Extremely Elevated Total Mercury and Methylmercury in Forage Plants in a World Large-scale Abandoned Hg Mining Site: A Potential Risk of Exposure to Grazing Animals

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Abstract

Ninety-five wild forage plants (belonging to 22 species of 18 families) and their corresponding rhizosphere soil samples were collected from wastelands of a world large-scale abandoned Hg mining region for total Hg (THg) and methylmercury (MeHg) analysis. The forage plant communities on the wastelands were dominated by the Asteraceae, Crassulaceae and Polygonaceae families. The THg and MeHg concentrations in the forage plants varied widely and were in the range of 0.10 to 13 mg/kg and 0.19 to 23 µg/kg, respectively. Shoots of Aster ageratoides showed the highest average THg concentration of 12±1.1 mg/kg, while those of Aster subulatus had the highest average MeHg concentrations of 7.4±6.1 µg/kg. Both the THg and MeHg concentrations in the aboveground plant parts exhibited positive correlations with the THg (r=0.70, P<0.01) and MeHg (r=0.68, P<0.01) concentrations in the roots but these were not correlated with the THg and MeHg concentrations in their rhizosphere soils. The species A. ageratoides, A. subulatus, and S. brachyotus showed strong accumulation of Hg and are of concern for herbivorous/omnivorous wildlife and feeding livestock. Taking the provisional tolerable weekly intake (PTWI) values for Hg recommended by the JECFA (2010) for human dietary exposure of 4 ng/g into account, grazing on 1.0 kg of forage (dry weight) by a 65 kg animal would mean that the daily intake of IHg was between 190-13200 µg, which reaches 3-5 order of magnitude higher than the permitted limit, suggesting a potential risk of exposure.

1. Introduction

Mercury (Hg) is a global pollutant and Hg exposure can increase the risk of cardiovascular disease and have neurological effects on humans, even at low concentrations (Driscoll et al., 2013; Peng et al., 2015). Mining and retorting of cinnabar ores are major sources of metal Hg, which is also one of the major sources of anthropogenic Hg to the environment (Xu et al., 2019). The areas impacted by historic Hg mining continue to be threatened by the heavy Hg pollution caused by the abandoned mine-waste calcines (ignited residues). These are enriched with water-soluble secondary Hg compounds, such as meta cinnabar, polymorphic sulfide Hg, sulphate Hg, chloride Hg, etc. Those large amounts of water-soluble Hg in effluent discharges from mine-waste calcines can be readily transformed into the more toxic methylmercury (MeHg) under suboxic conditions (Qiu et al., 2005; Lin et al., 2010). MeHg is considered the most harmful form of Hg due to its high lipophilicity (Agency for Toxic Substances and Disease Registry, 2013), so the transformation, bioaccumulation, and biomagnification of MeHg is of the greatest concern.

China has rich cinnabar deposits, and it ranks third in the world for its total reserves. Of these mines, the Wanshan Hg mine was once known as the “Mercury Capital”, and it was the largest elemental Hg production center in China. Activities are exploiting this area in China date back to the Qin Dynasty (220 B.C.). These activities have resulted in severe environmental Hg contamination and generated significant quantities of wastelands. THg concentrations as high as 4,400 mg/kg have been found in mine-waste calcines of the Wanshan Hg mine, as high as 790 mg/kg Hg in soils from paddies, and 10,000 ng/L Hg in surface water (Horvat et al., 2003; Qiu et al., 2005). The highest concentrations of Hg in the soils from the abandoned Hg mining region were approximately 2–3 orders of magnitude higher than the ‘probable effect concentration’ of 1.06 mg/kg Hg, above which harmful effects on organisms are likely to be observed (MacDonald et al., 2000; Conko et al., 2013), posing public concerns.

Many studies have shown that mine-waste calcines sites are favorable for Hg methylation in Hg mining areas and as high as 3,100 µg/kg MeHg has been reported in calcines (Gray et al., 2006). Owing to the extremely high Hg, particularly MeHg, mine-waste calcines have become major sources of Hg to the environment surrounding mines, causing elevated concentrations of both inorganic Hg (IHg) and MeHg in soils, water, and biota (Gibb et al., 2011; Xu et al., 2017; Qian et al., 2018; Li et al., 2020). Numerous investigations have revealed elevated high concentrations of THg in wild plants, for instance, concentrations in goosefoots (Chenopodium glaucum) and ferns (Pteris vittata L.) from the Wanshan Hg mining region reached 100 mg/kg (Wang et al., 2011; Qian, 2020), which is 3–4 orders of magnitude higher than the limit of 0.05 mg/kg in edible plants set by the Ministry of Health of China (2017). However, only a few studies have focused on plants MeHg, and most of them concentrated on rice, of which high levels of MeHg can accumulate in the grain (Li et al., 2017). Currently, data for MeHg in wild plants from Hg-contaminated sites is considerably lacking.

