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Influence of saliva, gastric and intestinal phases on the prediction of As relative bioavailability using the Unified Bioaccessibility Research Group of Europe Method (UBM)

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ABSTRACT

In this study, As-contaminated soils ($n = 12$) were assessed for As bioaccessibility using the Unified Bioaccessibility Research Group of Europe in vitro method (UBM) incorporating gastric, saliva–gastric or saliva–gastric–intestinal phases. Arsenic bioaccessibility was compared to previous published As relative bioavailability data for these soils to determine the correlation between in vitro and in vivo data. Comparison of in vitro and in vivo data indicated that the correlation between As bioaccessibility (UBM) and As relative bioavailability (swine assay) was similar irrespective of the in vitro phase used for its determination. The UBM incorporating all phases (saliva–gastric–intestinal) provided the best in vivo–in vitro correlation (slope = 1.08; $R^2 = 0.59$), however there was no significant difference in the goodness of fit (R^2 ranged from 0.48 to 0.59) or the slope of the lines (0.93–1.08) for either variation of the UBM ($P = 0.9946$). This indicates that there was no improvement in the As relative bioavailability predictive capabilities when the UBM was extended from a single gastric phase to saliva–gastric or saliva–gastric–intestinal phases.

1. Introduction

A major non-dietary exposure pathway for arsenic (As) is via the incidental ingestion of contaminated soil and dust. When assessing exposure for human health risk assessment, the conservative approach is to assume that all of the soil-borne As following ingestion is absorbed into systemic circulation. However, this assumption may overestimate As bioavailability due to physico-chemical and biological constraints which limit As dissolution and absorption in the gastrointestinal tract [1–3]. A more accurate estimate of exposure may be gained from the assessment of As relative bioavailability using in vivo assays or from surrogate in vitro assays (i.e. bioaccessibility) which predict As relative bioavailability. While a number of in vitro methodologies (e.g. Solubility Bioaccessibility Research Consortium assay [SBRC], In Vitro Gastrointestinal extraction method [IVG], Physiologically Based Extraction Test [PBET], Standardised German In Vitro Assay [DIN], Unified Bioaccessibility Research Group of Europe Method [UBM], Simulator of the Human Intestinal Ecosystem [SHIME], Dynamic Computer-Controlled Gastrointestinal Model [TIM]) are available as potential surrogate assays for predicting As relative bioavailability, limited information currently exists on the suitability of these in vitro methodologies to act as surrogate in vivo assays [4]. This is due to in part by the limited number of soils which has been evaluated for in vivo As relative bioavailability; the reference point for determining which method most closely approximates in vivo results.

Recently, Juhasz et al. [5] determined that the relative bioavailability of As in soils ($n = 12$) containing elevated concentrations of As (42–1114 mg As kg^{-1}) resulting from anthropogenic inputs (herbicide, pesticide inputs; mining activities) or geogenic (gossans) processes ranged from $6.9 \pm 5.0\%$ to $74.7 \pm 11.2\%$. In vitro assessment (SBRC, IVG, PBET and DIN) of the same soils found that As bioaccessibility varied depending on the methodology employed. However, when the correlation between in vivo As relative bioavailability and in vitro As bioaccessibility was assessed, As relative bioavailability could be predicted using gastric or intestinal phases of SBRC, IVG, PBET and DIN assays with varying degrees of confidence ($R^2 = 0.53$–0.75, Pearson correlation = 0.73–0.87) [4]. An in vitro methodology that was not included in the study of Juhasz et al. [4] was the unified Bioaccessibility Research Group of Europe (BARGE) method (UBM) which has been adopted as the standardised in vitro assay in Europe [6]. The assay is based on the a method developed by the Dutch Institute of Public Health (RIVM) [7] which

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Table 1
Selected properties of soils used in this study. The <250 μm particle size fraction was used for all analyses.

Sample no.	Soil properties Total As (mg kg^{-1})[a]	Total Al (g kg^{-1})[a]	Total Fe (g kg^{-1})[a,b]	Total *P* (mg kg^{-1})[a]	pH[a]
Railway corridors					
2	267	22.2	17.6 (10.6)	234	8.8
4	42	18.3	13.7 (8.5)	385	8.4
5	1114	16.3	68.3(16.6)	874	7.8
10	257	27.8	25.8 (9.9)	242	6.4
16	751	10.8	14.5 (11.4)	422	8.3
18	91	5.1	10.0 (3.2)	130	7.5
Dip sites					
24	713	94.7	98.6 (48.6)	3144	5.7
27	228	22.4	17.9 (12.5)	2941	5.2
Mine sites					
33	807	10.9	23.5 (7.1)	546	7.6
34	577	17.6	24.6 (12.5)	468	6.6
Gossans					
44	190	8.3	21.0 (15.6)	200	8.6
45	88	9.6	21.0 (13.2)	370	8.1

[a] Data represent the mean of duplicate analysis. Values varied by less than 5%.
[b] Values in parenthesis represent dithionite citrate bicarbonate extractable Fe (free Fe).

was considered the most suitable batch method for the assessment of contaminant bioaccessibility. The UBM is a physiologically based extraction test which utilises a more complex chyme composition compared to the simplistic SBRC, IVG, PBET and DIN assays. In addition, unlike the aforementioned in vitro assays, the UBM includes a saliva component prior to gastric phase extraction.

