Assessment of cadmium bioaccessibility to predict its bioavailability in contaminated soils

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Abstract

In vitro assays have been developed to determine metal bioaccessibility in contaminated soils; however, their application to Cd is limited. To assess their suitability to determine Cd relative bioavailability (RBA), Cd-RBA in 12 contaminated soils containing 3.00–296 mg kg −1 Cd were determined using a mouse model and compared with Cd bioaccessibility data based on four assays including the UBM, SBRC, IVG, and PBET. After being administered feed amended with soil or CdCl2 for 10-day, the Cd concentrations in the mouse liver and/or kidneys were used as biomarkers to estimate Cd-RBA. Cd-RBA was comparable at 34–90% and 40–78% based on mouse liver and kidneys with RSD of 7.10–8.99%, and 37–84% based on mouse liver plus kidneys with lower RSD of 5.8%. Cadmium bioaccessibility in soils varied with assays, with 61–99, 59–103, 54–107, and 35–97% in the gastric phase and 20–56, 38–77, 42–88, and 19–64% in the intestinal phase of the UBM, SBRC, IVG and PBET assays. Based on the combined biomarker of liver plus kidneys, better correlation was observed for PBET (r 2 =0.61–0.70) than those for IVG, UBM and SBRC assays (0.12–0.52). The monthly Cd intake in children was 0.24–23.9 μg kg −1 using total Cd concentration in soils, which was reduced by 43% to 0.18–12.3 μg kg −1 using bioavailable Cd. Our data suggest it is important to consider Cd-RBA to assess risk associated with contaminated soils and the PBET may have potential to predict Cd-RBA in contaminated soils.

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1. Introduction

With rapid urbanization and industrialization over the past three decades, heavy metal contamination in soils has become an issue in China (Li et al., 2014; Wang et al., 2001). Recent nationwide surveys showed that cadmium (Cd) contaminated soils account for up to 7% of contaminated soils based on the Chinese Soil Environmental Quality Standards, making it most serious problem in China (Zhao et al., 2015). This is partially due to the low Cd class II values (0.3 mg kg −1) for soils with pH < 7.5, which is to protect agricultural production and human health via food chain, and is applicable to agricultural, orchard and pasture land. Cadmium concentrations in soils in England and Wales are also high, with 45% > 0.3 mg kg −1, but they are within their guideline values (residential soil = 10 mg kg −1) (Rawlins et al., 2012). Cadmium in soils derives from both anthropogenic and geogenic origins. Mining and smelting of ores, atmospheric deposition from incineration, and burning of fossil fuels are the main contributors to Cd in contaminated sites (Alloway 1995).

Human exposure to Cd can result in obstructive pulmonary disease, emphysema, and kidneys disease (Farooq et al., 2012). Its exposure pathways include smoking, consumption of contaminated food or water, inhalation of dust, and incidental ingestion of Cd-contaminated soil and dust. For children living near contaminated sites, soil ingestion may be an important pathway (Schilderman et al., 1997). In vivo experiments based on mouse and swine models showed that not all the Cd in soils is absorbed into systemic circulation and becomes bioavailable, which means Cd bioavailability is often <100%. Therefore, using total Cd in soils to perform risk assessment may overestimate its risks.

To refine Cd risk from oral ingestion of contaminated soils, animal model using juvenile swine and mouse have been used to estimate Cd relative bioavailability (RBA) (Denys et al., 2012; Juhasz et al., 2010). However, animal models are costly and time consuming. As a result, UBM (unified BARGE method), SBRC (Solubility Bioaccessibility Research Consortium), IVG (in vitro gastrointestinal) and PBET (physiologically based extraction test) assays have been developed to simulate gastrointestinal process in humans (Denys et al., 2012; Juhasz et al., 2010; Ruby et al., 1996; Schroder et al., 2003). The fraction of Cd dissolved in gastrointestinal fluids represents the Cd potentially
available for absorption into the systemic circulation, i.e., bioaccessible Cd.