Plants provide the basic food energy for animals occupying the areas of former Hg mining. The consumption of the extremely Hg-contaminated forage-plants that may cause Hg accumulation and biomagnification in primary consumers, which could then enter terrestrial food chains. Mercury, as a long-term hazard in vegetated wastes, may have critical impacts on wildlife dependent on the region (Madejón et al., 2012; Basri et al., 2020). Our recent investigations have shown that MeHg has accumulated in the herbivorous...
wildlife in the Wanshan Hg mining region to levels that could cause health effects (Abeyesinghe et al., 2017; Xu et al., 2019). To better understand the potential risks of THg and MeHg exposure to herbivorous wildlife as well as livestock, the characterization of THg and MeHg in wild plants (forages) is an urgent necessity. It is important to know if forage-plants in contaminated regions are of concern for herbivores.

In the present study, dominant wild forage plants growing on wastelands in the Wanshan Hg mining district, Southwest China were investigated. The objectives were to (1) obtain basic information on the forage plants present and their THg and MeHg levels, (2) elucidate the transfer efficiency of THg and MeHg from the soil to the forage species and factors influencing this, and (3) clarify which species are of the greatest concern to herbivorous animals and assess the potential risk of exposure.

2. Materials And Methods

2.1. Study area

The Wanshan Hg mining district, on the eastern edge of the Yun-Gui Plateau in southwestern China (E: 109°07'-109°24'; N: 27°24'-27°38'), is the largest industrial metallic Hg production center in China. This region has a typical karst landscape with an average elevation of 850 m. The annual average temperature is 13.4°C, and the mean annual precipitation is 1,400 mm/year. Cinnabar is the main ore mineral associated with metacinnabar, natural metallic Hg, tiemannite, sphalerite, pyrite, and stibnite. The average Hg grade of the ore deposits is higher than 0.25%.

Extensive Hg mining and retorting occurred for 630 years and ceased in 2004. Approximately 125.8 million tonnes of mine-waste calcines were introduced into the environment between the early 1950s and the late 1990s (Qiu et al., 2005). The large historic Hg mining adits of Lengfengdong and Meizixi are headwaters of the rivers Xiaxi and Aozhai, the major aquatic systems in the Wanshan mining district. Large mine-waste calcine piles were placed adjacent to the corresponding adits and generated a significant amount of Hg-contaminated wastelands. In the present study, the wastelands generated by the calcine piles from Lengfengdong (LFD), Chongjiao (CJ), and Meizixi (MZX) were selected for investigation (Fig. 1; Table S1).

2.2. Sampling and preparation

Ninety-five samples of dominant forage plants belonging to 22 species of 18 families, which are favored by grazing animals, were collected from the wastelands. We preferentially sampled herbaceous plants rather than woody species. All of the plants were identified to the species level based on descriptions in the Flora of China (flora.huh.harvard.edu/china/mss/welcome.htm).

During sampling, dominant forage samples were randomly taken from the wastelands, within a sampling grid of 5×5 m. For each sample, three or more similarly sized individual plants of the same species were collected to ensure adequate amounts of tissue for analysis. Plant samples were dug out of the ground with a shovel and separated in situ into aboveground parts (shoots) and roots. In the laboratory, the plants were washed thoroughly with tap water and then with deionized water (DW) three times. Afterwards, the plants were frozen in a freezer and then placed in a vacuum freeze drier (-50°C) for drying. The dry plants were ground and sieved to fine powders using an analytical mill (IKA-A11 basic, IKA, Germany) and nylon sieve (mesh size of 0.18 mm). During processing, the lab equipment was rinsed three times with ethanol cleansing to control cross-contamination among the samples. The fine powder samples were stored in hermetic bags for analysis.

Corresponding rhizosphere soils were simultaneously collected with the plants. Approximately 0.5 kg of rhizosphere soil from the roots of each individual plant was shaken onto a piece of paper, and then the total 1.5 kg of soil collected from the 3 individual plant roots mentioned above was mixed as the final composite sample. The soils were stored in double polyethylene plastic bags to prevent any cross-contamination. After collection, all of the soil samples were air-dried in the laboratory, thoroughly mixed, and subsequently ground to fine powders using an agate mortar and nylon sieve (mesh size of 0.075 mm). A cleansing process similar to the plant samples preparation was applied to control cross-contamination among the samples. The fine powder samples were stored in double zip lock polyethylene plastic bags for analysis.

2.3. Sample analysis

2.3.1 Plant
For THg determination, approximately 0.1–0.2 g (accurate to 0.0001) samples were weighed into plastic tubes and digested with 5 mL \( \text{HNO}_3 \cdot \text{H}_2\text{SO}_4 = 4:1 (\nu/\nu) \) in a water bath at 95°C for 3 h. Afterwards, 5 mL DW and 0.5 mL BrCl were added to the solutions and they were digested for another 30 min. Finally, the digestion solution was brought to a fixed volume of 50 mL with DW. After leaving the digestion for 24 h, 400 µL NH\(_2\)OH·HCl was added, and 5.0 mL of the liquid supernatant was transferred to a bubble bottle. Then, 400 µL SnCl\(_2\) was added for Hg determination by atomic absorption spectroscopy (AAS, F732-V, Shanghai Huaguang, China) (Qiu et al., 2012).

