In vitro bioaccessibility and in vivo relative bioavailability in 12 contaminated soils: Method comparison and method development

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HIGHLIGHTS

• 12 As-contaminated soils were from different locations and contamination sources.
• Five in vitro assays (UBM, SBRC, IVG, DIN and PBET) were used to measure arsenic bioaccessibility in soils.
• A mouse blood model was used to measure arsenic relative bioavailability (RBA) in soils.
• Existing in vivo–in vitro correlation (IVIVC) models failed to predict As-RBA in soils.
• Strong IVIVC suggested IVG and UBM methods can potentially measure As-RBA in these soils.

ABSTRACT

Previous studies have established in vivo–in vitro correlations (IVIVC) between arsenic (As) relative bioavailability (RBA) and bioaccessibility in contaminated soils. However, their ability to predict As-RBA in soils outside the models is unclear. In this study, As bioaccessibility and As-RBA in 12 As-contaminated soils (22.2–4172 mg kg⁻¹ As) were measured using five assays (SBRC, IVG, DIN, PBET, and UBM) and a mouse blood model. Arsenic RBA in the soils ranged from 6.38 ± 2.80% to 73.1 ± 17.7% with soils containing higher extractable Fe showing lower values. Arsenic bioaccessibility varied within and between assays. Arsenic bioaccessibility was used as input values into established IVIVC to predict As-RBA in soils. There were significant differences between predicted and measured As-RBA for the 12 soils, illustrating the inability of established IVIVC to predict As-RBA in those contaminated soils. Therefore, a new IVIVC was established by correlating measured As-RBA and As bioaccessibility for the 12 soils. The strength of the predictive models varied from r² = 0.50 for PBET to r² = 0.83 for IVG, with IVG assay providing the best prediction of As-RBA. When IVIVC were compared to those of Juhasz et al. (2014a), slopes of the relationships were significantly higher possibly due to different As-RBA measurements. Our research showed that IVG has potential to measure As bioavailability in contaminated soils from China though UBM and SBRC assays were also suitable. More research is needed to verify their suitability to predict As-RBA in soils for refining health risk assessment.

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1. Introduction

As a toxic carcinogen, arsenic (As) is considered a priority pollutant in the USA (ATSDR, 2011; IARC, 2004). Soil ingestion is an important non-dietary pathway for human exposure to As through hand-to-mouth activities, especially for children (Ruby and Lowney, 2012). The estimated incidental daily soil ingestion rate is 23 mg for children (Wilson et al., 2013), but as high as 200 mg has been used (Van Wijnen et al., 1990). To accurately assess health risk, it is important to determine As bioavailability in contaminated soils, i.e., the amount of As in soil that enters the systemic circulation in humans (Ruby et al., 1996).

Arsenic bioavailability (RBA) measures the As uptake in the target organ from the soil matrix relative to the uptake from a readily soluble As salt (e.g., sodium arsenate). USEPA (2012) uses the default value of 60% for As-RBA in soils. However, As-RBA varies considerably with contamination sources and is influenced by soil properties such as Fe and P contents (Juhasz et al., 2014a; Smith et al., 2002). Therefore, it is important to measure site-specific As-RBA to refine human As exposure to contaminated soils (USEPA, 2012).

In vivo animal models including swine and mouse have been developed to measure As-RBA in contaminated soils (Bradham et al., 2011; Brattin and Casteel, 2013; Juhasz et al., 2007). However, animal assays are time-consuming and costly. As an alternative, in vitro assays have been developed to determine As bioaccessibility, i.e., the amount of dissolved As in simulated gastrointestinal solution, which is potentially available for absorption into the systemic circulation. Common bioaccessibility methods include the in vitro gastrointestinal (IVG) (Rodriguez et al., 1999), Solubility/Bioaccessibility Research Consortium (SBRC) (Kelley et al., 2002), Deutsches Institut für Normung e.V. (DIN, 2000), physiologically based extraction test (PBET) (Ruby et al., 1996), and unified BARGE method (UBM) (Wragg et al., 2011). These assays all include gastric phase (GP) and intestinal phase (IP), and they vary in gastrointestinal components and analysis parameters. As a result, As bioaccessibility in contaminated soils varies with in vitro assays (Oomen et al., 2002). For example, based on the gastric phase, SBRC and UBM assays provide higher As bioaccessibility in contaminated soils than IVG, DIN, and PBET assays due to their lower gastric pH (1.5 vs. 1.8–2.5) (Juhasz et al., 2009, 2014a).

