1996. Trace elements in human nutrition and health. World Health Organization, Geneva.

Williams M. 2001. Arsenic in mine waters: An international study. *Environ Geol* 40:267-278.

Williams RJ, Holladay SD, Williams SM, and Gogal Jr RM. 2018. Environmental lead and wild birds: A review. Pages 157-180 *in* P. de Voogt, E. R. Bennett, and P. S. Ross, editors. *Rev Environ Cont Tox*.

Yang D, Chen Y, Gunn JM, and Belzile N. 2008. Selenium and mercury in organisms: Interactions and mechanisms. *Environ Rev* 16:71-92.

Zhang L, Qin X, Tang J, Liu W, and Yang H. 2017. Review of arsenic geochemical characteristics and its significance on arsenic pollution studies in karst groundwater, Southwest China. *Appl Geochem* 77:80-88.

Figure 1 The 14 study sites on four rivers in Hechi Prefecture and one river in Chongzuo Prefecture, Guangxi Zhuang Autonomous Region, China. There were three study sites per river, except for the shortest river, the Qingshui. The most upstream site was near a source of pollution, which was either a mine (three rivers) or a center of smelting (Diaojiang). The Chengjiang was chosen as a control river with no known source of pollution. In general, the midstream site on the river was 10 km from the upstream site, and the downstream site 30-50 km from the midstream site.