Even though these assays have been used to determine metal bioaccessibility in soils, inconsistent results have been found using different methods. For example, different Cd bioaccessibility in the gastric phase of SRB (57–122%), IVG (43–107%), and PBET (30–110%) was found in 7 Cd-contaminated soils partially due to their different gastric pH values (Juhasz et al., 2010). Oomen et al. (2002) found different Cd bioaccessibility in 3 soils using 5 in vitro assays. Similar results were found for Pb bioaccessibility in contaminated soils by Li et al. (2015a) who reported that Pb bioaccessibility was significantly higher using the gastric phase of SRB (30–99%) than those of the UBM, IVG, and PBET assays (0.46–84%). This may be due to different compositions and extraction parameters in different assays such as pH and soil/solution ratio.

In addition, in vitro assays must be predictive of in vivo RBA before being used as an appropriate surrogate. To date, several studies showed good correlation between Cd bioaccessibility and Cd bioavailability in contaminated soils. For example, Schroder et al. (2003) found a good correlation ($r^2 = 0.64$) between Cd bioaccessibility based on the IVG assay and Cd bioavailability based on the Cd concentrations in the kidneys following 15-day of dosing of 10 soils to juvenile swine. Cadmium bioaccessibility using the UBM is strongly correlated ($r^2 = 0.77–0.94$) with Cd-RBA based on the Cd concentrations in the liver, kidneys, femur or urine of juvenile swine following 14-day of dosing of 10 soils (Denys et al., 2012). Bioaccessible Cd using the SRBC and PBET assays is also correlated ($r^2 = 0.72–0.91$) with Cd-RBA based on the Cd concentrations in the liver or kidneys using a mouse model (Juhasz et al., 2010). However, limited studies focus on the relationship between Cd bioaccessibility using different in vitro assays and Cd bioavailability in contaminated soils. Juhasz et al. (2010) correlated Cd bioavailability with Cd bioaccessibility based on different assays, however, only 7 soils were used in that study. Therefore, additional soils are needed to verify their ability to predict Cd bioavailability in contaminated soils.

In this study, 12 Cd-contaminated soils were collected from different locations and contamination sources in China. Four assays including UBM, SRBC, IVG, and PBET were used to determine Cd bioaccessibility in soils. In addition, a 10-day steady state dosing exposure was used to measure Cd bioaccessibility using a mouse model, with the Cd concentrations in the kidneys, liver, femur, or liver plus kidneys being used as biomarkers. The objectives of this study were to: 1) measure Cd relative bioavailability (RBA) in contaminated soils using a mouse model; 2) determine Cd bioaccessibility in contaminated soils using four in vitro assays; and 3) assess the suitability of in vitro assays to predict Cd bioavailability in contaminated soils by correlating Cd bioaccessibility with Cd-RBA in contaminated soils. This study may help to develop Cd bioaccessibility method to predict Cd bioavailability during risk assessment of Cd-contaminated soils.

### 2. Materials and methods

#### 2.1. Contaminated soils

Twelve Cd-contaminated soils were collected from different sites, which were impacted by farming, mining, smelting and residential activities in 5 provinces of China (Table 1). Air-dried soils were sieved to <250 μm particle size to obtain the fraction that is easily ingested by hand-to-mouth activities. Concentrated HNO$_3$ and 30% H$_2$O$_2$ were used to digest soils to obtain total Cd, Ca, Fe, Zn, and P concentrations following USEPA Method 3050B. Soil pH was determined in water extracts (1:5 soil: solution) after 2 h of shaking. Total organic carbon (TOC) content was determined as loss on ignition at 900 °C using an element analyzer (vario TOC select, Elementar, Germany) after removing carbonate carbon with HCl. Amorphous Fe, Al, and Mn oxides ($\text{Fe}_\text{AM}, \text{Al}_\text{AM}$, and $\text{Mn}_\text{AM}$) were extracted using acid ammonium oxalate (McKeague and Day 1966). A laser diffraction meter (Masterizer 2000, Malvern, UK) was used to obtain clay content. Inductively coupled plasma mass spectrometry was used to measure Cd concentration in extracts (ICP-MS, NexIONTM300X, Perkin Elmer, USA), while Fe, Al, Mn, and Ca were quantified using inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 5300, Perkin-Elmer, USA). The optimizing procedures and operating parameters were provided in supporting information (Tables S1 and S2). Soil reference material D061-540 from Environmental Resource Associates was included for QA/QC. The recovery for Cd, Fe, Ca, Zn, and P in the reference material was 97.6 ± 5.1, 102.3 ± 4.6, 96.9 ± 5.5, 110 ± 4.8, and 104.3 ± 8.7% ($n = 3$).

