Variability of arsenic bioaccessibility and metabolism in soils by human gut microbiota using different *in vitro* methods combined with SHIME

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HIGHLIGHTS

- Five *in vitro* gastrointestinal methods combined with SHIME (colon phase) were used.
- As bioaccessibility varied in the colon phase among these methods.
- Plenty of As(III) and methylated arsenicals by microbial transformation were observed.
- As bioaccessibility in PBET/SBRC-SHIME colon phase were closer to *in vivo* results for NIST 2710a.

GRAPHICAL ABSTRACT

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ABSTRACT

Arsenic (As) speciation analysis is essential when evaluating the risks upon oral exposure to As-contaminated soils. In this study, we first investigated the variability in the As bioaccessibility and speciation using a combination of five common *in vitro* methods (SBRC, PBET, DIN, UBM and IVG) (gastric and small intestinal phases) and the SHIME model (colon phase). Our results indicate that the As bioaccessibility varies in the colon phase. An increase in the As bioaccessibility for SBRC and PBET, and a decrease for UBM and IVG were observed in the colon phase. In addition, we found different extents of methylation and large amounts of arsenite [As(III)] due to microbial reduction in the colon digests. The UBM-SHIME method displayed a higher methylation percentage of 13.5–82.1%, but a lower methylation percentage of 0.2–21.8% was observed in the SBRC-SHIME method. Besides, The MMA^V levels in the colon digests were positively correlated with those of As(III) and DMA^V, so DMA^V can be considered an indicator to evaluate the As metabolic speed of *in vitro* cultured human gut microbiota. Based on the standard reference soil of NIST 2710a, the As bioaccessibility in the colon phase of PBET-SHIME and SBRC-SHIME were the closest to the *in vivo* results. Combining *in vitro* methods and SHIME will remarkably affect the accurate assessment of potential risks to human health associated with oral exposure to soil As.

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1. Introduction

Arsenic (As) is a common hazardous substance that is extensively distributed in the soil environment (Bhattacharya et al., 2007). Human exposure to soil As, particularly for children, is a major public concern and is associated with serious risks to human health because As is known as a carcinogen (Wang and Mulligan, 2008). Inadvertent oral ingestion has been considered an important exposure pathway for soil As, especially through outdoor hand-to-mouth activities by children (Ljung et al., 2006). Human health risk assessment depends mainly on *in vivo* bioavailability methods and *in vitro* bioaccessibility methods. Generally, bioaccessibility is defined as the fraction of As that is soluble in the gastrointestinal environment of human being and available for absorption, whereas bioavailability is determined as the fraction of As that is absorbed into the systemic circulation (Juhasz et al., 2009; Ruby et al., 1999). In view of the time, cost and ethical considerations associated with *in vivo* methods, *in vitro* methods are widely used in assessing human health risk. For As contaminated soils, a strong linear relationship between As relative bioavailability (RBA) and As bioaccessibility has been reported (Bradham et al., 2011; Juhasz et al., 2009, 2014; Rodriguez and Basta, 1999; Ruby et al., 1996). By establishing good *in vivo*-*in vitro* correlations, these *in vitro* bioaccessibility methods have been proposed as surrogates to predict the RBA for refining the risk to human health of inadvertent oral soil ingestion as an important exposure route. To date, numerous *in vitro* methods have been used to assess As bioaccessibility in soils (Kelly et al., 2002; Rodriguez and Basta, 1999; Ruby et al., 1996; Wragg et al., 2011). A respectable amount of information is available regarding the comparison of *in vitro* methods for the assessment of As bioaccessibility. Within the studies undertaken, researchers reported that As bioaccessibility varied between *in vitro* methods, in soils and household dust respectively (Juhasz et al., 2014; Li et al., 2014; Oomen et al., 2002). Moreover, variable As bioaccessibility has been observed with differences in the compositions of the gastric and small intestinal phases, *in vitro* pH and other parameters (e.g., soil/solution ratio, residence time) (Juhasz et al., 2009, 2011; Smith et al., 2014).

