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 fractionsattributed most to bioavailable Pb in soils

ABSTRACT

This studyinvestigatedthe relationship between Pbrelative bioavailability (RBA)and bioaccessibility, and their relationships with Pbin differentpools in soils.TwelvePb-contaminated soils representing different contamination sourcesfrom China were analyzed forPbbioaccessibilityusing four *in vitro*methods (UBM, SBRC, IVG, and PBET), Pb-RBAusing amouseblood 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 bioaccessibilityvaried with assays, with SBRC (3.0–99%)producing significantly higher bioaccessible Pbthan other assays (0.46–84%) in the gastric phase. However, Pb bioaccessibilityin the intestinal phase (IP) of all assays sharply decreased to 0.01–20%possibly due to Pbsorption to solid phase at higher pH. Lead bioaccessibility by UBM-GPassay 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 andthe sum of exchangeable and carbonate fractions. Our study suggested thatUBM-GP assay has potential to determine Pbbioaccessibility in contaminated soils in China.

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

1. Introduction

Lead (Pb) exposure has been ofmajor public concern due to its well-established adverse neuro-behavioral effects on children[1, 2].After phasingoutof Pb from gasoline and paint, child blood Pblevelshave significantly declinedworldwide[3, 4].However, in Pb-contaminated sites such as mining andsmelting areas, childhood Pbpoisoning 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]. Themean soil ingestion rate for children ranges from 1.2 to 23mg soil per day [9]. Therefore, assessment of Pb exposure through soil ingestion is receivingincreasing consideration [10]. However, risk assessmentsusually assume that allPb in soil isabsorbed into the blood systemic circulationfollowing oral ingestion (i.e., 100% bioavailable). In reality, Pb bioavailability in soil is often <100%, depending on soil properties and Pbspeciation[11].

At present, several *in vivo* animal modelsincluding mouse and swine have beenused to assess Pbrelative 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, whichmeasures the soluble Pb fraction from soil in simulatedhuman gastrointestinalfluids[10–11, 15–16]. Commonmethods includeSolubility/Bioavailability Research Consortium method (SBRC), in vitro gastrointestinalmethod (IVG), physiologically based extraction test (PBET), DeutschesInstitutfürNormunge.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 producedifferentbioaccessibility results [10, 15, 22]. Therefore, before these assays can be used to accurately predict Pb-RBAin contaminated soils, they need to be

correlated toPb-RBA determined via animal models.

To date, several studies have correlated Pb bioaccessibility in contaminated soils to Pb-RBAbased ondifferent 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 Pbbioaccessibilitybythe IVG gastric phase andPb-RBAbased on blood Pb following 15-dsoil dosing to juvenile swine.Strong correlations (r^2=0.78–0.90, n=16) were observed betweenPbbioaccessibilityby UBM and Pb-RBA using a swine model and Pb in the kidney after 14-dsoil dosing[16]. Smith et al.[11]showed strong correlations(r^2=0.78 and 0.88, n=12) betweenPbbioaccessibility by SBRC andPb-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 investigatedthe variability of IVIVC within assays.

Compared to limited *in vivo*Pb-RBA studies, manystudies investigated Pbfractions in soils using sequential extraction [23, 24]. The methodofTessier et al. [25]is the most widely used, which operationally separatesPb into5 fractions, i.e., exchangeable Pb, and Pb associated with carbonates, Fe/Mn oxides, organic matter, and residualfractions, showing decreasing bioavailability. For example, Pb inthe carbonate and Fe/Mn oxides bound fractionsis more bioavailable than Pbinthe residual fraction [26, 27].However, correlations between Pb fractionation by sequential extraction and Pb bioaccessibilityare rarely established [23, 28].

For this study, we used a mouseblood model viaareas under the blood Pb concentration time curve (AUC) to measure Pb-RBA in soilswithPb 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 wasto investigate the relationship between Pb-RBAand Pbbioaccessibility and their relationships with Pbin differentfractions in contaminated

soils.This was accomplished by 1)correlatingPb-RBAin 12 Pb-contaminated soils via a mousebloodmodelwith Pbbioaccessibilityby four assays, and 2) correlatingPb bioaccessibility and Pb-RBA in soils with Pbin different fractions via sequential extraction.Establishing Pb*in vivo–in vitro* correlations using contaminated soils fromChina provides an extensionof the current knowledge of Pbbioaccessibility andPb-RBA done in other parts of the world. Correlations between Pb-RBAand Pbin differentfractions provide an insightof how Pbfrom differentpools contributes toPb-RBA.