For MeHg determination, approximately 0.3–0.5 g (accurate to 0.0001) samples were weighed into Teflon tubes, and a 5 mL methanol solution with 25% KOH was added. The samples were digested for 3 h in a water bath at 75°C, and then, 1.5 mL of concentrated HCl was added to acidify the solution. Subsequently, 10 mL of CH\(_2\)Cl\(_2\) was added to the digestate, shaken for 30 min, and then separated. The lower solvent phase CH\(_2\)Cl\(_2\) was collected in a 50 mL Teflon bottle. Approximately 30 mL of DW was added to the solvent phase in the 50 mL Teflon bottle, and then the MeHg was back-extracted into the new water phase with a fixed volume of 50 mL. Approximately 10 mL samples were placed into a bubbler for MeHg determination by gas chromatography-cold vapor atomic fluorescence spectroscopy (GC-CVAFS) according to the US EPA method 1630 (Liang et al., 1996; USEPA, 2001) by using a Brooks Rand Model III mercury detector (Seattle, USA) followed with a progressive sequence of aqueous phase ethylation, addition of 2 M acetate buffer, ethylation with 1% sodium tetraethylborate. The methylethylmercury was purged onto Tenax traps, from which it was subsequently thermally desorbed and separated for MeHg detection.

The bioaccumulation of heavy metals from soil can be described using bioconcentration factors (BCFs) and transfer factors (TFs) (Yoon et al., 2006; Gonzaga et al., 2008). To calculate BCFs and TFs of the plants, inorganic Hg (IHg) was calculated. Here we defined IHg as the difference between THg and MeHg in both the plant roots and shoots according to Lin et al. (2008) and Shi et al. (2005a, b).

### 2.3.2 Soil

For THg determination, approximately 0.1–0.2 g (accurate to 0.0001 g) soil samples were weighed and placed into plastic tubes. Then, 5 mL DW and 5 mL fresh aqua regia (HCl : HNO\(_3\) = 3 : 1, \(\nu/\nu\)) were added. The samples were rested for 5 min, and then, 1 mL BrCl was added for water bath digestion at 95°C for 3 h. The digestate was left for 24 h. Following this, 400 µL NH\(_2\)OH·HCl was added to remove the free halogens, and the samples were brought to a fixed volume of 50 mL with DW. Approximately 5 mL of the digestate was taken for Hg analysis, similar to the methods used for the plants.

For MeHg determination, approximately 0.3–0.4 g (accurate to 0.0001) soil samples were weighed and placed into 50 mL plastic centrifuge tubes. Next, 1 mL of 2 mol/L CuSO\(_4\) and 4 mL concentrated HNO\(_3\) : H\(_2\)O = 1:3 (\(\nu/\nu\)) were added. Then, 5 mL ultra-pure CH\(_2\)Cl\(_2\) was added and shaken for 30 min to extract the MeHg into the solvent. Afterwards, the CH\(_2\)Cl\(_2\) solvent phase was collected in a 50 mL Teflon bottle. Approximately 30 mL of DW was added and then the MeHg was back-extracted into the new water phase. The extract was brought to a fixed volume of 50 mL with DW (Liang et al., 1996). Approximately 5 mL aliquots were taken for MeHg GC-CVAFS analysis, similar to the procedure used for the plants.

For the soil pH measurements, approximately 10 g soil samples were weighed and placed into plastic vials. Next, 25 mL of DW without CO\(_2\) was added, mixed for 2 min, and left to settle for 30 min (Lu, 2000). The soil pH was determined using a pH meter (PHS-3E, Shanghai Leici, China).

For soil organic matter (OM) determination, approximately 0.5-1.0 g of soil was weighed and placed into colorimetric tubes. Concentrated sulfuric acid was added and then OM measurement followed a water bath-potassium dichromate volumetric method (Lu, 2000).

### 2.4. Quality Assurance/Quality Control

Quality assurance/quality control (QA/QC) measures employed consisted of the use of a standard working curve, blanks, sample duplicates, matrix spikes, and the certified reference materials of lichen (BCR-482), lobster hepatopancreas (TORT-2), Chinese yellow-red soil (GBW07405), and estuarine sediment (ERM-CC580), as further described below and in the Supplementary Material (Table S2).

For THg, the method was validated using the reference materials BCR-482 and GBW07405. An average total Hg concentration of 0.475 ± 0.02 mg/kg (n = 5) was obtained for the lichen standard BCR-482, which was within the range of the certified value of 0.48 ±
0.02 mg/kg. For the soil, GBW07405 was used, and the measured concentration of $0.32 \pm 0.02$ mg/kg ($n = 5$) was within acceptable range of the certified value of $0.29 \pm 0.04$ mg/kg.

