Coupling bioavailability and stable isotope ratio to discern dietary and non-dietary contribution of metal exposure to residents in mining-impacted areas

Di Zhao, Jue-Yang Wang, Ni Tang, Dai-Xia Yin, Jun Luo, Ping Xiang, Albert L. Juhasz, Hong-Bo Li, Lena Q. Ma

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Both dietary and non-dietary pathways contribute to metal exposure in residents living near mining-impacted areas. In this study, bioavailability-based metal intake estimation coupled with stable Pb isotope ratio fingerprinting technique were used to discern dietary (i.e., rice consumption) and non-dietary (i.e., housedust ingestion) contribution to As, Cd, and Pb exposure in residents living near mining-impacted areas. Results showed that not only rice \((n = 44; \text{0.10–0.56, 0.01–1.77, and 0.03–0.88 mg kg}^{-1})\) but also housedust \((n = 44; 2.15–2380, 2.55–329, and 87.0–56,184 \text{mg kg}^{-1})\) were contaminated with As, Cd, and Pb. Based on in vivo mouse bioassays, bioavailability of As, Cd, and Pb in rice \((n = 11; 34 ± 15, 59 ± 13, and 31 ± 15\%)\) were greater than housedust \((n = 14; 17 ± 6.7, 46 ± 10, and 25 ± 6.8\%)\). Estimated daily intake of As, Cd, and Pb after incorporating metal bioavailability showed that As intake via rice was 5-fold higher than housedust for adults, whereas As intake via housedust was 3-fold higher than rice for children. For both adults and children, rice was the main source for Cd exposure, while housedust was the predominant Pb contributor. To ascertain the dominant Pb source from housedust ingestion, stable Pb isotope ratios \((207\text{Pb}/206\text{Pb} \text{and } 208\text{Pb}/206\text{Pb})\) of hair samples of local residents \((n = 27, 0.8481 ± 0.0049 \text{and } 2.0904 ± 0.0102)\) were compared to housedust \((n = 27, 0.8485 ± 0.0047 \text{and } 2.0885 ± 0.0107)\) and rice \((n = 27, 0.8369 ± 0.0057 \text{and } 2.0521 ± 0.0119)\), showing an overlap between hair and housedust, but not rice, confirming that incidental housedust ingestion was the main source of Pb exposure. This study coupled bioavailability and stable isotope techniques to accurately identify the source of metal exposure as well as their potential health risk.

1. Introduction

In mining-impacted areas, As, Cd, and Pb often exist as co-contaminants, resulting in adverse health effects including cardiovascular disease, decreased bone density, neuro-behavioral effects, and even cancers \((\text{Naujokas et al., 2013; Riederer et al., 2013; Navas-Acien et al., 2007; Oliver-Williams et al., 2018})\). For residents living near mining-impacted areas, both dietary and non-dietary pathways are important contributors to metal exposure \((\text{Li et al., 2011; Zheng et al., 2013})\).

Metal-contaminated dust particles from mining activities could deposit on indoor surfaces of nearby houses via atmospheric deposition, leading to incidental ingestion of housedust by residents especially for children with hand-to-mouth behavior \((\text{von Lindern et al., 2016})\). Li et al. \((\text{2015})\) reported the contribution of housedust ingestion to blood Pb in children living near industrial areas. In addition, studies also reported elevated As and Cd concentrations in foods from mining areas such as rice \((\text{Hu et al., 2016; Chen et al., 2018a, b})\). As a staple food for the vast population in Asia, rice is a significant contributor to As and Cd intake \((> 70\%)\) \((\text{Torres-Escribano et al., 2008; Zhu et al., 2016})\). However, for residents living near mining-impacted areas, the contribution of dietary and non-dietary pathways to metal exposure needs to be clarified, which helps to develop targeted interventions to reduce metal exposure and the associated health risk.