2. Materials and Methods

2.1. Leadcontaminated 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 sitesin 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 muchattentionrecently[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-driedand sieved to<250μmfor bioavailability and bioaccessibility assessment. This fraction most likely adheres to children's hands and is ingested via hand-to-mouth contact[31].

Soilsweredigested using USEPA Method 3050Band analyzed for totalPb concentrationsby 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±1.56% and 101±5.96% (n=3).

2.2. In vivo Pb bioavailability in soils

Adult Balb/c mice with a body weight (bw) of 20–25gwere 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 waterat all times. Mice werequarantined according to standard protocols at Nanjing University before starting the treatment.

Preliminary tests of Pb acetate absorption pharmacokinetics in the micewere conducted (Fig. S1). A single dose (0.5 mL) of two Pb acetate solutions(250 and 500 mg L^{-1}) wasadministered to fasted mice via gavage, which resulted in Pb dose levels of 5 and10 mg Pb kg^{-1} bw. The absorption kinetic curve was established by sacrificing 4 mice and collecting blood samples into heparin tubesat 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 Pbconcentration rangeadministered,suggesting that Pb absorption was linearly dose-dependent.

Similarly, toassessPb relative bioavailability (RBA) in soil, a single dose of soil suspensioncontaining 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 Pbconcentrationfollowing soil ingestion was established by sacrificing 4 mice at each time point (4, 8, 16, 24, and 48 h) after soil exposure and determiningPbconcentration in blood samples.Lead RBA was calculated using AUC withzero 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$AUC_{oralsoil}$ and AUC_{oralPb}= area under the blood Pb concentration time curve for Pb-contaminated soil and Pbacetate,and $DR_{oralsoil}$ and DR_{oralPb}=Pbdose for Pb-contaminated soil and Pbacetate (mg $Pbkg^{-1}$ mouse bw).

2.3. In vitro Pbbioaccessibility 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 soilwasmixed withthe gastric phase (GP) solution in high density polyethylene tubes at soil:solution ratio of1:37.5 (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 PBETusingconcentrated HCl. The mixtureswerehorizontallyshakenat 37°C and 150rpm for 1h. 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 usingconcentrated 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 (0.45μm), stored at 4°C before analysis of Pband Fe usingICP–MS and FAAS.

Afterthe 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(TableS1). The soil:solution ratio of UBM was increased to 1:97.5 in the intestinal phase. After 1h (IVG) or4 h (PBET, SBRC, and UBM) of extraction, thesoil slurry was centrifuged and supernatantsampleswere filtered (0.45μm),acidifiedby adding 200 μLconcentrated HNO_3,and stored at 4°Cbefore analysis

usingICP–MS and FAAS.

Leadbioaccessibilitywas calculated by dividing extractablePbin the gastric phase or intestinal phase of *in vitro* assays by total Pb in soil samples (<250 μm):

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

For QA/QC, a standard soil reference material(NISTSRM 2711a, National Institute of Standards and Technology) was included. BioaccessiblePb based on the gastric phase of UBM, SBRC, IVG, and PBET assays were 926±5.11, 1,089±91.0, 1,069±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 extractionof soils

Lead in the soils was fractionated according to Tessier et al. [25]. Lead fractions were operationally defined as follows: exchangeable, carbonate-bound, Fe/Mnoxides-bound, organic-bound, and residual fractions. Following each extraction, soil solutions were centrifuged at 10,000 rpm for10 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 (0.45μm), diluted with 0.1 M HNO_3, and analyzed using ICP–MS.In the sequential extraction procedure, recoveriesof Pb (ratios of sum of five fractions to total Pb concentrations) in soil samples ranged from 75.0 to 118%, averaging 97.0±11.4%.

