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Lead bioaccessibility in 12 contaminated soils from China: correlation to lead relative bioavailability and lead in different fractions

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Highlights

- Four in vitro assays were used to measure Pb bioaccessibility in contaminated soils
- A single dose mouse blood model was used to estimate Pb relative bioavailability
- UBM gastric phase correlated ($r^2=0.67$) with Pb relative bioavailability in soils
- Exchangeable and carbonate Pb fractions attributed most to bioavailable Pb in soils

ABSTRACT

This study investigated the relationship between Pb relative bioavailability (RBA) and bioaccessibility, and their relationships with Pb in different pools in soils. Twelve Pb-contaminated soils representing different contamination sources from China were analyzed for Pb bioaccessibility using four *in vitro* methods (UBM, SBRC, IVG, and PBET), Pb-RBA using a mouse blood model, and Pb fractionation using sequential extraction. Lead bioaccessibility in the gastric phase (GP) and Pb-RBA was generally lower in mining soils (0.46–29% and 7.0–26%) than smelting (19–92% and 31–84%) and farming soils (13–99% and 51–61%), with more Pb in the residual fraction in mining soils. Lead bioaccessibility varied with assays, with SBRC (3.0–99%) producing significantly higher bioaccessible Pb than other assays (0.46–84%) in the gastric phase. However, Pb bioaccessibility in the intestinal phase (IP) of all assays sharply decreased to 0.01–20% possibly due to Pb sorption to solid phase at higher pH. Lead bioaccessibility by UBM-GP assay was best correlated with Pb-RBA ($r^2=0.67$), followed by IVG-GP ($r^2=0.55$). Among different Pb fractions, strong correlation was found between Pb bioaccessibility/Pb-RBA and the sum of exchangeable and carbonate fractions. Our study suggested that UBM-GP assay has potential to determine Pb bioaccessibility in contaminated soils in China.

Keywords: lead; contaminated soil; bioaccessibility; bioavailability; sequential extraction

1. Introduction

Lead (Pb) exposure has been of major public concern due to its well-established adverse neuro-behavioral effects on children [1, 2]. After phasing out of Pb from gasoline and paint, child blood Pb levels have significantly declined worldwide [3, 4]. However, in Pb-contaminated sites such as mining and smelting areas, childhood Pb poisoning is still of concern [5, 6]. Lead exposure to children near Pb-contaminated areas include incidental ingestion of soils via hand-to-mouth behaviors and inhalation of resuspended soil particles [7, 8]. The mean soil ingestion rate for children ranges from 1.2 to 23 mg soil per day [9]. Therefore, assessment of Pb exposure through soil ingestion is receiving increasing consideration [10]. However, risk assessments usually assume that all Pb in soil is absorbed into the blood systemic circulation following oral ingestion (i.e., 100% bioavailable). In reality, Pb bioavailability in soil is often <100%, depending on soil properties and Pb speciation [11].

At present, several *in vivo* animal models including mouse and swine have been used to assess Pb relative bioavailability (RBA, relative to water soluble lead acetate) [11–14]. However, *in vivo* assays are costly and time consuming, therefore not suitable to measure site-specific Pb-RBA on a large scale. As a result, various *in vitro* assays to determine Pb bioaccessibility have been developed, which measure the soluble Pb fraction from soil in simulated human gastrointestinal fluids [10–11, 15–16]. Common methods include Solubility/Bioavailability Research Consortium method (SBRC), *in vitro* gastrointestinal method (IVG), physiologically based extraction test (PBET), Deutsches Institut für Normung V. method (DIN), and unified BARGE method (UBM) [17–21]. However, due to variation in gastrointestinal fluid components and assay parameters (e.g., pH, soil/solution ratio, and extraction time), different assays often produced different bioaccessibility results [10, 15, 22]. Therefore, before these assays can be used to accurately predict Pb-RBA in contaminated soils, they need to be

correlated to Pb-RBA determined via animal models.

To date, several studies have correlated Pb bioaccessibility in contaminated soils to Pb-RBA based on different models (swine, mouse, or rat) and methods (single versus multiple doses). For example, using 18 contaminated soils, Schroder et al. [17] showed a strong correlation ($r^2=0.79$) between Pb bioaccessibility by the IVG gastric phase and Pb-RBA based on blood Pb following 15-day soil dosing to juvenile swine. Strong correlations ($r^2=0.78-0.90$, $n=16$) were observed between Pb bioaccessibility by UBM and Pb-RBA using a swine model and Pb in the kidney after 14-day soil dosing [16]. Smith et al. [11] showed strong correlations ($r^2=0.78$ and 0.88 , $n=12$) between Pb bioaccessibility by SBRC and Pb-RBA based on a mouse model and a single gavage dose. These studies demonstrated the predictive ability of a given assay for the tested soils. However, few studies have established *in vivo-in vitro* correlations (IVIVC) for different bioaccessibility assays using the same soils and investigated the variability of IVIVC within assays.

Compared to limited *in vivo* Pb-RBA studies, many studies investigated Pb fractions in soils using sequential extraction [23, 24]. The method of Tessier et al. [25] is the most widely used, which operationally separates Pb into 5 fractions, i.e., exchangeable Pb, and Pb associated with carbonates, Fe/Mn oxides, organic matter, and residual fractions, showing decreasing bioavailability. For example, Pb in the carbonate and Fe/Mn oxides bound fractions is more bioavailable than Pb in the residual fraction [26, 27]. However, correlations between Pb fractionation by sequential extraction and Pb bioaccessibility are rarely established [23, 28].

For this study, we used a mouse blood model via areas under the blood Pb concentration time curve (AUC) to measure Pb-RBA in soils with Pb acetate as a reference [11]. For bioaccessibility, we selected 4 common *in vitro* assays, including UBM, SBRC, IVG, and PBET. The overall objective of this study was to investigate the relationship between Pb-RBA and Pb bioaccessibility and their relationships with Pb in different fractions in contaminated

soils. This was accomplished by 1) correlating Pb-RBA in 12 Pb-contaminated soils via a mouse blood model with Pb bioaccessibility by four assays, and 2) correlating Pb bioaccessibility and Pb-RBA in soils with Pb in different fractions via sequential extraction. Establishing Pb *in vivo*–*in vitro* correlations using contaminated soils from China provides an extension of the current knowledge of Pb bioaccessibility and Pb-RBA done in other parts of the world. Correlations between Pb-RBA and Pb in different fractions provide an insight of how Pb from different pools contributes to Pb-RBA.