To differentiate dietary from non-dietary pathways, one method is to estimate daily metal intakes from foods and environmental media using bioavailability-based methods. Bioavailability assessment is critical to determine the fraction of metals in food and environmental matrices that are solubilized in gastrointestinal fluid and absorbed...
across the gastrointestinal epithelium into the systemic circulation (Ruby et al., 1999). The inclusion of metal bioavailability into human health exposure assessment helps to refine the default assumption that external dose is equivalent to the internal dose. In reality, the physio-chemical parameters of contaminants and the matrix both influence metal dissolution and absorption, thereby impacting the extent of the internal dose. However, assessment of metal bioavailability in contaminated housedust and rice samples from mining-impacted areas is not routinely undertaken. While studies have developed in vivo mouse bioassays to assess As and Cd relative bioavailability (RBA, relative to sodium arsenate and cadmium chloride) in rice samples (Li et al., 2017; Zhao et al., 2017a, 2017b), there is no report of Pb-RBA in rice. Though studies reported As- and Pb-RBA of 22–86% and 29–60% in housedust from urban areas (Li et al., 2014a, 2014b), there is a lack of measurement of As-, Cd-, and Pb-RBA in mining-contaminated housedust. More importantly, co-contaminants in rice or housedust may interact with each other, thereby impacting their RBA (Ollson et al., 2017). To gain a comprehensive understanding of metal exposure and the contribution of dietary and non-dietary pathways, assessment of As-, Cd-, and Pb-RBA in rice and housedust is essential.

Although the approach of estimating daily intakes could discriminate between dietary and non-dietary contribution, it also has uncertainties with the assumption of daily rice consumption and housedust ingestion rates, which could vary considerably among individuals. In addition, the internal metal dose is also influenced by individuals’ nutrient status (e.g., intake of minerals like Ca, Fe, and Zn), which is not considered by bioavailability-based metal intake method. Furthermore, stable isotope ratio technique can be used as a supplementary measure to identify the source of metal exposure from foods and environmental media using internal biomarkers (e.g., blood, hair, and urine). Li et al. (2015) used stable Pb isotope ratio technique to identify the dominant source of Pb in children’s blood. By coupling bioavailability-based intake estimation and stable isotope ratio method, dominant exposure sources (dietary vs. non-dietary) can be more accurately assessed.

Therefore, the objective of this study was to assess the contribution of dietary and non-dietary pathways to metal exposure in residents living near mining-impacted areas by coupling bioavailability-based metal intake estimation and stable isotope ratio fingerprinting. Typical mining-impacted areas in Hunan, China were selected and housedust, rice, and hair samples were collected from local residents. Rice and housedust were selected as the representative of dietary and non-dietary contributors to metal exposure. Following determination of metal concentrations, rice and housedust samples were measured for As-, Cd- and Pb-RBA using in vivo mouse assays. Estimated daily intake of As, Cd, and Pb via rice consumption and housedust ingestion was calculated to identify the dominant metal exposure source. In addition, to verify Pb source, stable isotope ratios of Pb in residents’ hair were compared to those of rice and housedust samples. This study coupled bioavailability with stable isotope techniques to accurately identify the source of metal exposure as well as their potential health risk assessment, which is valuable to effectively reduce metal exposures to residents living near mining-impacted areas.

2. Materials and methods

2.1. Sampling and processing of rice, housedust, and human hair

Mining-impacted areas in Chenzhou and Hengyang cities in Hunan, China were chosen as the study site (Fig. 1). Hunan is a major rice producing area with > 4000 ha of paddy fields (Williams et al., 2009), however, it is also the heartland of mining/smelting activities. Numerous studies documented elevated As, Cd, and Pb concentrations in paddy soils and rice in Hunan (Zhu et al., 2008; Liao et al., 2005). Williams et al. (2009) reported that As, Cd, and Pb concentrations in rice grains from mining districts in Hunan were 0.15–0.59, 0.03–2.76, and 0.05–0.78 mg kg\(^{-1}\), while Liu et al. (2005) reported As, Cd, and Pb concentrations at 82.0–1227, 1.7–11.1, 42.2–1444 mg kg\(^{-1}\) in soils from Pb/Zn mine areas of Hunan. A recent paper also showed high As, Cd, and Pb concentrations in farmland soils at 23.7–148.7, 0.24–3.83, and 35.1–173.7 mg kg\(^{-1}\) from mining-impacted areas of Hunan, suggesting that metal contamination at mining areas (Zhuang et al., 2018).

From the two cities, six locations were chosen as study sites, i.e., Wugaishan Mountain, Matian, Zilong, and Dengjiatang villages from Chenzhou, and Zhipu and Yancheng villages from Hengyang (Fig. 1). From the 6 sites, paired rice (polished, locally-grown) and housedust samples (n = 44) were collected. Rice was selected to represent dietary contributor to metal exposure, while vegetable samples were not. This was because most residents consumed vegetables not locally-grown. In addition, drinking water was not contaminated with metals at the sites so it was not included. All rice samples were locally-grown and were consumed by local residents.

