

1 *In vitro* model insights into the role of human gut microbiota on
2 arsenic bioaccessibility and its speciation in soils

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4 Haifeng Chi ^{a,b}, Yanwei Hou ^c, Guofeng Li ^{a,b}, Youchi Zhang ^a, Frédéric Coulon ^d,
5 Chao Cai ^{a,*}

6 a. State Key Laboratory of Urban Environment and Health, Institute of Urban
7 Environment, Chinese Academy of Sciences, Xiamen 361021, China

8 b. University of Chinese Academy of Sciences, Beijing 100049, China

9 c. Department of Environmental Science and Engineering, Huaqiao University,
10 Xiamen 361021, China

11 d. School of Water, Energy and Environment, Cranfield University, Cranfield MK43
12 0AL, UK

13 * Corresponding author. E-mail: ccai@iue.ac.cn

14 Address: No. 1799 Jimei Road, Jimei District, Xiamen, Fujian, 361021, P. R. China.

15 Tel: +86-0592-6190997; Fax number: +86-0592-6190977

16 **Abstract**

17 The bioaccessibility of arsenic and its speciation are two important factors in
18 assessing human health risks exposure to contaminated soils. However, the effects of
19 human gut microbiota on arsenic bioaccessibility and its speciation are not well
20 characterized. In this study, an improved *in vitro* model was utilized to investigate the
21 bioaccessibility of arsenic in the digestive tract and the role of human gut microbiota
22 in the regulation of arsenic speciation. For all soils, arsenic bioaccessibility from the
23 combined *in vitro* model showed that it was < 40% in the gastric, small intestinal and
24 colon phases. This finding demonstrated that the common bioaccessibility approach
25 assuming 100% bioaccessibility would overestimate the human health risks posed by
26 contaminated soils. Further to this, the study showed that arsenic bioaccessibility was
27 22% higher in the active colon phase than that in the sterile colon phase indicating
28 that human colon microorganisms could induce arsenic release from the solid phase.
29 Only inorganic arsenic was detected in the gastric and small intestinal phases, with
30 arsenate [As(V)] being the dominant arsenic species (74%-87% of total arsenic).
31 Arsenic speciation was significantly altered by the active colon microbiota, which
32 resulted in the formation of methylated arsenic species, including monomethylarsonic
33 acid [MMA(V)] and dimethylarsinic acid [DMA(V)] with low toxicity, and a highly
34 toxic arsenic species monomethylarsonous acid [MMA(III)]. Additionally, a high
35 level of monomethylmonothioarsonic acid [MMMTA(V)] (up to 17% of total arsenic
36 in the extraction solution) with unknown toxicological properties was also detected in
37 the active colon phase. The formation of various organic arsenic species
38 demonstrated that human colon microorganisms could actively metabolize inorganic

39 arsenic into methylated arsenicals and methylated thioarsenicals. Such transformation
40 should be considered when assessing the human health risks associated with oral
41 exposure to soil.

42 **Keywords:** soils, arsenic bioaccessibility, human gut microbiota, *in vitro* models,
43 health risk assessment

44 **Main findings:** The human colon microbes could actively metabolize soil inorganic
45 arsenic into highly toxic MMA(III) and unknown toxicological MMMTA(V).

1. Introduction

Arsenic is a ubiquitous element in the environment presenting high toxicity and carcinogenicity (Zhu et al., 2014). Soils have been proven to be important sinks for arsenic, and the chemical fractionations of arsenic feature differential labile phases and bioavailability in soils. Generally, arsenic in soil is dominantly associated with iron (Fe) oxides, amorphous manganese (Mn) and aluminum (Al) that can pose detrimental health effects to humans (Niazi et al., 2011). An increasing body of evidence establishes a clear correlation between arsenic and human diseases, such as Blackfoot disease (Tseng, 2005), neonatal death (Milton et al., 2005) and even cancers (Lin et al., 2013; Zhou and Xi, 2018). Although inhalation of arsenic-containing particles contributes negligibly to arsenic exposure (Meacher et al., 2002), incidental oral ingestion of soil is, however, an important exposure route for arsenic, especially for children (Ljung et al., 2006). The reported human soil ingestion rates generally range between 37 and 207 mg d⁻¹ for children (Davis and Mirick, 2006). Considering the notable ingestion of soil, the human health risk associated with oral exposure to soil arsenic is becoming a public issue (Luo et al., 2012).

Several human health risk assessments for heavy metals contaminated sites rely on the use of over conservative estimation based on the total concentration of the element considered (Liao et al., 2005; Wcisło et al., 2016). However, recent studies have demonstrated that the physiological and mineralogical properties of the soils influence element dissolution and gastrointestinal absorption (Frau and Arda, 2004; Ruby et al., 1999; Stýblo et al., 2002). Thus, to overcome this risk overestimation, several studies developed *in vivo* models, using rodents, rabbits and swine, to quantify element bioavailability, especially for arsenic (Juhasz et al., 2007; Ng et al., 1998; Rodriguez et al., 1999; Li et al., 2019). However, the use of *in vivo* models is time-consuming and expensive and also poses ethical