#### 2.2. Cadmium relative bioavailability in soils

Female Balb/C mice weighing 18–22 g were used to determine Cd relative bioavailability (RBA) in contaminated soils. Animal care followed the standard procedures of Nanjing University. Mice were housed in metabolic cages with 12/12 light/dark cycles, with rodent diet and Milli-Q water being supplied ad libitum. After acclimation for 7 days, mice were randomly assigned to metabolic cages with one mouse per cage and three mice per group. Soils were incorporated into mouse basal diet at 1:50 mass ratio in soil-amended diet with Cd concentrations of 0.06–5.92 mg kg$^{-1}$ dry weight (dw). Similarly, CdCl$_2$-amended diet with 0.5–5.0 mg kg$^{-1}$ Cd was prepared. All diets were molded into pellets and freeze-dried.

After fasting overnight, mice were weighed and fed with ~4 g of prepared diet daily over a 10-day period. Mice fed with basal diet were used as control. Dosed concentrations at 0–800 μg Cd Kg BW day$^{-1}$ (CdCl$_2$) were used to establish a dose–response curve for Cd. For soil-amended food, Cd exposure doses were 11–1082 μg Cd Kg BW day$^{-1}$. At the end of 10 days, mice were fasted overnight again, weighed and

<table>
<thead>
<tr>
<th>ID</th>
<th>Location (province)</th>
<th>Land uses</th>
<th>Cd (mg kg$^{-1}$)</th>
<th>Ca (g kg$^{-1}$)</th>
<th>Fe (g kg$^{-1}$)</th>
<th>Zn (mg kg$^{-1}$)</th>
<th>P (mg kg$^{-1}$)</th>
<th>pH</th>
<th>TOC (%)</th>
<th>Clay (%)</th>
<th>$\text{Al}_\text{AM}^a$ (g kg$^{-1}$)</th>
<th>$\text{Fe}_\text{AM}$ (g kg$^{-1}$)</th>
<th>$\text{Mn}_\text{AM}$ (mg kg$^{-1}$)</th>
</tr>
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<tbody>
<tr>
<td>S1</td>
<td>Hunan Mining</td>
<td>3.00 ± 0.50$^b$</td>
<td>7.37 ± 0.20</td>
<td>314.3 ± 1.7</td>
<td>834 ± 84</td>
<td>205 ± 18</td>
<td>2.8 ± 0.64</td>
<td>13.1 ± 0.24</td>
<td>2.82 ± 0.23</td>
<td>45.5 ± 1.6</td>
<td>2.58 ± 0.12</td>
<td></td>
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</tr>
<tr>
<td>S2</td>
<td>Yunan Mining</td>
<td>2.29 ± 0.38</td>
<td>8.13 ± 0.20</td>
<td>10.8 ± 0.71</td>
<td>310 ± 1.7</td>
<td>205 ± 15</td>
<td>2.8 ± 0.72</td>
<td>13.1 ± 0.24</td>
<td>2.82 ± 0.23</td>
<td>45.5 ± 1.6</td>
<td>2.58 ± 0.12</td>
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$^a$ AM = amorphous.

$^b$ Values represent mean and standard deviation of triplicates.
scarified to collect the liver, kidneys and femur. For kidneys and liver, samples were immediately stored at −80 °C. Femur samples were collected by removing the hind limbs from the trunk of the body and the knee joint, immediately steamed to remove muscle and connective tissue from the femur and then stored at −80 °C. These samples were freeze-dried and digested using USEPA 3050B to determine Cd by ICP-MS.

The dose response of Cd accumulation in the liver, kidneys and femur was established following administration of CdCl₂-amended feed to mice. Then Cd-RBA was calculated as the ratio of dose-normalized Cd concentration in the liver, kidneys, femur or liver plus kidneys (LKF) of mouse following soil-Cd exposure to that of CdCl₂ exposure (Eq. 1). All concentrations were based on dry weight.

\[
\text{Cd relative bioavailability (RBA)}(\%) = \left( \frac{\text{LKF Cd}_{\text{soil}}}{\text{LKF Cd}_{\text{CdCl2}}} \times \frac{\text{Cd dose}_{\text{CdCl2}}}{\text{Cd dose}_{\text{soil}}} \right) \times 100
\]

where LKF Cd_{soil} and LKF Cd_{CdCl2} = Cd concentration in the liver, kidneys, femur or liver plus kidneys (LKF) of mice exposed to soil and CdCl₂, and Cd dose_{soil} and Cd dose_{CdCl2} = Cd dose level in mice exposed to soil and CdCl₂.