2.5. Data processing and statistical analysis

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

deviations.Differences in Pbbioaccessibilityamong *in vitro* methods were performed using variance analysis (ANOVA) based on Tukey's multiple comparisonsusingSAS version 9.1.3. Linear regression analysis between Pb bioaccessibility and Pb-RBAwasconducted 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 Pbcontaminated soils

The 12 Pb-contaminated soils were collected from different locations in China, representingPbcontamination from agricultural, smelting, and mining activities. The soilsvariedconsiderably in total Pb and Fe(Table 1). Lead concentrations in the soils varied by 2 fold, rangingfrom 215to 25,329 mgkg^{-1}.Soils 8 and 9 with the highest Pbconcentrations(9,958 and 25,329 mgkg^{-1}) werefrom asmelting area. Total Fe concentrations were 20.7–219 g kg^{-1}, with the highestconcentration 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 Pbconcentrationsindifferentfractions (Fig. 1). Lead in the firstfraction 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), averaging10, 22, 35, 13, and 20%, with F3>C2>R5>O4>E1.Similar Pbdistribution in contaminated soils was obtained by Jalali and Khanlari[34]withC2>R5>O4>F3>E1.In allsoilsexcluding 11, C2+F3 accounted for 41–76%, indicating that Pbwasmainly associated withcarbonate and Fe/Mnoxide fractions. For soils from farming and smelting areas, 20–51%of the Pbwas in E1+C2 fraction, however, they accounted for much less(3.4–9.3%) in soilsfrom mining area. Soil 11 from mining

areacontained97% Pb in the residual fraction, suggesting low Pbbioavailability. Based on fractionation data, Pbbioavailabilitycouldvary greatly with soils.

3.2. Pb bioaccessibility and relative bioavailability varied with soils

Lead bioaccessibilityand Pb-RBA varied withthe 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 strickingly similar.Based on the gastric phase, Pbbioaccessibility 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 Pbbioaccessibility [16, 18]. Likewise, Pb-RBAusing 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). ThesePb-RBA values fall within the Pb-RBArange 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-RBAcompared to other soils.

3.3. Pb bioaccessibility in the gastric phase

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

phase, Pbbioaccessibilityin soils using UBM, SBRC, IVG, and PBET was 1.1–84, 3.0–99, 0.46–71, and 0.85–60%, averaging44, 69, 46, and 28%with the order of SBRC>UBM= IVG>PBET. Generally, the highest Pbbioaccessibility was obtained by SBRC andthe lowest by PBET. Similar Pbbioaccessibilityin mining/smelting impacted soils was reported byDenys 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 Pbbioaccessibility between methods arises from the differences in assayparameters. As gastric pH strongly influencesPbsolubility in soils [18],lowergastric pH in SBRC (pH 1.5) than IVG and PBET (pH 1.8, and 2.5) partiallyattributed to thehigherPbbioaccessibilityby SBRC. However, pH couldnot explain the results for UBM, which has lower gastric pH value (pH 1.2)than SBRC, butlower Pbbioaccessibility. 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.

Lowsoil:solution ratiomay underestimatePbbioaccessibilityin soils due to limited metal solubility [18]. Compared to SBRC (1:100), UBM usessoil:solution ratio at 1:37.5, which might inhibit Pb dissolution from soils,contributing to its lowerPbbioaccessibility.To test this hypothesis, we increasedsoil:solution ratio of UBM from 1:37.5 to1:100 and used soils2, 7, and 12 for extractions, which represented farming, smelting and mining soils. For all 3 soils, Pbbioaccessibilityusing the modified UBM was increased from 65, 57, and 23% to 88, 82, and 30%,averaging 18% increase(Fig. S2A). These resultssuggestedthat although UBM used a lower gastric pH, its ability to solubilizePb from soils was inhibited by its low soil:solution ratio, leading to lower Pbbioaccessibilitythan 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 Pbbioaccessibility among assays. To test this hypothesis, we used 10 g $L^{-1}$of glycine, pepsin, and mucin solution at pH 1.5 toextract soil 8. Lead bioaccessibilityusing glycine (93%) was significantlyhigher than thatusing pepsin (72%) and mucin(77%) (Fig. S2B). The increased Pbbioaccessibility with glycine was probably related to its strong pH buffering ability [35]. We observed that more HCl (290 μL) was needed to adjust 20 mL of glycine solution to pH 1.5 than thoseneeded for adjusting pepsin and mucin solution (80 and 105 μL) and Milli-Q water (70 μL). Therefore, glycinein the gastric phase ofSBRC is another reason for itshighestPbbioaccessibilityamong the 4 assays, in addition to its low gastric pHand high soil:solution ratio.