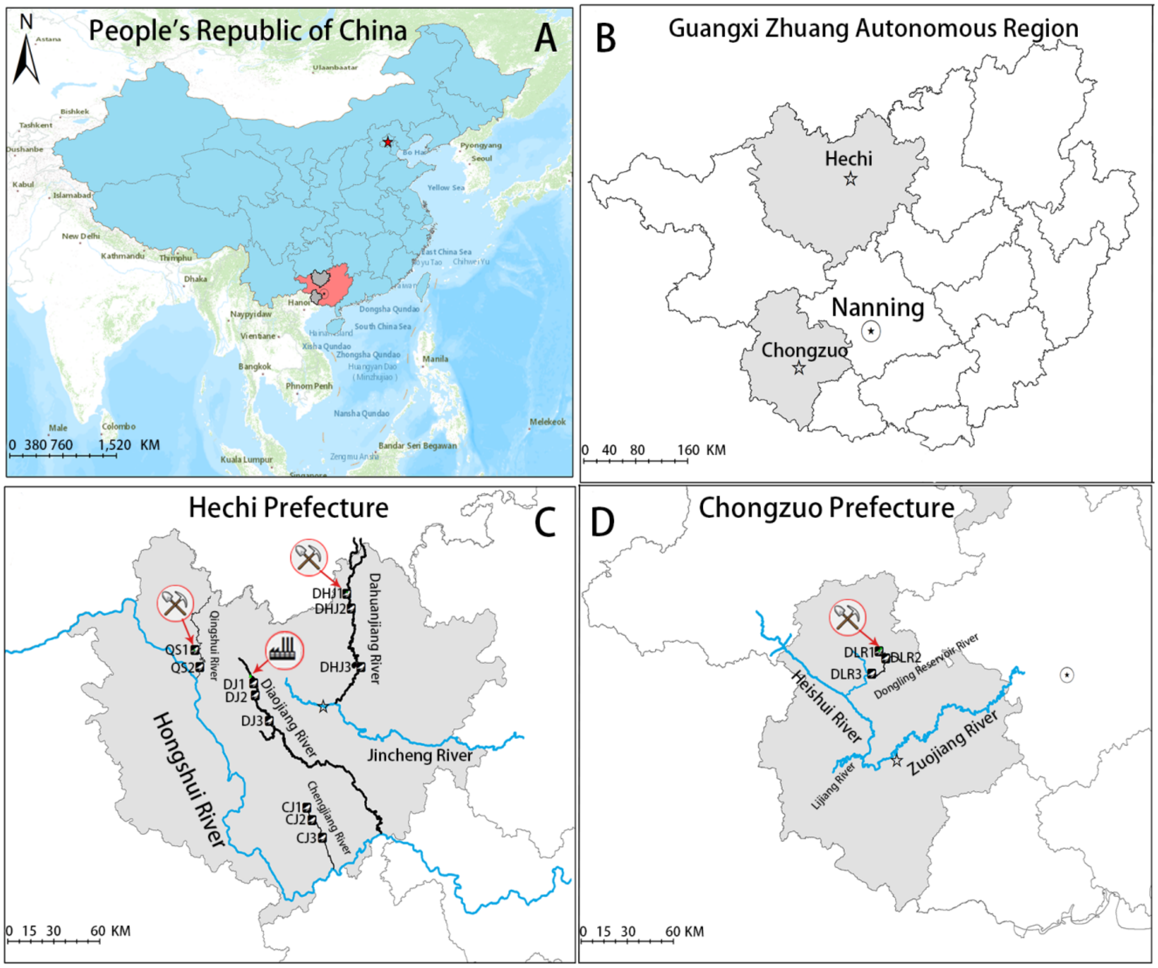

Figure 2 Diet was a significant influence on the concentrations of heavy metals for four metals, Hg, Se, Zn and Sn. For Hg, the effect was strong ($P < 0.001$ for contrast between carnivores and other guilds) and in the direction of biomagnification, with carnivores and insectivores having the highest concentrations; a similar pattern was shown for Se. Whereas for Zn and Sn, there was a weaker effect in the opposite direction.

