



Effects of Selenium on Mercury Bioaccumulation in a Terrestrial food Chain from an Abandoned Mercury Mining Region

Guangle Qiu¹ · Kasun S. Abeyasinghe^{1,2,3} · Xiao-Dong Yang³ · Zhidong Xu^{1,2} · Xiaohang Xu^{1,2} · Kang Luo³ · Eben Goodale⁴

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Abstract

Few reports of the relationship exist between mercury (Hg) and selenium (Se) from locations of severe Hg contamination in terrestrial environments. Here, we report the concentrations of Hg and Se as well as Se:Hg molar ratios in biotic samples collected from a region with a long history of Hg mining. Nitrogen isotopes ($\delta^{15}\text{N}$) were analyzed to confirm the trophic levels. Results showed that bird feathers at the top trophic level exhibited the highest Hg concentrations, while the lowest concentrations were found in herbivorous insects, demonstrating a significant biomagnification across the food chain. In contrast, herbivorous insects of different types (generalists vs. specialized rice pests) exhibited both the highest and the lowest concentrations of Se, indicating a lack of biomagnification. Indeed, Se was correlated positively with Hg when Se:Hg ratios were greater than one, suggesting Se:Hg molar ratios can be a controlling influence on Hg in terrestrial organisms.

Keywords Mercury · Selenium · Interaction effects · Terrestrial food chain

Mercury (Hg) is a highly toxic element (Clarkson 1993; Mergler et al. 2007), ranking third on the list of substances developed by the Agency for Toxic Substances and Disease Registry (ATSDR 2017). Nonferrous metal smelting and fossil fuel combustion are currently considered as two major anthropogenic Hg emission sources. Once introduced into the environment, Hg can be transformed into a more toxic organic form, methylmercury (MeHg), and eventually

bioaccumulated in living organisms and biomagnified via food chains. Selenium (Se) is essential for living organisms at low concentrations and toxic at elevated concentrations. Adverse biological effects of deficiency and toxicity may occur when the bioavailable Se in the biota is too low or too high, causing lethal deformities (Lemly 2014). As a micronutrient and antioxidant, it is necessary for proper functioning of important antioxidant enzymes. Many seleno-proteins or Se-containing protein subunits have been detected in animal and plant cells.

That Se can reduce Hg toxicity was first reported by Pařízek and Ošťádalová (1967). Later, Koeman et al. (1973) found that a 1:1 Se:Hg molar ratio plays an important role in this detoxification process. Up to now, a large number of studies on the antagonistic effects of Se against Hg toxicity have been documented in mammals and aquatic organisms (e.g. Rudd et al. 1980; Paulsson and Lundberg 1991; Yang et al. 2008; Khan and Wang 2009). Further, an increasing number of studies on the Se:Hg molar ratio have assessed potential toxicity and bioaccumulation of Hg from aquatic biota (Belzile et al. 2006; Peterson et al. 2009a; Yang et al. 2010; Squadrone et al. 2015; Johnson et al. 2018). These intensive studies on the toxicity of Hg and Se have shed much light on the widespread Hg-Se antagonism (Tran et al. 2007; Agusa et al. 2008). However, most of these studies

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✉ Guangle Qiu
qiuguangle@vip.skleg.cn

- ¹ State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550081, China
- ² University of Chinese Academy of Sciences, Beijing 100049, China
- ³ Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla 666303, China
- ⁴ Guangxi Key Laboratory of Forest Ecology and Conservation, College of Forestry, Guangxi University, Nanning 530004, Guangxi, China

have been done with laboratory-based mammals and aquatic organisms (Khan and Wang 2009). To date, very few studies have addressed the combined cycle of Se and Hg in terrestrial ecosystems, particularly the possible interaction between Hg and Se in the biota dwelling at sites heavily contaminated by both Hg and Se.

Wanshan Hg mine, the world's third-largest mine that was closed in 2004, located in eastern Guizhou Province, has experienced a long history of cinnabar ores' mining and retorting. Significant quantities of irrigated residues (calcines), deposited on nearby river banks, are continually introducing Hg into the environment via the processes of weathering, leaching, and wind blowing, causing heavy Hg contamination throughout the mining region (Horvat et al. 2003; Qiu et al. 2005, 2008, 2009). On the other hand, the cinnabar ore of Hg mines in the region always contains high quantities of Se, which was thoroughly released into the environment during ore processing and resulted in elevated levels of Se in the surroundings (Horvat et al. 2003). Hence, the coexistence of Se and Hg in ecosystems in the Wanshan mining region provides an excellent opportunity to better understand their interaction.