issues (Basta et al., 2007). Thus, simple, fast and inexpensive *in vitro* models such as the physiologically based extraction test (PBET), *In vitro* gastrointestinal method (IVG), simple bioaccessibility extraction test (SBET) and unified BARGE method (UBM) models have been developed to measure the fraction of arsenic that is released from the soil for intestinal tract absorption (the bioaccessible fraction) (Ruby et al., 1996; Sarkar et al., 2007). Furthermore, these *in vitro* models have been validated for predicting arsenic relative bioavailability by establishing the *in vivo-in vitro* correlations (IVIVC) (Juhasz et al., 2009; Li et al., 2015). Given that bioaccessibility is one of the principal factors limiting arsenic assimilation, such insight is invaluable in the assessment of exposure risk. Assessing the health risks from ingesting arsenic-contaminated soil requires data on the arsenic ingestion rate, arsenic bioaccessibility in the gastrointestinal tract as well as the speciation of arsenic following gastrointestinal digestion of the soil, as its speciation largely determines its toxicity (Zhu et al., 2014). Although inorganic arsenic may be the major species in soil, arsenic speciation in the digestive tract is not well characterized (Alava et al., 2012). Furthermore, the colon, as one of the digestive organs, represents a highly reducing environment and provides a vast (up to 10^{14} bacterial cells) and diverse (above 1,000 speciation) microbial community (Eckburg et al., 2010), which could influence arsenic bioaccessibility and speciation (Van de Wiele et al., 2010). However, most *in vitro* models only consider the digestive process that takes place in the stomach and small intestine (Oomen et al., 2002; Rodriguez et al., 1999; Ruby et al., 1996; Xia et al., 2016). Indeed, many compounds including arsenic could be transported across the epithelium in the colon, and health modulation by the human gut microbial community should not be underestimated (Diaz-Bone and Van de Wiele, 2010; Roggenbeck et al., 2016). In this case, exclusion of the

colon from these *in vitro* models may be a shortcoming, as the colon represents a contrasting environment to the stomach and small intestine.

A dynamic human gastrointestinal simulator known as the simulator of the human intestinal microbial ecosystem (SHIME) has been used to investigate the measurement of contaminants bioaccessibility by mimicking the physiological parameters of the human gastrointestinal tract (Ruby et al., 1993; Chi et al., 2018). Unlike other *in vitro* models, the SHIME model is seeded with microbial community cultures obtained from the human feces. Results obtained with the SHIME model have proven that the human colon microbiota can transform inorganic arsenic into organic arsenicals (Van de Wiele et al., 2010). To further explore arsenic transformation in all regions of the digestive tract, other *in vitro* models such as UBM, IVG, and PBET, were combined with SHIME to study arsenic bioaccessibility and speciation changes during passage through the gastrointestinal tract (Sun et al., 2012; Yin et al., 2015; Yin et al., 2016). Yin et al. (2015) found that a large amount of toxic arsenite [As(III)] was observed as a result of human gut microbial reduction by using a combined PBET-SHIME model, and various organic arsenic species, such as monomethylarsonic acid [MMA(V)], dimethylarsinic acid [DMA(V)] and monomethylmonothioarsonic acid [MMMTA(V)], were also observed in the active human colon stage. Furthermore, arsenic bioaccessibility varied in the colon phase among these methods (Yin et al., 2016). Sun et al. (2012) also found highly toxic monomethylarsonous acid [MMA(III)] in colon digests of arsenic-contaminated rice, resulting in a higher human health risk. These findings indicated that various arsenic transformations have occurred in the digestive tract, especially with the involvement of human gut microorganisms, which resulted in the complexity of human health risk assessment. However, studies of the effects of human gut microbiota on arsenic bioaccessibility and speciation in soils are limited. In the current study, an improved *in vitro*

117 model, UBM-SHIME, was employed to evaluate (1) arsenic bioaccessibility and its
118 relationship with arsenic fractionation in soils and (2) arsenic metabolism in soils by human
119 gut microbiota. This study provides new insight into health risk assessments related to oral
120 exposure to soils.

2. Materials and Methods

2.1 Chemicals

Ultrapure 18 mΩ water (DDI; Millipore, Bedford, MA, USA) was used to prepare the stock standard solutions and chromatographic mobile phase. Sodium arsenate ($\text{Na}_2\text{HAsO}_4 \cdot 12\text{H}_2\text{O}$) and sodium arsenite (NaAsO_2) were purchased from BAL (Beijing, China), MMA(V) and DMA(V) were purchased from AccuStandard Inc (New Haven, CT), MMA(III) was purchased from Sigma Chemicals (Belgium), and MMMTA(V) was synthesized using a mixture of MMA(V) and an H_2S solution (Sergio et al., 2014). Detailed information about the method used to synthesize MMMTA(V) is provided in Supporting Information. Chromatographic confirmation of the MMMTA(V) is shown in Figure S1.

2.2 Soil collection and characterization

Surface soil samples (0-20 cm) were collected from different types of sites, including mining land, chemical land, and battery plants. All collected soil samples were placed in nylon woven bags and transported back to the laboratory. Samples were then freeze-dried, crushed and sieved to 250 μm for *in vitro* gastrointestinal incubation. This reflects the size of particles that most likely stick to the hands and thereby provide a route of exposure to humans (Zagury, 2007). The dissolved organic carbon (DOC) fraction of the soil samples was extracted with ultrapure water (Yu et al., 2012), and a total organic carbon analyzer (TOC-L CPH, Shimadzu, Japan) was utilized for DOC measurement. The soils were digested using an HNO_3 and HClO_4 method for arsenic and other metals analysis (Lee et al., 2006). Then, the concentrations of Fe and Mn were quantified by inductively coupled plasma-optical emission spectroscopy (ICP-OES, Optima 7000DV, PerkinElmer, USA) and inductively coupled plasma-mass spectrometry (ICP-MS, 7500a, Agilent Technologies, USA) was used to quantify the concentration of arsenic. The physicochemical properties of the

soils were presented in Table 1. Arsenic speciation in soils was extracted by using a 300 mM phosphate solution of pH 6.0 at 40 °C (Alam et al., 2001), and HPLC-ICP-MS was utilized for arsenic speciation analysis. In the digestion process, blank and standard reference materials (GSS-1 and GSS-3, National Institute of Metrology, China) were employed to ensure the accuracy and recovery rates of arsenic (90.2%-118.9%).