In this study, the As-contaminated soils ($n = 12$) from Juhasz et al. [5] were utilised for the assessment of As bioaccessibility using the UBM incorporating both saliva–gastric (S–G) and saliva–gastric–intestinal phases (S–G–I). In addition, in vitro assays were conducted using gastric (G-) phase extraction alone to determine the impact of the saliva phase on As bioaccessibility. Furthermore, As bioaccessibility (G-, S–G and S–G–I phases) and As relative bioavailability data were compared to determine the correlation between in vitro and in vivo data.

2. Materials and methods

2.1. Soils

Soils used in this study were sourced from a variety of peri-urban locations and have previously been assessed for As bioaccessibility (using alternative in vitro assays) and relative bioavailability (using the swine model) [2,4,5]. Table 1 shows selected soils properties for each of the soils. For further information on As-contaminated soils, please refer to Juhasz et al. [2].

2.2. Assessment of contaminant bioaccessibility

A number of different As bioaccessibility methodologies (e.g. SBRC, IVG, PBET, DIN) have been evaluated for their ability to act as surrogate assays to predict As relative bioavailability [3–5,8]. These methodologies may be utilised as single phase procedures (e.g. gastric phase extraction) or may encompass dual phases (i.e. gastro–intestinal extraction). The Unified BARGE Method (UBM) differs from these methodologies by the inclusion of a saliva phase prior to gastro–intestinal extraction in order to simulate dissolution processes that may occur in the mouth following incidental soil ingestion.

Initially, As bioaccessibility was assessed in triplicate using the three phase (saliva, gastric and intestinal) UBM bioaccessibility assay (see Supplementary material). Bioaccessibility assessment was performed according to Wragg et al. [6], however, due to the large tolerance in gastric phase pH (1.2–1.7), a more stringent approach was utilised whereby the gastric phase pH was maintained at pH 1.5 ± 0.05. Following saliva and gastric phase extraction (termed S–G phase) or saliva, gastric and intestinal phase extraction (termed S–G–I phase), samples were centrifuged (3000 *g* for 5 min) and 1.0 ml of the supernatant diluted with 0.1 M HNO_3 (9 ml). Extracts were stored at 4 °C prior to the determination of As bioaccessibility by ICP-MS. For further details of UBM constituents and operating procedures, please refer to Wragg et al. [6].

In addition, As bioaccessibility was also assessed using the UBM gastric phase only (termed G-phase) at the prescribed soil:solution ratio of 1:37.5 (termed G-37.5) and at an increased soil:solution ratio of 1:100 (termed G-100). The increased soil:solution ratio was assessed to determine whether solubility issues may limit As bioaccessibility in the gastric phase.

Arsenic bioaccessibility was calculated by dividing As extracted by G-, S–G or S–G–I phases by the total soil As concentration in the <250 μm soil particle size fraction (Eq. (1)).

$$\text{In vitro bioaccessibility}\,(\%) = \left(\frac{\text{In vitro As}}{\text{Total As}}\right) * 100 \quad (1)$$

where:

In vitro As (μg) extracted from soil following G-, S–G or S–G–I phases treatment.

Total As = As (μg) present in contaminated soil prior to in vitro treatment.

2.3. Comparison of UBM bioaccessibility and relative bioavailability

For the As contaminated soils studied, relative bioavailability data, derived from in vivo assays was compared to As bioaccessibility determined using G-, S–G or S–G–I phases of the UBM. For further details regarding the assessment of in vivo relative As bioavailability, readers are referred to Juhasz et al. [5]. Bioaccessibility-bioavailability best fit models were determined using stepwise multiple regression. SPSS 16.0.1 (2007) was used for the determination of all models.

2.4. Quality assurance and quality control

In vitro extracts were analysed by ICP-MS. During the determination of As concentration in in vitro extracts, duplicate analysis, spiked sample recoveries and check values were included. The

Table 2
Arsenic bioaccessibility in contaminated soils determined using the UBM.

Sample #	As bioaccessibility (%)			
	UBM		UBM gastric phase only	
	S–G extraction	S–G–I extraction	G-37.5 extraction	G-100 extraction
2	61.5 ± 2.1^{a,b}	59.3 ± 2.0^{a}	61.9 ± 3.0^{a,b}	66.7 ± 2.5^{b}
4	47.4 ± 1.6^{a}	48.3 ± 1.9^{a}	40.5 ± 0.6^{b}	48.6 ± 1.5^{a}
5	19.4 ± 1.2^{a,c}	20.4 ± 0.9^{a}	13.7 ± 0.4^{b}	17.6 ± 0.8^{c}
10	20.2 ± 0.6^{a}	21.7 ± 0.5^{b}	13.1 ± 0.3^{c}	14.4 ± 0.2^{d}
16	35.7 ± 3.6^{a,b}	40.3 ± 2.8^{a}	33.5 ± 0.8^{b}	41.0 ± 0.5^{a}
18	42.6 ± 0.8^{a}	49.7 ± 4.9^{b}	33.3 ± 0.3^{c,d}	37.5 ± 0.1^{a,d}
24	23.5 ± 1.2^{a}	33.6 ± 1.5^{b}	14.6 ± 0.5^{c}	24.5 ± 0.2^{a}
27	57.8 ± 0.9^{a}	58.5 ± 2.9^{a}	35.3 ± 0.6^{b}	43.1 ± 1.8^{c}
33	19.6 ± 1.1^{a}	20.0 ± 1.4^{a}	12.9 ± 0.3^{b}	14.0 ± 0.5^{b}
34	6.5 ± 0.4^{a}	7.2 ± 0.3^{b}	4.4 ± 0.0^{c}	4.8 ± 0.0^{c}
44	36.9 ± 1.9^{a}	31.3 ± 1.2^{b,c,d}	27.4 ± 0.8^{c}	32.2 ± 2.3^{d}
45	18.7 ± 1.3^{a}	20.0 ± 0.5^{a}	14.8 ± 0.2^{b}	18.1 ± 0.6^{a}