Among those *in vitro* methods, the Simulator of Human Intestinal Microbial Ecosystem (SHIME) describes the colon microbial community of humans (Van de Wiele et al., 2004b) based on other *in vitro* methods. Van de Wiele et al. (2010) first reported the importance of the metabolism of soil As by human gut microbiota based on *in vitro* experiments. Other recent studies have shown that human gut microbiota actively metabolize As, and affect As bioaccessibility in food and contaminated soils (Laird et al., 2007; Sun et al., 2012). Speciation analysis is essential when evaluating risks from soil As exposure. Arsenic species that have previously been detected in the colon digests, include arsenate [As(V)], arsenite [As(III)], low-toxicity monomethylarsonic acid (MMA^V) and dimethylarsinic acid (DMA^V), highly toxic monomethylarsonous acid (MMA^{III}), and monomethylmonothioarsonic acid (MMMTA^V) of unknown toxicity (Van de Wiele et al., 2010). In addition, some studies on urine analysis by As thiolation using animal models revealed sulfur-containing methylated As metabolites, dimethylmonothioarsonic acid (DMMTA^V), and dimethyldithioarsonic acid (DMDTA^V) (Kubachka et al., 2009; Naranmandura et al., 2007; Raml et al., 2007). As bioaccessibility in the gastric and small intestinal phases is used to estimate As potential harm to human health.

Nevertheless, As biotransformation by human gut microbiota must be considered an essential part of the risk assessment for *in vitro* methods for oral exposure to soil As.

This is necessary for the refinement of human health risk assessments associated with soil As exposure, when As metabolism is taken into account. Unfortunately, there are limited data of the variability in soil As metabolism by human gut microbiota regarding the combination of different *in vitro* methods (gastric and small intestinal phases) and SHIME (colon phase). The between-method variability can result in the indeterminacy of the As metabolism by human gut microbiota and As distribution in the colon digest and soil solid phase, and thereby of the changes in the As speciation and bioaccessibility. It was hypothesized that As metabolism by human gut microbiota in the colon phase would differ as a consequence of the variability in these *in vitro* methods. Therefore, in this study, five common *in vitro* methods (SBRC, PBET, DIN, UBM and IVG) (gastric and small intestinal phases) combined with the SHIME (colon phase) were used to (1) investigate the variability of soil As bioaccessibility in the gastric, small intestinal and colon phases; (2) determine the As speciation in the colon phase and assess differences in soil As transformation by human gut microbiota; and (3) investigate the relationship between As bioaccessibility in the colon phase and the reported *in vivo* results based on the reference soil (NIST 2710a).

2. Materials and methods

2.1. Arsenic-contaminated soils

In our previous study, Yin et al. (2015b) determined the transformation of As species by human gut microbiota using a combination of PBET and SHIME in five contaminated soils from mining and farmland sites in China with high As concentrations ranging from 110.9 to 802.5 mg kg⁻¹. In this study, six soils including four previous soils, one Chinese reference material (GSS-5, National Institute of Metrology, China) and one reference material (NIST 2710a), were used to evaluate As bioaccessibility and speciation using a combination of different commonly used *in vitro* methods and SHIME. These soil samples cover a range of values for soil physicochemical properties and different concentrations of total As (Table 1). All soil samples were air-dried and sieved to a <250 µm particle size fraction for *in vitro* studies; this size of particle is most likely to stick to the hands of exposed humans (Kelly et al., 2002). Table 1 and Table S1 detail the physicochemical properties of the selected soils, including the concentrations of key metal elements, soil pH, organic matter, clay, and oxalate-extractable iron (Fe), aluminum (Al) and manganese (Mn).

2.2. Dynamic SHIME

The *in vitro* colon microbial community utilized in the experiments was cultured and maintained in a modified SHIME as previously described (Van de Wiele et al., 2004a), which consisted of five compartments simulating the stomach, small intestine, ascending colon, transverse colon and descending colon. In previous studies about SHIME, fresh fecal microorganisms were obtained from one volunteer (Laird et al., 2007; Sun et al., 2012; Van de Wiele et al., 2010), and we chose one Chinese volunteer. Briefly, fresh fecal microorganisms

Table 1
Physicochemical properties of the soils (n = 3).