For MeHg, the obtained value of $75.0 \pm 3.1$ µg/kg ($n = 5$) met the certified value of $75.5 \pm 3.7$ µg/kg for the ERMCC-580 soil standard. In addition, the obtained value of $155 \pm 25$ µg/kg ($n = 5$) met the certified value of $152 \pm 13$ µg/kg for the TORT-2 plant standard. The recovery of THg and MeHg in the solid samples was in the range of 95–109%, and 87–108%, respectively.

### 2.5. Calculations of BCFs and TFs of IHg and MeHg

In the present study, the IHg and MeHg BCFs were defined as the ratios of their concentration in the plant roots to that in the soil ($[\text{IHg or MeHg}]_{\text{root}}/[\text{IHg or MeHg}]_{\text{soil}}$), reflecting the capability of the plant's roots to absorb and accumulate IHg and MeHg from the soil. The TFs were defined as the ratio of the IHg and MeHg concentration in the shoots of the plants to that in their roots ($[\text{IHg or MeHg}]_{\text{shoot}}/[\text{IHg or MeHg}]_{\text{root}}$), referring to the capability of the plant to transport IHg and MeHg from the roots to the shoots.

### 2.6. Statistical analysis

Data analyses were performed using Microsoft Excel 2010 (Microsoft Co. Ltd., USA). CorelDRAW Graphics Suits X8 (Corel Corporation, USA) was used to draw a map of the sampling sites. Other figures and one-way ANOVA followed by Dunnett's multiple comparisons test were performed using GraphPad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, USA) and R v3.6.1 (R Core Team, 2019).

### 3. Results And Discussion

#### 3.1. Plant species

All of the samples belonged to 22 species of 18 families. The colonizing plants on the CJ wasteland consisted of 11 species *Aster ageratoides*, *Aster subulatus*, *Buddleja davidii*, *Cibotium barometz*, *Conyza canadensis*, *Corydalis edulis Maxim*, *Gynura bicolor*, *Herba artimisiae sieversianae*, *Rumex japonicus*, *Sonchus brachyotus*, and *Sonchus oleraceus*. These plants had high coverage and biomass, and the *Asteraceae* family accounted for 50.0% of them. Plants such as *C. canadensis* are amphibious plants, which have various growth habits but have a strong reproductive capacity and can grow well in places with high Hg concentrations. On the LFD and MZX wastelands, the dominant plants included *A. ageratoides*, *B. davidii*, *H. artimisiae sieversianae*, *Houttuynia cordata*, *Oenanthe javanica*, *Primula sikkimensis*, *Portulaca oleracea*, *Primula sikkimensis*, *R. japonicas*, *A. subulatus*, *Brassica campestris*, *C. canadensis*, *H. cordata*, *Ipomoea batatas*, *Mentha canadensis*, *Plantago asiatica*, *Rumex acetosa*, *R. japonicus*, *Sedum bulbiferum*, *Sedum emarginatum*, and *S. oleraceus*. Of these plants, the three families *Asteraceae*, *Crassulaceae* and *Polygonaceae* accounted for 69.2% of the total.

The wastelands used to be arable lands but currently contain a significant amount of mine-waste calcine, resulting in poor nutrient content and extremely high Hg concentrations. These conditions are conducive only to certain plant species with the capacity to grow in disturbed environments and with a high Hg tolerance. The herbaceous *Asteraceae* species accounted for 40% of the total investigated plants. These species exhibit particular characteristics such as abundant seeds, fast bud and growth rates, high biomass, and resilience, allowing them to become the dominant plant colonies on the wastelands.

#### 3.2. Concentrations of THg and MeHg

##### 3.2.1 THg

THg exhibited a wide range of concentrations in the roots and shoots of the 22 species, ranging from 0.10 to 4.4 mg/kg and 0.19 to 13 mg/kg, respectively (Table 1; Fig. 2a). Compared with the average THg values among the different species, the shoots of *A. ageratoides* showed the highest THg level, ranging $12 \pm 1.1$ mg/kg, followed by *P. sikkimensis* with $6.7 \pm 1.2$ mg/kg, while the lowest THg level was in *A. subulatus* with $0.39 \pm 0.055$ mg/kg. The *A. ageratoides* roots also showed the highest THg level of $3.8 \pm 0.69$ mg/kg, on average, while the lowest THg level of 0.13 ± 0.052 mg/kg was in *B. campestris*. One-Way ANOVA tests showed no differences in the THg concentrations in both the roots and shoots of the plants among the different wastelands, even though significant differences in soil THg were observed between CJ and MZX ($P = 0.0007$), and between LFD and MZX ($P = 0.0108$) (Table 3; Figure S1). A positive correlation was detected between the shoots and roots ($r^2 = 0.48$, $P < 0.0001$; Fig. 3a).
### Table 1
Concentrations of THg and its BCFs and TFs in wild plants inhabiting the wastelands of the Wanshan Hg mining region, Guizhou Province, Southwest China (mg/kg)