2. Materials and Methods

2.1. Lead contaminated soils

Twelve soils from different contaminated sites in farmland and near mining and smelting areas were collected from China (Table 1). These sites represent Pb-contaminated sites in China, including west-central Hunan, northwestern Henan, the junction of Yunnan, Guizhou, and Sichuan Provinces, central Guangxi Province, and the border between Shanxi and Gansu Provinces [29]. Due to its potential risk to human health, Pb contamination in these contaminated areas has received much attention recently [30]. The farming soils were probably contaminated through application of fertilizers and pesticides as well as reused wastewater. The rest of soils were collected from typical mining/smelter sites in southern China. All soils were air-dried and sieved to <250 µm for bioavailability and bioaccessibility assessment. This fraction most likely adheres to children's hands and is ingested via hand-to-mouth contact [31].

Soils were digested using USEPA Method 3050B and analyzed for total Pb concentrations by inductively coupled plasma mass spectroscopy (ICP–MS, PerkinElmer NexION 300, USA). Total iron (Fe) concentrations were quantified using flame atomic absorption spectrometry (FAAS, PinAAcle 900T, PerkinElmer, USA). A certified soil reference material (D056) was included for quality assurance and quality control (QA/QC). The recovery

of Pb and Fe in D-056 was $95.0 \pm 1.56\%$ and $101 \pm 5.96\%$ ($n=3$).

2.2. *In vivo Pb bioavailability in soils*

Adult Balb/c mice with a body weight (bw) of 20–25 g were used. Animals were acclimatized in groups of 4, receiving a 12/12 light/dark photocycle at 20–22°C. Mice had free access to rodent diet obtained from Qinglongshan Experimental Animal Breeding Farm (Nanjing, China) and Milli-Q water at all times. Mice were quarantined according to standard protocols at Nanjing University before starting the treatment.

Preliminary tests of Pb acetate absorption pharmacokinetics in the mice were conducted (Fig. S1). A single dose (0.5 mL) of two Pb acetate solutions (250 and 500 mg L⁻¹) was administered to fasted mice via gavage, which resulted in Pb dose levels of 5 and 10 mg Pb kg⁻¹ bw. The absorption kinetic curve was established by sacrificing 4 mice and collecting blood samples into heparin tubes at different time intervals (4, 8, 16, 24, and 48 h) after exposure. Blood samples were digested using USEPA Method 3050B and measured for Pb concentrations using ICP–MS. Areas under the blood Pb concentration time curve (AUC) were 4,284 and 8,342 for the two doses of Pb acetate, indicating a linear dose-response in Pb concentration range administered, suggesting that Pb absorption was linearly dose-dependent.

Similarly, to assess Pb relative bioavailability (RBA) in soil, a single dose of soil suspension containing 0.01–0.25 g of soil in 0.75 mL of Milli-Q water was administered to fasted mice via gavage, which resulted in soil Pb dose levels of 2.15–10.7 mg Pb kg⁻¹ bw. The control group received only Milli-Q water. The time curve of mouse blood Pb concentration following soil ingestion was established by sacrificing 4 mice at each time point (4, 8, 16, 24, and 48 h) after soil exposure and determining Pb concentration in blood samples. Lead RBA was calculated using AUC with zero correction and dose normalization [11]:

$$\text{Pb relative bioavailability (\%)} = \left(\frac{\text{AUC}_{\text{oral soil}}}{\text{AUC}_{\text{oral Pb}}} \times \frac{\text{DR}_{\text{oral Pb}}}{\text{DR}_{\text{oral soil}}} \right) \times 100 \quad (1)$$

where $\text{AUC}_{\text{oral soil}}$ and $\text{AUC}_{\text{oral Pb}}$ = area under the blood Pb concentration time curve for Pb-contaminated soil and Pbacetate, and $\text{DR}_{\text{oral soil}}$ and $\text{DR}_{\text{oral Pb}}$ = Pb dose for Pb-contaminated soil and Pbacetate (mg Pb kg^{-1} mouse bw).

2.3. *In vitro* Pb bioaccessibility in soils

Four *in vitro* methods (UBM, SBRC, IVG, and PBET), which have been widely used and correlated with *in vivo* data, were selected [11, 16–18]. Their composition and analysis parameters are provided in Table S1. In short, 0.3 g of soil was mixed with the gastric phase (GP) solution in high density polyethylene tubes at soil:solution ratio of 1:37.5 (UBM), 1:100 (PBET and SBRC), or 1:150 (IVG). The pH was adjusted to 1.2, 1.5, 1.8, and 2.5 for UBM, SBRC, IVG, and PBET using concentrated HCl. The mixtures were horizontally shaken at 37°C and 150 rpm for 1 h. The use of shaking rate at 150 rpm was to prevent soil particles sticking to the bottom of tubes and maximize the contact between sample and fluid. During GP extraction, solution pH was continuously monitored and adjusted using concentrated HCl. After GP extraction, the soil suspension was centrifuged at 4,000 rpm for 10 min and supernatant samples (1 mL for UBM, 3 mL for SBRC and PBET, and 4.5 mL for IVG) were pipetted and filtered (0.45 μm), stored at 4°C before analysis of Pb and Fe using ICP–MS and FAAS.

After the gastric phase, the solution was modified to simulate the intestinal phase (IP) by adjusting pH to 5.5 (IVG) and 7.0 (PBET) with NaHCO_3 , or 6.3 (UBM) and 7.0 (SBRC) with NaOH, and then adding bile and pancreatin (Table S1). The soil:solution ratio of UBM was increased to 1:97.5 in the intestinal phase. After 1 h (IVG) or 4 h (PBET, SBRC, and UBM) of extraction, the soil slurry was centrifuged and supernatant samples were filtered (0.45 μm), acidified by adding 200 μL concentrated HNO_3 , and stored at 4°C before analysis.

using ICP–MS and FAAS.