Given the variation among in vitro assays, a bioaccessibility method needs to be correlated with in vivo animal data before being used as a surrogate of in vivo assay. Several studies have established in vitro–in vivo correlations (IVIVC) between As-RBA and As bioaccessibility using different in vitro assays. For example, Rodriguez et al. (1999) demonstrated a good correlation ($r^2 = 0.67–0.69$) between As bioaccessibility by IVG method and As-RBA using a swine model in 15 contaminated soils from the USA. Similarly, based on a swine model, Juhasz et al. (2009) correlated As bioaccessibility by the gastric phase of SBRC method ($r^2 = 0.75$) with As-RBA in 12 contaminated soils from Australia. Denys et al. (2012) validated the UBM method to assess As-RBA in 16 contaminated soils from Europe ($r^2 = 0.8$). In addition to swine model, a mouse model has also been used to validate in vitro assays. Bradham et al. (2011) showed a strong correlation between As bioaccessibility by the gastric phase of SBRC method and As-RBA by a mouse urine model in contaminated soils from the USA ($r^2 = 0.88$). These studies demonstrated that As-RBA in contaminated soils can be predicted using in vitro methods and the established in vivo–in vitro correlations (IVIVC).

However, established IVIVCs are often soil specific, varying with the soils used. For example, when established IVIVC for SBRC, IVG, DIN, PBET, and UBM assays using Australian soils (Juhasz et al., 2009) were compared to those using American soils (Juhasz et al., 2014a), significant differences in slope and y-intercept of the relationships were observed. The large difference makes the prediction of As-RBA using established IVIVC less accurate. In addition, established IVIVC have rarely been validated against soils outside those used to establish IVIVC except for the gastric phase of SBRC method by Juhasz et al. (2014b). Therefore, it remains unclear whether established IVIVC have the ability to predict As-RBA in contaminated soils outside the models. Furthermore, to date, no study tested the feasibility of in vitro assays to predict As-RBA in contaminated soils from China (Zhao et al., 2015).

The objective of this study was to 1) investigate the feasibility of established in vitro–in vivo correlations (IVIVC) in predicting As-RBA in contaminated soils in China, and 2) develop IVIVC for As-contaminated soils from China. Arsenic bioaccessibility in 12 contaminated soils from China based on five in vitro assays (UBM, SBRC, IVG, DIN, and PBET) was used as input values into established IVIVC using contaminated soils from Australia (Juhasz et al., 2009, 2011) and the USA (Juhasz et al., 2014a) to calculate As-RBA. Calculated As-RBA values were then compared to the As-RBA based on a mouse blood model to determine the applicability of established IVIVC to contaminated soils from China. In addition, an IVIVC was established based on our data by correlating As-RBA with As bioaccessibility in 12 contaminated soils. Our results suggest the importance of choosing suitable in vitro assays based on an in vivo animal model to assess As-RBA in soils from different countries.

2. Materials and methods

2.1. Soil collection and characterization

Twelve As-contaminated soil samples were collected from 8 cities in China, representing different geographical locations and contamination sources (farming, mining, and smelting sites; Table 1). Soils were air-dried and sieved to <250 μm for bioavailability and bioaccessibility assessment. Citrate dithionite extractable Fe contents were determined using the method of Holmgren (1967) and measured using flame atomic absorption spectrometry (FAAS, PerkinElmer, Pinnacle 900T, USA). Soil was digested using USEPA Method 3050B (USEPA, 1996) and analyzed for total As using inductively coupled plasma mass spectroscopy (ICP-MS, PerkinElmer NexION 300, USA) and total Fe and Mn using FAAS.