Housedust on indoor surfaces including floors, window sills, and furniture was brushed into plastic sample bags and sealed. Outdoor soil was not considered since adults and children spend more time indoors with their propensity for hand to mouth contact with housedust compared to soils. In addition, hair samples were collected from 27 participants aged from 3 to 80 years who consumed rice as a staple (Table S1), with scalp hair being sampled from the occipital region of the head using scissors and stored in sealed plastic bags. Although there are limitations in using element concentrations in hair as biomarkers of exposure to metals (Skröder et al., 2017), hair has been used widely as a biomonitor after careful washing (Hinwood et al., 2003; Cui et al., 2013). Participants were asked to complete a self-administered questionnaire including information regarding age, sex, and body weight (Table S1).

To prepare samples for analysis, rice was washed thoroughly with Milli-Q water three times to remove dust particles. Following washing, rice was cooked using an electric cooker (CFXB50YB7F-65, Supor, China) at a ratio of rice to water of 1:2. After cooking, rice was stored at –80 °C and freeze-dried (Freezone 6 Plus, Labconco, USA) prior to grinding to a powder for homogenization. Housedust was freeze-dried, thoroughly mixed, and sieved to < 150 μm for further analysis. This fraction was selected since it is most likely to adhere to children’s hands and be ingested via hand-to-mouth contact (Ruby and Lowney, 2012).

Hair samples were cut into 5 mm pieces, washed in three steps using Milli-Q water, methanol, and Milli-Q water, then dried at 60 °C. The water-methanol washing method is efficient to remove surface hair contamination without leaching internal metals of interest out of the hair (Hinwood et al., 2003; Cui et al., 2013). Concentrations of As, Cd, and Pb in rice, housedust, and hair were determined using inductively coupled plasma mass spectrometry (ICP-MS, NexION300X, PerkiñElmer, USA) following digestion using a Hot Block digestion system (Environmental Express, Mt. Pleasant, SC) according to USEPA Method 3050B.

In addition to total As analysis, As speciation in cooked rice was determined according to Zhu et al. (2008). Briefly, cooked rice powder (0.5 g) was extracted using 1% nitric acid (10 mL) in a microwave-accelerated reaction system (ETHOS UP, Milestone Srl, Italy). Samples were heated to 55 °C for 10 min, then 75 °C for 10 min, and finally 95 °C for 30 min. After cooling to room temperature, extracts were centrifuged, filtered through 0.22 μm filters, diluted with 1% nitric acid, and assessed for As speciation using HPLC-ICP-MS. An anion exchange chromatography column (Hamilton, PRP-X100, 250 mm × 4.1 mm, 10 μm particle size) was used as the mobile phase to separate As species using 8.0 mM (NH₄)₂HPO₄ and 8.0 mM NH₄NO₃ (pH = 6.2). Standard solutions of arsenite (As(III), arsenate (As(V), dimethylarsinic acid (DMA), and monomethylarsonic acid (MMA) were prepared daily from stock solutions. A standard of 10 μg As L\(^{-1}\) was measured every 10 samples to monitor the stability of HPLC-ICP-MS, showing recovery of 95.1 ± 3.5%. The extraction efficiency of the 1% nitric acid method was 78.2–95.7%, averaging 87.2 ± 7.46%. The sum of As(III) and As(V)
was reported as inorganic As (iAs) to avoid changes in iAs species during extraction and analysis.

2.2. Assessment of As, Cd, and Pb relative bioavailability in rice and housedust

To refine human exposure via rice consumption and incidental dust ingestion, an in vivo mouse bioassay was used to determine metal relative bioavailability (RBA) in rice and housedust. Considering the ethical issues coupled with the high cost and difficulty in performing in vivo mouse bioassay, samples for mouse experiment were chosen based on metal concentrations and the amount of samples collected from local residents. Finally, 11 cooked rice (0.17–0.49 mg kg\(^{-1}\) As, 0.41–1.67 mg kg\(^{-1}\) Cd, and 0.09–0.88 mg kg\(^{-1}\) Pb) and 14 housedust samples (37.2–1051 mg kg\(^{-1}\) As, 3.70–329 mg kg\(^{-1}\) Cd, 138–56,185 mg kg\(^{-1}\) Pb) were studied.