Lead bioaccessibility was calculated by dividing extractable Pb in the gastric phase or intestinal phase of *in vitro* assays by total Pb in soil samples (<250 µm):

$$\text{In vitro Pb bioaccessibility (\%)} = \left(\frac{\text{extractable Pb}}{\text{total Pb}} \right) \times 100 \quad (2)$$

For QA/QC, a standard soil reference material (NIST SRM 2711a, National Institute of Standards and Technology) was included. Bioaccessible Pb based on the gastric phase of UBM, SBRC, IVG, and PBET assays were 926 ± 5.11 , $1,089 \pm 91.0$, $1,069 \pm 36.5$, and 610 ± 22.0 mg kg⁻¹, consistent with 1,044 mg kg⁻¹ (SBRC) [32] and 1,068, 1,066, and 554 mg kg⁻¹ (SBRC, IVG, and PBET) by Li et al. [33].

2.4. Sequential extraction of soils

Lead in the soils was fractionated according to Tessier et al. [25]. Lead fractions were operationally defined as follows: exchangeable, carbonate-bound, Fe/Mn oxides-bound, organic-bound, and residual fractions. Following each extraction, soil solutions were centrifuged at 10,000 rpm for 10 min to retrieve the supernatant. The remaining soil residues were washed twice with Milli-Q water before continuing with the next step. After extraction of the organic-bound fraction, soil residues were digested using USEPA Method 3050B to determine Pb in the residual fraction. The supernatants and digested solutions were filtered (0.45 µm), diluted with 0.1 M HNO₃, and analyzed using ICP–MS. In the sequential extraction procedure, recoveries of Pb (ratios of sum of five fractions to total Pb concentrations) in soil samples ranged from 75.0 to 118%, averaging $97.0 \pm 11.4\%$.

2.5. Data processing and statistical analysis

In vitro assays and sequential extractions were performed in triplicate and animal experiments were with four replicates. The results were presented as mean values and standard

deviations. Differences in Pb bioaccessibility among *in vitro* methods were performed using variance analysis (ANOVA) based on Tukey's multiple comparisons using SAS version 9.1.3. Linear regression analysis between Pb bioaccessibility and Pb-RBA was conducted using a repeated-median approach using R statistical analysis [16]. Simple linear correlations between Pb bioaccessibility/RBA and Pb in different fractions were established. All graphs were drawn using SigmaPlot 10.0.

3. Results and discussion

3.1. Characterization of Pb contaminated soils

The 12 Pb-contaminated soils were collected from different locations in China, representing Pb contamination from agricultural, smelting, and mining activities. The soils varied considerably in total Pb and Fe (Table 1). Lead concentrations in the soils varied by 2 fold, ranging from 215 to 25,329 mg kg⁻¹. Soils 8 and 9 with the highest Pb concentrations (9,958 and 25,329 mg kg⁻¹) were from a smelting area. Total Fe concentrations were 20.7–219 g kg⁻¹, with the highest concentration in soil 9. Soils 11 and 12 from mining areas also had high Pb (1,073 and 4,164 mg kg⁻¹) and Fe concentrations (143 and 115 g kg⁻¹).

In addition to variation in total metals, the soils also varied in Pb concentrations in different fractions (Fig. 1). Lead in the first fraction was 0.70–20.3% (exchangeable, E1), the second fraction 0.38–41% (carbonate, C2), the third fraction 0.02–55% (Fe/Mn oxides, F3), the fourth fraction 0.19–28% (organic, O4), and the fifth fraction 4.7–97% (residual, R5), averaging 10, 22, 35, 13, and 20%, with F3 > C2 > R5 > O4 > E1. Similar Pb distribution in contaminated soils was obtained by Jalali and Khanlari [34] with C2 > R5 > O4 > F3 > E1. In all soils excluding 11, C2 + F3 accounted for 41–76%, indicating that Pb was mainly associated with carbonate and Fe/Mn oxide fractions. For soils from farming and smelting areas, 20–51% of the Pb was in E1 + C2 fraction, however, they accounted for much less (3.4–9.3%) in soils from mining area. Soil 11 from mining

area contained 97% Pb in the residual fraction, suggesting low Pb bioavailability. Based on fractionation data, Pb bioavailability could vary greatly with soils.

3.2. Pb bioaccessibility and relative bioavailability varied with soils

Lead bioaccessibility and Pb-RBA varied with the soils. The range of Pb-RBA was narrower (7.0–84%, averaging 46%) than that of Pb bioaccessibility (0.46–99%, averaging 47%), but the means for the two were strikingly similar. Based on the gastric phase, Pb bioaccessibility was the highest in farming soils (13–99%, averaging 64%), followed by smelting soils (19–92%, averaging 57%) and mining soils (0.46–29%, averaging 9.0%) (Fig. 2). Similar results were obtained by others with mining soils having the lowest Pb bioaccessibility [16, 18]. Likewise, Pb-RBA using the mouse blood model varied with soils, with farming soils being the highest (51–61%, averaging 57%), followed by smelting soils (31–84%, averaging 55%) and mining soils (7.0–26%, averaging 17%) (Table 1). These Pb-RBA values fall within the Pb-RBA range of 1.0–108% that measured in Pb-contaminated soils from various sources (shooting range, mining, and smelting) [12, 17].

Lower Pb bioaccessibility and Pb-RBA have been reported for mining soils mainly due to the presence of insoluble Pb minerals (e.g., PbS) [18]. The differences in Pb bioaccessibility and Pb-RBA among soils can be explained by their different Pb fractionations in soils. Lead in the mining soils was primarily associated with less available forms including F3, O4, and R5 (Fig. 1). Particularly, >90% of the Pb in soil 11 was in the residual fraction (R5). The lower Pb in the more bioavailable fractions (E1 and C2) in mining soils was consistent with their lower Pb bioaccessibility and Pb-RBA compared to other soils.

3.3. Pb bioaccessibility in the gastric phase

Fig. 2 shows the variability in Pb bioaccessibility among 4 methods. Based on the gastric

phase, Pb bioaccessibility in soils using UBM, SBRC, IVG, and PBET was 1.1–84, 3.0–99, 0.46–71, and 0.85–60%, averaging 44, 69, 46, and 28% with the order of SBRC > UBM = IVG > PBET. Generally, the highest Pb bioaccessibility was obtained by SBRC and the lowest by PBET. Similar Pb bioaccessibility in mining/smelting impacted soils was reported by Denys et al. [16] using UBM (9.2–83%), by Smith et al. [11] using SBRC (0.50–99%), by Schroder et al. [17] using IVG (0.05–37%), and by Ruby et al. [18] using PBET assay (3.80–41.0%).