2.3. Cadmium bioaccessibility in soils

To simulate processes occurring in humans, various in vitro assays have been developed to measure contaminant bioaccessibility. In this study, four common assays including UBM, SBRC, IVG, and PBET were used to measure Cd bioaccessibility in contaminated soils (Denys et al., 2012; Ruby et al., 1996; Schroder et al., 2004; Smith et al., 2011). The UBM includes saliva, gastric, and intestinal phases whereas the others have the gastric and intestinal phases. Detailed information on the procedure, fluid compositions and analysis parameters can be found in Li et al. (2015b) and Table S3. In vitro assays were performed in triplicate. Cadmium bioaccessibility was calculated by dividing extractable Cd in the gastric or intestinal phase by total Cd in soils.

2.4. Statistical analysis

All Cd-RBA and Cd bioaccessibility data are shown as means and standard deviations of triplicate analyses. One-way ANOVA based on the least significant difference was applied to determine differences in Cd bioaccessibility in four vitro assays and Cd-RBA using different biomarkers in a mouse model. Origin 9.0 was used for linear correlation analysis to correlate Cd bioaccessibility with Cd RBA in contaminated soils.

3. Results and discussion

3.1. Soil characteristics

Cadmium concentrations in 12 contaminated soils varied from 3.00 to 296 mg kg⁻¹, which was much higher than the Class II of Chinese Soil Environmental Quality Standards (0.3 mg kg⁻¹ for soils with pH < 7.5). The high Cd concentrations in soils ensured accurate measurement Cd-RBA in soils. For example, Cd concentration in mouse liver was 31 μg g⁻¹ for soil 1 containing 3.00 mg kg⁻¹ Cd, which was close to the background exposure at 29 μg g⁻¹ Cd (data not shown). In previous studies, soils with high Cd concentrations were also employed to determine Cd-RBA. For example, soil Cd concentrations were 22–184 mg kg⁻¹ in Denys et al. (2012) and 24–465 and 11–267 mg kg⁻¹ in Schroder et al. (2003) and Juhasz et al. (2010). In addition, soil properties known to influence Cd bioavailability also varied among 12 soils including pH (2.8–8.9), total organic carbon (0.64–5.26%), clay content (2.91–13.4%), and amorphous Al, Fe, and Mn oxides (0.74–5.88 g kg⁻¹, 1.76–45.5 g kg⁻¹, and 2.58–8463 mg kg⁻¹) (Pelfrêne et al., 2012; Tang et al., 2006). Elements including Ca (2.22–48.1 g kg⁻¹), Fe (21.1–134 g kg⁻¹), Zn (50.7–2404 mg kg⁻¹), and P (170–1317 mg kg⁻¹) may also interact with Cd during its intestinal absorption (Table 1; Ohta and Cherian 1995).

3.2. Cadmium relative bioavailability in contaminated soils

Cadmium relative bioavailability (RBA) in contaminated soils was determined based on the Cd concentrations in the liver, kidneys, femur or liver plus kidneys after a 10-day steady state dosing exposure using a mouse model.

Before Cd-RBA measurement, linear Cd dose response curve was established using different biomarkers. The Cd concentrations in the liver, kidneys, or femur were highly correlated with Cd dose after 10-d exposure (Fig. 1A; r² = 0.93–0.99), illustrating good linear response of Cd using these biomarkers. The slope was the highest in the kidneys (0.77), followed by liver (0.42) and femur (0.018), indicating the Cd in the kidneys and liver were good biomarkers for Cd accumulation in mice. Previous studies used Cd accumulation in the liver and kidneys as biomarkers to determine Cd-RBA, with limited use of femur (Denys et al., 2012; Juhasz et al., 2010; Schroder et al., 2003). In this study, with Cd dose increasing from 80 to 800 μg Cd Kg BW day⁻¹, Cd concentrations became more scattered in the liver and kidneys (standard deviation increased from 2.89 to 68.9 μg kg⁻¹ and 5.94 to 38.7 μg kg⁻¹) (Fig. 1A). This may be due to the individual response differences among mice at high Cd loading (Klaassen et al., 2009). Casteel et al. (2006) found...
similar trend using the liver, kidneys, or femur Pb as biomarker in a swine model when determining Pb-RBA in soils.