3.4. Pb bioaccessibility in the intestinal phase

As pH increased from1.2–2.5 inthe gastric phase to 5.5–7.0 in the intestinal phase, solublePbin the gastric phase isprobably absorbed by soilsand Fe oxides [23, 36]. In the intestinal phase , average Pbbioaccessibilityin soils using UBM, SBRC, IVG, and PBETdecreased from 45 to 2.1%,69 to 9.3%, 46 to 6.0%, and 28 to 8.1%, i.e. by20, 7.4, 7.8, and 3.5 fold, respectively (Fig. 2). Similar decrease in Pbbioaccessibilityby 2–45foldin the intestinal phase of SBRC and IVG has been observed in contaminated soils [17, 37].

Atneutral intestinal solutions, soluble Fe inthe gastric phase precipitates as Fe oxides, which adsorbssolublePb, decreasingPb solubility [11].In this study,for UBM, SBRC, and IVG, decreased Pb concentration in the intestinal phase was accompanied by a sharpdecrease in soluble Fe (Fig. S3A–C), suggestingprecipitation 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 Pbbioaccessibility 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 notprecipitated(Fig. S3D). This may beattributed to citrate in the UBM fluid, inhibiting Fe precipitation in the intestinal phase [33]. However,

Pbbioaccessibilityin the UBM-IP in the 3 soils still sharply decreased from 19–60to 3.1–9.0% (Fig. S3D). This wasattributed to Pbsorption onto soil matrix at pH 7 [11]. Li et al.[33] confirmed that Pb spiked inthe intestinal solutionsisabsorbed ontosolid 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 Pbbioaccessibilityby 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-RBAmeasurements, 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, Pbbioaccessibilityinthe gastric phasewas 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.TheUBM-GP provided satisfactorycorrelation with r^2 >0.6as 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 inthe 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 Pbbioaccessibility in contaminated soils from China. Future studiesare 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 ofPbin different fractionstobioaccessible and bioavailable Pb. Simple linear correlations between Pbbioaccessibility 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, Pbbioaccessibility in the gastric phasewasmore 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 Pbbioaccessibilityinthe 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 Pbbioaccessibility 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 Pbbioaccessibilityby the gastric phasewere examined, y-interceptswere close to 0 (–1.99 to 10.3) (Table 3). Among 4 assays, Pb in E1+C2 was best

correlated with Pbbioaccessibility by SBRC-GP (r^2=0.87) with a slope of 1.87, indicating Pbin E1+C2 was lower thanbioaccessible Pb measured by SBRC-GP.When relationships wereexaminedfor 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 inthe gastric phaseof all 4 assays could bepredicted using Pb inE1+C2, though E1+C2 underestimatedbioaccessible 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-RBAwas better correlated with Pb pools in E1+C2 (r^2=0.52) than Pbin E1, E1+C2+F3, and E1+C2+F3+O4 (r^2=0.32–0.42) (Table 3). This suggested that bioavailablePb also mainly came from Pb in the exchangeableand carbonate fractions.

4.Conclusion

Results from this study suggestedthatPb 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 phaseassay was the best among the four*in vitro* assays tested to predict Pb-RBAin 12 contaminated soils fromChina, though other assays also showed predictive ability.Hence, *in vitro*assays can beuseful in risk assessment of contaminated soils. To reduce human health risk associated with oral ingestion of contaminated soil, remediation measures are neededto reduce exchangeable and carbonate associatedPb 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 foundin the online version.