Sample	Site	Type	pH	Fe (g kg ⁻¹)	Mn (g kg ⁻¹)	Al (g kg ⁻¹)	As (mg kg ⁻¹)
Soil 1	Chifeng, Inner Mongolia	Mining	7.4	21.1	1.5	23.8	110.9
Soil 2	Dalian, Liaoning	Mining	3.5	191.1	0.1	10.8	802.5
Soil 3	Shangyu, Zhejiang	Farmland	7.4	35.6	0.8	48.4	323.9
Soil 4	Chenzhou, Hunan	Farmland	7.6	27.5	0.5	53.8	143.3
Soil 5		GSS-5		75.4	1.4	97.2	412.0
Soil 6		NIST 2710a	4.0	34.0	1.7	10.0	1400.0

obtained from a Chinese 28-year-old male volunteer with no history of antibiotic treatment in the six months before the study, were inoculated in the three colon compartments. Feed solution was added three times per day to provide digested nutrition for the colon microorganisms. The temperature (37 °C) and pH (5.6–5.9, 6.15–6.4, and 6.7–6.9 in the ascending colon, transverse colon and descending colon, respectively) (Molly et al., 1994) were controlled automatically. The SHIME reactors were continuously stirred and kept under anaerobic conditions by regularly flushing with nitrogen. After 3–4 weeks of adaptation, stable microbial communities were obtained in the respective colon compartments. The distal colon microbial fermentation activity (short chain fatty acid production and ammonium production) and community composition were investigated, and found to be consistent with those of previous SHIME runs and the *in vivo* situation (Molly et al., 1994; Yin et al., 2015b). For the dynamic SHIME, the feed solution contained (in grams/liter) arabinogalactan (1.0), pectin (2.0), xylan (1.0), starch (4.0), glucose (0.4), yeast extract (3.0), peptone (3.0), mucin (1.0), and cysteine (0.5). After autoclaving and cooling down, the feed was adjusted at pH 2.0 ± 0.1. The pancreatic juice contained (in grams/liter) NaHCO₃ (12.5), bile salts (6.0), and pancreatin (0.9). Autoclave distilled water and then when it cooled down, put everything inside to prepare pancreatic juice.

2.3. Estimation of bioaccessible As

A variety of commonly used *in vitro* methods were selected to assess the As bioaccessibility in contaminated soils. In this study, we compared the soil As bioaccessibility investigated in the gastric, small intestinal and colon phases by five *in vitro* methods combined with SHIME. The *in vitro* methods evaluated were the Solubility Bioaccessibility Research Consortium (SBRC) assay (Kelly et al., 2002), the physiologically based extraction test (PBET) (Ruby et al., 1996), the German standard bioaccessibility methodology (DIN) (Deutsches Institut für Normung e.V., 2000), the Unified Bioaccessibility Research Group of Europe (BARGE) method (UBM) (Wragg et al., 2011), and the *in vitro* gastrointestinal extraction method (IVG) (Rodriguez and Basta, 1999), with details provided in Table S2. In brief, soils were added to polypropylene conical centrifuge tubes with the gastric and small intestinal digests at specific soil/solution (s/s) ratios. Following the small intestinal phase, these digests were transferred into 100 mL anaerobic serum bottles and corresponding volumes of colon solution from the colon compartment of the dynamic SHIME system were added with double ratios (s/s) of the small intestinal phase (Van de Wiele et al., 2010; Yin et al., 2015b). The bottles were capped with butyl rubber stoppers and immediately flushed with nitrogen gas for 20–30 min to obtain anaerobic conditions. The digests were shaken at 150 rpm and incubated at 37 °C for 48 h (Sun et al., 2012; Yin et al., 2015b). The experiments were conducted in triplicate. For the assessment of the As bioaccessibility, the digests of the gastric, small intestinal and colon phases were filtered (0.45 µm) and analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES, Optima-2000, Perkin-Elmer Co. Ltd., USA) or inductively coupled plasma mass spectrometry (ICP-MS, 7500a; Agilent, Palo Alto, CA, USA). During ICP-MS measurement of As concentration in sample supernatants, As recovery ($n = 18$) was 93.6–109.2% (mean value 103.5%) with a standard solution of 20 µg L⁻¹.