| Species (acronym)          | Soil  | Root    | Shoot  | BCFs  | TFs  |
|----------------------------|-------|---------|--------|-------|------|
| Aster ageratoides (A.a)    | 32±5.6| 3.8±0.69| 12±1.1 | 0.12±0.028| 3.1±0.37 |
| Aster subulatus (A.s)      | 34±6.5| 2.3±0.76| 0.39±0.055| 0.065±0.018| 0.19±0.09 |
| Brassica campestris L. (B.c)| 24±7.1| 0.13±0.052| 1.4±0.34| 0.006±0.002| 11±1.9 |
| Buddleja davidii (B.d)     | 46±14 | 0.58±0.26| 2.5±0.91| 0.013±0.004| 4.5±1.4 |
| Cibotium barometz L. (C.b) | 37±3.5| 1.6±0.26| 3.4±0.46| 0.043±0.011| 2.2±0.072 |
| Conyza canadensis (C.c)    | 31±15 | 1.2±0.50| 1.4±0.71| 0.040±0.012| 1.3±0.47 |
| Corydalis edulis Maxim. (C.e)| 24±5.7| 0.15±0.038| 0.69±0.30| 0.006±0.003| 4.5±1.1 |
| Gynura bicolor (G.b)       | 43±5.5| 0.86±0.11| 0.76±0.11| 0.019±0.001| 0.90±0.22 |
| Herba artimisiae (H.a)     | 60±28 | 1.7±1.1| 0.92±0.27| 0.029±0.014| 0.72±0.40 |
| Houttuynia cordata (H.c)   | 179±39| 1.3±0.57| 1.5±0.53| 0.007±0.003| 1.4±0.86 |
| Ipomoea batatas (I.b)      | 355±113| 3.4±0.92| 3.7±0.49| 0.010±0.003| 1.22±0.58 |
| Mentha canadensis (M.c)    | 132±40| 1.1±0.32| 1.2±0.34| 0.009±0.004| 1.1±0.56 |
| Oenanthe javanica (O.j)    | 61±21 | 0.92±0.040| 0.84±0.041| 0.02±0.0008| 0.92±0.08 |
| Plantago asiatica (Pa)     | 159±68| 0.56±0.034| 0.45±0.070| 0.004±0.002| 0.81±0.17 |
| Portulaca oleracea (P.o)   | 72±18 | 0.83±0.13| 0.84±0.13| 0.012±0.002| 1.0±0.045 |
| Primula sikkimensis (Ps)   | 135±17| 2.6±0.12| 6.7±1.2| 0.019±0.002| 2.7±0.64 |
| Rumex acetosa (R.a)        | 85±3.9| 0.57±0.36| 0.40±0.20| 0.007±0.003| 0.73±0.18 |
| Rumex japonicus (R.j)      | 47±18 | 0.30±0.13| 0.93±0.57| 0.007±0.002| 3.2±1.4 |
| Sedum bulbiferum (S.bu)    | 252±23| 0.69±0.090| 0.51±0.24| 0.003±0.0002| 0.72±0.27 |
| Sedum emarginatum (S.e)    | 53±23 | 0.82±0.21| 0.61±0.067| 0.017±0.007| 0.81±0.32 |
| Sonchus brachyotus (S.br)  | 45±4.9| 0.58±0.29| 0.60±0.12| 0.013±0.008| 1.3±0.77 |
| Sonchus oleraceus (S.o)    | 121±27| 0.67±0.22| 0.41±0.12| 0.006±0.003| 0.69±0.44 |

The plants in the present study exhibited a comparable level of THg (9.9 mg/kg on average) to that recently reported from artisanal and small-scale mining in the Bombana, Indonesia, though this was much higher than the THg levels from the Alacrán gold mining area in the USA, and those observed in agricultural plants of the same region (Marrugo-Negrete et al., 2016; Basri et al., 2020). In the study area, fresh forage-plants were the main feedstock for cattle and goats, hence, such elevated levels of Hg leads to high risks for potential of exposure to livestock and wildlife that consume these plants. Among the investigated forage-plant species, *A. ageratoides* recorded the highest THg concentrations both in the shoots and roots, which may be of great concern for herbivorous/omnivorous grazing animals.

### 3.2.2 MeHg

Forage-plants showed broad ranges of MeHg concentrations in the roots and shoots, ranging from 0.19 to 23 µg/kg and 0.28 to 11 µg/kg, respectively (Table 2; Fig. 2b). The highest average MeHg concentration was found in the shoots of *A. subulatus* at 7.4 ± 6.1 µg/kg, followed by *S. brachyotus* with 3.5 ± 2.5 µg/kg, while the lowest was in *B. campestris* with 0.49 ± 0.11 µg/kg. *S. brachyotus* exhibited the highest average MeHg concentration in its roots with 13 ± 10 µg/kg, followed by *A. subulatus* at 9.4 ± 8.0 µg/kg, while the lowest was in *A. ageratoides* at 1.1 ± 0.76 µg/kg. As expected, the root MeHg exhibited a significant positive correlation to the MeHg in the shoots ($r^2 = 0.46, P < 0.0001$; Fig. 3b), suggesting a strong transport of MeHg from the roots to the aboveground parts of the plants.
Table 2
Concentrations of MeHg and its BCFs and TFs in wild plants inhabiting the wastelands of the Wanshan Hg mining region, Guizhou Province, Southwest China (μg/kg)