When mice were administered with basal diet, low Cd concentrations were found in the kidneys (40.6–58.5 μg kg⁻¹), liver (27.3–31.0 μg kg⁻¹), and femur (0.55–0.61 μg kg⁻¹). The detection of low Cd in mice indicated that they were probably exposed to Cd via basal diet, which were 0.23 mg kg⁻¹ Cd (data not shown). The results were consistent with Juhasz et al. (2010) and Schilderman et al. (1997) who also detected Cd in control mice, with higher Cd concentrations being in the kidneys than liver. After incorporating 0.5–5 mg kg⁻¹ Cd into mouse diet, Cd concentrations in the kidneys, liver, and femur increased to 86.0–815, 49.3–349, and 2.71–15.8 μg kg⁻¹, which was 1.7–16, 1.7–12, and 4.8–28 fold higher than those in control mice. In addition, the ratios of Cd concentration in the kidneys to liver decreased from 2.5 to 2.0 in this study, which was negatively correlated with Cd exposure dose ($r = -0.6$). Our data showed that at high Cd dose, Cd preferred to accumulate in the liver, consistent with Juhasz et al. (2010). It is known that Cd has high affinity for metallothionein. At high dose, it was possible that metallothionein in the kidneys was saturated with Cd, leaving more Cd to be accumulated in the liver (Lehman and Klaassen 1986).

After establishing the dose response curve in the liver, kidneys, and femur in mice, Cd-RBA in contaminated soils was determined (Eq. 1). Based on the Cd concentrations in the liver and kidneys, Cd-RBA was similar to 33.5–89.8% and 39.6–77.8% (Fig. 2), consistent with Juhasz et al. (2010). For 9 out of 12 soils, no significant difference was found between Cd-RBA based on Cd in the liver or kidneys, consistent with their strong correlation ($r^2 = 0.81$; Fig. 1B). To increase the confidence in RBA measurement, combing two or more biomarkers has been recommended (Casteel et al., 2006). For example, biomarkers by combining the liver, kidneys, femur, and blood for Pb-RBA and the liver and kidneys for Cd-RBA in soils have been recommended (Casteel et al., 2006; Juhasz et al., 2010). In this study, the Cd concentrations in the liver plus kidneys was used as a biomarker. Strong correlation between Cd in the liver plus kidneys and different doses of CdCl₂ to mice was found, indicating its suitability as a biomarker ($r^2 = 0.99$; Fig. 1A). Cadmium RBA based on the liver plus kidneys may overcome the issue of unequal Cd distribution in different tissues. The Cd-RBA based on the liver plus kidneys was 36.6–83.8%, with relative standard deviation of 5.78%, which was lower than that for liver (8.99%) or kidneys (7.10%) (Fig. 2). The Cd-RBA based on the liver plus kidneys was comparable to the liver or kidneys with no significant difference being observed ($p > 0.05$). It is known that elemental concentrations influence Cd-RBA in soils, however, no relationship between Cd-RBA based on Cd in the liver plus kidneys and concentrations of amorphous Fe, Al, or Mn was observed ($r^2 < 0.1$, Fig. S1).

The Cd-RBA based on femur in soils 10, 11, and 12 with high Cd concentrations (204–296 mg kg⁻¹) were 76.5 ± 12.6, 43.9 ± 14.3, and 39.9 ± 5.56% (data not shown). However, the Cd-RBA in soils 1–9 was inaccurate as the measured Cd concentration was below the method detect limit (0.008 μg kg⁻¹), implying femur was not a good biomarker for measuring Cd-RBA in low Cd soils based on a mouse model. Similarly, femur Cd is not a good biomarker for low Cd soils either in a swine model (20–106 mg kg⁻¹) (Denys et al., 2012).