2.3 Sequential extraction of soil arsenic from soil

Two sequential extraction procedures (SEPs), including the Tessier and Wenzel SEPs, were compared for arsenic fractionation in soils. Tessier SEP is a classical sequential extraction method for the partitioning of heavy metals into the exchangeable fraction (F1), the fraction bound to carbonates (F2), the fraction bound to Fe and Mn oxides (F3), the fraction bound to organic matter (F4) and the residual fraction (F5) (Tessier et al., 1979). The Wenzel SEP is an improved sequential extraction procedure specially developed for arsenic fractionation. With this procedure, the arsenic in soil is divided into the nonspecifically sorbed fraction (NS1), the specifically sorbed fraction (SS2), the amorphous and poorly crystalline hydrous oxides of Fe and Al fraction (AF3), the well-crystallized hydrous oxides of Fe and Al fraction (CF4) and the residual phases (RS5) (Wenzel et al., 2001). The extracted supernatant was centrifuged and filtered through 0.22 µm filters for further analysis using ICP-MS (Agilent 7500a, USA).

2.4 Production and characterization of colon microbiota for SHIME

The colon microbial community utilized in this experiment was cultured and maintained in an improved SHIME model (Chi et al., 2018). The SHIME consisted of five double-jacketed vessels maintained at a temperature of 37 °C, which simulated the stomach, small intestine, and the ascending, transverse and descending colon, respectively. The colon vessel pH controllers maintained the pH in the ascending colon, transverse colon and

169 descending colon at 5.6-5.9, 6.1-6.4 and 6.6-6.9, respectively. The SHIME reactors were
170 continuously stirred and kept under anaerobic conditions by regularly flushing with nitrogen.
171 After three weeks of adaptation, stable microbial communities were obtained from the
172 descending colon compartments for further study. The total DNA extraction was conducted
173 according to the manufacturer's instructions for the FastDNA[®] Spin Kit for Soil (MP
174 Biomedicals Inc, Santa Ana, USA). The general bacterial primers 338F-GC and 518R were
175 used in PCR amplification (Figure S4).

176 **2.5 Arsenic bioaccessible assessment**

177 The *in vitro* approach was adapted from a UBM-SHIME method (Wragg et al., 2011;
178 Chi et al., 2018). For the stomach phase: 0.36 g of soil was accurately added into a 100 mL
179 brown serum bottle, and 5.4 mL of simulated saliva was added via a pipette and then
180 manually shaken to thoroughly mix the soil and simulated fluids. Subsequently, simulated
181 gastric fluid (8.1 mL) was added to each bottle. The solution pH was adjusted to 2.0 using
182 HCl (1.0 M), high purity nitrogen gas was flushed into the bottles, which were then capped
183 with a rubber stopper to ensure an anaerobic environment. Then, bottles were shaken (100
184 rpm) at 37 °C for 1 h. In the small intestine phase, after 1 h of incubation, 16.2 mL of
185 simulated duodenal fluid and 5.4 mL of simulated bile fluid were added into each bottle, and
186 the pH was adjusted to 6.0 with NaOH (1.0 M) and flushed with nitrogen gas to ensure an
187 anaerobic environment. These bottles were returned to the shaker for an additional 4 h. In the
188 colon phase, 35.1 ml of colon SHIME solution from the descending compartment of the
189 dynamic SHIME system was added to the bottles. Then, the bottles were capped with a
190 rubber stopper, flushed with nitrogen gas for 30 min to replace the headspace and assure
191 anaerobic conditions, placed in a shaker and incubated at 37 °C for an additional 48 h. A
192 sterilized colon suspension was used to investigate the effect of colon microbes on arsenic

speciation modulation. Destructive sampling was carried out. The experiment was conducted in quadruplicate. To avoid contamination, all glassware, storage bottles, and centrifuge tubes were kept in 10% nitric acid for at least 24 h, rinsed three times with ultrapure water and dried before use.

2.6 Chemical analysis

To preserve the speciation of arsenic in the digestive phases, all samples from the stomach, small intestine and colon phases were immediately flash-frozen with liquid nitrogen and subsequently stored at -80 °C. The supernatants were centrifuged at 10,000 g for 10 min and then passed through a 0.22 µm filter before analysis. The arsenic speciation was determined by high-performance liquid chromatography coupled with inductively coupled plasma-mass spectrometry (HPLC-ICP-MS). A Hamilton PRP-X100 column (250×4.6 mm, 10 µm) (Yan et al., 2017) and a Phoenix C18 column (250×4.6 mm, 10 µm) (Yan et al., 2015) were used. The chromatographic condition details are provided in Table S1. Arsenic speciation in the digestive tract solution was identified by comparing their retention time to those of standards [As(III), As(V), MMA(III), MMA(V), DMA(V) and MMMTA(V)] and quantified by external calibration curves of DMA(V) (Xu et al., 2012). The chromatograms are presented in Figure S2. The sum of the arsenic speciation in the filtrate was considered the bioaccessible fraction (Sun et al., 2012). Bioaccessibility was calculated using the following equation:

$$\text{Arsenic bioaccessibility (\%)} = \frac{(\text{Arsenic})_{\text{filtrate}} \times \text{fluid volume}}{(\text{Arsenic})_{\text{soil}} \times \text{soil mass}} \times 100\%$$

where (Arsenic)_{filtrate} is the total arsenic concentration (mg L⁻¹) in the 0.22 µm-filtered, fluid volume is the total volume of the gastric, small intestinal and colon fluid (L), (Arsenic)_{soil} is the total arsenic concentration (mg kg⁻¹) in the soil, and soil mass is the total mass (kg) of the soil used in the *in vitro* test.