The disparity in Pb bioaccessibility between methods arises from the differences in assay parameters. As gastric pH strongly influences Pb solubility in soils [18], lower gastric pH in SBRC (pH 1.5) than IVG and PBET (pH 1.8, and 2.5) partially attributed to the higher Pb bioaccessibility by SBRC. However, pH could not explain the results for UBM, which has lower gastric pH value (pH 1.2) than SBRC, but lower Pb bioaccessibility. Differences in other assay parameters (e.g., soil:solution ratio) and gastric fluid components (e.g., chyme composition) were probably responsible. By comparing five *in vitro* methods, Van de Wiele et al. [15] reported that less Pb was dissolved under lower soil:solution ratio condition.

Low soil:solution ratio may underestimate Pb bioaccessibility in soils due to limited metal solubility [18]. Compared to SBRC (1:100), UBM uses soil:solution ratio at 1:37.5, which might inhibit Pb dissolution from soils, contributing to its lower Pb bioaccessibility. To test this hypothesis, we increased soil:solution ratio of UBM from 1:37.5 to 1:100 and used soils 2, 7, and 12 for extractions, which represented farming, smelting and mining soils. For all 3 soils, Pb bioaccessibility using the modified UBM was increased from 65, 57, and 23% to 88, 82, and 30%, averaging 18% increase (Fig. S2A). These results suggested that although UBM used a lower gastric pH, its ability to solubilize Pb from soils was inhibited by its low soil:solution ratio, leading to lower Pb bioaccessibility than SBRC assay.

In addition, different components used in gastric fluids of the 4 methods (pepsin and mucin for UBM, glycine for SBRC, and pepsin for IVG and PBET, Table S1) may have

contributed to the variability in Pb bioaccessibility among assays. To test this hypothesis, we used 10 g L⁻¹ of glycine, pepsin, and mucin solution at pH 1.5 to extract soil. Lead bioaccessibility using glycine (93%) was significantly higher than that using pepsin (72%) and mucin (77%) (Fig. S2B). The increased Pb bioaccessibility with glycine was probably related to its strong pH buffering ability [35]. We observed that more HCl (290 μ L) was needed to adjust 20 mL of glycine solution to pH 1.5 than those needed for adjusting pepsin and mucin solution (80 and 105 μ L) and Milli-Q water (70 μ L). Therefore, glycine in the gastric phase of SBRC is another reason for its highest Pb bioaccessibility among the 4 assays, in addition to its low gastric pH and high soil:solution ratio.

3.4. Pb bioaccessibility in the intestinal phase

As pH increased from 1.2–2.5 in the gastric phase to 5.5–7.0 in the intestinal phase, soluble Pb in the gastric phase is probably absorbed by soils and Fe oxides [23, 36]. In the intestinal phase, average Pb bioaccessibility in soils using UBM, SBRC, IVG, and PBET decreased from 45 to 2.1%, 69 to 9.3%, 46 to 6.0%, and 28 to 8.1%, i.e. by 20, 7.4, 7.8, and 3.5 fold, respectively (Fig. 2). Similar decrease in Pb bioaccessibility by 2–45 fold in the intestinal phase of SBRC and IVG has been observed in contaminated soils [17, 37].

At neutral intestinal solutions, soluble Fe in the gastric phase precipitates as Fe oxides, which adsorb soluble Pb, decreasing Pb solubility [11]. In this study, for UBM, SBRC, and IVG, decreased Pb concentration in the intestinal phase was accompanied by a sharp decrease in soluble Fe (Fig. S3A–C), suggesting precipitation of soluble Fe as Fe oxides. Soluble Pb was probably absorbed onto Fe oxides and soils at elevated pH. However, for the PBET, decrease in Pb bioaccessibility was not always accompanied by decrease in soluble Fe. For example, soluble Fe in soils 4, 5, and 9 did not decrease from the gastric to intestinal phase (204–3,248 to 209–3,029 mg kg⁻¹), suggesting Fe was not precipitated (Fig. S3D). This may be attributed to citrate in the UBM fluid, inhibiting Fe precipitation in the intestinal phase [33]. However,

Pbbioaccessibility in the UBM-IP in the 3 soils still sharply decreased from 19–60 to 3.1–9.0% (Fig. S3D). This was attributed to Pb sorption onto soil matrix at pH 7 [11]. Li et al. [33] confirmed that Pb spiked in the intestinal solution is absorbed onto solid matrix during intestinal phase extraction with house dust.

3.5. Relationships between relative bioavailable Pb and bioaccessible Pb

The linear *in vivo*–*in vitro* correlations (IVIVC) between Pbbioaccessibility by the 4 assays and Pb-RBA by a mouse model were established for the 12 soils using a repeated-median approach (Fig. 3). Compared to simple linear regression, this method considers the uncertainty of both Pb bioaccessibility and Pb-RBA measurements, providing a distribution of values (mean and 95% confidence interval) for regression descriptive statistics [16]. Summary of IVIVC statistics including r-square (r^2), slope, and y-intercept and their 95% confidence intervals are shown in Fig. 4 and Table 2.

The IVIVC varied with *in vitro* assays. In general, Pbbioaccessibility in the gastric phase was better correlated with Pb-RBA than that in the intestinal phase, with r^2 values of 0.67, 0.43, 0.55, and 0.38 for UBM, SBRC, IVG, and PBET assays. The UBM-GP provided satisfactory correlation with $r^2 > 0.6$ as suggested by Wragg et al. [21]. In addition to r^2 , Wragg et al. [21] stated that IVIVC slope should be 0.8–1.2 and y-intercept not significantly different from 0. While the UBM-GP and PBET-GP met the slope criteria (0.80 and 0.87), SBRC and IVG did not (0.40 and 0.77). The y-intercepts for the gastric phase of UBM, SBRC, and IVG were similar at ~10, but PBET-GP was much higher at ~20. Considering all three parameters, we concluded that the UBM-GP provided the best correlation with Pb-RBA for the 12 soils. Poor IVIVC were found in the intestinal phase of the 4 assays, with r^2 of 0.01–0.24, primarily due to low soluble Pb.