Cooked rice powder was mixed with Milli-Q water to form a paste from which rice pellets were produced (SI). The freeze-dried rice pellets served as the exposure media and food source of mice during metal-RBA assessment in rice. In addition, a non-polluted rice was spiked individually with different concentrations of NaH\(_2\)AsO\(_4\) (0.2, 0.5, and 1.0 mg kg\(^{-1}\) As), CdCl\(_2\) (0.5, 1.0, and 2.0 mg kg\(^{-1}\) Cd), and Pb(Ac)\(_2\) (0.5, 1.0, and 2.0 mg kg\(^{-1}\) Pb) and fed to mice \((n = 3)\) for 10-d to assess the linear response \((R^2 = 0.97–0.98)\) of As excretion in urine, and Cd and Pb accumulation in the kidneys and liver to serve as reference doses to determine rice As-, Cd-, and Pb-RBA (Fig. S1).

For housedust, samples were incorporated into mouse basal diet at dust:diet ratio of 1:10 to achieve As, Cd, and Pb concentrations of 3.72–105, 0.37–32.9, and 13.8–5618 mg kg\(^{-1}\), respectively. Prior analysis of the unamended mouse basal diet confirmed low concentrations of As, Cd, and Pb \((0.32 \pm 0.01, 0.09 \pm 0.01\text{and } 0.24 \pm 0.03\text{ mg kg}^{-1}\) respectively). Diets were also prepared by incorporating NaH\(_2\)AsO\(_4\), CdCl\(_2\), and Pb(Ac)\(_2\) to achieve As, Cd, and Pb concentrations of 5–150, 1–20, and 10–1500 mg kg\(^{-1}\), respectively. These diets were administered to mice to determine the linearity of the dose response \((R^2 = 0.94–0.99, \text{ Fig. S1})\) and to calculate the RBA of As, Cd, and Pb in housedust samples.

Rice pellets or dust-amended basal diets were supplied to mice over 10 d \((Zhao et al., 2017a, 2017b; Li et al., 2014a)\). Urine excreted from mice during the 10-d period was collected, while mouse liver and kidney samples were obtained after 10-d exposure. For housedust samples, As, Cd, and Pb accumulation in the kidneys and liver were used as the exposure endpoints to calculate As-, Cd, and Pb-RBA. For rice samples, to overcome the limitation of relatively low As exposure, cumulative As urinary excretion during exposure was used as As exposure endpoint, while Cd and Pb accumulation in the kidneys and liver were used as Cd and Pb exposure endpoints. Similar mouse bioassays have been used to determine RBA of As and Cd in contaminated rice and RBA of As, Cd, and Pb in soils \((Li et al., 2016a,b,c; 2017; Zhao et al., 2017a, 2017b)\). showed consistency in As-RBA determination when different endpoints were used, suggesting little influences of endpoint selection on metal-RBA determination. Information on contaminated matrices, their preparation, reference materials \((\text{NaH}_2\text{AsO}_4, \text{CdCl}_2, \text{and Pb(Ac)}_2)\), exposure parameters, urine/organ collection, and their analyses for RBA assessment are detailed in SI.

For rice exposure, As urinary excretion factors (UEF) for As doses were calculated by dividing cumulative As excreted in the urine (\(\mu\text{g}\)) by cumulative As dietary intake (\(\mu\text{g}\)) (Eqs. (1) and (2)). Arsenic RBA in rice was determined by comparing UEF for rice exposure \((\text{UEFrice})\) to that for NaH\(_2\)AsO\(_4\) \((\text{UEFAs})\) (Eq. (3)).

\[
\text{UEF}_{\text{rice}} = \frac{\text{cumulative urinary excreted As via rice}}{\text{cumulative As intake via rice}}
\]  

\[
\text{UEF}_{\text{As}} = \frac{\text{cumulative urinary excreted As via NaH}_2\text{AsO}_4}{\text{cumulative As intake via NaH}_2\text{AsO}_4}
\]  

\[
\text{As-RBA in rice (\%)} = \frac{\text{UEF}_{\text{rice}}}{\text{UEF}_{\text{As}}} \times 100
\]  

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\[
\text{UEF}_{\text{rice}} = \frac{\text{cumulative urinary excreted As via rice}}{\text{cumulative As intake via rice}}
\]  

\[
\text{UEF}_{\text{As}} = \frac{\text{cumulative urinary excreted As via NaH}_2\text{AsO}_4}{\text{cumulative As intake via NaH}_2\text{AsO}_4}
\]  

\[
\text{As-RBA in rice (\%)} = \frac{\text{UEF}_{\text{rice}}}{\text{UEF}_{\text{As}}} \times 100
\]  