The *in vitro* As bioaccessibility was calculated as follows:

$$\text{As bioaccessibility (\%)} = (\text{invitro As} / \text{total As}) \times 100$$

where *in vitro* As = concentration of bioaccessible As in the gastric, small intestinal and colon phase, and total As = total As concentration in contaminated soils. The experiments were conducted in triplicate. One-way ANOVA was performed to determine significant differences in the As bioaccessibility for each soil using SPSS 18.0.0 (IBM).

2.4. Arsenic speciation analysis

All samples taken from the colon phase were centrifuged at 10,000 × g for 10 min. The supernatants were filtered (0.45 µm) and stored at -80 °C until analysis. Arsenic speciation was determined by high-performance liquid chromatography coupled with ICP-MS as described. Chromatographic columns were obtained from Hamilton and consisted of a pre-column (11.2 mm, 12–20 µm) and a PRP-X100 10-µm anion-exchange column (250 mm × 4.1 mm). The mobile phase consisted of 10 mM (NH₄)₂HPO₄ and 10 mM NH₄NO₃, adjusted to pH 6.2 using nitric acid or ammonia. The flow rate was 1 mL min⁻¹. As species in the colon digests were identified by comparing their retention times with those of standards [As(III), As(V), DMA^V, MMA^V and MMTA^V] as quantified by external calibration curves with peak areas. MMTA^V was synthesized using a mixture of MMA^V and H₂S solutions by this method (Alava et al., 2012; Rubin et al., 2014). 100 mL of saturated H₂S solution was added to 900 mL of a 40 mg As mL⁻¹ MMA^V solution in a glass vial. The mixture was shaken overnight on a mechanical shaker. The sum of all As species in the filtrates (0.45 µm) observed chromatographically was considered the bioaccessible As. The chromatographic recovery by this method has been validated (Zhu et al., 2008). Hereon, the chromatographic recovery was calculated using the total As concentration in the colon digest measured by ICP-MS divided by the sum of the concentrations of chromatographically detected As species. The recoveries were satisfactorily high: 102.2 ± 6.9% (mean ± SD) on average (Table S3).

3. Results and discussion

3.1. Arsenic bioaccessibility in the gastric and small intestinal phases

Fig. 1 shows comparisons of the As bioaccessibility values (0.9–41.2%) for six soils determined for the gastric (G) and small intestinal (I) phases using the SBRC, PBET, DIN, UBM, and IVG *in vitro* methods. Synchronously, As bioaccessibility data illustrate the considerable variability between *in vitro* methodologies for all studied soils. For four of the six soils evaluated, the SBRC-G or UBM-G methods gave dramatically higher assessments of the As bioaccessibility than those acquired from other *in vitro* methods. The disparity in the As bioaccessibility may be in part reflective of the differences in parameters (i.e., gastric pH, and constituents) for the *in vitro* methods. The lower pH in the gastric phase with higher As bioaccessibility leads to an increase in the dissolution of As and Fe (Juhász et al., 2009; Ruby et al., 1996). In addition, organic constituents in the gastric phase can influence the solubility of As and Fe (Smith et al., 2014). Nevertheless, soil properties (i.e., total As concentration, particle size, and mineral phases) may also affect the As bioaccessibility, as reported in previous studies (Cui and Chen, 2011; Juhász et al., 2009; Smith et al., 2014; Yang et al., 2003; Yin et al., 2015a). Presumably, the differences in As bioaccessibility are a result of synergistic effects between the *in vitro* parameters and soil properties.