### 3.3. Cadmium bioaccessibility based on four in vitro assays

Four common in vitro methods (UBM, SBRC, IVG, and PBET) were used to determine Cd bioaccessibility in 12 contaminated soils. As expected, Cd bioaccessibility varied with soils and assays used, ranging from 18.6 to 107%. Among the four assays, Cd bioaccessibility in the gastric phase of SBRC, UBM, IVG, and PBET assays were 59.4–103% (averaging 86.5%), 60.9–99.4% (79.0%), 54.4–107% (75.4%), and 34.7–97.3% (68.9%). Generally, Cd bioaccessibility was the highest based on the SBRC assay and the lowest based on the PBET, probably due to their pH differences (Table S3). Similar trend was observed by Li et al. (2015a) when determining Pb bioaccessibility in soils. However, the pH in the gastric phase of SBRC (1.5) was higher than that in UBM (1.2), so other factors probably played a role besides fluid pH. Van de Wiele et al. (2007) reported higher Pb dissolution in the gastric phase under lower soil:solution ratio, which is consistent with our data as SBRC (1:100) has lower soil:solution ratio than UBM (1:37.5), thereby higher Cd-RBA. The Cd bioaccessibility in the gastric phase obtained in this study was comparable with others, which were 56–92, 21–96, 8.4–88, and 53–77% using the SBRC, IVG, UBM, and PBET assays (Denys et al., 2012; Juhasz et al., 2010; Tang et al., 2006).

Among the two phases, Cd bioaccessibility based on the gastric phase (34.8–107%, averaging 77.5%) was greater than those based on the intestinal phase (18.6–87.9%, averaging 47.4%) (Fig. 3). Extending from the gastric phase to intestinal phase, the fluid pH increased from 1.2–25 to 5.5–7.0 (Table S3). As a result, Cd bioaccessibility decreased to 37.6–77.3, 19.8–56.3, 41.9–87.9, and 18.6–63.5% for the SBRC, UBM, IVG, and PBET assays (Fig. 3). Similar trend has been found by others (Tang et al., 2006; Wragg et al., 2011), Juhasz et al. (2010) suggested the decrease is probably due to Cd co-precipitation with Fe or absorption onto Fe oxides, which is supported by the concurrent decrease in
Cd and Fe concentrations in the intestinal fluid. Formation of ferrihydrite has been observed in the intestinal phase of the SBRC assay, which has high affinity for Cd under neutral conditions (Swedlund et al., 2003).

3.4. Correlating Cd bioaccessibility with Cd-RBA in soils

Cadmium bioaccessibility varied with in vitro assays. To determine the suitability of in vitro assays to predict Cd-RBA in soils, correlation was performed between Cd bioaccessibility and Cd-RBA based on Cd in the liver plus kidneys (Fig. 4). The strongest correlation ($r^2$) was observed for the PBET (gastric: 0.70 and intestinal: 0.61), followed by the IVG assay (0.52 and 0.42), with weak correlation for the UBM and SBRC assays (0.12–0.30). Among the four assays, the PBET was the only one meeting the criterion of $r^2 > 0.6$ (Wragg et al., 2011). Juhasz et al. (2010) also reported better correlation for the PBET (0.75 and 0.83) than IVG assay (0.42 and 0.58) based on Cd in the liver plus kidneys in a mouse model, which is consistent with our data. However, using a swine model, Schroder et al. (2003) reported better correlation for the IVG assay (0.74 and 0.29) using Cd in the kidneys while Juhasz et al. (2010) and Denys et al. (2012) reported better correlation for the SBRC (0.58 and 0.80) and UBM assays ($r^2 = 0.88$ and 0.94) using Cd in the kidneys and liver. The data suggest additional research is needed to select the most proper in vitro method to predict Cd-RBA in contaminated soils.