| Species (acronym)                  | Soil   | Root   | Shoot  | BCFs   | TFs    |
|-----------------------------------|--------|--------|--------|--------|--------|
| Aster ageratoides (A.a)           | 1.4±0.15 | 1.6±0.65 | 1.8±1.1 | 1.2±0.58 | 1.1±0.45 |
| Aster subulatus (A.s)             | 0.78±0.055 | 11.4±2.8 | 11±0.53 | 14±2.5 | 0.97±0.18 |
| Brassica campestris L. (B.c)      | 5.8±2.7   | 0.79±0.18 | 0.49±0.11 | 0.16±0.088 | 0.63±0.006 |
| Buddleja daviddii (B.d)           | 2.8±2.1   | 2.6±0.69 | 2.2±0.81 | 1.3±0.68 | 0.98±0.56 |
| Cibotium barometz L. (C.b)        | 16±11    | 1.1±0.18 | 1.2±0.67 | 0.16±0.21 | 1.1±0.45 |
| Conyza canadensis (C.c)           | 2.9±1.3  | 1.9±1.22 | 2.1±0.87 | 0.82±0.71 | 1.7±1.4 |
| Corydalis edulis Maxim. (C.e)     | 10±1.2  | 6.3±0.57 | 4.9±0.42 | 0.63±0.038 | 0.77±0.066 |
| Gynura bicolor (G.b)              | 11±0.99 | 3.8±0.77 | 1.2±0.21 | 0.34±0.10 | 0.32±0.10 |
| Herba artimisiae (H.a)            | 5.0±2.4  | 2.4±1.2 | 1.5±1.0 | 0.76±0.73 | 0.62±0.20 |
| Houttuynia cordata (H.c)          | 4.9±2.3  | 2.6±2.1 | 1.3±0.71 | 0.73±0.72 | 0.77±0.54 |
| Ipomoea batata (I.b)              | 18±2.2  | 2.3±0.71 | 4.2±1.4 | 0.13±0.049 | 1.9±1.1 |
| Mentha canadensis (M.c)           | 2.9±0.90 | 0.97±0.34 | 1.1±0.36 | 0.36±0.18 | 1.1±0.34 |
| Oenanthe javanica (O.j)           | 2.9±2.3  | 1.7±0.057 | 2.9±0.70 | 0.80±0.42 | 1.8±0.37 |
| Plantago asiatica (Pa)            | 4.4±1.6  | 1.2±0.79 | 0.59±0.19 | 0.38±0.36 | 0.67±0.43 |
| Portulaca oleracea (Po)           | 2.8±0.58 | 6.6±1.6 | 2.3±0.62 | 2.4±0.48 | 0.37±0.10 |
| Primula sikkimensis (Ps)          | 3.8±0.87 | 2.1±1.1 | 1.5±0.18 | 0.52±0.18 | 0.85±0.38 |
| Rumex acetosa (R.a)               | 4.0±1.5  | 1.8±2.5 | 0.75±0.27 | 0.38±0.44 | 1.1±0.79 |
| Rumex japonicus (R.j)             | 2.2±1.7  | 3.8±2.5 | 1.5±0.57 | 2.4±2.3 | 0.66±0.49 |
| Sedum bulbiferum (S.bu)           | 4.4±0.82 | 2.2±0.77 | 1.3±0.22 | 0.50±0.18 | 0.66±0.23 |
| Sedum emarginatum (S.e)           | 2.1±0.30 | 3.2±1.8 | 0.94±0.38 | 1.5±0.66 | 0.33±0.15 |
| Sonchus brachyotus (S.br)         | 5.9±2.2 | 19±2.6 | 6.5±1.1 | 3.8±1.8 | 0.34±0.088 |
| Sonchus oleraceus (S.o)           | 2.5±1.5  | 1.9±1.1 | 2.9±1.9 | 0.79±0.26 | 1.5±0.45 |

Different species exhibited different capabilities for MeHg bioaccumulation. In addition to A. subulatus, which recorded the highest levels of MeHg concentration (greater than 10 μg/kg on average) in both the shoots and roots, species S. brachyotus, C. edulis, and P. oleracea also showed high MeHg concentrations, particularly in their roots, with a range of 6.3–19 μg/kg on average. Those values were comparable to those observed in rice in the same region (Zhao et al., 2016; Xu et al., 2017). Such significantly high levels of MeHg in both the shoots and roots may result in heavy MeHg body burdens in herbivores as well as their predators because of how easily it is absorbed and accumulated by organisms. This may be an explanation of the high MeHg levels in herbivores identified in the study region in the report by Abeysinghe et al. (2017).
### 3.3. BCFs and TFs for IHg and MeHg