Data sharing the same letter do not differ significantly ($P > 0.05$).

average deviation between duplicate samples ($n = 6$) was 2.6%, the average recovery from spiked samples ($n = 6$) was 105.5% whereas check value recoveries ($n = 14$) ranged from 96.8% to 108.9% (102.9% average recovery).

3. Results and discussion

3.1. Assessment of As bioaccessibility using the UBM

When the UBM was used to assess As bioaccessibility in contaminated soils, As bioaccessibility ranged from 6.5% to 61.5% following S–G extraction (Table 2). Arsenic bioaccessibility was <25% for soil containing elevated concentrations of geogenic As (#45), mine impacted soil (#33 and #34) in addition to two soils (#5 and #10) impacted through the historical use of As herbicides. In contrast, As bioaccessibility was elevated (>50%) in soils impacted through herbicide (#2) and pesticide (#27) usage.

As detailed by Juhasz et al. [2] and Yang et al. [9], As bioaccessibility in herbicide and pesticide impacted soils may range considerably due to the influence of soil properties and ageing effects. As a result of the dominance of surface sorbed As in these soils, Fe (concentration and crystalline nature) plays an important role in controlling As bioaccessibility [2,9–12]. Yang et al. [9] attributed 75% of the variability in arsenate bioaccessibility to the soil's pH and Fe oxide content while Juhasz et al. [2] showed that total As, Fe and free Fe (dithionite citrate bicarbonate extractable Fe) were the descriptive variables best able to describe As bioaccessibility in herbicide and pesticide impacted soils. In contrast, As bioaccessibility in mine impacted soils and gossans will predominantly be influenced by mineralogical composition. Recent studies by Meunier et al. [13] showed decreasing As bioaccessibility with As-sulphides (e.g. arsenopyrite, realgar), iron arsenates (e.g. scorodite, kankite, pharmacosiderite), arsenic bearing Fe oxyhydroxides (e.g. goethite, lepidocrocite, akaganeite), roaster iron oxides (e.g. hematite, maghemite), As-sulphates (e.g. tooeleite, jarosite, schwertmannite), clay minerals and calcium-Fe-arsenates (e.g. yukonite) [13].

When the in vitro assay was extended to include the intestinal phase (S–G–I extraction), the range in As bioaccessibility for the 12 contaminated soils was similar (7.2–59.3%) to data obtained using S–G extraction. Although there was no significant difference ($p < 0.05$) between As bioaccessibility using S–G or S–G–I extraction for 8 of the 12 soils, some variability in As bioaccessibility data was observed between these extraction phases for samples #10, #24, #34 and #44 (Table 2). Extending the in vitro assay to accommodate the intestinal phase resulted in an increase in As bioaccessibility for herbicide (#10), pesticide (#24) and mine site (#34) impacted soils, however, for sample #45 (gossan), As bioaccessibility decreased following intestinal phase inclusion. Previous studies have reported an increase in As bioaccessibility following inclusion of an intestinal phase [4,14–17] presumably due to pH induced desorption of As from metal oxide-As complexes in the intestinal phase [18]. In contrast, reduction in As bioaccessibility following modification of gastric to intestinal phase conditions has also been reported [4,19] as a consequence of sorption of dissolved As to, and precipitation of, amorphous Fe from the increased pH in the intestinal phase (Fig. 1).

3.2. Assessment of As bioaccessibility using UBM gastric phase extraction

In previous studies, Basta et al. [3], Juhasz et al. [5] and Rodriguez et al. [8] demonstrated that in vivo As relative bioavailability could be accurately predicted using the gastric phase of IVG or SBRC assays. In a follow up study by Juhasz et al. [4], gastric phase extraction also provided a superior As relative bioavailability predictive capability over gastro–intestinal extraction for the DIN assay. These results suggest that a simplified in vitro assay incorporating gastric phase extraction may be suitable for the assessment of As bioaccessibility. In this study, the UBM in vitro assessment was simplified to determine As bioaccessibility in the 12 contaminated soils using only the gastric phase at a soil:solution ratio (1:37.5) and pH (1.5) commensurate to the UBM protocol. In addition, As bioaccessibility was also assessed using a soil:solution ratio of 1:100 to determine whether solubility issues limit As bioaccessibility in the gastric phase. This soil:solution ratio was selected as it is a standard operating parameter for the SBRC and PBET assays.