2.7 Statistical analysis

Statistical analysis was performed using SPSS Software 16.0. All statistical tests were considered significant at $p < 0.05$. Graphs were generated with SigmaPlot 12.5 and Origin 8.0.

3. Results and Discussions

3.1 Arsenic bioaccessibility and fractionation

The arsenic bioaccessibility of soils was highly variable, ranging between 11.5-18.3%, 14.7-32.5% and 19.7-36.9% in the gastric, small intestinal and colon phases, respectively (Figure 1). Arsenic bioaccessibility was $< 40\%$ in the digestive fluids, which confirmed that the arsenic could not be dissolved completely from the soil matrix. Previous studies demonstrated that chemical-form-oriented procedures could sufficiently define the mobile arsenic fraction in soils (Smith et al., 2008; Wan et al., 2017). To better understand the effects of the arsenic fraction on its bioaccessibility, two sequential extraction procedures (SEPs), including Tessier and Wenzel SEPs, were compared for arsenic fractionation in soils (Figure 2). The residual fractions extracted by the Tessier SEP (F5, accounting for 51.9%~74.2% of total arsenic) and Wenzel SEP (RS5, accounting for 35.7%~51.7% of total arsenic) were dominant in soils, which implied that the residual fraction of arsenic in soils could be one of the reasons for the low arsenic bioaccessibility. The residual fraction is mainly composed of oxyanions, which are tightly bound to the mineral components of the soil, such as conichalcite $[\text{CaCu}(\text{AsO}_4)\text{OH}]$, arsenopyrite (FeAsS) and realgar (As_4S_4) . All of these arsenic-containing ores in soils are indeed known to be less soluble than other forms of arsenic (Harvey et al., 2006; Meunier et al., 2010; Kim et al., 2014), resulting in a low level of arsenic bioaccessibility.

240 During the gastric digestive process, the bioaccessibility of arsenic in soil from the
241 mining land (soil 1 and soil 2), chemical plants (soil 3 and soil 4) and battery plants (soil 5
242 and soil 6) were 14.6%-14.8%, 16.0%-18.3%, and 11.5%-13.6%, respectively. The chemical
243 plant soils showed a higher arsenic bioaccessibility than that of the mining and battery plant
244 soils. The arsenic bioaccessibility was generally dependent on the type of soil. The battery
245 plant soils exhibited significantly ($p<0.05$) higher arsenic bioaccessibility values
246 (15.3%-32.5%) in the small intestinal phase than in the gastric phase. In contrast, the arsenic
247 bioaccessibility in the mining and chemical plant soils remained constant during the
248 digestive process from the gastric phase to the small intestinal phases. The bioaccessibility of
249 arsenic was associated with arsenic fractionation (Kim et al., 2014; Palumbo-Roe et al.,
250 2015). A significant correlation was observed between arsenic bioaccessibility in the gastric
251 phase and NS1+SS2+AF3 ($r^2=0.74$, $p<0.05$), which was consistent with previous studies
252 (Smith et al., 2008; Li et al., 2015). Li et al. (2015) compared the sequential extractable
253 arsenic fractions with bioaccessible arsenic based on four assays, and they indicated that the
254 exchangeable and outer-sphere (NS1), inner-sphere (SS2) and part of the amorphous and
255 poorly crystalline hydrous oxides of Fe and Al fractions (AF3) were considered to be
256 bioaccessible. The well-crystallized hydrous oxides of Fe and Al fraction (CF4) was thought
257 to be relatively immobile. However, the first four fractions (AF1+SS2+AF3+CF4) extracted
258 by the Wenzel SEP showed a strong correlation ($r^2=0.76$, $p<0.05$) with arsenic
259 bioaccessibility in the small intestinal phase, which implied that the CF4 fraction may
260 contribute to bioaccessible arsenic. Furthermore, soil physicochemical properties, including
261 the particle size fraction, soil organic matter (SOM), dissolved organic carbon (DOC), soil
262 pH, and total manganese (Mn) and total iron (Fe) concentrations, were selected to explore
263 the key soil parameters that might significantly affect arsenic bioaccessibility (Table S2).

DOC ($r^2 = 0.92$, $p < 0.01$) and SOM ($r^2 = 0.74$, $p < 0.01$) were identified as the two major physicochemical parameters influencing arsenic bioaccessibility in the gastric phase. The F4 fraction (bound to organic matter) extracted by the Tessier SEP also showed a strong correlation with arsenic bioaccessibility in the gastric phase ($r^2=0.87$, $p<0.01$). Arsenic is usually present as oxyanions in acidic environments, and organic matter carrying a negative charge could increase arsenic mobility by forming aqueous complexes, competing for adsorption sites or through electrostatic interactions (Wang and Mulligan, 2009).