2.2. Assessment of As bioaccessibility based on five assays

Juhasz et al. (2009, 2014a) established in vivo–in vitro correlations (IVIVC) between As-RBA and As bioaccessibility assessed using five in vitro assays, i.e., UBM, SBRC, IVG, DIN, and PBET. To investigate whether the established IVIVC are suitable to predict As-RBA in contaminated soils from China, we used the same assays to determine As bioaccessibility. The composition of gastrointestinal solution and operation protocols of the assays are summarized in Table S1. Arsenic bioaccessibility assessment was conducted according to Wragg et al. (2011), Kelley et al. (2002), Rodriguez et al. (1999), DIN (2000), and Ruby et al. (1996). Detailed information was provided in the Supporting Information.

2.3. Assessment of As relative bioavailability based on a mouse blood model

In vivo studies were conducted using adult female Balb/c mice with body weight (BW) of 18–23 g. Animals were acclimated in groups of 4 in clear plastic cages with shaving bedding and received a 12/12 light/dark photocycle at 20–22 °C. Mice had free access to Milli-Q water and rodent diet obtained from Qinglongshan Experimental Animal Breeding Farm (Nanjing, China). All mice were cared according to the Guide for the Care and Use of Laboratory Animals, which were approved by the Ethics Committee of Animal Experiments of Nanjing University. Before the in vivo test, mice were fasted for 24 h.

Initially, As absorption pharmacokinetics in mouse blood after sodium arsenate ingestion was investigated by administering 0.5 mL of 5, 25, and 250 mg L$^{-1}$ As to mice of ~20 g of BW via gavage. This resulted in As doses of 0.125, 0.625, and 6.25 mg As kg$^{-1}$ BW. To assess
As RBA in a soil, a single dose of soil suspension containing 0.25 g of soil in 0.75 mL of Milli-Q water was administered to mice via gavage. The control group received only Milli-Q water without soil. At each time interval (4, 8, 16, 24, and 48 h) after sodium arsenate and soil exposure, 4 mice were sacrificed and blood samples were collected into heparin tubes and digested using USEPA Method 3050B. Following digestion, samples were analyzed for As using ICP–MS to establish the blood As concentration time curve. Calculations of the area under the blood As concentration time curve (AUC) indicated a linear dose response to concentration time curve. Calculations of the area under the blood As concentration curve (AUC) were 557, 2928, and 27,607 for As-contaminated soil and sodium arsenate, respectively; and DRoral-soil As dose of orally administered soil and sodium arsenate (mg As kg−1 BW), respectively.

where AUCoral-soil = AUC for an oral dose of As-contaminated soil and sodium arsenate, respectively; and DRoral-soil As dose of orally administered soil and sodium arsenate (mg As kg−1 BW), respectively.

2.4. Application of established in vivo–in vitro correlations to contaminated soils

The in vivo–in vitro correlations (IVIVC) using 12 contaminated soils from Australia (Juhasz et al., 2009, 2011) and 10 contaminated soils from the USA (Juhasz et al., 2014a) were established (Table S2). To predict As-RBA in 12 As-contaminated soils from China, As bioaccessibility data were used as input values into the established IVIVC. The predicted values were expressed as an average of three replicate assays. To verify the feasibility of the established IVIVC to predict As-RBA in contaminated soils from China, the predicted values were compared to the measured As-RBA. In this study, we chose mouse as the animal model and As absorption in the blood after a single dose as the biomarker to determine As-RBA in soil samples. This method has been used to assess lead-RBA in contaminated soils (Smith et al., 2011).

2.5. Quality assurance and quality control

During analyses of As concentrations and As bioaccessibility in soil samples, a soil Standard Reference Material (SRM NIST 2711a, National Institute of Standards and Technology) was included for quality assurance/quality control (QA/QC). The total As was 86.0 ± 0.81 mg kg−1 using USEPA 3050B method, within the certified range of 81.0–110 mg kg−1 (Mackey et al., 2010). Bioaccessible As in the SRM in gastric and intestinal phases was 60.7 ± 1.63 and 46.8 ± 0.60 mg kg−1 for SBRC assay, 50.0 ± 3.42 and 43.9 ± 1.63 mg kg−1 for IVG assay, and 49.4 ± 1.15 and 49.3 ± 2.94 mg kg−1 for PBET assay, consistent with a previous result of 54.4 ± 3.00 mg kg−1 based on SBRC gastric phase (Li et al., 2014a).