When gastric phase extraction was undertaken at a soil:solution ratio of 1:37.5, As bioaccessibility decreased ($p < 0.05$) compared to S–G extraction with the exception of samples #2 and #16 where As bioaccessibility values were comparable (Table 2). Increasing the gastric phase soil:solution ratio to 1:100 increased As bioaccessibility for all soils, albeit significantly ($p < 0.05$) for 9 of the 12 soils tested (Table 2) compared to the lower soil:solution ratio (1:37.5). Low soil:solution ratios (1:5–1:25) has been shown to underestimate the bioaccessible fraction due to limited metal solubility at these ratios [19]. However, other research has indicated that there is little differences in metal bioaccessibility from soil:solution ratios of 1:100–1:5000 [20]. However, at a soil:solution ratio of 1:100, As bioaccessibility was significantly lower ($p < 0.05$) for 5 of the 12 soils compared to S–G extraction. While the mechanisms responsible for the increase in As bioaccessibility are unclear, presumably saliva phase constituents play a role in the enhancement of As solubilisation.

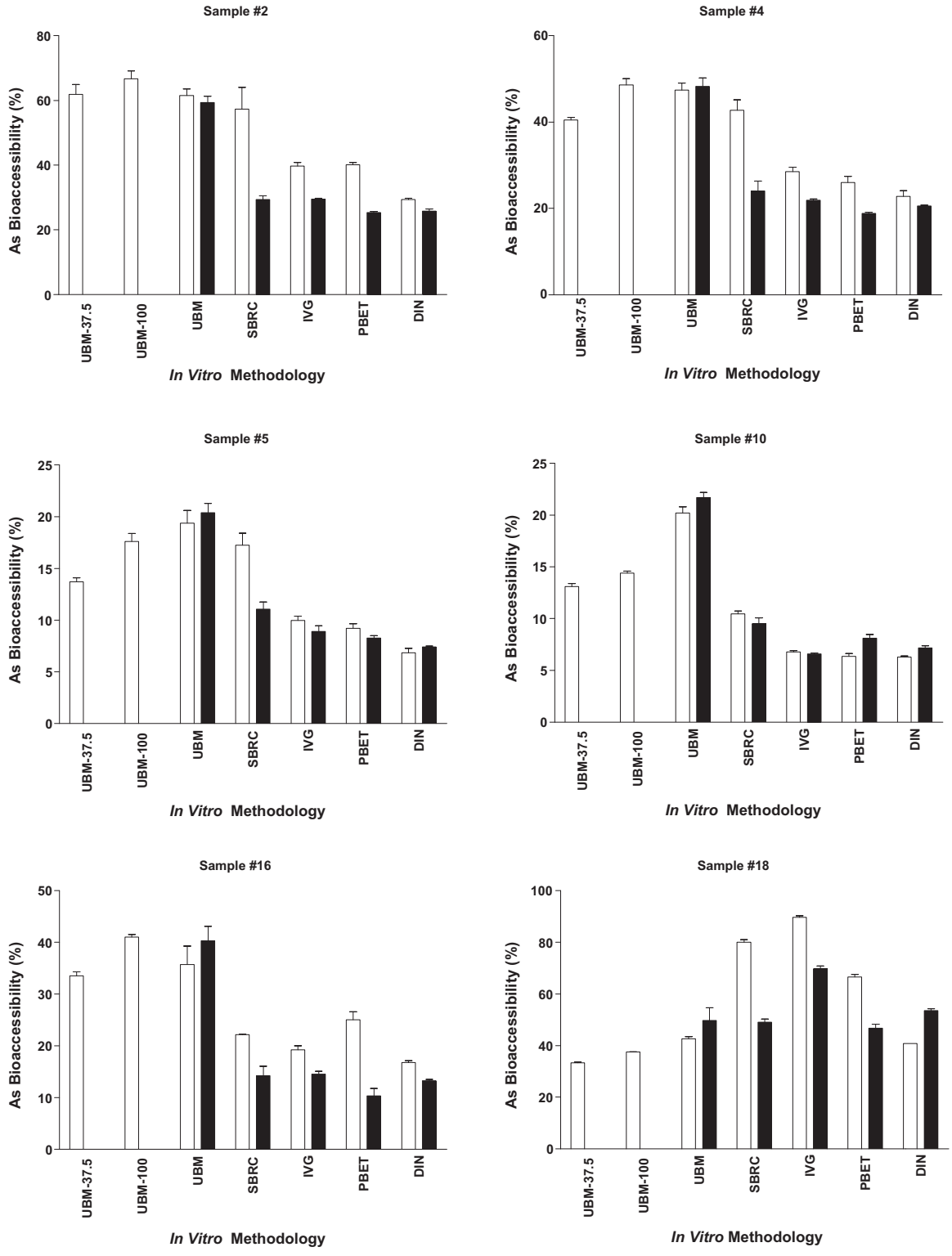

Fig. 1. Comparison of As bioaccessibility for herbicide impacted soils using UBM (G-, S–G, S–G–I), SBRC, IVG, PBET and DIN in vitro assays (Juhasz et al. [4]). Bars represent the mean and standard deviation of triplicate gastric (□) and intestinal phase (■) extractions.