The objectives of this study were to (1) reveal the correlation between the levels of these two elements and their molar ratios in terrestrial animals of different trophic levels; (2) understand the implications of these correlations for the protective mechanism of Se against Hg assimilation in the terrestrial food chain.

Materials and Methods

The Wanshan Hg mining District (E: 109°07′–109°24′; N: 27°24′–27°38′) is the largest Hg producing center in China (Qiu et al. 2005). It lies on the eastern edge of the Yun-Gui Plateau, southwest China, with a typical karst landscape. The climate in the region is characterized as humid subtropical, with an average temperature of 13.4 °C and annual precipitation of 1400 mm. Cinnabar ore is the dominant mineral, in which Se reaches 0.85% (Bao and Bao 1995). Approximately 250 tons of metal Hg has been released into the environment during a long history of Hg mining and retorting activities (Qiu et al. 2005). Elevated concentrations of both Hg and Se occur in water, soil, and air (Horvat et al. 2003; Qiu et al. 2005, 2009; Zhang et al. 2012, 2015), making the site an ideal study area to investigate the possible antagonistic phenomenon between Se and Hg. Another important aspect of the region is that rice-growing is common and is an exposure route for humans (Qiu et al. 2008) and wildlife (Abeyasinghe et al. 2017) in the region.

The biotic species were collected from the abandoned Wanshan Hg mining region between April 2014 and October 2015. A total of 368 individual biota samples covering

19 species of terrestrial biota were obtained (see detailed information in Supplemental Materials and Abeyasinghe et al. 2017). In the present study, the 19 species were further classified into the following subgroupings: rice pest type I (RI), representing herbivorous insects that feed on plants other than rice (generalists: short-horned grasshopper (*Oxya sp.*, [n = 33]), long-horned grasshopper (*Phaneroptera falcata*, [n = 30]) and green stink bug (*Nezara viridula*, [n = 37])), rice pest type II (RII), representing herbivorous insects that specifically feed on rice (specialized rice pests: fall armyworm moth (*Spodoptera frugiperda*, [n = 32]) and rice horned caterpillar butterfly (*Melanitis leda ismene*, [n = 38])), carnivorous invertebrates, including dragonflies (CI, *Sympetrum flaveolum* [n = 40]) and spiders (CII, *Leucauge sp.* [n = 15]; *Tetragnatha nitens* [n = 9]; *Nephila pilipes* [n = 9]), and birds, including insectivorous and omnivorous species (BI, n = 42 and 34, respectively), and frugivorous and granivorous species (BII, n = 30 and 19, respectively). The details of bird species can be found in Supplemental Materials.

Bird feather samples were stored in clean polyethylene plastic bags at –20 °C after being thoroughly washed with distilled water and dried on tissue paper. All other biotic samples were washed after being starved 24 h and then freeze dried at –80 °C. The dried samples were ground with porcelain mortar and pestle, put into polyethylene bags and labeled for analysis.

Mercury in samples was determined using thermal decomposition with a portable Hg vapor analyzer (Lumex, Model RA915+/Pyro-915+, St. Petersburg, Russia), following method 7473 of the USEPA (2007). Approximately 0.01–0.07 g of sample was subjected to the sample boat into the PYRO-915+ attachment for direct Hg analysis. The instrument is based on the Zeeman cold vapor atomic absorption spectrometry technique with a detection limit of 0.5 µg/kg. Selenium in samples was determined, following an acidic digestion of HNO₃ with H₂O₂. The Se in biotic digested solutions was quantified using an atomic fluorescence spectrometer (AFS) equipped with a sequential injection hydride generation (HG) sampling system (Beijing Haiguang Instrumentals Co. Ltd., China), with a detection limit of 0.02 µg/kg.

Quality-control measures consisted of method blanks, triplicates, and certified reference materials. The method was validated using certificated reference material (TORT-2, lobster hepatopancreas, NRC, Canada), and the found concentrations of both Hg and Se were 0.267 ± 0.07 mg/kg and 5.71 ± 0.58 mg/kg, which were in good agreement with the certified values of 0.27 ± 0.06 mg/kg and 5.63 ± 0.67 mg/kg, respectively. The relative percentage difference was less than 8% for triplicate samples. The concentration of Hg and Se in the feather is presented on a fresh weight (FW) basis, whereas the concentration of Hg and Se in all other sample

types is presented as a dry weight (DW) concentration. The molar ratios of Se:Hg were calculated by dividing the element’s concentration by the molecular weight of 78.96 and 200.59 for Se and Hg, respectively.