During ICP–MS measurement, triplicate analyses, check standard and spiked solutions were analyzed every 20 samples. Check recovery was 97.2 ± 1.31%, while spike recovery was 95.1 ± 4.60%. The relative standard deviation of triplicate analyses was 3.70%.
2.6. Data processing and statistics

All in vitro assays were performed in triplicate and animal experiments were with four replicates. The results were presented as mean values ± standard deviation. Differences in bioaccessibility among in vitro methods were performed using variance analysis based on Tukey’s multiple comparisons using the software SAS version 9.1.3. Linear regressions between As bioaccessibility and As relative bioavailability were developed. The differences in slopes and intercepts of in vivo–in vitro correlations among in vitro assays were performed using the software GraphPad Prism 5. All graphs were drawn using SigmaPlot 10.0.

Differences between predicted and measured As-RBA were quantified by calculating mean bias error (MBE) and root mean square error (RMSE) (Juhasz et al., 2014b):

\[
MBE = \frac{1}{n} \sum_{i=1}^{n} (RBA_{predicted} - RBA_{measured})
\]

\[
RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^{n} (RBA_{predicted} - RBA_{measured})^2}
\]

3. Results and discussion

3.1. Characteristics of As-contaminated soils

Table 1 shows selected properties of the 12 As-contaminated soils, which represent different locations and contamination sources in China. Total As concentrations ranged from 22.2 to 4172 mg kg⁻¹, with farming soils generally had lower As concentrations (36.4–171 mg kg⁻¹) than soils from mining (75.2–1470 mg kg⁻¹) and smelting areas (22.2–4172 mg kg⁻¹). Mining soils generally contained significantly higher total Fe and citrate–dithionite extractable Fe than farming and smelting soils. High Fe concentrations (120–294 g kg⁻¹) have been reported in smelting soils from the USA (Bradham et al., 2011). Total Mn ranged from 142 to 9807 mg kg⁻¹.

3.2. As bioaccessibility varied among in vitro assays

Reliable assessment of human health exposure to incidental ingestion of As-contaminated soils depends on accurate measurement of As bioavailability. Since in vivo animal tests are time-consuming and costly, in vitro assays have been developed to determine As bioaccessibility in 12 contaminated soil samples (S1–S12) determined using the gastric and intestinal phases of UBM, SBRC, IVG, DIN, and PBET methods. Data are shown in mean ± standard deviation of 3 replicates. Means marked with different letters indicate significant (p < 0.05) differences in As bioaccessibility for each soil.
bioaccessibility in soils. However, they need to be correlated with in vivo data before being valid. Juhasz et al. (2009, 2014a) have established correlations between five in vitro assays (UBM, SBRC, IVG, DIN, and PBET) to an in vivo test. Therefore, we used the same five assays to determine As bioaccessibility in 12 contaminated soils from China (Fig. 2).

As detailed in Fig. 2, As bioaccessibility varied considerably with assays. Based on gastric phase, As bioaccessibility was 7.59–59.4% by UBM (averaging 29.4%), 2.33–66.1% by SBRC (25.2%), 7.26–57.5% by IVG (20.2%), 0.30–83.1% by DIN (21.5%), and 1.32–50.1% by PBET method (16.1%), with the order of UBM > SBRC > IVG > DIN > PBET. The average As bioaccessibility was similar to those by Juhasz et al. (2009) at 32.5, 31.7, 22.0, 15.4, and 19.8%. Higher values by UBM and SBRC assays were attributed to their lower pH at 1.2–1.5 compared to 1.8–2.5 (Juhasz et al., 2009, 2014a). However, in some soil samples, variation of As bioaccessibility did not follow this trend. For example, for soils 3 and 8, the gastric phase of IVG or PBET assay provided the most conservative results, while for soil 11, UBM was the lowest. This might be related to their differences in soil properties, for example, soils 3 and 8 had the lowest extractable Fe concentrations while soil 11 had the highest (Table 1).