From the gastric to small intestinal phases, the As bioaccessibility also differed. For the SBRC method, the As bioaccessibility was significantly reduced by 2.0 to 14.5-fold in soils 1, 2, and 6. The importance of soil Fe in controlling the As dissolution and bioaccessibility has been evidenced (Mercer and Tobiasson, 2008). In contrast, increases in the As bioaccessibility using PBET-I and DIN-I for all soils except soil 1 were observed, by 1.1 to 1.7-fold and 1.1 to 2.8-fold, respectively. Masscheleyn et al. (1991) showed that under alkaline conditions, the As solubility is determined by solubility of Fe oxides/hydroxides. With pH values up to 7.0 in the small intestinal phase, the dissolved Fe concentrations increased, which can result in As desorption. Besides, the As bioaccessibility was either congruent, slightly decreased or increased following the transition from the gastric to small intestinal phases in the UBM and IVG methods.

Fig. 1. Comparison of As bioaccessibility values (%) for 6 soils using gastric, small intestinal and colon phases. For each soil, Means marked with the same letter indicate that data are not significantly different ($P > 0.05$).

3.2. Arsenic bioaccessibility in the colon phase

In this study, we first aimed to detail the variability in the As bioaccessibility and speciation in the colon phase. For the SBRC-SHIME method, following the transition from the small intestinal (SBRC-I) to the colon phase (SBRC-SHIME-C), As bioaccessibility was 1.9–19.3 times than that of SBRC-I (Fig. 1). A similar increase (1.4 to

3.4-fold) in the PBET-SHIME-C As bioaccessibility was observed for all soils (Fig. 1). An increase in the As bioaccessibility in PBET-SHIME-C compared to PBET-I has previously been reported (Yin et al., 2015b). In addition, the DIN-SHIME-C As bioaccessibility was greater than the DIN-I values for four of the six soils evaluated (Fig. 1). Researchers have found that As mobilization is enhanced by As(V) reduction from As-bearing minerals in the presence of As-

reducing bacteria (Smeaton et al., 2012; Smith et al., 2014). As detailed by Tian et al. (2015) microbial Fe(III) reduction may have two opposite consequences on the fate of associated As: mobilization (Cummings et al., 1999; Islam et al., 2004) and sequestration (Coker et al., 2006; Islam et al., 2005; Jiang et al., 2013). In anaerobic environments, the adsorption ability for As is weaker due to the reductive dissolution of Fe(III) oxides in the presence of Fe(III)-reducing bacteria; consequently, the As bioaccessibility is greatly increased. However, in the opposite case, the formation of secondary Fe-minerals during the Fe(III) reductive dissolution, can sequester As, resulting in reduced As bioaccessibility (Coker et al., 2006; Islam et

al., 2005). As mentioned, As(III) oxidation and As(V) sequestration by Mn oxides can also play a small but influential role in As bioaccessibility and speciation in the colon phase (Ying et al., 2011). In this study, the As bioaccessibility was reduced by 1.1 to 12.4-fold in five of six soils following the modification of IVG-I to IVG-SHIME-C (Fig. 1). The decrease in As bioaccessibility from the small intestinal to the colon phases has previously been reported by uniting IVG with SHIME (Van de Wiele et al., 2010). Furthermore, when the UBM method was modified to reflect the colon phase condition, the As bioaccessibility decreased in the colon phase except for some soils (i.e., soils 4 and 5).

Fig. 2. Comparison of the concentrations (mg kg^{-1}) of different species [As(III), DMA^{V} , MMA^{V} , As(V), and MMMTA^{V}] for 6 soils in the colon phase.

Fig. 3. Percentages (%) of As(III), methylarsenicals, and As(V) in the colon phase (total percentages are 100%) for 6 soils.

3.3. Arsenic speciation in the colon phase

Generally, As bioaccessibility and RBA are used for human health risk assessments, but it cannot be ignored that there are different As species with varying toxicities, considering As metabolism by human gut microbiota. Accordingly, compared to As bioaccessibility in the gastric and small intestinal phases, As speciation analysis can result in more accurate risk assessment to human health associated with soil As exposures. As detailed in Fig. 2, As speciation varies considerably with regard to the impacts of different constituents between methods.