Previous studies have correlated Pbbioaccessibility by gastric phase of the 4 assays to Pb-RBA [11, 16–18]. Compared to previous IVIVC ($r^2 = 0.78–0.93$), correlations were weaker

($r^2=0.38-0.67$) in this study (Table 2). However, when IVIVC of UBM-GP and PBET-GP were compared to those by Denys et al. [16] and Ruby et al. [18] based on different animal models (swine and rat), there were no significant differences in slope and y-intercept ($p=0.08-0.37$). However, significant differences in slope ($p<0.001$) were observed when IVIVC of SBRC-GP and IVG-GP were compared to those of Drexler and Brattin[38] and Schroder et al.[17] using a swine blood Pb model. Smith et al. [11] established IVIVC for SBRC-GP in contaminated soils using mouse blood model. However, when compared to Smith et al.[11], significant difference was observed in slope ($p=0.04$). The different animal models and soil properties may have caused the different results. Our results confirmed the ability of UBM-GP to assess Pb bioaccessibility in contaminated soils from China. Future studies are needed to investigate the impacts of selected animal models on Pb-RBA determination.

3.6. Relationship between bioavailable Pb and sequentially extracted Pb

Few studies have quantified the contributions of Pb in different fractions to bioaccessible and bioavailable Pb. Simple linear correlations between Pb bioaccessibility and Pb-RBA in 12 soils and Pb in different fractions, including the first fraction E1, sum of the first and second fractions ($E1+C2$), the first three fractions ($E1+C2+F3$), and the first four fractions ($E1+C2+F3+O4$), were examined (Table 3). In general, Pb bioaccessibility in the gastric phase was more strongly correlated with Pb fractionation data than that in the intestinal phase. Among different fractions, satisfactory correlation of $r^2>0.6$ was found between Pb bioaccessibility in the gastric phase and Pb in $E1+C2$ ($r^2=0.68-0.87$) and $E1+C2+F3$ ($r^2=0.65-0.76$). These results suggested that variation in Pb bioaccessibility among soils could be best explained by Pb pools in $E1+C2$, followed by $E1+C2+F3$.

Here, we used the benchmark for IVIVC to assess the correlation. When relationships between Pb in $E1+C2$ and Pb bioaccessibility by the gastric phase were examined, y-intercepts were close to 0 (-1.99 to 10.3) (Table 3). Among 4 assays, Pb in $E1+C2$ was best

correlated with Pb bioaccessibility by SBRC-GP ($r^2=0.87$) with a slope of 1.87, indicating Pb in E1+C2 was lower than bioaccessible Pb measured by SBRC-GP. When relationships were examined for UBM, IVG, and PBET, slopes became lower to 0.94–1.28. Considering all three parameters (r^2 , slope, and y-intercept), we concluded that bioaccessible Pb in the gastric phase of all 4 assays could be predicted using Pb in E1+C2, though E1+C2 underestimated bioaccessible Pb by SBRC-GP. When Pb in E1+C2+F3 was correlated to bioaccessible Pb by SBRC-GP ($r^2=0.75$), the slope decreased to 1.29, suggesting that bioaccessible Pb by SBRC-GP was better reflected by Pb in the first 3 fractions.

Similar to bioaccessible Pb, Pb-RBA was better correlated with Pb pools in E1+C2 ($r^2=0.52$) than Pb in E1, E1+C2+F3, and E1+C2+F3+O4 ($r^2=0.32-0.42$) (Table 3). This suggested that bioavailable Pb also mainly came from Pb in the exchangeable and carbonate fractions.

4. Conclusion

Results from this study suggested that Pb bioaccessibility and Pb bioavailability in contaminated soils depended on soil types and methods used, supporting the hypothesis that site-specific approach is necessary to accurately determine Pb bioavailability. The UBM gastric phase assay was the best among the four *in vitro* assays tested to predict Pb-RBA in 12 contaminated soils from China, though other assays also showed predictive ability. Hence, *in vitro* assays can be useful in risk assessment of contaminated soils. To reduce human health risk associated with oral ingestion of contaminated soil, remediation measures are needed to reduce exchangeable and carbonate associated Pb fractions, which contributed the most to bioaccessible and bioavailable Pb in Pb-contaminated soils.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version.

Abbreviations

Pb	lead
RBA	relative bioavailability
GP	gastric phase
IP	intestinal phase
SBRC	solubility/bioavailability research consortium method
IVG	<i>in vitro</i> gastrointestinal method
PBET	physiologically based extraction test
DIN	Deutsches Institut für Normung e.V. method
UBM	unified BARGE method
DRC	dose response curve
AUC	area under the blood Pb time curve
E1	exchangeable fraction
C2	carbonate fraction
F3	Fe/Mn oxides fraction
O4	organic fraction
R5	residual fraction

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Table1. Selected properties and Pb relative bioavailability (RBA) of 12 contaminated soils from China

Sample	Contamination Source	Location	Total Pb (mg kg ⁻¹)	Total Fe (g kg ⁻¹)	RBA (%)
1	Farming	Huangshi, Hubei	215±0.62	28.8±0.84	51.4±16.2
2		Jiyuan, Henan	734±4.24	30.4±0.33	59.7±19.8
3		Jiyuan, Henan	1,306±19.4	26.5±0.58	55.8±16.5
4		Jiyuan, Henan	1,543±16.0	27.9±0.48	60.5±20.1
5	Smelting	Fengxian, Shanxi	250±4.19	30.7± 0.58	56.9±18.7
6		Fenglan, Gansu	515±11.0	37.5±0.29	84.3±12.1
7		Shuikou, Hunan	1,174±12.6	22.7±0.55	62.3±25.3
8		Zhuzhou, Hunan	9,958±243	41.0± 0.53	39.6±5.28
9		Zhuzhou, Hunan	25,329±213	219±2.00	30.8±9.39
10	Mining	Hechi, Guangxi	516±7.03	20.7±0.19	7.00±1.80
11		Shimen, Hunan	1,073±4.89	143±1.31	16.4±8.48
12		Gejiu, Yunnan	4,164±77.6	115±5.11	26.0±5.35