3.3. Comparison of UBM As bioaccessibility and other in vitro methodologies

Figs. 2 and 3 show a comparison of As bioaccessibility data generated for the 12 contaminated soils using a variety of in vitro assays. Arsenic bioaccessibility determined using SBRC, IVG, PBET and DIN assays (gastric and intestinal phases) was previously undertaken by Juhasz et al. [4] in a study to determine the suitability of these methodologies for predicting As relative bioavailability in contaminated soils. As detailed in Fig. 1 (herbicide-impacted soils) and Fig. 2 (pesticide-, mine site-impacted soils and gossans) considerable variability exists between methodologies for

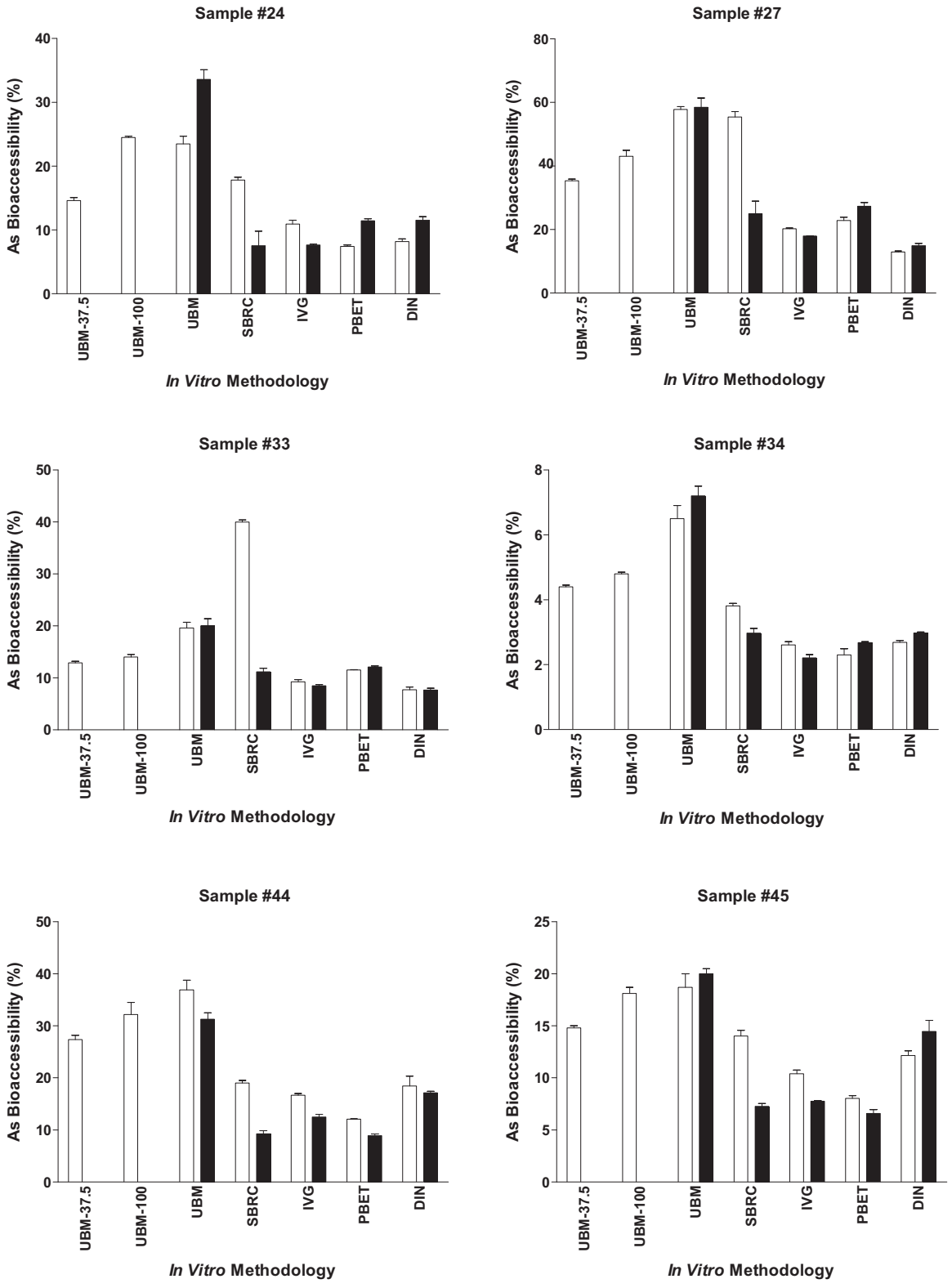

Fig. 2. Comparison of As bioaccessibility for pesticide impacted (sample #24 and #27), mine site impacted (sample #33 and #34) and gossan soils (sample #44 and #45) using UBM (G-, S–G, S–G–I), SBRC, IVG, PBET and DIN in vitro assays (Juhasz et al. [4]). Bars represent the mean and standard deviation of triplicate gastric (□) and intestinal phase (■) extractions.

all soils studied. In most cases (except samples #18 and #33), assessment of As-contaminated soils using the UBM (S–G or S–G–I extraction) gave the highest value for As bioaccessibility (i.e. most conservative value for human health exposure assessment) compared to SBRC, IVG, PBET and DIN assays. Arsenic bioaccessibility was up to 4.6- and 4.4-fold greater following S–G and S–G–I assessment compared to gastric and intestinal phase extraction of SBRC, IVG, PBET and DIN assays respectively. The conservative As bioaccessibility values determined using the UBM, compared to other in vitro assays, may be in part reflective of the