#### 3.3.1 BCFs

Plants exhibited a wide range of BCFs for IHg and MeHg, ranging from 0.0023 to 17, respectively. The BCFs for IHg in all species were less than 1, with the highest average value in *A. ageratoides* at 0.12 ± 0.028. For MeHg, seven species, *A. ageratoides, A. subulatus, B. daviddii, P. oleracea, R. japonicus, S. emarginatum*, and *S. brachyotus* exhibited peak values exceeding 1.0, ranging from 1.3 to 14 on average. The *A. subulatus* showed the highest MeHg BCF of 14 ± 2.5, on average, followed by *S. brachyotus* with 3.8 ± 1.8, while the lowest value of 0.13 ± 0.049 was in *I. batatas* (Tables 1 & 2; Fig. 4). The extent of bioaccumulation of MeHg in plants is likely dependent on the species. Previous studies have reported that BCFs of THg and/or IHg in plants are usually lower than 0.5 (Zhang et al., 2010; Cosio et al., 2014), while the BCFs of MeHg were usually higher than that of THg and/or IHg (Schwesig and Krebs, 2003; Tong et al., 2013), which was in agreement with our data.

#### 3.3.2 TFs

The TFs for IHg in the plants varied widely, ranging between 0.12 and 13. The lowest TF value differed from the highest by more than 100 times. Most of the species exhibited average values of TF greater than 1, and among them, *B. campestris* exhibited the highest value of 11 ± 1.9 on average. For MeHg, eight species exhibited peak values exceeding 1.0, ranging from 1.1 to 1.9, with the highest value observed in *I. batatas* (Tables 1 & 2; Fig. 4). Five species, *A. ageratoides, C. barometz, C. canadensis, I. batatas*, and *M. canadesis* showed high TFs for both IHg and MeHg with peak values exceeding 1.0 on average, indicating their increased ability to accumulate IHg and MeHg compared with that of other species.

For all of the species, the MeHg in the roots was at higher concentrations than that observed in the shoots, and the MeHg showed significantly higher BCFs than that of IHg, confirming that MeHg is more easily absorbed by the roots. Plant roots can absorb MeHg from the soil and the total amount of MeHg in the soil plays a critical role in controlling MeHg concentrations in rice (Meng et al., 2011). At highly Hg-contaminated sites, high amounts of MeHg can be generated due to the active methylation occurring in the water and soil, which could eventually lead to increased MeHg accumulation in plants.

Although all of the plants were capable of growing in a heavily Hg-contaminated environment, the significant variation in the Hg concentrations may be due to the different physiological characteristics of the plants (Marrugo-Negrete et al., 2016). Mercury is a unique heavy metal that can exist in its elemental form in the atmosphere, so mining and retorting activities resulted in an even further increase in the atmospheric Hg (i.e., 1–3 orders of magnitude higher than in the remote regions; Zhang et al., 2016; Xu et al., 2020). Hence, the finding that the shoots of the plants exhibited higher IHg concentrations than those of the roots, in the present study, may be attributed to the elevated levels of atmospheric Hg, which can also explain the elevated TFs for IHg (Fig. 5).

### 3.4. Correlations among Factors affecting IHg and MeHg in plants
Rhizosphere soil Hg, as well as soil pH and OM, can affect Hg levels in plants (Zhao et al., 2016; Tang et al., 2018). The average THg concentrations in the investigated plants’ rhizosphere soil ranged from 18 to 261 mg/kg THg and from 1.4 to 19 µg/kg MeHg. Soil pH values were all higher than 7.5, due to the carbonate buffering of the surrounding rock and the introduction of large amounts of MgO and CaO during the retorting of cinnabar ores.

Spearman correlation coefficients of the Hg concentrations with the bioaccumulation factors are shown in Fig. 6. Roots IHg had the most significant correlations with the shoots IHg (r = 0.7, P < 0.01) and IHg BCFs (r = 0.66, P < 0.01), as well as positive correlations with soil THg (r = 0.31, P < 0.01) and MeHg (r = 0.24, P < 0.05), and negative correlation with IHg TFs (r=-0.22, P < 0.05), signifying their contributions to the accumulation of Hg in the roots. Shoot IHg had fewer correlations with other parameters, showing positive correlations only with IHg BCFs (r = 0.7, P < 0.01) and IHg TFs (r = 0.22, P < 0.05). The concentrations of root MeHg also exhibited a strong positive correlation to MeHg BCFs as well as shoot MeHg. Interestingly, soil pH exhibited positive correlations to both root MeHg (r = 0.25, P < 0.05) and shoot MeHg (r = 0.34, P < 0.01), and likewise to MeHg TFs (r = 0.22, P < 0.05) as well as MeHg BCFs (r = 0.17, P < 0.05), suggesting that pH was an important factor for controlling the processes of plant MeHg uptake and transport from the soil into the body. In contrast, soil OM showed negative correlations to both MeHg BCFs (r=-0.29, P < 0.01) and MeHg TFs (r=-0.32, P < 0.01), as well as shoot MeHg (r=-0.34, P < 0.01).