Table 2. Comparison of *in vivo*–*in vitro* correlations (IVIVC) between Pb relative bioavailability and Pb bioaccessibility for gastric (GP) and intestinal phases (IP) of UBM, SBRC, IVG, and PBET assays in the current study and previous studies in contaminated soils

<i>In vitro</i>		IVIVC parameters			Pb	No. of	<i>In vivo</i> assay			
assay	Phase	slope	y-intercept	r ²	concentration	soil	animal	biomarker	method	Reference
					(mg/kg)	samples				
<i>This study</i>										
UBM	GP	0.80	9.99	0.67	214–25,329	12	mouse	blood	AUC	This study
	IP	1.26	47.8	0.01						
SBRC	GP	0.40	14.0	0.43						
	IP	2.54	26.3	0.21						
IVG	GP	0.77	6.36	0.55						
	IP	4.17	22.7	0.24						
PBET	GP	0.87	18.9	0.38						
	IP	2.38	29.6	0.20						
<i>Previous studies</i>										
UBM	GP	1.07	−6.25	0.88	1,630–40,214	16	swine	kidney	DRC	[16]
SBRC	GP	0.88	−0.03	0.92	1,270–14,200	19	swine	blood	DRC	[38]
	GP	0.94	−21.7	0.78	576–2,248	12	mouse	blood	AUC	[11]
IVG	GP	1.53	2.22	0.79	1,270–14,200	11	swine	blood	DRC	[17]
PBET	GP	1.41	3.19	0.93	1,388–10,230	7	rat	blood		[18]

AUC: area under blood Pb time curve; DRC: dose response curve.

Table 3. Linear correlations between Pb bioaccessibility determined using the gastric (GP) and intestinal phases (IP) of four assays (UBM, SBRC, IVG, and PBET) and Pb in different fractions and between Pb relative bioavailability (RBA) and Pb in different fractions in 12 Pb-contaminated soils.

Pb bioaccessibility		E1*			E1+C2			E1+C2+F3			E1+C2+F3+O4		
Assay	Phase	slope	y-intercept	r ²	slope	y-intercept	r ²	slope	y-intercept	r ²	slope	y-intercept	r ²
		/RBA			t			t			t		
UBM	GP	2.73	19.4	0.44	1.16	10.3	0.68	0.89	-9.66	0.65	0.72	-10.0	0.52
	IP	-0.03	2.45	0.01	0.06	0.29	0.13	0.03	-0.12	0.08	0.03	-0.50	0.08
SBRC	GP	3.59	32.2	0.38	1.87	10.1	0.87	1.29	-17.5	0.75	1.07	-16.6	0.58
	IP	0.60	3.12	0.30	0.28	0.33	0.56	0.21	-4.79	0.55	0.15	-2.60	0.31
IVG	GP	2.76	17.9	0.45	1.28	5.55	0.82	0.92	-15.5	0.76	0.76	-14.1	0.57
	IP	0.68	-1.02	0.67	0.22	-1.01	0.59	0.15	-3.99	0.49	0.11	-2.92	0.31
PBET	GP	2.25	4.85	0.51	0.94	-1.99	0.75	0.66	-16.6	0.67	0.50	-11.5	0.42
	IP	0.24	5.63	0.05	0.27	-0.38	0.52	0.17	-3.57	0.39	0.10	-3.12	0.29
RBA		2.35	21.8	0.42	0.90	17.3	0.52	0.60	5.76	0.41	0.50	6.04	0.32

*E1 =exchangeable fraction; C2 =carbonate fraction; F3 =Fe/Mn oxides fraction; O4 =organic fraction; and R5 =residual fraction.

r squared > 0.60 was in bold.