Abbreviations

Pb	lead
RBA	relative bioavailability
GP	gastric phase
IP	intestinal phase
SBRC	solubility/bioavailability research consortium method
IVG	*in vitro*gastrointestinalmethod
PBET	physiologically based extraction test
DIN	DeutschesInstitutfür Normunge.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) betweenPb relative bioavailabilityand Pb bioaccessibility for gastric (GP) and intestinal phases (IP) of UBM, SBRC, IVG, and PBET assaysin the currentstudy and previous studies in contaminated soils

In vitro assay	Phase	IVIVC parameters			Pb concentration (mg/kg)	No. of soil samples	*In vivo* assay			Reference
		slope	y-intercept	r^2			animal	biomarker	method	
This study										
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						
Previous studies										
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 Pbbioaccessibilitydetermined usingthe gastric (GP) and intestinal phases (IP) of four assays (UBM, SBRC, IVG, and PBET) and Pbin different fractionsand between Pb relative bioavailability (RBA) and Pbin different fractionsin 12 Pb-contaminated soils.

Pb bioaccessibility /RBA		E1*			E1+C2			E1+C2+F3			E1+C2+F3+O4		
		slope	y-intercept	r^2	slope	y-intercept	r^2	slope	y-intercept	r^2	slope	y-intercept	r^2
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 =exchangeablefraction; C2 =carbonatefraction; F3 =Fe/Mn oxidesfraction; O4 =organicfraction; and R5 =residual fraction.
r squared> 0.60 was in bold.