In the present study, though soil Hg played an important role in the absorption and enrichment of Hg in the plants, it was found that the rhizosphere soil pH and OM may also play important roles in the process of Hg uptake and transfer in plants. Our results indicate that there is a complex mechanism for Hg uptake, particularly for plants growing in heavily Hg-contaminated sites.

### 3.5. Potential risks for herbivores

The forage plants investigated in the present study are usually consumed by domestic animals such as cows, goats, and poultry. Based on the ecotoxicological effects of Hg on organisms, De Vries et al. (2002) recommended a set of critical limits for Hg in plants, and categorized Hg concentrations into three hazard levels: high hazard (>3 mg/kg), low-moderate hazard (0.1-3.0 mg/kg), and low hazard (<0.1 mg/kg). Considering the shoots, all of the investigated species of forage plant were at a low-moderate or greater hazard level, with approximately 15.8% of them falling into the high hazard level with greater than 3 mg/kg Hg. Moreover, compared to the national permitted limit of 0.1 mg/kg THg in vegetables (GB 2762–2017), the average THg concentration in the shoots of most the forage-plant species was elevated by 2–3 orders of magnitude.

Four species, *A. ageratoides*, *P. sikkimensis*, *I. batatas*, and *C. barometz*, exhibited THg greater than 3 mg/kg in their shoots, suggesting that these plants had high Hg accumulation capabilities. These species are generally characterized by cold, drought-tolerance, large biomass, and long growing period (Xing et al., 2010; Wang et al., 2011), resulting in their strong growth on the wastelands. Hence, the high concentrations of Hg in their shoots may cause potentially high Hg exposure risks to herbivorous/omnivorous wildlife and feeding livestock.

Currently, critical limits for Hg in plants (grass) for grazing animals are not available. Therefore, JECFA PTWI values for human intake were cited in the risk assessment for herbivores (Gramss and Voigt, 2014). Taking the provisional tolerable weekly intake (PTWI) value 4 ng/g for IHg recommended by the JECFA (2010) for human dietary exposure from foods other than fish and shellfish into account, daily ingestion of 0.037 µg IHg by a 65 kg animal is acceptable. Grazing on 1.0 kg of the shoots of the forage-plants (dry weight) would mean that the daily intake of IHg and MeHg was between 190-13200 µg, which reaches 3–5 orders of magnitude higher than the permitted limit. Moreover, grazing animals, such as cows and sheep are highly sensitive to contamination due to the ingestion of soil along with grass intake, hence, the heavily contaminated soil with both THg and MeHg is another point of concern.

### 4. Conclusions

The dominant forage plants collected from the wastelands of this world large-scale Hg mine exhibited high concentrations of both THg and MeHg, ranging from 0.085 to 13 mg/kg and 0.059 to 24 µg/kg, respectively. Species *A. ageratoides*, *C. barometz*, *I. batatas*, and *P. sikkimensis* exhibited THg greater than the high hazard level of 3 mg/kg in their shoots. Moreover, comparable levels of MeHg in *A. subulatus*, *C. edulis*, *S. brachyotus*, and *P. oleracea* to that found in rice were observed. Among of those species, *A. ageratoides*, *C. barometz*, and *I. batatas* showed high abilities to accumulate and transfer both IHg and MeHg to their shoots, with their TFs being greater than 1.0. The species *A. ageratoides*, *A. subulatus*, and *S. brachyotus* in the present study may pose the highest risks for THg and MeHg exposure to biota due to their strong Hg accumulation abilities and abundant biomass. Because the investigated forage
plants were widely consumed by herbivorous/omnivorous wildlife and feeding livestock, the high THg and MeHg concentrations could directly result in Hg accumulation and biomagnification in the terrestrial food chain. Thus, future studies on the plant-herbivorous-carnivorous food chain are urgently needed to clarify the risks from THg, and particularly MeHg, exposure to wildlife and feeding livestock.

**Declarations**

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**Declaration of Interest**

The authors declare that they have no conflict of interests or personal relationships that could have influenced the work reported in the manuscript — “Extremely elevated total mercury and methylmercury in forage plants in a world large-scale abandoned Hg mining site: A potential risk of exposure to grazing animals”

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Figures
Figure 1

Study area and sampling sites Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.
Figure 2

THg and MeHg in the roots and shoots of the forage plants
Figure 3

Correlation of THg and MeHg between the roots and shoots of the forage plants
Figure 4

Distribution of the BCFs and TFs for IHg and MeHg in the forage plants
Figure 5

Differences in IHg, MeHg, BCFs, and TFs between the roots and shoots of the forage plants (*P<0.05, ****P<0.0001)
Figure 6

Pearson correlation analysis of the THg and MeHg

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