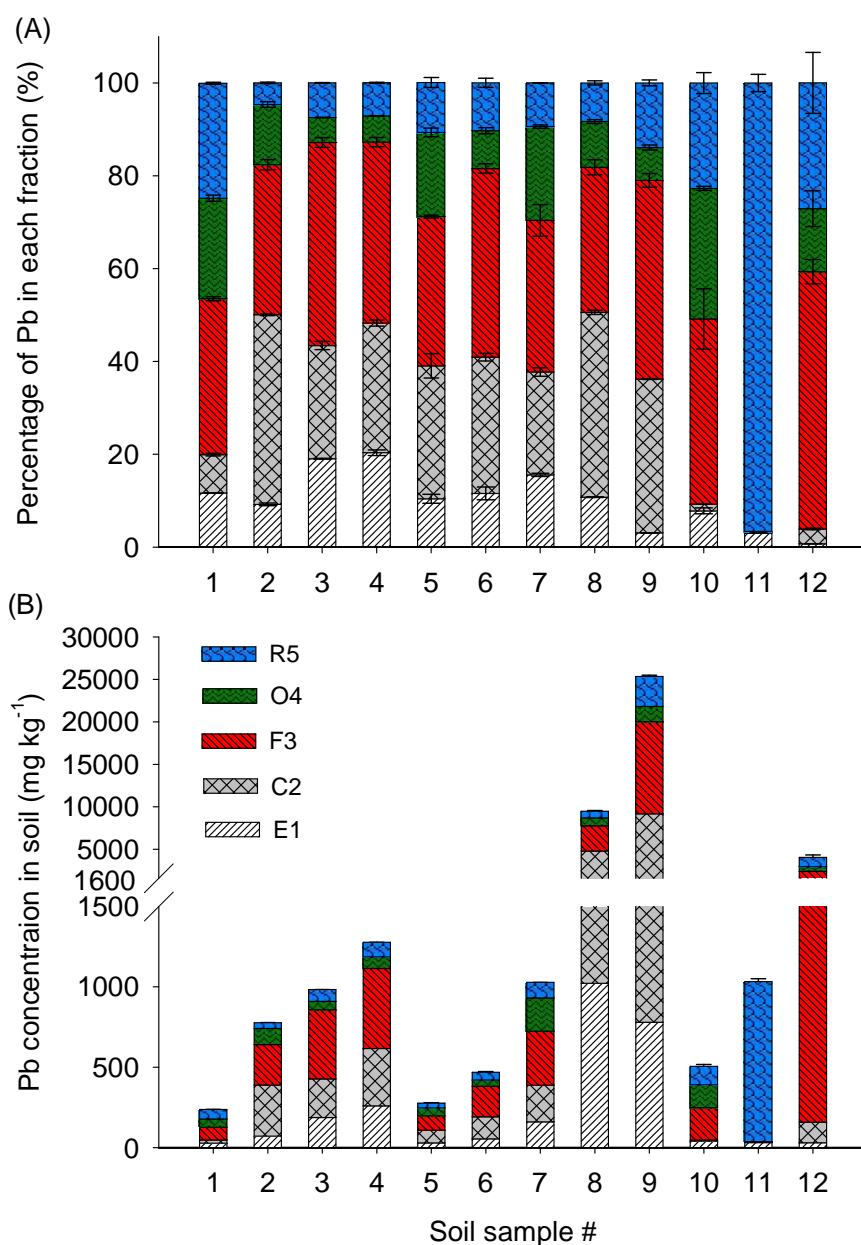


Fig.1.(A)Pbfractionation as % of the sum of 5 fractions and (B) Pb concentration in each fraction in 12 contaminated soils. E1 =exchangeable fraction; C2 =carbonate fraction; F3 =Fe/Mn oxides fraction; O4 =organic fraction; and R5 =residual fraction.Bars represent the mean and standard deviations of triplicates.

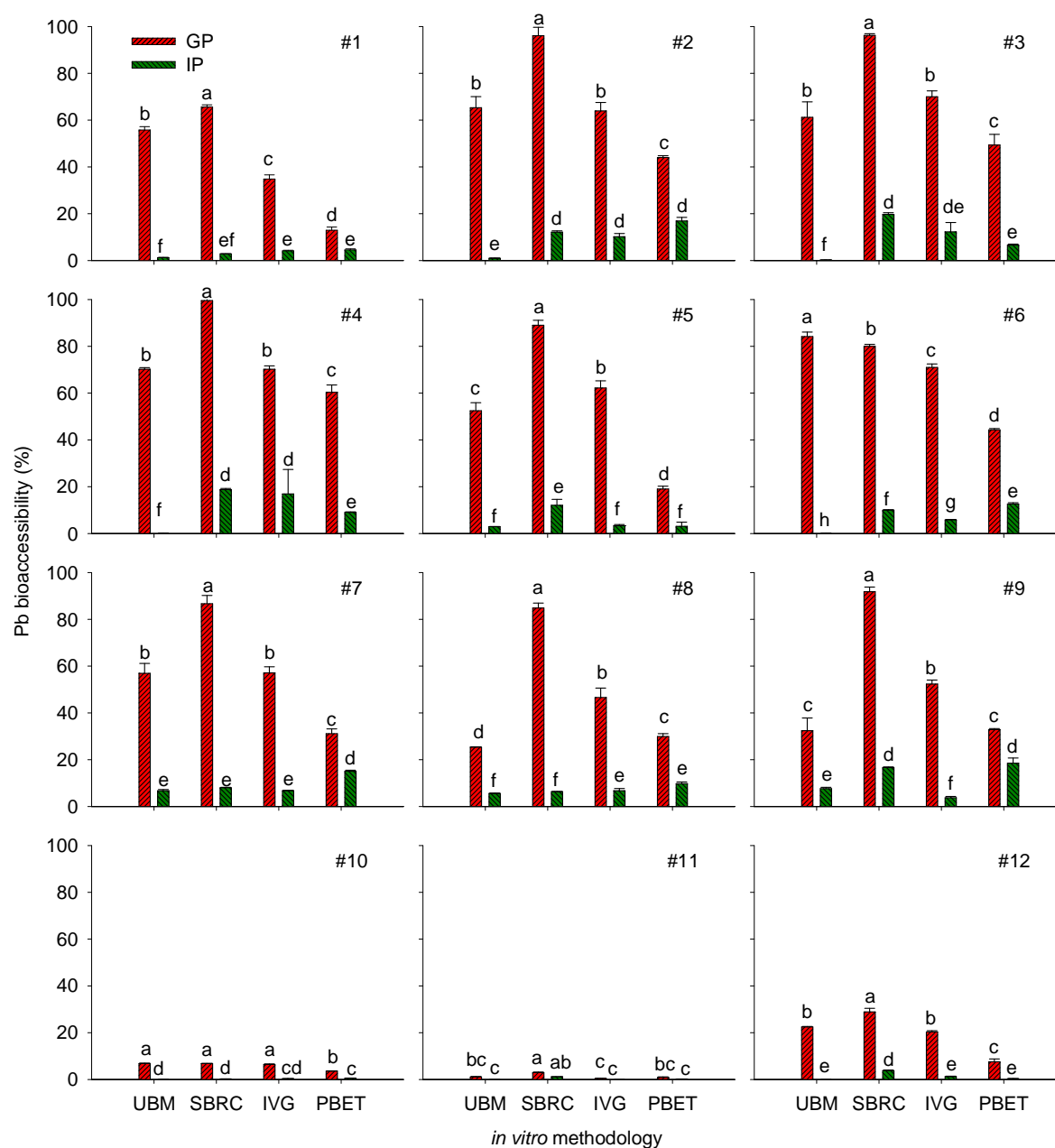


Fig. 2. Lead bioaccessibility in 12 soils based on the gastric (GP) and intestinal phases (IP) of UBM, SBRC, IVG, and PBET assays. Bars represent the mean and standard deviations of triplicates. Data with different letters indicate significant ($p < 0.05$) differences in Pb bioaccessibility for a given soil.