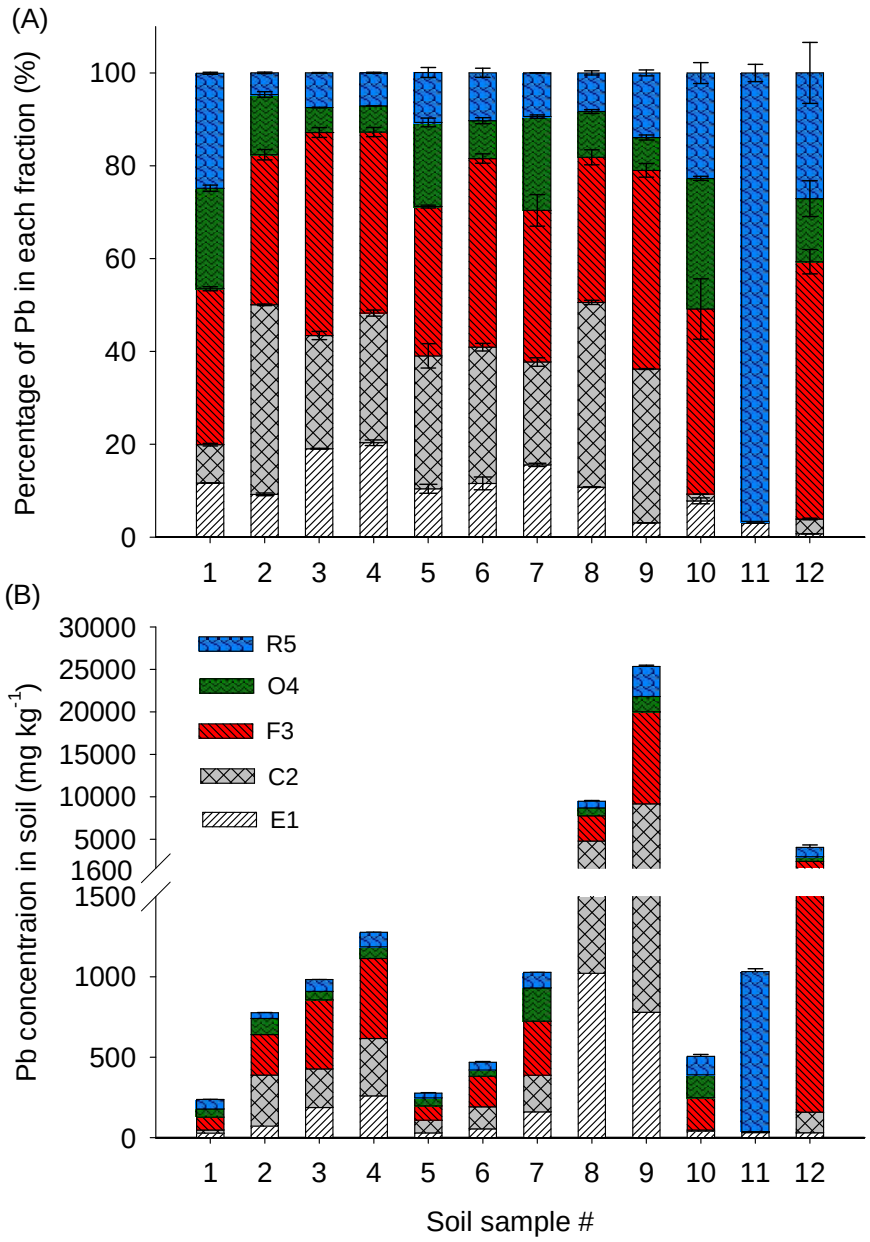

Fig.1.(A)Pbfractionation as % of the sum of 5 fractions and (B) Pb concentration in each fraction in 12 contaminated soils. E1 =exchangeable fraction; C2 =carbonate fraction; F3 =Fe/Mn oxides fraction; O4 =organic fraction; and R5 =residual fraction.Bars represent the mean and standard deviations of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