For stable isotopes of nitrogen analysis, approximately 0.4–0.5 mg of sample was packed into a tin container and fully combusted, and then the gases were separated by a purge and trap column to an isotope ratio mass spectrometer for analysis. The relative abundance of nitrogen stable isotopes was determined with a continuous-flow mass spectrometer (Thermo Scientific MAT 253) connected to an elemental analyzer (Thermo Scientific Flash EA 2000 HT). Stable isotope composition was expressed in the conventional delta notation (‰): $\delta^{15}\text{N}_{\text{sample}} = (\text{R}_{\text{sample}}/\text{R}_{\text{reference}} - 1) \times 1000$ with $\text{R} = {}^{15}\text{N}/{}^{14}\text{N}$. The reference used was standard atmospheric nitrogen for $\delta^{15}\text{N}$. The $\delta^{15}\text{N}$ mean percent recoveries were in the range of 99.8% to 100.2%. Analysis uncertainty was less than 0.2‰. Data were presented as mean \pm standard deviation (SD) in the manuscript.

Results and Discussion

Mercury concentrations in all samples exhibited a wide range of concentrations, 0.21 – 190 mg/kg (n = 368). The BI concentrations showed the highest Hg value, reaching 61 ± 50 mg/kg, followed by CII with 11 ± 8.3 mg/kg, and the lowest in RI of 0.55 ± 0.26 mg/kg. The BII concentrations were of the same order as those in CII, RII and CI, indicating bioaccumulation among a wide variety of species by historic Hg mining and retorting activities. The concentrations of Se was not as variable, ranging in all species from 0.50 to 6.1 mg/kg with an average of 2.3 ± 1.2 mg/kg (n = 368). The highest average Se concentration was found in RII at 3.5 ± 1.3 mg/kg, followed by BII at 3.0 ± 1.2 mg/kg, and the lowest in RI at 1.3 ± 0.33 mg/kg. Owing to the large variation of Hg

concentrations, a broad range of Se:Hg molar ratios in samples was observed, ranging from 0.01 to 16. Herbivorous insects in both RI and RII exhibited the highest Se:Hg molar ratios, reaching 6.9 ± 2.9 and 4.8 ± 2.0 , respectively. The BII and CI also exhibited high Se:Hg molar ratios, greater than 1. The lowest average Se:Hg molar ratios were found in BI at 0.17 ± 0.19 and CII at 0.77 ± 0.54 . From experimental studies, Hg toxicity may occur when the molar ratio of Se:Hg is less than 1 (Ralston and Raymond 2010; Yang et al. 2010; Peterson et al. 2009b). Both insectivorous and omnivorous birds (BI), and spiders (CII), had average molar ratios of Se:Hg < 1, therefore, these taxa are vulnerable to health risks, considering the protective effect of Se on Hg toxicity. All statistic data are listed in Table 1.

Nitrogen isotopic composition $\delta^{15}\text{N}$ are commonly used to determine trophic levels, since they increase 3–4‰ from prey to predator (Amezcuca et al. 2015). Results for $\delta^{15}\text{N}$ compositions are listed in Table 1. The herbivorous species all had low $\delta^{15}\text{N}$ concentrations, and carnivorous species had high $\delta^{15}\text{N}$ concentrations. Among all species, spider (CII) and carnivorous dragonflies (CI) recorded the highest means for $\delta^{15}\text{N}$, and the insectivorous and omnivorous birds showed high values of $\delta^{15}\text{N}$ as well. The lowest concentrations were detected in herbivorous species of RI, RII, and frugivorous and granivorous birds (BII).