As-RBA in dust was calculated as the ratio of dose normalized As accumulation in the liver and kidneys following dust exposure to that following NaH\(_2\)AsO\(_4\) exposure (Eq. (4)).
Fig. 2. As RBA in housedust (%) = \left( \frac{\text{Organ As} \text{ housedust}}{\text{As dose} \text{ housedust}} \times \frac{\text{As dose} \text{ NaH}_2\text{AsO}_4}{\text{Organ As} \text{ NaH}_2\text{AsO}_4} \right) \times 100 \tag{4}

where, \text{Organ As} \text{ housedust} and \text{Organ As} \text{ NaH}_2\text{AsO}_4 are As concentrations in mouse liver and kidneys following exposure to housedust and Na-H_2\text{AsO}_4. \text{As dose} \text{ housedust} and \text{As dose} \text{ NaH}_2\text{AsO}_4 are As dose levels following exposure to housedust and Na-H_2\text{AsO}_4.

Cadmium and Pb RBA in rice or housedust was calculated as the ratio of dose normalized Cd and Pb accumulation in the liver and kidneys following rice or housedust exposure to that following CdCl_2 and Pb(\text{Ac})_2 exposure (Eqs. (5) and (6)).

Cd RBA in rice/housedust (%) = \left( \frac{\text{Organ Cd} \text{ rice/housedust}}{\text{Cd dose} \text{ rice/housedust}} \times \frac{\text{Cd dose} \text{ CdCl}_2}{\text{Organ Cd} \text{ CdCl}_2} \right) \times 100 \tag{5}

Pb RBA in rice/housedust (%) = \left( \frac{\text{Organ Pb} \text{ rice/housedust}}{\text{Pb dose} \text{ rice/housedust}} \times \frac{\text{Pb dose} \text{ Pb(\text{Ac})}_2}{\text{Organ Pb} \text{ Pb(\text{Ac})}_2} \right) \times 100 \tag{6}

where, \text{Organ Cd} \text{ rice/housedust} and \text{Organ Pb} \text{ rice/housedust} are Cd and Pb concentrations in mouse liver and kidneys following rice or housedust exposure; \text{Organ Cd} \text{ CdCl}_2 and \text{Organ Pb} \text{ Pb(\text{Ac})}_2 are Cd and Pb concentrations in mouse liver plus kidneys following CdCl_2 and Pb(\text{Ac})_2 exposure; \text{Cd dose} \text{ rice/housedust} and \text{Pb dose} \text{ rice/housedust} are Cd and Pb dose levels following rice or housedust exposure; and \text{Cd dose} \text{ CdCl}_2 and \text{Pb dose} \text{ Pb(\text{Ac})}_2 are Cd and Pb dose levels following CdCl_2 and Pb(\text{Ac})_2 exposure.

2.3. Daily As, Cd, and Pb intake estimation

Following determination of As, Cd, and Pb RBA in both rice and housedust, daily metal intake (DI, \mu g kg^{-1} bw d^{-1}) via the two sources was calculated (Eq. 7):

\[ DI = \frac{C \times IR \times RBA}{BW} \tag{7} \]

where, C represents total As, Cd, or Pb concentration (mg kg^{-1}) in rice or housedust, IR represents daily rice intake rates (371 g day^{-1} for adults, and 56 g day^{-1} children aged 1–6 years) or daily housedust ingestion rates (100 mg day^{-1} for adults, and 200 mg day^{-1} for children aged 1–6 years) (Li et al., 2011; Zheng et al., 2013), and BW represents body weight (70 kg for adults and 16 kg for children aged 1–6 years) (Zhang et al., 2008). For RBA, measured As, Cd- and Pb-RBA values were used when they were measured whereas for samples not assessed, mean value of measured RBA was used for daily As, Cd, and Pb intake estimation.

2.4. Stable Pb isotope ratio analyses

To ascertain that incidental ingestion of housedust was the dominant Pb exposure source for the residents identified by daily Pb intake calculation, stable Pb isotope ratio analyses were performed for matched rice, housedust, and hair samples (n = 27). Stable Pb isotope ratios (^{207}\text{Pb}/^{206}\text{Pb} and ^{208}\text{Pb}/^{206}\text{Pb}) of the rice, housedust, and hair samples were measured using ICP-MS following diluting the digest to \approx 15 mg L^{-1} Pb using high-purity 0.1 M \text{HNO}_3. Procedure blanks were measured before samples, and the standard reference material SRM NIST 981 (15 mg L^{-1}) was measured every five samples to determine the ratio correction factors to compensate for mass bias. Each sample was measured in 10 replicates with RSD of < 0.5% for ^{207}\text{Pb}/^{206}\text{Pb} and ^{208}\text{Pb}/^{206}\text{Pb} ratios. Measured ^{207}\text{Pb}/^{206}\text{Pb} and ^{208}\text{Pb}/^{206}\text{Pb} ratios of SRM NIST 981 (0.9157 ± 0.0014 and 2.1693 ± 0.0018, n = 6) were in agreement with the certified values of 0.9146 and 2.1681.