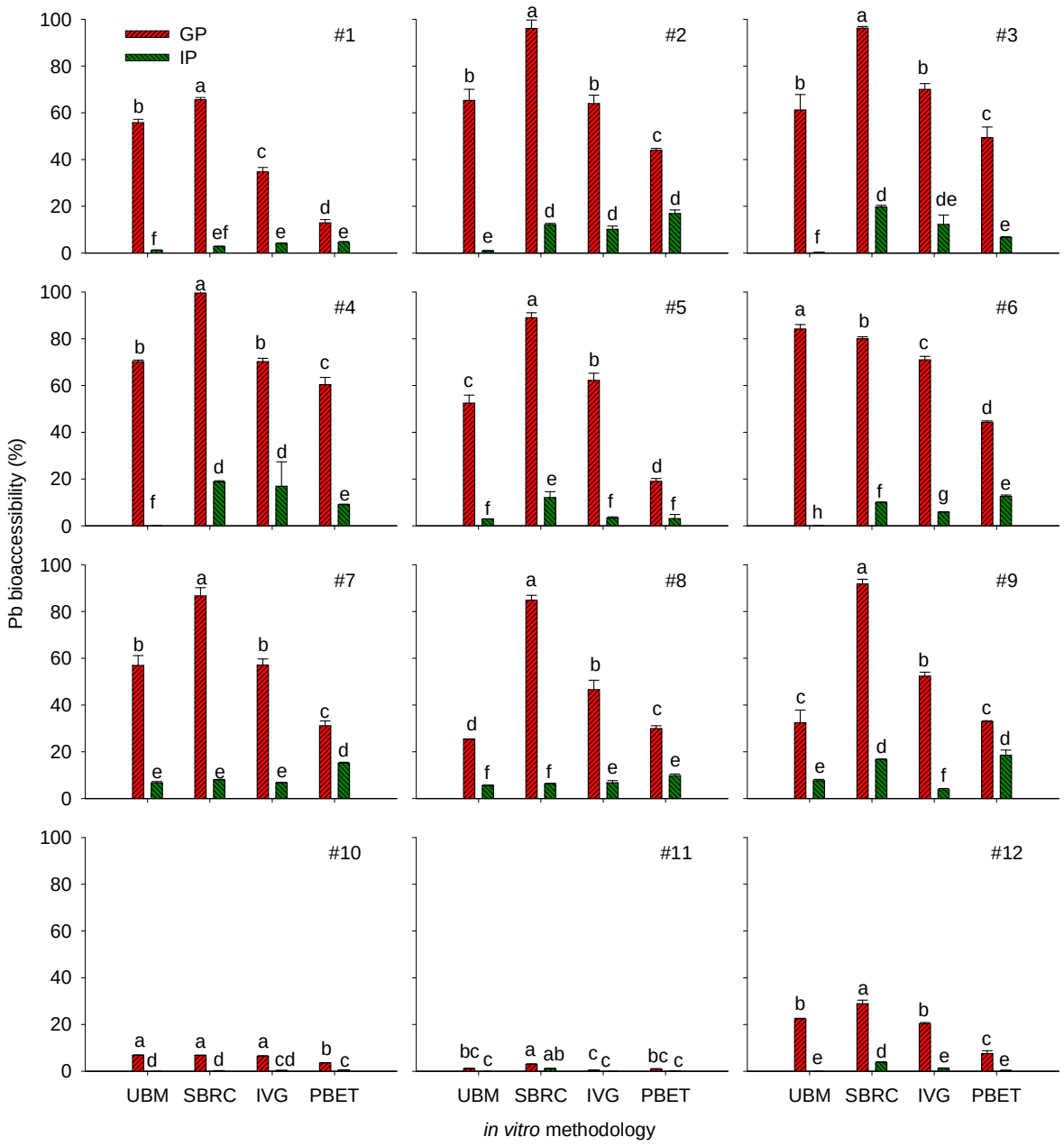

Fig. 2. Lead bioaccessibility in 12 soils based on the gastric (GP) and intestinal phases (IP) of UBM, SBRC, IVG, and PBET assays. Bars represent the mean and standard deviations of triplicates. Data with different letters indicate significant ($p < 0.05$) differences in Pb bioaccessibility for a given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