Among all species of the terrestrial food chain in the present study, the insectivorous and omnivorous birds (BI), spiders (CII) and dragonflies (CI) recorded high $\delta^{15}\text{N}$, but only BI exhibited high Hg concentrations (Fig. 1a). Other two species of CII, particularly CI accumulated low Hg, showing no Hg biomagnification with the enhancement of trophic level. Previous studies indicated that bulk $\delta^{15}\text{N}$ in a growing body can change in response to food sources as well as other factors such as starvation and water stress in terrestrial organisms, which makes the $\delta^{15}\text{N}$ analysis of individual amino acids rather than bulk material as an unambiguous trophic level indicator (Gannes et al. 1997;

Table 1 Concentrations of Hg and Se, as well as Se:Hg molar ratios, and $\delta^{15}\text{N}$ in the biotic samples (Data are presented as mean +/- standard deviation)

Species	Subgroup	Hg (mg/kg)		Se (mg/kg)		Se:Hg		$\delta^{15}\text{N}$ (‰)	
		Range	Average	Range	Average	Range	Average	Range	Average
Rice pests	RI (short-horned grasshopper, long-horned grasshopper, green stink bug)	0.21~1.2	0.55 ± 0.26	0.56~2.0	1.3 ± 0.33	2.4~16	6.9 ± 2.9	1.1~2.6	1.9 ± 0.38
	RII (fall armyworm moth, rice horned caterpillar butterfly)	0.73~4.3	2.0 ± 0.59	1.6~6.0	3.5 ± 1.3	1.5~9.5	4.8 ± 2.0	1.1~3.7	2.1 ± 0.94
Carnivorous Invertebrates	CI (dragonfly)	1.2~3.7	2.5 ± 0.55	1.1~4.7	2.5 ± 0.81	1.1~5.1	2.6 ± 0.93	7.0~7.6	7.4 ± 0.23
	CII (spider)	1.8~25	11 ± 8.3	0.98~5.3	2.1 ± 1.0	0.21~1.9	0.77 ± 0.54	5.3~9.1	7.2 ± 1.2
Bird feathers	BI (insectivorous, omnivorous)	5.1~190	62 ± 50	0.50~3.7	1.7 ± 0.75	0.010~0.82	0.16 ± 0.17	3.0~6.6	5.2 ± 0.76
	BII (frugivorous, granivorous)	0.62~42	10 ± 11	1.3~6.1	3.0 ± 1.2	0.15~12	2.6 ± 2.8	1.4~3.6	2.0 ± 0.55

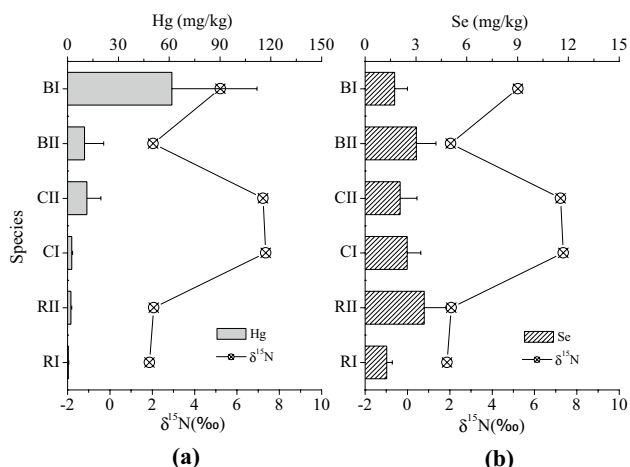


Fig. 1 Concentrations of total Hg and Se in different biotic samples found in the Wanshan Hg mining region, Guizhou Province, China (Data are presented as mean \pm standard deviation)

McClelland and Montoya, 2002). This may be associated with the high $\delta^{15}\text{N}$ with low Hg concentrations in biota.

Generally, when the feeding habits for the species were considered, the carnivorous species were observed to accumulate higher levels of Hg than the non-carnivorous species. The carnivorous species of BI were about two orders of magnitude higher than the non-carnivorous species of RI in terms of Hg. In contrast, Se did not show biomagnification across the terrestrial food chain. The highest concentrations of Se occurred in RII and the lowest concentrations in RI, both of which exhibited low $\delta^{15}\text{N}$ (Fig. 1b). Concentrations of Se in non-carnivorous species of RI and RII were comparable to that observed in carnivorous species of CI, CII, BI, and BII.