In the colon phase, the bioaccessibility of arsenic ranged from 19.7% to 36.9%, which was 1.3 to 2.1 times higher than that in the small intestinal phase, respectively. A sterile colon suspension from the dynamic SHIME was utilized to explore the effects of the colon microbial community on arsenic bioaccessibility (Figure S2). The arsenic bioaccessibility in the active colon phase was higher than that in the sterile colon phase. Similar results indicated that human colon microorganisms could increase arsenic bioaccessibility (Oremland and Stolz, 2005; Laird et al., 2007; Yin et al., 2015). Under the anaerobic conditions of the colon phase, there was abundant gut microbiota responsible for the reduction of As(V) to As(III), which possesses a lower affinity for sorption to iron oxides. Additionally, human gut microbiota could catalyze the reduction of iron oxides bearing arsenic. Both of these reductions could be reasons for the increase in arsenic bioaccessibility by gut microbiota.

3.2 Arsenic speciation in the digestive tract

Only inorganic arsenic was detected in all the soils, and As(V), accounting for 86.0%~99.0% of the total extractable arsenic, was the dominant species in soils (Figure S5). After incubation of the active colon microbes, organoarsenicals, including two pentavalent methylated species [MMA(V) and DMA(V)], a trivalent methylated species [MMA(III)] and

a methylated thioarsenical species [MMMTA(V)], were detected simultaneously, which accounted for 17.8-41.5% of the total soluble arsenic in the colon phase (Figure 3c). Arsenic speciation in the sterile colon phase was also analyzed to confirm the contribution of gut microbiota to arsenic metabolism. Only inorganic arsenic, including As(III) and As(V), was found, with As(V) being dominant (Figure 3d). The amount of As(V) accounted for 46.7% and 76.8% of the total arsenicals in the active and sterile colons, respectively, suggesting the significant reduction of As(V) by gut microbes. Previous studies demonstrated that As(III) showed a lower affinity for sorption to iron oxides than that of As(V) (Cao et al., 2003; Dixit and Hering, 2003), and the high proportion of As(III) in the active colon phase could increase bioaccessibility (Yin et al., 2015). These results suggested that the presence of colon microorganisms not only increased the bioaccessibility of arsenic but also had the potential to actively metabolize inorganic arsenic into methylated arsenical and thioarsenical species, as reported in previous studies (Laird et al., 2007; Van de Wiele et al., 2010; Yin et al., 2015). The colon microbial suspensions were collected from the improved SHIME model for 16S rDNA extraction and then high throughput sequencing. The results showed that the average abundances of the Enterobacteriaceae and Bacteroides genera were 38.0% and 22.3% respectively (Figure S4). Previous studies have shown that some species in the Enterobacteriaceae and Bacteroides genera can methylate arsenic (Isokpehi et al., 2014; Li et al., 2016; Yu et al., 2016). Thus, it is reasonable to hypothesize that human colon microbes have a high level of arsenic methylation potential. Compared with the diverse arsenic speciation in the colon phase, only inorganic arsenic was detected in the stomach and small intestinal phases (Figure 3a, 3b), and the dominant form was As(V), which accounted for 77.6-87.4% and 73.9-77.1% in the stomach and small intestinal phases, respectively.

Overall, As(III) rather than As(V) was the substrate for arsenic methylation, thus, the high reduction of As(V) in the active colon phases suggests the possibility of subsequent arsenic methylation in our study. Although the detailed mechanism of arsenic methylation is still a highly controversial topic, arsenic methylation has been proven to be catalyzed by the enzyme As(III) *S*-adenosylmethionine (SAM) methyltransferase (named ArsM in microbes and AS3MT in mammals) (Ajees and Rosen, 2015; Cai et al., 2018). It is also clear that the products of the enzyme are all trivalent and that the pentavalent species are the result of non-enzymatic oxidation in the air (Yang and Rosen, 2016). Thus, we proposed that intracellular As(III) was methylated by ArsMs from gut microbes to MMA(III) and DMA(III) and then oxidized to MMA(V) and DMA(V) by oxygen in the air during sample preparation and measurement. At the same time, dissolved oxygen is naturally present in gut fluids, and the oxidation process could also occur in the colon phase. This is further supported by the fact that DMA(III) is more sensitive to oxygen than MMA(III), and only MMA(III) but no DMA(III) was detected in the samples. Furthermore, because most intracellular As(III) is bound to intracellular thiols or thiols in proteins to yield trivalent protein-bound arsenicals under the exposure of glutathione (GSH), thiolation could be a competitive reaction to form stable pentavalent protein-bound arsenicals under the exposure of H₂S, which can be further hydrolyzed to MMMTA(V) and DMMTA(V) (Sergio et al., 2014). In this study, due to the abundant presence of sulfate-reducing bacteria that produce H₂S in the human fecal and colon microbiota, a considerable amount of MMMTA(V) was detected in the samples. However, the reason why no DMMTA(V) was detected needs further study.