UBM-SHIME-C displayed the highest methylation percentage of 13.5–82.1% (mean value 49.1%) for all soils. However, the methylation percentage in SBRC-SHIME-C was lower by comparison, 0.2–21.8%. No As methylated species were detected in a few cases (Fig. 2). A similar between-method variability in determining the As methylation percentage for any of the six soils (Fig. 3) showed adequately different methylation abilities among these methods. Arsenic methylation and thiolation by human gut microbiota have been found previously in SHIME experiments (Rubin et al., 2014; Van de Wiele et al., 2010). Studies have indicated that MMA^V can be regarded as an intermediate in the production of MMTA^V by microbial thiolation whose main sulfide source can be H₂S by sulfate-reducing bacteria (SRB) (Rubin et al., 2014). The disparity in As methylation can be attributed to the impacts of different organic constituents for these methods. The organic constituents can be regarded as nutritional supplements to significantly

increase the microbial activity (Laird et al., 2009). Huang et al. (2012) demonstrated that organic matter addition (*i.e.*, clover or dried distillers grain) increases As methylation and volatilization from paddy soils. In our study, the presence of more or fewer organic constituents for the UBM and SBRC methods resulted in corresponding As methylation percentages for the colon digests. Moreover, for soil 1, the As bioaccessibility and methylation for all *in vitro* methods were generally lower. Presumably, there were higher amounts of crystalline Fe oxides and residual phase by soil As sequential extraction, which can restrict As mobility (Yin et al., 2015b).

In our study, large amounts of As(III) were observed in the colon digests due to the microbial reduction of As(V). The As(III) percentage in the colon phase varied, being 2.3–31.5% (mean value 20.2%, SBRC-SHIME), 11.4–31.0% (20.3%, PBET-SHIME), 1.3–34.0% (12.3%, DIN-SHIME), 2.5–25.0% (12.7%, UBM-SHIME), and 1.7–21.5% (13.3%, IVG-SHIME). Interestingly, by the Spearman correlation analysis of the data for all *in vitro* methods (Table 2), we found that the MMA^V levels in the colon digests were positively correlated with those of As(III) ($r^2 = 0.42$, $P < 0.05$) and DMA^V ($r^2 = 0.60$, $P < 0.01$), whereas the correlation between the levels of As(III) and DMA^V was much lower ($r^2 = 0.01$, $P > 0.05$). Additionally, in the colon phase, the As(III) percentages negatively correlated with those of methylarsenicals ($r^2 = -0.53$, $P < 0.01$). This seems to indicate the interconversion among As(III), MMA^V, and DMA^V. During the As methylation process, As(III) may be primarily converted into MMA^V, and then the MMA^V

Table 2

Correlation matrix between the concentrations of different species, bioaccessible As concentrations, and percentages of As(III) and methylation ($n = 30$).

	As(III)	DMA ^V	MMA ^V	As(V)	Bioaccessible As	As(III) (%)	Methylarsenicals	Methylation (%)
As(III)	1.000	0.013	0.415*	0.919**	0.906**	0.775**	0.112	-0.544**
DMA ^V		1.000	0.599**	0.096	0.222	-0.405*	0.874**	0.669**
MMA ^V			1.000	0.337	0.468*	0.170	0.800**	0.334
As(V)				1.000	0.939**	0.564**	0.036	-0.626**
Bioaccessible As					1.000	0.504**	0.243	-0.443*
As(III) (%)						1.000	-0.119	-0.525**
Methylarsenicals							1.000	0.680**
Methylation (%)								1.000

As(III), DMA^V, MMA^V, As(V), bioaccessible As, and methylarsenicals: the concentrations (mg kg⁻¹); As(III) and methylation (%): As(III) and methylarsenicals concentrations were divided by bioaccessible As concentrations in the colon extractions.

* $P < 0.05$.