Interestingly, the relationships between Hg and Se concentrations in samples exhibited a significant positive correlation ($r=0.70$, $p<0.0001$) when $\text{Se:Hg}>1$, while there was no significant correlation ($r=-0.14$, $p=0.13$) when $\text{Se:Hg}<1$ (Fig. 2). The enhancement of Hg uptake in presence of Se probably reflects the protection process of Se and Hg forming non-toxic compounds within the body of the biota (Khan and Wang 2009). Similar correlations between these elements were reported in aquatic food webs (Burger et al. 2001; Ouédraogo and Amyot 2013). Strong positive correlations between Se and Hg seemed confined to species with Hg concentrations lower than 10 mg/kg, including rice pests (RI, RII), carnivorous invertebrates (CI, CII), and frugivorous and granivorous birds (BII). However, this was not consistent with Palmisano et al. (1995) who found that the co-accumulation of Se and Hg could only occur above a Hg level of 100 mg/kg wet weight in dolphin liver, probably indicating not enough Se to form the HgSe compounds while $\text{Hg}>10$ mg/kg in the present study.

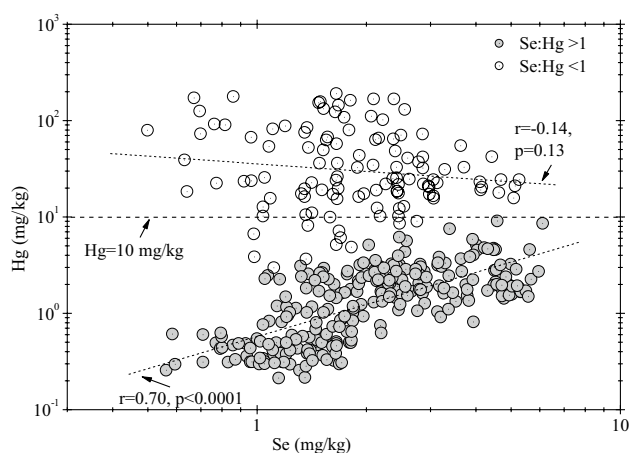


Fig. 2 Correlations between Hg concentrations and Se:Hg molar ratios as well as Se concentrations

A 1:1 molar ratio between Se and Hg has often been reported in organs of mammals and the formation of an inert HgSe precipitate is more readily expected owing to its low solubility product (K_{sp}) of ca. 10^{-58} . When Se in biota is high, so is Hg in them, suggesting a prominent controlling of Se to exert a protective effect on the accumulation of Hg (Koeman et al. 1973; Dietz et al. 2000). However, some observations made in studies on aquatic organisms suggested that Se played an important role in limiting the assimilation of Hg at aquatic food chains (Chen et al. 2001; Belzile et al. 2006; Yang et al. 2010). In the present study, the highly elevated level of Hg at the top of terrestrial food chains inversely correlated to Se concentration is explained by the lack of bioaccumulation of Se through the terrestrial food chain. The mechanisms contributing to the protective effect are unclear, but the significant positive correlation between Se and Hg concentrations when $\text{Se:Hg}>1$ as well as no significant correlations existed between Se and Hg concentrations when $\text{Se:Hg}<1$ suggested that there might be an antagonistic effect between Se and Hg, highly dependent on their molar ratios (Arcagni et al. 2017).

Since all the species investigated lived within heavily Hg-contaminated sites, which was also Se contaminated, their Hg and Se exposure was derived from local anthropogenic pollution sources. Previous studies have reported that the chemical interaction between Hg and Se is the possible mechanism behind Hg detoxification. Many authors have suggested that the protective effect of Se on the toxicity of Hg is due to the in vivo formation of mercuric selenide (HgSe), a stable and biologically inert complex, a process that has been confirmed (Cuvin-Aralar and Furness 1991; Belzile et al. 2006; Gailer 2007). Other possible mechanisms may also be involved in the antagonism between Se compounds and CH_3Hg^+ , as well as Hg-induced Se deficiency (Watanabe et al. 1999; Raymond and Ralston 2004). The

molar ratio of Se:Hg negatively correlated to Hg concentrations in all trophic groups suggests an important role in the accumulation of Hg in the terrestrial food chain but the protective role of Se is not trophic dependence. The relationship between Se and Hg being positive when Se:Hg ratio greater than 1 suggests that Se and Hg forms non-toxic compounds within the body of biota. At low trophic position, in the present study, the more Se in the biota, the more Hg is. But at high trophic position, Hg rather than Se can be efficiently biomagnified across the food chain, hence comparing to Se, more Hg was accumulated in the biota. However, a better understanding of the mechanism of antagonistic effects of Se on Hg in biota of the terrestrial food chain is necessary in the future.

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