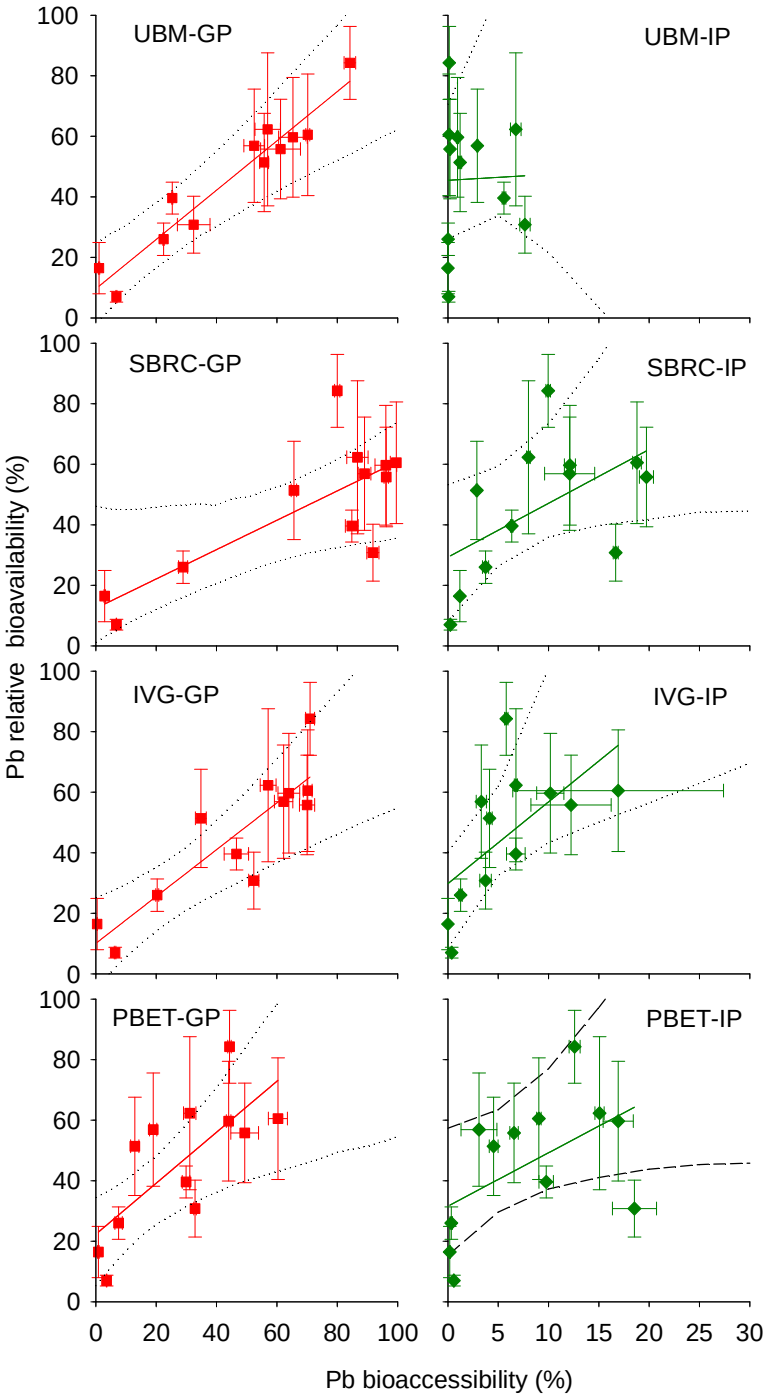

Fig.3. Comparison of *in vitro* Pbbioaccessibility based onthe gastric (GP) and intestinal phases (IP) of UBM, SBRC, IVG, and PBET assays with *in vivo*Pbrelative bioavailability usinga mousebloodassay in 12 contaminated soils. The solid lines show the best line of fit, while dottedlines show the 95% confidence intervals.

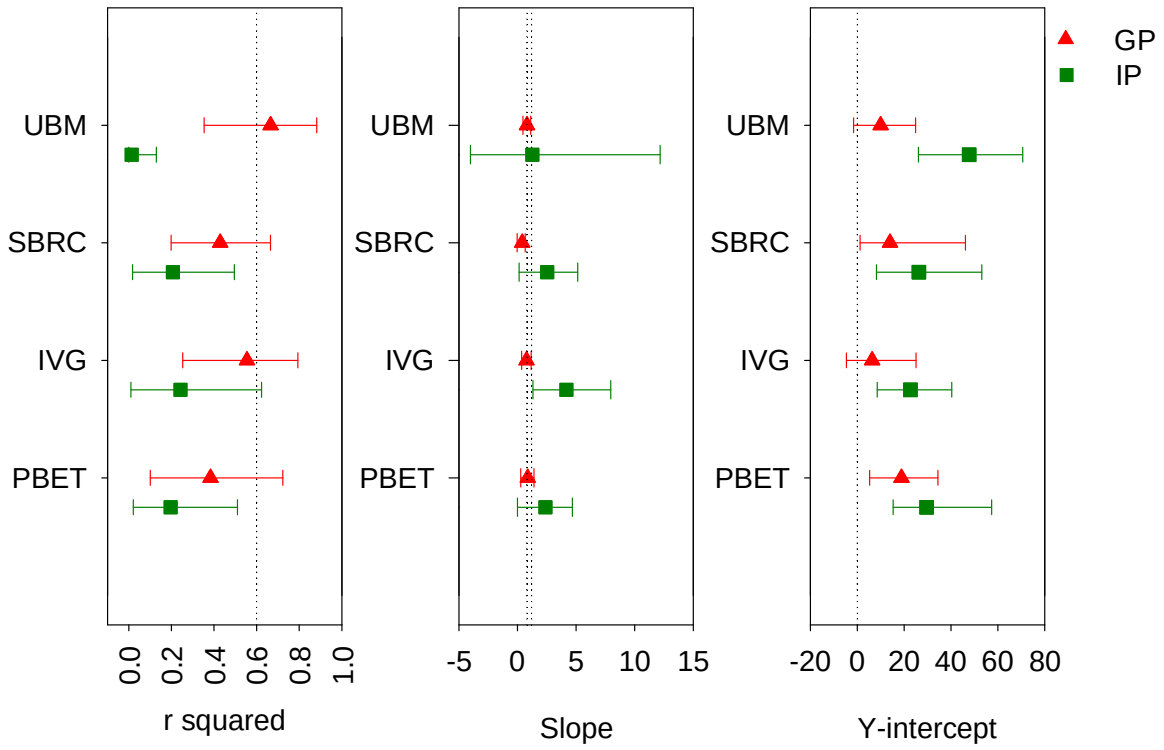

Fig.4. Mean and 95% confidence limits of regression statistics (i.e., r squared, slope, and y intercept) betweenPb relative bioavailability andPbbioaccessibility for UBM, SBRC, IVG, and PBET gastric (GP) and intestinal phase (IP). Error bars represent 95% confidence limits, dotted lines show benchmark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