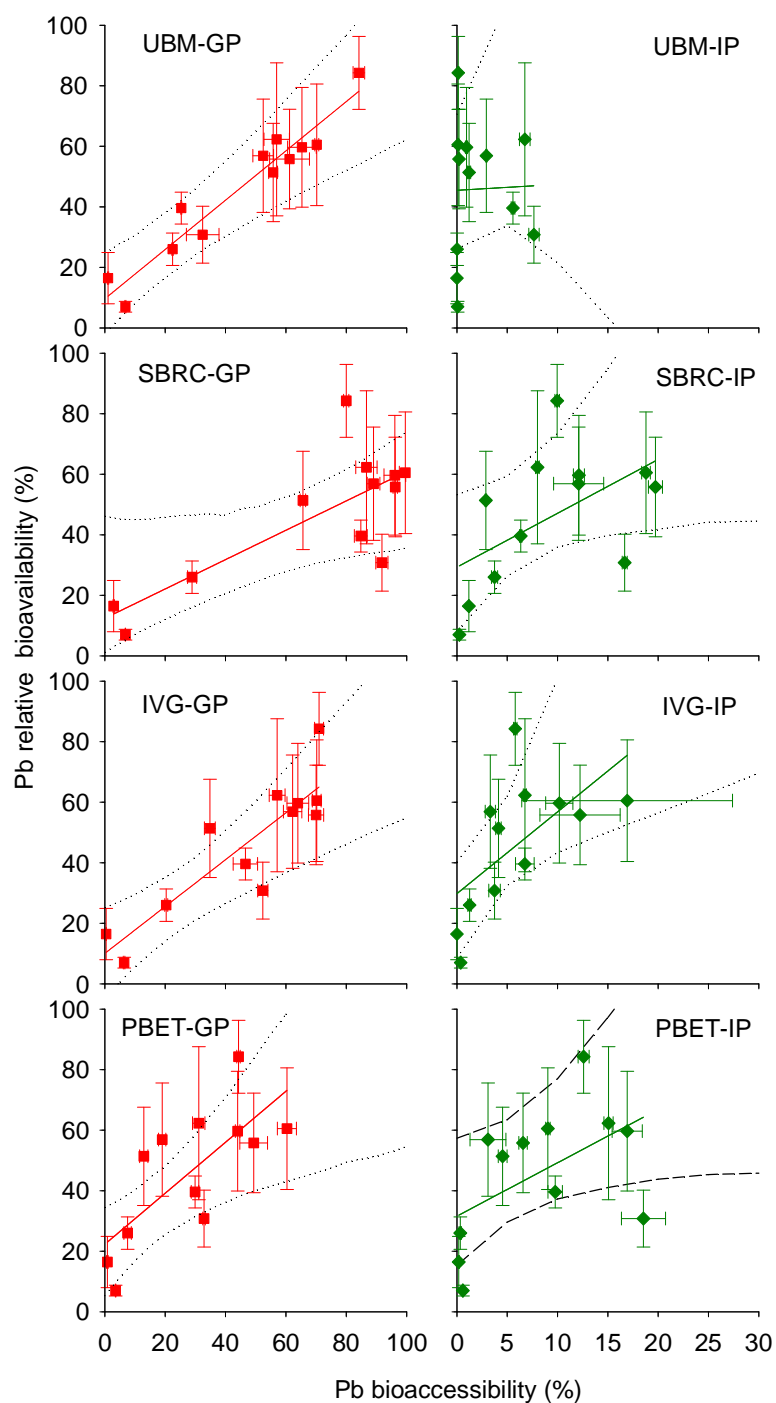


Fig.3. Comparison of *in vitro* Pb bioaccessibility based on the gastric (GP) and intestinal phases (IP) of UBM, SBRC, IVG, and PBET assays with *in vivo* Pb relative bioavailability using a mouse blood assay in 12 contaminated soils. The solid lines show the best line of fit, while dotted lines show the 95% confidence intervals.

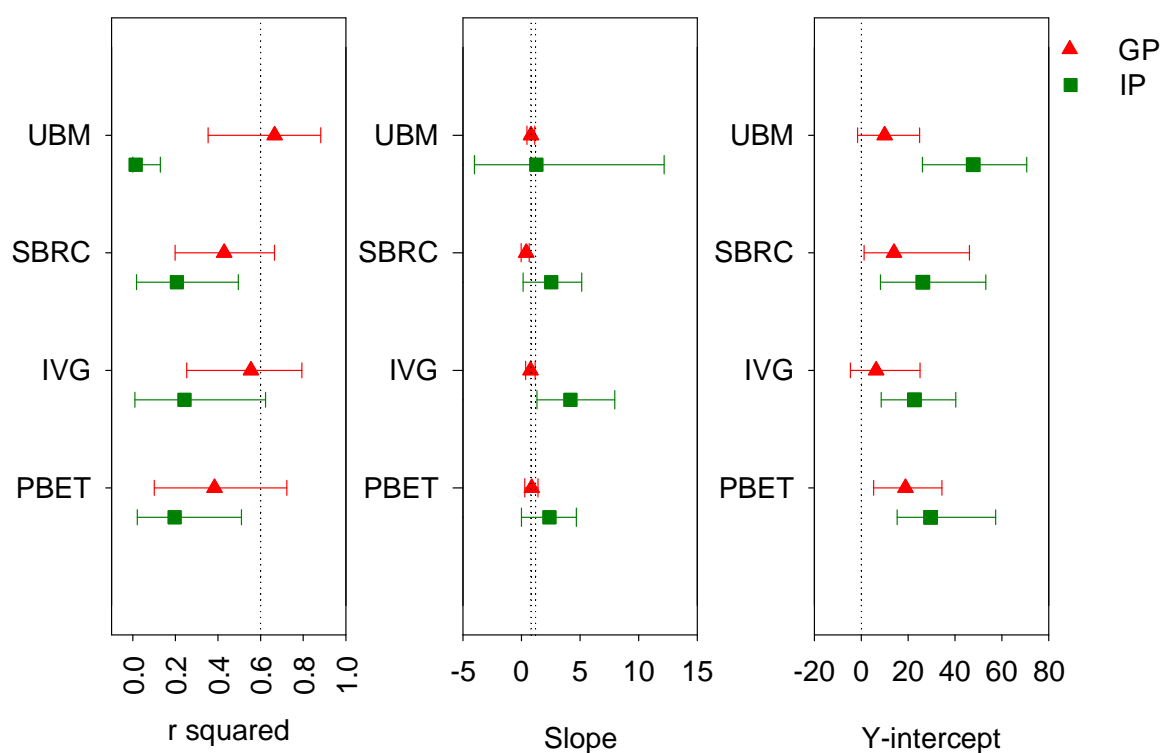


Fig.4. Mean and 95% confidence limits of regression statistics (i.e., r^2 , slope, and y intercept) between Pb relative bioavailability and Pb bioaccessibility for UBM, SBRC, IVG, and PBET gastric (GP) and intestinal phase (IP). Error bars represent 95% confidence limits, dotted lines show benchmark values ($r^2 > 0.6$; $0.8 < \text{slope} < 1.2$, y intercept of ~ 0) suggested by Wragg et al.[21].