** $P < 0.01$.

Fig. 4. As bioaccessibility (%) using the combination of SBRC, PBET, DIN, UBM, and IVG (gastric and small intestinal phases) with SHIME (colon phase) and RBA (%) in animal models [A1 (Bradham et al., 2011) and A2 (Juhász et al., 2014): mouse; A3: swine (Brattin et al., 2013)] for NIST 2710a.

may be transformed into DMA^V. Zhu et al. (2014) summarized the As species and their biotransformation in a detailed diagram showing known enzymes for biotransformation and representative protein structures. Researchers have clearly demonstrated that gut microbiota significantly affect the As metabolism by, for example, reduction, methylation, and thiolation (Lu et al., 2013). The previous study showed that As(V) is the predominant form (nearly >99%) for the small intestinal digest in contaminated soils (Yin et al., 2015b). As(V) reduction and As(III) methylation should be two vital processes in As metabolism by human gut microbiota. In general, As(III) as an intermediary is converted into methylated species that eventually end up as low-toxicity MMA^V and DMA^V through a detoxification process. Therefore, we infer that DMA^V can partly be considered to evaluate the As metabolic speed of *in vitro* cultured human gut microbiota, and the transformation rate can be fast with high DMA^V levels. Nevertheless, the highly toxic MMA^{III} detected as a metabolite by human colon microbiota indicates that As methylation will not lead to detoxification (Van de Wiele et al., 2010). Due to As thiolation by SBR, some thiolated arsenicals, DMMTA^V with high toxicity and harmless DMDTA^V, are often found in the urine after inorganic As exposure (Kubachka et al., 2009; Naranmandura et al., 2007; Raml et al., 2007).

3.4. Arsenic relative bioavailability and As bioaccessibility of NIST 2710a

In previous studies, animal models (e.g., mouse, swine, and monkey) were utilized for the determination of As RBA in contaminated soils (Bradham et al., 2011; Brattin et al., 2013; Roberts et al., 2007). For NIST 2710a, these studies investigated the relationship between the As RBA determined in mouse and swine *in vivo* models and the As bioaccessibility by multiple *in vitro* methods (e.g., SBRC, PBET, DIN, UBM, and IVG). The As RBA values for NIST 2710a were 42.9% (Bradham et al., 2011), 42.1% (Juhász et al., 2014), and 41.8% (Brattin et al., 2013), respectively.

From Fig. 4, we find that the As bioaccessibility values in SBRC-SHIME-C, DIN-I, UBM-G, UBM-I, and PBET-SHIME-C, are relatively close (<15%) to that of the As RBA. Comparing the results of the As bioaccessibility in the colon phase with that of the As RBA, almost all of them were lower except for the PBET-SHIME method (1.2 times the As RBA). The values are approximately 7% (IVG-SHIME), 22% (UBM-SHIME), 47% (DIN-SHIME) and 86% (SBRC-SHIME) of the mean As RBA value; the results of PBET-SHIME and SBRC-SHIME are the closest to As RBA. Based on the variation in results between different *in vitro* (values in the colon phase) and *in vivo* methods, two aspects should

attract considerable attention, animal experiments may not completely simulate the human digestive process, and the results in the colon phase can be considered accurate values for assessing the As risk in some soils. A comparison of different *in vitro/in vivo* methods and considering As metabolism by human gut microbiota can improve the accuracy of risk assessment.

4. Conclusions

In the colon phase, the As bioaccessibility varies and one highlighted result is that different extents of As methylation and large amounts of As(III) due to microbial reduction are observed. Additionally, we inferred forcefully that DMA^V can be considered an indicator to estimate the As metabolic speed of *in vitro* cultured human gut microbiota. Another highlighted result is that the As bioaccessibility values in the colon phase of the PBET-SHIME and SBRC-SHIME methods are closest to the *in vivo* results based on the standard reference soil of NIST 2710a compared to UBM-SHIME, IVG-SHIME and DIN-SHIME. Lacking data on *in vivo-in vitro* (colon phase) correlations in soils, it is difficult to confirm which method can most accurately predict the As RBA. It is therefore critical to establish As *in vivo-in vitro* (colon phase) predictive models to evaluate the As RBA in contaminated soil.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2016.06.071>.

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