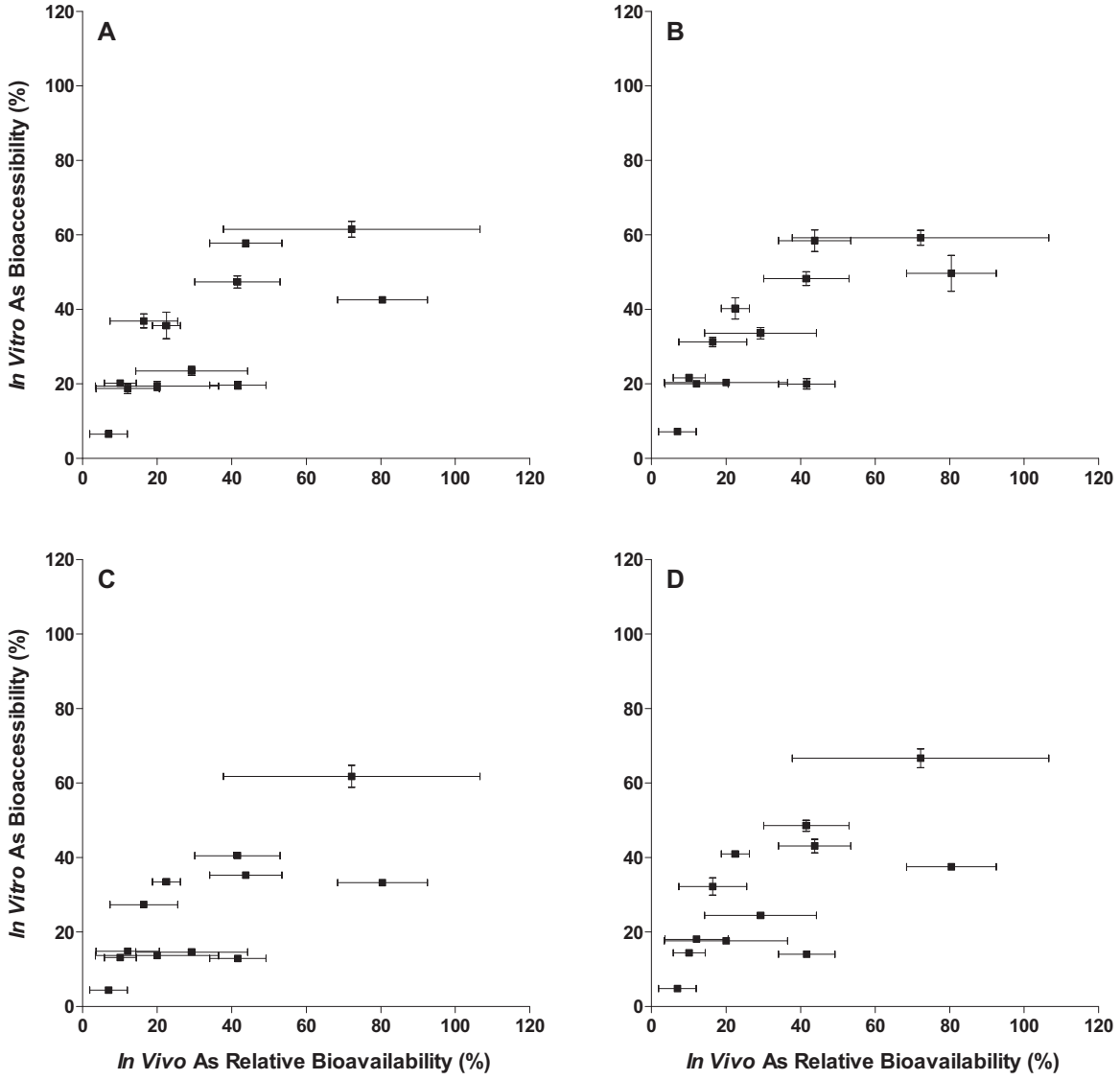

Fig. 3. Comparison of As bioaccessibility (in vitro) and relative As bioavailability (in vivo) for gossan soils and herbicide, pesticide and mine site impacted soil: (a) S–G phase, (b) S–G–I phase, (c) G-37.5 phase, (d) G-100 phase.

pH of gastric phase extraction. The lower pH of the UBM gastric phase (pH 1.5) compared to gastric phase conditions in IVG, PBET and DIN assays (1.8, 2.5 and 2.0 respectively) resulted in an increase in the dissolution of As and Fe in the gastric phase. It is well know that As retention is strongly related to the presence of Fe mineral phases in soils [21,22]. Therefore enhanced Fe dissolution at the lower pH value resulted in increased As concentrations in gastric phase solutions. Increased As bioaccessibility with decreasing gastric phase pH have been reported for mine wastes [19] and drinking water treatment residuals [23]. In the study of Ruby et al. [19], As bioaccessibility in mine waste was 1.1- to 1.4-fold greater when the gastric phase pH was reduced from 2.2 to 1.3. Similarly, Makris et al. [23] determined that the bioaccessibility of As associated with amorphous Fe-water treatment residuals increased from <10% at pH 3.5 to >50% at pH 1.0.

Although the gastric phase of UBM and SBRC assays are both pH 1.5, As bioaccessibility was greater in 6 of the 12 contaminated soils when assessed using the UBM. While the gastric phase pH is an important parameter influencing the solubilisation of As and Fe, it is also evident from these results that gastric phase constituents must also play a small, but influential role in As bioaccessibility. UBM and SBRC gastric phase solutions vary considerably in their organic constituents and their concentration. Presumably, the difference in As bioaccessibility data obtained for in vitro assays was a result of interactions between the different organic constituents and soil Fe mineral phases during gastric phase extraction. Previous studies have demonstrated that the nature of organic constituents will influence the solubilisation of Fe from a variety of matrices [24]. Even though gastric phase pH is a predominant factor influencing the solubility of As and Fe, the differences in As and Fe solubility in gastric phases demonstrates the influence of chyme composition on As bioaccessibility.