2.5. QA/QC and data processing

During metal concentration analyses using ICP-MS, an indium isotope (^{114}\text{In}) was used as an internal standard. Quality assurance and control was conducted using SRM for rice (GSB-21, Chinese Geological Reference Materials) and soil (SRM 2711a, NIST). The accuracy of USEPA Method 3050B was acceptable with measured As, Cd, and Pb concentrations of 0.112 ± 0.004, 0.012 ± 0.002 and 0.067 ± 0.008 mg kg^{-1} in GSB-21 (0.114 ± 0.018, 0.012 ± 0.003, and 0.075 ± 0.025 mg kg^{-1}) and 91.6 ± 11.3, 47.9 ± 5.1, and 1354 ± 62 mg kg^{-1} in NIST 2711a (107 ± 5, 54.1 ± 0.5, and 1400 ± 10 mg kg^{-1}). All samples were above the ICP-MS detection limits for As, Cd, and Pb (0.005, 0.006, and 0.008 mg L^{-1}). During analyses, spiked and check samples (1–10 mg L^{-1}) were included every 20 samples, with recoveries being 101 ± 7% and 99 ± 8% (n = 30).

Metal concentrations in both rice and housedust are reported on dry weight basis. Animal experiments were performed using three replicates with results presented as mean values ± standard deviation of 3 mice. Statistical differences in rice or housedust samples were analyzed using variance analysis based on Tukey’s multiple comparisons using software SAS, version 9.1.3.

3. Results and discussions

3.1. Arsenic, Cd, and Pb concentrations in rice and housedust

In this study, 44 paired rice and housedust samples were collected from residents living near mining-impacted areas. The samples were numbered #1–44 based on increasing Pb concentration in rice samples (Table S2). Elevated As (0.10–0.56 mg kg^{-1}; mean = 0.29), Cd (0.01–1.76 mg kg^{-1}; mean = 0.48) and Pb concentrations (0.03–0.88 mg kg^{-1}; mean = 0.20) were found in rice samples (Fig. 2A, Table S2). Of the 44 rice samples, 66, 41, and 25% had As, Cd, and Pb concentrations exceeding the Chinese regulatory limit in rice respectively.
For the 11 samples with Pb concentrations > 0.20 mg kg$^{-1}$; Chinese Food Standards Agency, 2012). For the 11 samples with Pb concentrations > 0.20 mg kg$^{-1}$, their As and Cd concentration also exceeded regulatory limits (Table S2), indicating that rice from these locations was co-contaminated. Metal concentrations in rice reported here were 3–10 times higher than mean Cd and Pb concentrations in 712 milled rice (0.05 and 0.06 mg kg$^{-1}$) from other locations in China (Qian et al., 2010). Studies from smelting locations in Wenzhou, China, also showed elevated Cd concentrations in rice (up to 0.51 mg kg$^{-1}$; Hu et al., 2016), while As concentrations of 0.66–2.05 mg kg$^{-1}$ were in rice in the US and Bangladesh (Zavala et al., 2008).

In addition to dietary exposure via rice consumption, residents may also be exposed to metals through incidental ingestion of housedust. Arsenic, Cd, and Pb concentrations in housedust were 2.15–2380 mg kg$^{-1}$ (mean = 453), 2.55–329 mg kg$^{-1}$ (mean = 65.4), and 87.0–56,184 mg kg$^{-1}$ (mean = 3725) (Fig. 2B, Table S2). Strong linear correlations ($R^2 = 0.42$–0.82) were observed between As, Cd, and Pb concentrations in housedust, indicating high As contamination was accompanied with high Cd and Pb contamination (Fig. S2). Housedust in this study contained higher As concentrations than those from homes near mining areas in southwest England (43.0–486 mg kg$^{-1}$, n = 20) and a mining district of Bolivia (12.9–636 mg kg$^{-1}$, n = 57; Rieuwerts et al., 2006; Fonturbel et al., 2011). Similar high Pb concentrations were reported in housedust from homes in Sydney (16–16,600 mg kg$^{-1}$) and from areas near metal recycling facilities (22,900–206,000 mg kg$^{-1}$; Leung et al., 2008; Chattopadhyay et al., 2003). However, values reported in this study were significantly higher than mean Pb (208 ± 328 mg kg$^{-1}$, n = 122) and Cd concentrations (0.26–4.50 mg kg$^{-1}$, n = 69) in housedust from non-mining impacted areas of China (Han et al., 2012; Zhu et al., 2013).