Besides better correlation, parameters including y-intercept (close to zero) and slope (0.8–1.2) of the correlations also need to be considered (Wragg et al., 2011). The slopes using the IVG (0.62 and 0.72) and PBET assays (0.53 and 0.71) were closer to 1 than those of UBM (0.65 and 0.49) and SBRC assays (0.33 and 0.51) with the y-intercepts varied from 9.77 to 47.7. When the slopes were compared with those of Schroder et al. (2003), Juhasz et al. (2010), and Denys et al. (2012), excluding the intestinal phase of the SBRC assay ($p = 0.016$), no significant difference was found for the four assays ($p = 0.11–0.40$). When the intercepts were compared, excluding the intestinal phase of the IVG assay, significant difference was found for the four assays ($p = 0–0.02$). For the predictive strength of the four assays, correlation efficient is a more important parameter than slopes and intercepts. The PBET had a slope of 0.53 (gastric) and 0.71 (intestinal), which is outside the recommended range. Though not ideal, considering the strong correlation (0.70 and 0.61), Cd bioaccessibility based on the PBET was probably suitable to predict Cd-RBA in contaminated soils. Juhasz et al. (2010) also reported that the PBET was more suitable than the SBRC and IVG assays in predicting Cd-RBA in contaminated soils, consistent with our data. However, more work is needed to verify the suitability of the PBET.

Among various factors determining the correlation between in vitro and in vivo assays, animal models and dosing schemes are important. For example, both swine and mouse have been used to determine Cd-RBA, but they have notable differences in physiological parameters (Bradham et al., 2013; Juhasz et al., 2014). In addition, difference in
The dosing scheme is also important. In this study, soils were incorporated into basal feed, which was fed daily for 10 days. In the study of Denys et al. (2012), swine was exposed to Cd for two weeks in a dough ball that was given at 9 a.m. daily after an overnight fasting, which represents a worst-case scenario exposure. However, incorporation of soils into feed may change Cd bioavailability. For example, Ca, Fe and Zn in food may interact with Cd during intestinal absorption (Ohta and Cherian 1995). Our data suggested that more research is needed to improve the relationship between in vivo and in vitro data. These include comparison of Cd-RBA using different animal models (i.e., mouse vs. swine), and different exposure scenarios (stead state exposure via diet vs. single exposure via gavage). In addition, collecting more Cd-contaminated soils to represent different contamination sources to improve the strength of the established model to predict Cd-RBA is also needed.

3.5. Cadmium intake via oral ingestion based on bioavailable Cd

To demonstrate the importance of incorporating bioavailable Cd into risk assessment, we compared Cd intake based on total and bioavailable Cd (Cd in the liver plus kidneys) assuming child body weight of 18.6 kg.

Fig. 4. Correlation of Cd relative bioavailability based on Cd in the liver plus kidneys, and Cd bioaccessibility based on the gastric (G) and intestinal (I) phase of the UBM, SBRC, IVG, and PBET assays. Regression equation is expressed as $y = (\text{slope} \pm \text{SD}) x + (\text{intercept} \pm \text{SD})$. Data are expressed as mean and standard deviation (SD) of triplicates and dash lines represent 95% confidence interval.
Based on total Cd, Cd intake from 12 soils was 0.24–23.9 μg kg⁻¹ and contributed 0.97–95.5% (averaging 27.4%) of the provisional tolerable monthly intake (PTMI at 25 μg kg⁻¹ BW month⁻¹) (WHO 2011). As expected, the Cd intake contribution to PTMI was much less at 0.18–12.3 μg kg⁻¹ (0.73–49.1%, averaging 15.7%) based on bioavailable Cd, a reduction of 43%. For example, for soils 11 and 12 containing 212 and 296 mg kg⁻¹ Cd, significant difference in their contribution of Cd intake to PTMI was found based on total Cd (68.4 ± 1.2% vs. 95.5 ± 6.7%; p < 0.05). However, there was no difference when employing bioavailable Cd (47.1 ± 3.5% vs. 49.2 ± 0.4%; p > 0.05). This was due to the higher Cd-RBA in soil 11 than that in soil 12 (68.8% vs. 51.5%). Therefore, assessing Cd intake based on total Cd concentration may overestimate Cd exposure in samples with low Cd-RBA, so it is important to consider Cd-RBA in contaminated soils to assess Cd risk associated with incidental soil ingestion.

Based on our data, steady state dosing exposure for 10-days can be used to measure Cd-RBA based on Cd concentrations in the kidneys, liver or kidneys plus liver using a mouse model with kidneys plus liver being more robust. In addition, based on the strong correlation between Cd bioaccessibility and Cd-RBA using Cd concentrations in the kidneys plus liver, the PBET may have a potential to predict Cd-RBA in contaminated soils. Risk assessment based on total Cd in soils can overestimate the risk associated with incidental soil ingestion, so it is important to incorporate Cd-RBA into risk assessment.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2016.06.022.

References