Although Figs. 1 and 2 demonstrate the variability in As bioaccessibility when different assays are utilised, the true indication of the performance of an in vitro assay is the correlation between in vitro As bioaccessibility and in vivo As relative bioavailability. As the inclusion of As bioaccessibility data (as a surrogate for As relative bioavailability) in exposure assessment has the potential to make significant impacts on estimated risk and remediation targets [25], in vitro methodologies need to be robust and provide defensible data that can ensure accurate estimation of contaminant relative bioavailability.

Table 3
Comparison of linear regression models for predicting in vivo relative As bioavailability in contaminated soils using UBM, SBRC, IVG, PBET and DIN in vitro assays.

In vitro assay	Phase	In vivo–in vitro predictive model	Pearson correlation
UBM	S–G	In vivo As relative bioavailability (%) = (0.99) UBM S–G (%) + 0.80 R^2 = 0.52	0.66
	S–G–I	In vivo As relative bioavailability (%) = (1.08) UBM S–G–I (%) − 3.73 R^2 = 0.59	0.69
	G-37.5	In vivo As relative bioavailability (%) = (1.05) UBM G-37.5 (%) + 6.29 R^2 = 0.52	0.65
	G-100	In vivo As relative bioavailability (%) = (0.93) UBM G-100 (%) + 5.07 R^2 = 0.48	0.62
SBRC[a]	Gastric	In vivo As relative bioavailability (%) = (0.99) SBRC-Gastric (%) + 1.69 R^2 = 0.75	0.87
	Intestinal	In vivo As relative bioavailability (%) = (1.64) SBRC-Intestinal (%) + 5.63 R^2 = 0.65	0.81
IVG[a]	Gastric	In vivo As relative bioavailability (%) = (0.85) IVG-Gastric (%) + 14.32 R^2 = 0.57	0.76
	Intestinal	In vivo As relative bioavailability (%) = (1.11) IVG-Intestinal (%) + 13.97 R^2 = 0.57	0.75
PBET[a]	Gastric	In vivo As relative bioavailability (%) = (1.16) PBET-Gastric (%) + 10.10 R^2 = 0.64	0.80
	Intestinal	In vivo As relative bioavailability (%) = (1.76) PBET-Intestinal (%) + 5.68 R^2 = 0.67	0.82
DIN[a]	Gastric	In vivo As relative bioavailability (%) = (1.77) DIN-Gastric (%) + 5.73 R^2 = 0.55	0.74
	Intestinal	In vivo As relative bioavailability (%) = (1.46) DIN-Intestinal (%) + 9.20 R^2 = 0.53	0.73

[a] Data from Juhasz et al. [4].

3.4. Comparison of UBM bioaccessibility and relative bioavailability

In order to determine the correlation between in vivo and in vitro assays, the relationship between As bioaccessibility determined using G-37.5, G-100, S–G and S–G–I extraction and As relative bioavailability measured using an in vivo swine assay [5] was calculated using linear regression and Pearson correlation methods (Table 3). Comparison of in vitro and in vivo results (Fig. 3; Table 3) indicated that the correlation between As bioaccessibility and As relative bioavailability was similar irrespective of the in vitro phase used for its determination. The UBM incorporating all phases (S–G–I) provided the best in vivo–in vitro correlation (slope = 1.08; R^2 = 0.59), however there was no significant difference in the goodness of fit (R^2 ranged from 0.48 to 0.59) or the slope of the lines (0.93–1.08) for either variation of the UBM (P = 0.9946). This indicates that there was no improvement in the As relative bioavailability predictive capabilities when the UBM was extended from a single gastric phase to saliva–gastric or saliva–gastric–intestinal phases. Similarly, Juhasz et al. [4] and Rodriguez et al. [8] reported that extending the IVG, SBRC and DIN methods beyond the gastric phase did not improve the ability of the assays to predict As relative bioavailability. For the UBM, similar As in vivo–in vitro correlations have been reported by Caboche [26]. Arsenic relative bioavailability was assessed using an in vivo swine assay and urine analysis following 14 days exposure to contaminated soils (n = 15; 18–25,000 mg As kg^{-1}) while As bioaccessibility was determined using S–G and S–G–I phases of the UBM. Both in vitro phases were able to accurately predict As relative bioavailability with linear relationships (R^2 = 0.99) and slopes of 0.969 and 0.908 for S–G and S–G–I phases respectively. A linear relationship between in vivo As relative bioavailability and in vitro As bioaccessibility with a slope of unity is advantageous as it demonstrates the ability of the in vitro methods to act as a surrogate assay for predicting As relative bioavailability.