3.3 Implication for the health risk assessment

Incidental soil ingestion is a potentially main route for non-dietary exposure to arsenic. Based on the soil ingestion rate, arsenic concentration in soil and arsenic bioaccessibility, the

daily amount of bioaccessible arsenic can be calculated by the following equation: the daily amount of bioaccessible arsenic ($\mu\text{g d}^{-1}$) = soil ingestion rate (g d^{-1}) $\times$ arsenic concentration in soil ($\mu\text{g g}^{-1}$) $\times$ arsenic bioaccessibility (%). The variability of arsenic bioaccessibility using different *in vitro* models results in the conservative approach of assuming the bioaccessibility of arsenic is 100%. Nevertheless, our results showed that only a low level of arsenic could be dissolved from the soil matrix, and Yin et al. (2016) demonstrated that arsenic bioaccessibility values in the gastric and small intestinal fluids of the UBM-SHIME combined model were closed to that of the arsenic relative bioavailability. In this case, the low levels of arsenic bioaccessibility in our study suggested that the health risk assessment based on the total concentration would be overestimated.

Furthermore, arsenic toxicity is one of the primary parameters for assessing the health risks associated with arsenic exposure. The toxicity of arsenic is highly dependent on its speciation (Bissen and Frimmel, 2003). Previous studies demonstrated that the LD_{50} of MMA(III) was 12 times lower than that of As(III), which indicated that MMA(III) is a much more toxicant and potent enzyme inhibitor than As(III) (Drobná et al., 2005; Petrick et al., 2001). This is also reflected by a larger cellular uptake and accumulation of MMA(III) compared to As(III) in human urothelial cells and rat hepatocytes (Drobná et al., 2005; Styblo et al., 2000). Furthermore, the finding of MMMTA(V) formation by the human colon microorganisms raises questions about its toxicological importance. Hinrichsen et al. (2015) demonstrated that the cellular retention of MMMTA(V) was lower than that of DMA(V), but the intestinal transport of MMMTA(V) was similar to that of As(V) in Caco-2 cell assays. Although the absorption kinetics of MMMTA(V) across the epithelium is not well characterized, there is evidence that some methylated thio-arsenicals elicit a more efficient uptake than that of As(V), which is essential in causing the toxicity (Naranmandura et al.,

2007). In this case, the formation of MMMTA(V) and MMA(III) in the colon phase will increase the uncertainty of the human health risk assessment. Our observations emphasized the need to investigate the behavior of MMA(III) and MMMTA(V) in the gut lumen. Considering that the formation of highly toxic MMA(III) and MMMTA(V) with unknown toxicokinetic properties by colon microorganisms, the arsenic metabolism by human colon microorganisms should be considered seriously while assessing human health risk after oral exposure to soil.

Conclusions

We presented detailed results on the bioaccessibility of arsenic ranging between 11.5% and 18.3% in the stomach, 14.7% and 32.5% in the small intestine and 19.7% and 36.9% in the colon, respectively. The low level of arsenic bioaccessibility values demonstrated that human health risks are overestimated by using the total concentration. However, the formation of highly toxic MMA(III) and MMMTA(V) with unknown toxicokinetic properties in the colon phase implied an increase in uncertainty of human health risk. The formation of various organic arsenic species demonstrated that human colon microorganisms had the potential to actively metabolize soil inorganic arsenic into methylated arsenicals and methylated thioarsenicals. Herein, the arsenic metabolism by human colon microorganisms should be considered seriously while assessing the human health risk after oral exposure to soil.

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382 ***Notes***

383 The authors declare no competing financial interest.

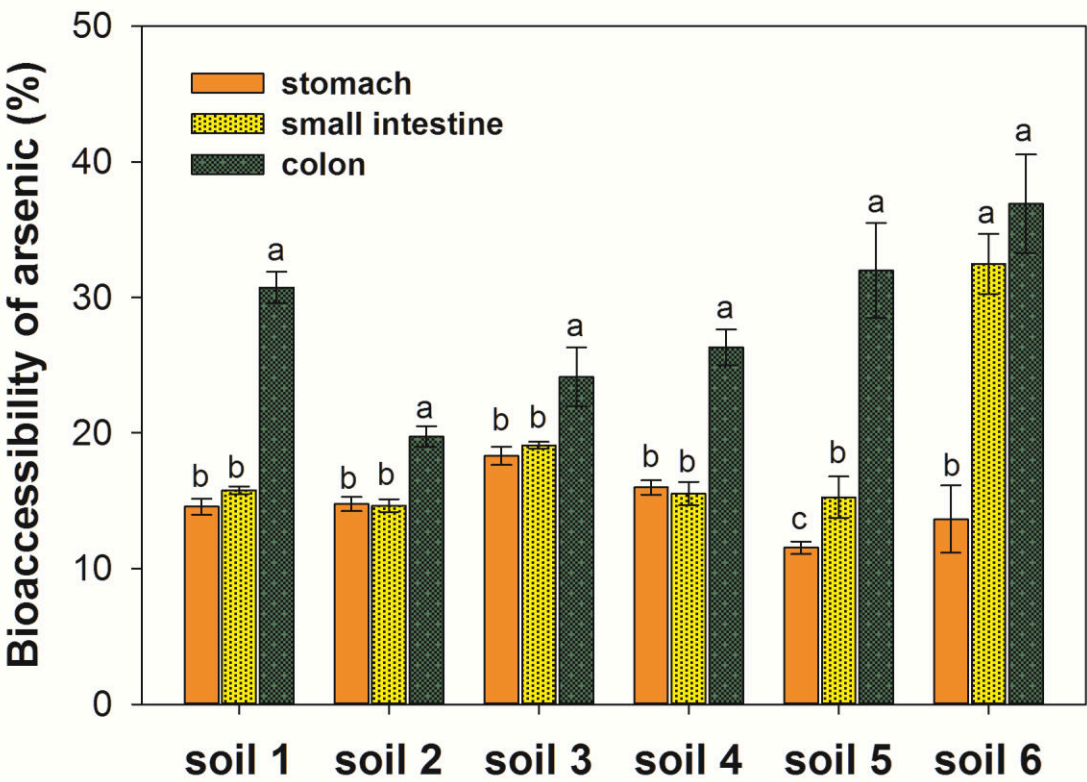
Figure captions

Figure 1 The bioaccessibility of arsenic in the digestive tract. (n=3). The different letters indicate significant differences between the samples at $p < 0.05$ using a one-way ANOVA test.