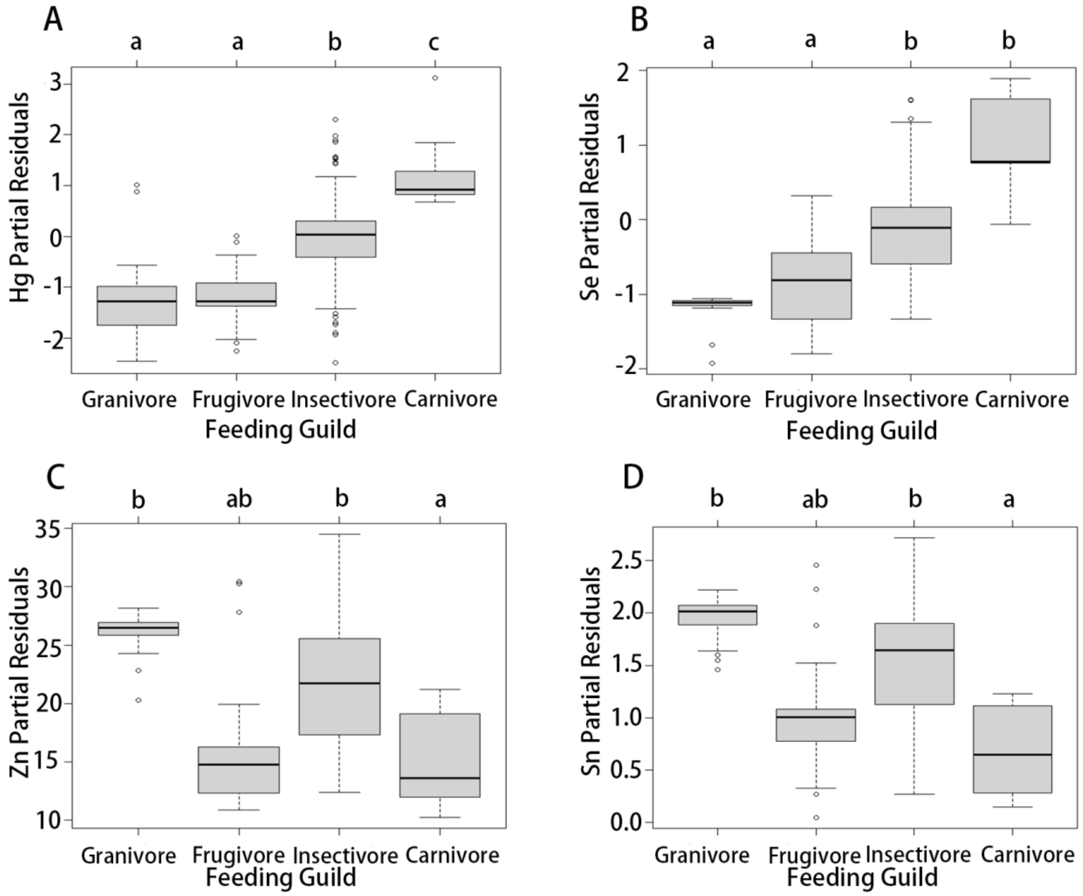

Figure 3 Pearson correlations, and their associated significance, between the concentrations of different metals. For each species we averaged the values of individuals to produce 139 mean values for a species at a site. The sample size for Se was smaller (65 mean values), so it was analyzed separately.

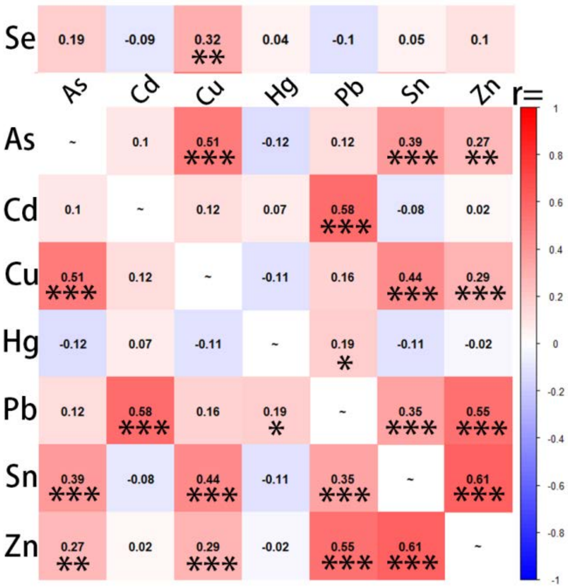

**Table 1.** Average concentrations of different metals/metalloids in feathers (wet weight) for all samples (top six lines), and for sites (and in some metals, diet categories) that are particularly elevated

| | | As (ppm) | Cd (ppm) | Cu (ppm) | Hg (ppm) | Pb (ppm) | Se (ppm) | Sn (ppm) | Zn (ppm) |
|---|---|---|---|---|---|---|---|---|---|
| Total Resident Birds | Max | 193.78 | 25.21 | 223.86 | 22.36 | 209.19 | 30.37 | 42.41 | 2526.65 |
| | Min | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 88.89 |
| | Mean | 4.78 | 1.12 | 9.71 | 1.27 | 17.18 | 1.60 | 2.83 | 506.29 |
| | SD | 17.11 | 2.64 | 11.87 | 2.02 | 24.49 | 3.07 | 3.32 | 329.81 |
| | CV | 3.58 | 2.35 | 1.22 | 1.59 | 1.43 | 1.92 | 1.17 | 0.65 |
| | N | 622 | 622 | 622 | 625 | 622 | 235 | 622 | 622 |
| Highest Site/Diet Group* | Location | DHJ1 All Species | DLR1 All Species | DHJ2 All Species | QS1 Insectivores | QS1 All Species | DLR3 Insectivores | DJ1 Insectivores | DJ1 Granivores |
| | Max | 79.84 | 25.21 | 64.28 | 14.16 | 138.94 | 14.67 | 7.43 | 1478.15 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Min | 0.00 | 0.00 | 6.22 | 0.39 | 1.90 | 1.81 | 0.79 | 538.46 |
| | Mean | 20.89 | 3.37 | 17.63 | 6.39 | 35.37 | 5.10 | 5.37 | 811.42 |
| | SD | 27.57 | 5.57 | 11.83 | 3.96 | 28.60 | 3.87 | 2.17 | 378.26 |
| | N | 13 | 71 | 73 | 36 | 41 | 11 | 10 | 7 |

* For metals/metalloids that did not vary by diet class (As, Cd, Cu, Pb), we report the values for the site with the greatest average concentrations. For metals/metalloids in which the concentrations varied by diet class (Hg, Se, Sn, Zn; see Figure 2), we report values for the diet class that had the greatest concentrations, at the site that had the greatest concentrations (n > 5 samples).