As suggested by Cave (pers. comms.), an alternative approach for assessing the suitability of in vitro assays for predicting contaminant relative bioavailability is to assess in vivo and in vitro data using Bland–Altman plots [27]. This method, commonly utilised in clinical medicine, may be used to compare new measurement techniques (e.g. in vitro assays) to those of a 'gold standard' (e.g. in vivo assays) to determine how well the two methods of measurement agree. In addition, Bland–Altman plots provide a visual representation of any discrepancies between measured values, clearly identifying outlying observations. Fig. 4a shows a Bland–Altman plot of the difference between As relative bioavailability and UBM (S–G–I) measurements for each soil against their average including mean bias with 95% limits of agreement. Data for Bland–Altman comparisons included the mean of replicate analyses (n = 3) for As relative bioavailability and As bioaccessibility for each soil. The mean bias when the difference between methodologies was plotted against their average was −1.09% indicating that on average in vitro values were similar to those determined using in vivo assays. Parity in As relative bioavailability and As bioaccessibility measurement was obtained for 2 soils (#5 and #34), however, significant variability between methods was observed for the remaining soils (Fig. 4a). For 3 of the 12 soils (#2, #18 and #33), measurement of As relative bioavailability provided considerably higher values compared to As bioaccessibility (S–G–I). In contrast, the conservatism of the in vitro assay as a surrogate for As relative bioavailability was demonstrated for the majority of soils (7 of 12) assessed (Fig. 4a).

In Fig. 4b, the relationship between in vivo and in vitro methodologies is expressed as the ratio of As relative bioavailability and As bioaccessibility versus their average. Again, the Bland–Altman plot clearly illustrates that for 2 soils, there is strong agreement between the two methodologies, however, for 7 of the 12 soils, As relative bioavailability was exceeded when measured using UBM. For soil #10 (herbicide impacted), As relative bioavailability was over-predicted by 53% when assessed using the UBM. In contrast,

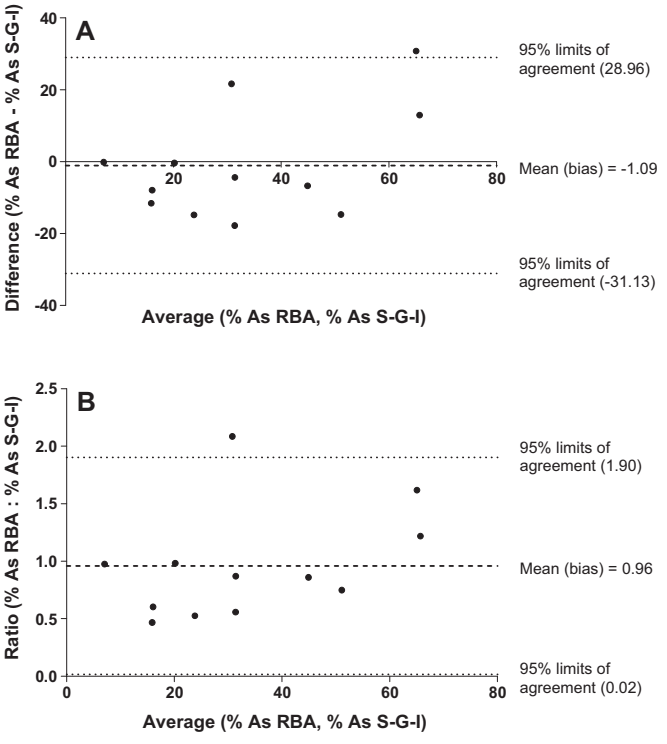

Fig. 4. Bland–Altman plots of (a) difference between As relative bioavailability and UBM (S–G–I) measurements for each soil (n = 12) against their average and (b) ratio of As relative bioavailability and UBM (S–G–I) measurements for each soil (n = 12) against their average. Mean bias with 95% limits of agreement are also shown.

for the remaining 3 soils (#2, #18 and #33) As relative bioavailability was 1.22-, 1.62- and 2.08-fold greater compared to in vitro measurement and as a result, the in vitro measurement significantly under-predicted As relative bioavailability.

While Bland–Altman plots provide a statistical and graphical approach for assessing the agreement between two methods, an issue with this type of analysis, especially for relative bioavailability-bioaccessibility research is the small data set used in the analysis. Unlike clinical medicine where 100s to 1000s of patients and measurements are taken, assessment of in vivo–in vitro correlations is restricted by the expense of undertaking relative bioavailability studies. Repeatability coefficients were 35% higher for in vivo assays compared to in vitro assays indicating greater variability in measurements for As relative bioavailability. As highlighted by Rees et al. [28], the standard deviations for As relative bioavailability measurement may be large when replicate in vivo assessment is undertaken using multiple animals due to intra-species variability. This may impact on method comparison unlike in clinical medicine where repeat measurements may be undertaken on the same patient. In addition, in vivo–in vitro comparisons may be influenced by inter-laboratory variability associated with the in vitro methodology. Round robin studies have identified considerable inter-laboratory variability for As bioaccessibility when measured using SBRC, IVG and PBET with relative standard deviations ranging up to 46% (Koch pers. comms.). While these in vitro methodologies are relatively simplistic, the tri-phasic UBM accommodates a more complex operational procedure which may add inter-laboratory variability to As bioaccessibility measurements.

4. Conclusions

In this study, As bioaccessibility in contaminated soil was assessed using the UBM and compared to As relative bioavailability values determined using an in vivo swine model. The UBM incorporating S–G–I phases provided the best in vivo–in vitro correlation (slope = 1.08; R^2 = 0.59), however, there was no significant difference in the goodness of fit (R^2 ranged from 0.48 to 0.59) or the slope of the lines (0.93–1.08) when G- or S–G phases were utilised. While linear regression models provided a 1 to 1 relationship between in vivo and in vitro measurements, significant variability was observed between methods for 10 of the 12 soils tested. Further validation of the UBM is recommended for bioaccessibility data to be used appropriately for evaluating the relative bioavailability of As in contaminated soil.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.09.068.

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