Figure 2 The distribution of arsenic fractionations by Wenzel SEP (a) and Tessier SEP (b). Non-specifically sorbed fraction (NS1), the specially-sorbed fraction (SS2), the amorphous and poorly-crystalline hydrous oxides of Fe and Al fraction (AF3), well-crystallized hydrous oxides of Fe and Al (CF4) and the residual phases (RS5). Exchangeable fraction (F1), bound to carbonates fraction (F2), bound to iron and manganese fraction (F3), bound to organic matter fraction (F4), the residual fraction (F5).

Figure 3. Contents of chromatographically detected arsenic speciation [As(III), As(V), MMA(V), DMA(V), MMA(III) and MMMTA(V)] in gastric (a), small intestinal (b), active colon (c) and sterile colon (d) phases. Values are represented as averages $\pm$ standard deviation (n=3). Note that arsenic contents were presented as a bioaccessible fraction in digestive phases. N.D. represented as Not Detected.

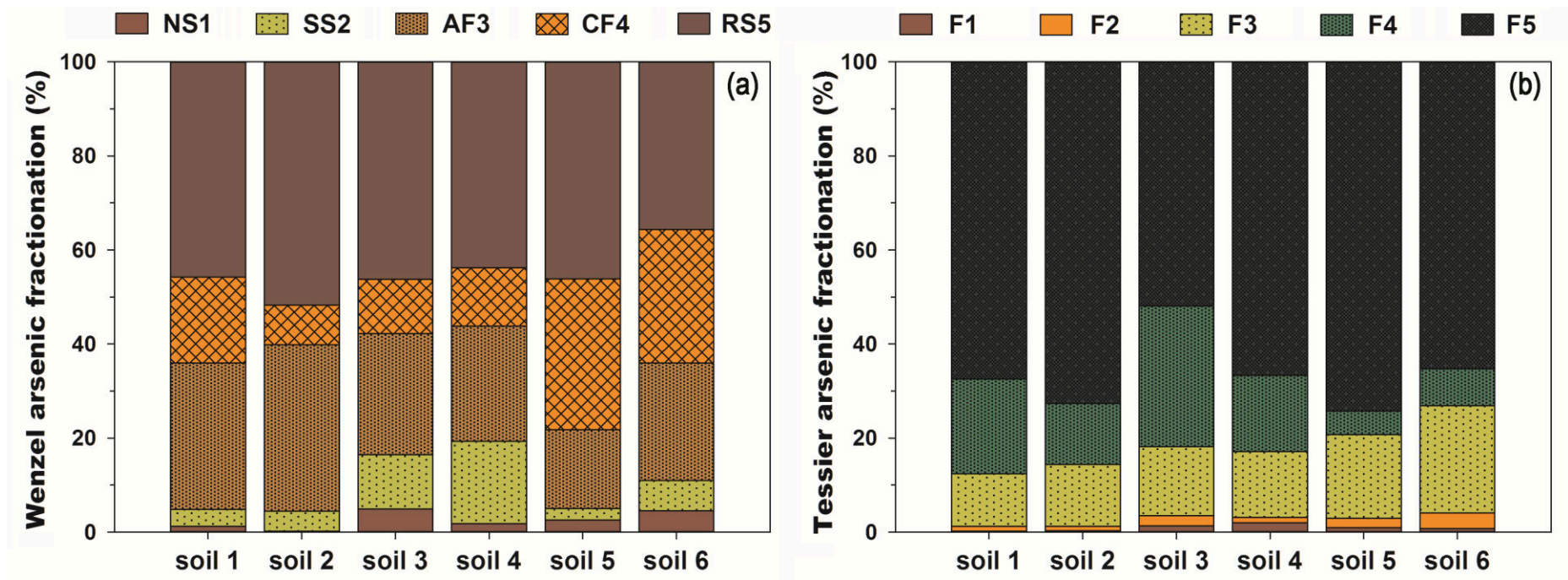
401 Figure 1



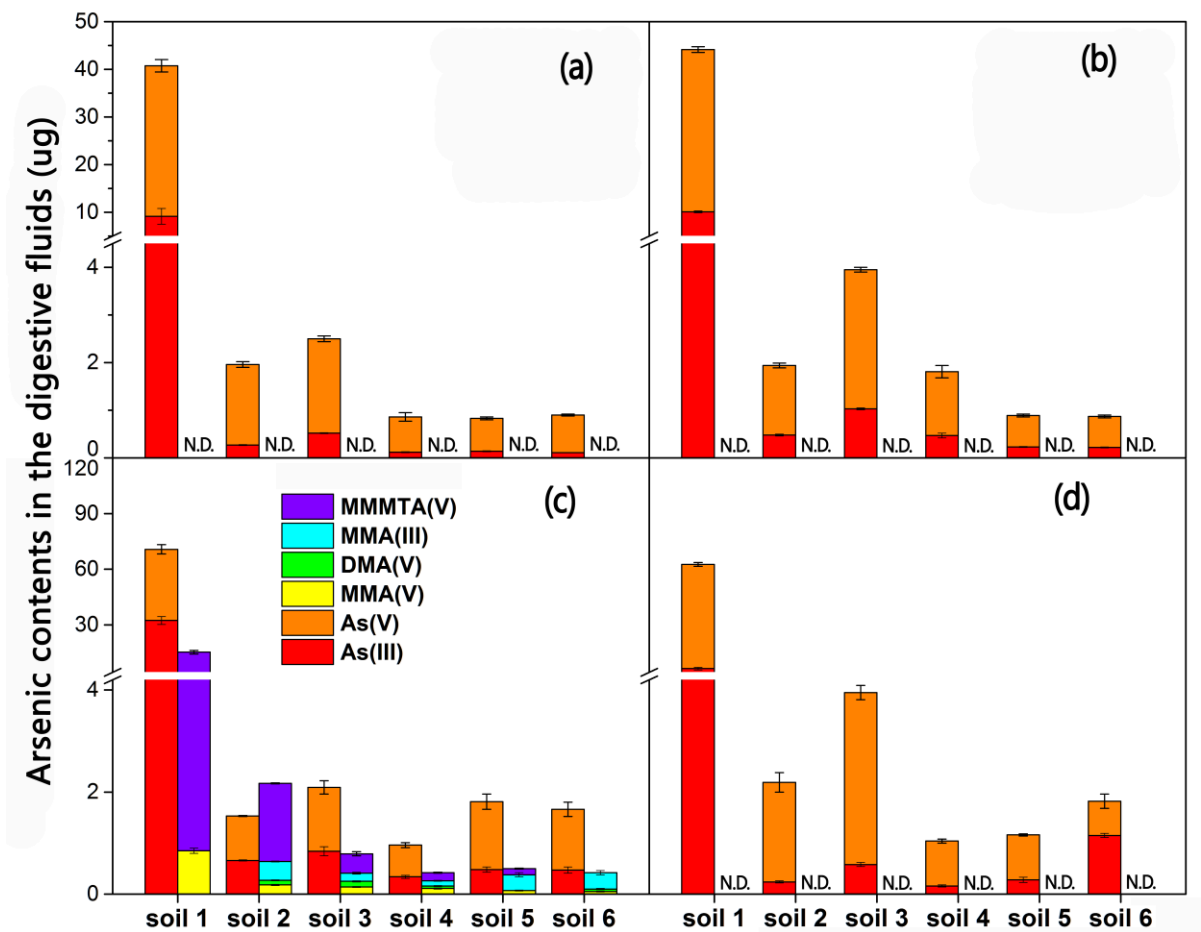
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403

404 Figure 2



407 Figure 3



410 **Tables**

411 Table 1 Physicochemical properties of the soils (values are represented as averages $\pm$ standard deviation, n=3)