### 3.2. Arsenic, Cd, and Pb relative bioavailability in rice

Reliable assessment of metal exposure depends on the determination of both metal concentration and bioavailability. However, limited studies have determined oral RBA of As and Cd in rice with no report of Pb-RBA (Li et al., 2017; Zhao et al., 2017a, 2017b). In this study, 11 rice samples contaminated with As, Cd, and Pb were assessed for metal RBA simultaneously using in vivo mouse models. With the exception of 3 samples with bioavailable Pb lower than quantifiable limits, As-, Cd-, and Pb-RBA were 11–65% (mean = 34%), 41–84% (mean = 59%), and 11–59% (mean = 31%), respectively (Fig. 3A–C).

Our study is the first report of Pb-RBA in rice using an in vivo bioassay, being lower than Cd-RBA while similar to As-RBA, indicating that Pb in rice was poorly absorbed following consumption. Only two studies assessed As- and Cd-RBA in rice, with As-RBA (n = 11; 0.13–0.32 mg kg$^{-1}$) being 13–54% (Li et al., 2017), and Cd-RBA (n = 10; 0.29–1.09 mg kg$^{-1}$) being 17–57% (Zhao et al., 2017a). While As-RBA in this study was comparable to previous measurements, Cd-RBA was significantly higher than that reported by (Zhao et al., 2017a).
Unlike As, the mechanisms underlying the variability in Cd- and Pb-RBA in rice was unclear. A weak correlation was observed between Cd-RBA in rice and contamination source, and interaction between co-contaminants, which warrant further studies.

3.3. Arsenic, Cd, and Pb relative bioavailability in housedust

In addition to rice, 14 housedust samples contaminated with As, Cd, and Pb were assessed for metal-RBA (Fig. 3D–F). Similar to rice, Cd-RBA in contaminated housedust (28–68%, mean = 46%) was higher than As (8.5–37%, mean = 17%) and Pb-RBA (11–34%, mean = 25%), suggesting Cd in housedust and rice was more bioavailable than As and Pb. A previous study proposed that the sorption sites in solid matrices occupied by metals depend on their affinity to the sites. Loganathan et al. (2012) reported that Cd had lower affinity to sorption sites than As and Pb, leading to higher Cd absorption.

Limited studies determined RBA of As, Cd, and Pb in housedust. Our study is the first to report Cd-RBA in housedust, while Li et al. (2014a, 2014b) determined As-RBA of 22–86% (mean = 51%) and Pb-RBA of 29–60% (mean = 50%) in housedust from different cities. Compared to Li et al. (2014a, 2014b), As- and Pb-RBA of this study was significantly lower (p < 0.05), which may be related to differences in contamination source (mining vs. coal combustion) and contaminant speciation. For housedust impacted through mining activities, Pb may present as less soluble minerals (e.g. Pb sulfide), while more Pb in housedust from different cities may exist as readily-soluble Pb oxide (Bi et al., 2015; MacLean et al., 2011). In addition, interactions between contaminants may also contribute to lower As- and Pb-RBA. Reduction in Pb-RBA following Pb and As co-exposure was reported, presumably due to the formation of Pb arsenate (Diacomanolis et al., 2013; Han et al., 2012), while Olsson et al. (2017) suggested that decrease in As-RBA may occur due to disruption of phosphate transporters through the influence of Cd.

3.4. Source identification based on daily As, Cd, and Pb intake estimation

Following determination of RBA of As, Cd and Pb in both rice and housedust, daily intakes via rice consumption and housedust ingestion were estimated based on total and bioavailable concentrations to identify dominant sources of metal exposure (Fig. S5, Tables S3 and S4).

When As, Cd and Pb daily intake was estimated based on bioavailable concentrations, values were significantly lower (p < 0.05) compared to total metal concentration (Fig. 5). For example, estimated daily Pb intake via incidental ingestion of housedust was ~5 times higher when total Pb concentrations were used compared to bioavailable Pb concentrations, values were significantly lower (p < 0.05), which may be related to differences in contaminant speciation. However, when Pb-RBA was considered, this value was reduced to 11 samples (1.50–8.58 μg kg⁻¹ bw d⁻¹) compared to bioavailable Pb (9.53 ± 14.2 μg kg⁻¹ bw d⁻¹) (Table S4). As a consequence, refining exposure through the incorporation of metal-RBA may significantly impact estimated daily intake values and the associated risks.