In the intestinal phase, As bioaccessibility in the soils was 5.70–58.8% by UBM (averaging 30.6%), 0.63–37.1% by SBRC (15.4%), 2.31–45.7% by IVG (19.8%), 2.10–37.7% (18.0%) by DIN, and 0.86–45.2% by PBET assay (18.7%), with the SBRC assay generally providing the lowest values (Fig. 2). Compared to gastric phase, As bioaccessibility by the intestinal phase of SBRC assay was significantly lower for most samples possibly due to the precipitation of dissolved Fe in the neutral condition at pH 7 in the intestinal phase (Fig. 3) and/or As adsorption onto the newly formed amorphous Fe (Juhasz et al., 2009, 2014a). In contrast to SBRC assay, As bioaccessibility didn’t decrease in the intestinal phase for UBM, IVG, DIN, and PBET assays (Fig. 2). For example, As bioaccessibility in soil 2, 5, 6, 9, and 12 was increased by 1.1–2.8 fold from the gastric to intestinal phase of PBET assay, which corresponded to increased soluble Fe concentrations of 2.3–6.3 fold (Fig. 3). The increased As bioaccessibility in the intestinal phase of PBET has been attributed to elevated Fe dissolution in PBET’s intestinal fluid due to organic acids (Juhasz et al., 2014a). The organic acids such as citrate in the PBET intestinal fluid probably inhibited Fe precipitation in the intestinal phase (Li et al., 2014b). However, unlike SBRC assay, As bioaccessibility by UBM and IVG didn’t show a corresponding decrease with decreased soluble Fe (Figs. 2 and 3), which may be attributed to their different

**Fig. 3.** Amount of Fe extracted from soils during the gastric and intestinal phase extractions of 5 in vitro assays (UBM, SBRC, IVG, DIN, and PBET). Data are shown in mean ± standard deviation of 3 replicates. Means marked with different letters indicate significant (p < 0.05) differences in soluble Fe for each soil.
gastrointestinal constituents (pepsin and mucin) compared to the SBRC assay (glycine) (Table S1) (Smith et al., 2014).

3.3. Assessment of As relative bioavailability based on a mouse blood model

To test whether an in vitro assay is a suitable alternative to in vivo test, they need to be correlated to As-RBA based on animal model. In this study, we used a mouse blood AUC single gavage method (Smith et al., 2011). Compared to swine, mouse is relatively inexpensive and easier to take care of. In addition, As absorption in mouse blood was linearly dose-dependent (Fig. 1). The AUC values for sodium arsenate doses of 0.125, 0.625, and 6.25 mg As kg\(^{-1}\) BW mice were 557, 2928, and 27,607. Another advantage of the methodology is that mice are fasted before gavage. Compared to mouse steady state urinary excretion model, which measured As concentration in urine following multiple doses of soil or sodium arsenate in feed (Bradham et al., 2011), measuring mouse blood As concentration following a single gavage dose eliminates the potential effects of feed on As-RBA in soils. As a consequence, As-RBA measurement using the single gavage dose and mouse blood AUC approach may represent a worst-case scenario for As exposure.

Arsenic RBA in the 12 soils was 6.38–73.1%, averaging 29.0% (Table 1). Our study was the first to determine As-RBA in contaminated soils based on the mouse blood AUC single gavage method. But, the values were similar to those in contaminated soils measured using swine blood AUC single gavage model (6.98–80.5%, averaging 33.1%) and mouse steady state urinary excretion multiple dose model (11.1–52.8%, averaging 32.3%) (Bradham et al., 2011; Juhasz et al., 2007). Among the 12 soils, arsenic RBA was significantly lower in the 3 mining soils. In addition, soil 2 (farming soil), and soils 10 and 11 (smelting soils) also contained low As-RBA (Table 1). The low As-RBA for these soils was possibly due to their higher extractable Fe contents (25.3–50.9 vs. 8.29–16.7 g kg\(^{-1}\)). Similar association of low As-RBA with Fe oxides has been reported (Bradham et al., 2011; Juhasz et al., 2014a).