sample	site	type	pH	SOM (%)	DOC (mg kg ⁻¹)	particle size (%)		As (mg kg ⁻¹)	Fe (g kg ⁻¹)	Al (g kg ⁻¹)	Mn (g kg ⁻¹)
						clay	silt				
soil 1	Chenzhou, Hunan	mining land	7.33 $\pm$ 0.08	1.83 $\pm$ 0.02	133.52 $\pm$ 23.15	1.69 $\pm$ 0.04	36.92 $\pm$ 1.94	777.25 $\pm$ 20.51	22.48 $\pm$ 1.28	28.33 $\pm$ 2.84	2.85 $\pm$ 0.17
soil 2	Longyan, Fujian	mining land	5.25 $\pm$ 0.02	3.34 $\pm$ 0.09	74.97 $\pm$ 5.26	0.71 $\pm$ 0.03	30.73 $\pm$ 1.73	36.92 $\pm$ 1.13	12.41 $\pm$ 0.17	20.65 $\pm$ 1.23	3.22 $\pm$ 0.10
soil 3	Suzhou, Jiangsu	chemical land	6.76 $\pm$ 0.06	16.79 $\pm$ 0.13	372.23 $\pm$ 45.99	6.32 $\pm$ 0.23	34.22 $\pm$ 0.93	33.09 $\pm$ 0.72	17.17 $\pm$ 0.65	28.05 $\pm$ 1.09	0.28 $\pm$ 0.01
soil 4	Suzhou, Jiangsu	chemical land	5.13 $\pm$ 0.06	3.32 $\pm$ 0.17	335.75 $\pm$ 53.59	9.03 $\pm$ 0.10	52.32 $\pm$ 2.00	14.64 $\pm$ 0.95	14.97 $\pm$ 0.70	21.69 $\pm$ 1.24	0.23 $\pm$ 0.01
soil 5	Chongqing	battery plant	7.60 $\pm$ 0.06	2.10 $\pm$ 0.08	10.24 $\pm$ 4.03	7.43 $\pm$ 0.17	28.06 $\pm$ 0.55	20.06 $\pm$ 1.57	22.19 $\pm$ 1.02	23.06 $\pm$ 1.68	0.40 $\pm$ 0.02
soil 6	Chongqing	battery plant	7.60 $\pm$ 0.02	1.53 $\pm$ 0.05	16.93 $\pm$ 1.61	8.77 $\pm$ 0.12	30.45 $\pm$ 3.75	15.68 $\pm$ 0.73	19.72 $\pm$ 0.23	22.90 $\pm$ 0.81	0.39 $\pm$ 0.01

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