When exposure was refined based on measured RBA, mean As intake via rice consumption (0.51 ± 0.24 μg kg⁻¹ bw d⁻¹) for adults was ~5-fold higher compared to exposure via housedust ingestion (0.11 ± 0.13 μg kg⁻¹ bw d⁻¹) (Table S3). However, for children, due to differences in exposure parameters (e.g., values for rice consumption and incidental housedust ingestion), the results were reversed with daily As intake via incidental housedust ingestion (0.97 ± 1.14 μg kg⁻¹ bw d⁻¹) being 3-fold higher compared to rice consumption (0.34 ± 0.16 μg kg⁻¹ bw d⁻¹). The data suggested that non-dietary exposure was an important contributor to childhood As exposure, while dietary exposure was the dominant As source for adults.
living near mining-contaminated areas. The exposure scenario was based on the assumption that metal bioavailability was similar between adults and children, which may not be true. However, this assumption would not influence the contribution of housedust ingestion and rice consumption to metal exposure to adults or children. The data highlighted the importance to develop targeted measures to protect the health of adults and children, i.e., strategies to decrease non-dietary As exposure for children and to decrease dietary As exposure for adults.

In terms of Cd exposure, for both adults and children, rice consumption was the major pathway. Compared to Cd intake via incidental housedust ingestion, Cd intake via rice consumption was 35- and 2-fold higher for adults (1.52 ± 1.64 vs. 0.04 ± 0.05 μg kg⁻¹ bw d⁻¹) and children (1.01 ± 1.08 vs. 0.37 ± 0.46 μg kg⁻¹ bw d⁻¹) (Tables S3 and S4).

In contrast to Cd, for both adults and children, incidental housedust ingestion was the dominant pathway. Estimated daily Pb intake via housedust ingestion was 3- and 46-fold higher than rice consumption for both adults (1.09 ± 1.63 vs. 0.32 ± 0.38 μg kg⁻¹ bw d⁻¹) and children (9.53 ± 14.2 vs. 0.21 ± 0.25 μg kg⁻¹ bw d⁻¹). The data suggested that non-dietary source was more important in contributing to Pb exposure whereas dietary source was more important for Cd exposure for residents living near contaminated areas.

Similar results were reported by Li et al. (2015) and Zhao et al. (2017a), showing that incidental housedust ingestion and rice consumption contributed 55% and 71% to Pb and Cd exposure when RBA was included in daily intake calculations. For both adults (46 vs. 6.3%) and children (37 vs. 27%) living in an e-waste cycling area, rice was a more important contributor to Cd intake than housedust ingestion (Zheng et al., 2013), while Ohno et al. (2007) reported that rice contributed 56% to total As intake. However, those studies either evaluated human exposure to individual metal or only one exposure source, with little attention to co-contaminant exposure. Another strength of this study is that we considered the RBA of As, Cd, and Pb from both dietary and non-dietary sources, which helps to improve exposure estimation compared to the use of total metal concentration.

### 3.5. Source identification based on stable Pb isotopic fingerprinting

Daily metal intake calculations provide an estimation of the dominant exposure pathway for metals. To ascertain Pb exposure from housedust was the dominant pathway, 27 hair samples were collected from local residents who provided paired rice and housedust samples and analyzed for stable Pb isotopic composition (²⁰⁷Pb/²⁰⁶Pb and ²⁰⁸Pb/²⁰⁶Pb). Arsenic, Cd, and Pb concentrations in hair were 0.15–9.40, 0.10–18.2 and 1.72–283 mg kg⁻¹ (Fig. 6A and Table S1). Of the 27 participants, 21 and 25 individuals had As and Pb hair concentration exceeding the background level of 1 and 5 mg kg⁻¹ (Sanna et al., 2003; Cui et al., 2013).

Stable Pb isotope ratios ²⁰⁷Pb/²⁰⁶Pb and ²⁰⁸Pb/²⁰⁶Pb in hair (0.8401–0.8596 and 2.0742–2.1149; mean: 0.8481 ± 0.0049 and
Even though our study was conducted on a specific region in China, the findings could also be helpful for places with similar contaminations around the world. In addition, the coupled bioavailability and stable isotope techniques could be used in future studies to accurately identify the sources of metal exposure. Further studies are warranted to understand the variations in Cd- and Pb-RBA among individual samples.

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Appendix A. Supplementary data

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References