3.4. Prediction of As-RBA using established in vivo–in vitro correlations

Since As bioaccessibility varied considerably with assays used, in vitro assays need to be validated before they can be used as a surrogate of in vivo test. Several studies have established in vivo–in vitro correlations (IVIVC) between As-RBA and As bioaccessibility in contaminated soils, showing the potential of in vitro assays to predict As-RBA (Juhasz et al., 2009, 2014a). However, these models have rarely been validated using soils outside the model (Juhasz et al., 2013). Therefore, we performed a validation test of the predictive ability of established IVIVC by Juhasz et al. (2009, 2011, 2014a) for As-RBA in 12-contaminated soils from China. Arsenic bioaccessibility measured using 5 in vitro assays was entered into the two established IVIVC to obtain predicted As-RBA, then a comparison between predicted and measured As-RBA was made. As detailed in Fig. 4, predicted As-RBA using established IVIVC was significantly different from measured As-RBA for most soil samples, although some soils fell on the 1:1 measured:predicted line.

The accuracy of established in vivo–in vitro correlations (IVIVC) to predict As-RBA in 12 soils in this study was quantified by calculating mean bias error and root mean square error (Eqs. (2) and (3)). As shown in Table S3, when established IVIVC by Juhasz et al. (2009) for the intestinal phase of SBRC was used to predict As-RBA in 12 contaminated soils from China, an underestimation occurred with mean bias error = −12 (Fig. 4). However, when IVIVCs of other assays were used, As-RBA predictions were generally higher than measured As-RBA with mean bias error being 0.31–15. In contrast, predictions using established IVIVC by Juhasz et al. (2014a) were generally lower, with mean bias error being −6.0 to −2.1.

The prediction root mean square error of established IVIVC by Juhasz et al. (2009) for 12 contaminated soils from China varied with assays, with IVIVC of the IVG assay showing lower root mean square error than other assays (12–13 vs. 9.8–30). Similar variation in root mean square error was observed when established IVIVC of Juhasz et al. (2014a) was tested. However, the root mean square error was generally >10, while some IVIVC showed root mean square error ≥20, indicating that established IVIVC could not accurately predict As-RBA in contaminated soils from China. This may be due to the fact that established IVIVCs were based on a different animal model (swine, Juhasz et al., 2009, 2011) and/or different As-RBA methodology (mouse ISSUE, Juhasz et al., 2014a).

3.5. In vivo–in vitro correlation for As in contaminated soils from China

Since using established in vivo–in vitro correlations (IVIVC) and As bioaccessibility data could not predict As-RBA in our soils, a new IVIVC was developed based on As bioaccessibility using 5 assays and As-RBA using a mouse blood model (Fig. 5). Table 2 details the goodness of fit \(r^2\), slope of the relationship, and y-intercept for IVIVC. Several criteria, including \(r^2 > 0.6\), slope of 0.8–1.2, and y-intercept close to zero, have been recommended to evaluate validity of IVIVC (Wragg et al., 2011). The IVIVC varied depending on the phases of in vitro assays used. The
The strength of the predictive models varied from $r^2 = 0.50$ (intestinal phase of PBET) to $r^2 = 0.83$ (gastric phase of IVG) with IVG assay providing the best prediction of in vivo relative bioavailability ($r^2 = 0.83$ and 0.81 for gastric phase and intestinal phase). For all IVIVC, the y intercepts were close to zero ($b_0$). As for the slopes (1.3–1.4), IVIVC of the IVG assay did not meet the criteria of 0.8–1.2, while IVIVC’s slopes for UBM, and gastric phase of SBRC and DIN assays were within the criteria. However, there was no significant difference in the slopes between gastric phase of IVG and UBM and SBRC ($p = 0.27–0.45$). Therefore, the high correlation ($r^2 = 0.83$ and 0.81) and low y-intercept ($−1.06$)

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**Fig. 5.** In vivo–in vitro correlations between measured As relative bioavailability (RBA) in 12 contaminated soils from China using a mouse model and As bioaccessibility determined using gastric (GP) and intestinal (IP) phases of five in vitro assays (UBM, SBRC, IVG, DIN, and PBET).
Table 2
Linear relationship between in vivo relative As relative bioavailability (RBA) and in vitro As bioaccessibility in 12 contaminated soils from China based on gastric (GP) and intestinal (IP) phases of UBM, SBRC, IVG, DIN, and PBET assays.

<table>
<thead>
<tr>
<th>In vitro assay</th>
<th>Phase</th>
<th>In vivo–in vitro predictive model</th>
<th>r²a</th>
</tr>
</thead>
<tbody>
<tr>
<td>UBM</td>
<td>Gastric</td>
<td>RBA = -4.40 + 0.97 (UBM-GP)</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>Intestinal</td>
<td>RBA = -4.43 + 1.09 (UBM-IP)</td>
<td>0.80</td>
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<tr>
<td>SBRC</td>
<td>Gastric</td>
<td>RBA = -0.60 + 0.96 (SBRC-GP)</td>
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<td></td>
<td>Intestinal</td>
<td>RBA = 8.22 + 1.35 (SBRC-IP)</td>
<td>0.57</td>
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<tr>
<td>IVGb</td>
<td>Gastric</td>
<td>RBA = -1.06 + 1.29 (IVG-GP)</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>Intestinal</td>
<td>RBA = 2.28 + 1.35 (IVG-IP)</td>
<td>0.81</td>
</tr>
<tr>
<td>DIN</td>
<td>Gastric</td>
<td>RBA = 12.1 + 0.79 (DIN-GP)</td>
<td>0.61</td>
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<tr>
<td></td>
<td>Intestinal</td>
<td>RBA = 4.52 + 1.36 (DIN-IP)</td>
<td>0.53</td>
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<tr>
<td>PBET</td>
<td>Gastric</td>
<td>RBA = 8.59 + 1.08 (PBET-GP)</td>
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<td></td>
<td>Intestinal</td>
<td>RBA = 9.46 + 1.04 (PBET-IP)</td>
<td>0.50</td>
</tr>
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</table>

a r² should be greater than 0.60.
b the bold represented the strongest linear relationship.

Table 3
Comparison of in vivo–in vitro correlations (IVIVC) for gastric (GP) and intestinal (IP) phases of UBM, SBRC, IVG, and PBET assays between this study and previous studies.

<table>
<thead>
<tr>
<th>In vitro assay</th>
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<th>Soil sample</th>
<th>In vivo assay</th>
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<td></td>
<td></td>
<td>Animal</td>
<td>Slope</td>
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<td>SBRC-GP</td>
<td>Juhasz et al. (2009)</td>
<td>12</td>
<td>Swine</td>
<td>Blood AUC</td>
</tr>
<tr>
<td></td>
<td>Juhasz et al. (2014a)</td>
<td>10</td>
<td>Mouse</td>
<td>Urinary excretion</td>
</tr>
<tr>
<td>This study*</td>
<td></td>
<td>12</td>
<td>Mouse</td>
<td>Blood AUC</td>
</tr>
<tr>
<td>SBRC-IP</td>
<td>Juhasz et al. (2009)</td>
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a and ** indicate significant differences in slope or y-intercept between this study and previous study at p < 0.05 and < 0.01 for each assay, respectively.
b because the slope differ so much, it is not possible to test whether the intercepts differ significantly.

4. Conclusions

Results from this study suggested that As bioaccessibility and As relative bioavailability in contaminated soils depended on soil types and methods used, supporting the hypothesis that site-specific approach is necessary to accurately determine As bioavailability in contaminated soils. The IVG assay was the best among the five in vitro assays tested to predict As-RBA in 12 contaminated soils from China though UBM and SBRC assays were also suitable. There was significant difference in slope when established IVIVC for gastric (GP) and intestinal (IP) phases of UBM, SBRC, IVG, and PBET assays were compared to previous studies. This might be attributed to different methodologies used for measuring As-RBA. Hence, future research is needed to compare As bioavailability values measured using different biomarkers and different animal models.
Abbreviations

As         arsenic
AUC        area under the blood Pb time curve
DIN        Deutsches Institut für Normung e.V.
IP         intestinal phase
IVG        in vitro gastrointestinal
IVIVC      in vivo–in vitro correlations
GP         gastric phase
MBE        mean bias error
PBET       physiologically based extraction test
RBA        relative bioavailability
RMSE       root mean square error
SBRC       solubility/bioavailability research consortium
SSUE       steady state urinary excretion
UBM        unified BARGE method

Acknowledgments

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Appendix A. Supplementary data

A description of 5 in vitro assays to determine As bioaccessibility (Table S1), previously established IVIVC models (Table S2), and a comparison of IVIVI between this study with previous studies (Table 3) can be found in the supplementary data. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.scitotenv.2015.05.113.

References


