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

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Abstract

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

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

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

Main findings: The human colon microbes could actively metabolize soil inorganic 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 Ardau, 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

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

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

95 A dynamic human gastrointestinal simulator known as the simulator of the human
96 intestinal microbial ecosystem(SHIME) has been used to investigate the measurement of
97 contaminants bioaccessibility by mimicking the physiological parameters of the human
98 gastrointestinal tract (Ruby et al., 1993; Chi et al., 2018). Unlike other *in vitro* models, the
99 SHIME model is seeded with microbial community cultures obtained from the human feces.
100 Results obtained with the SHIME model have proven that the human colon microbiota can
101 transform inorganic arsenic into organic arsenicals (Van de Wiele et al., 2010). To further
102 explore arsenic transformation in all regions of the digestive tract, other *in vitro* models such
103 as UBM, IVG, and PBET, were combined with SHIME to study arsenic bioaccessibility and
104 speciation changes during passage through the gastrointestinal tract (Sun et al., 2012; Yin et
105 al., 2015; Yin et al., 2016). Yin et al. (2015) found that a large amount of toxic arsenite
106 [As(III)] was observed as a result of human gut microbial reduction by using a combined
107 PBET-SHIME model, and various organic arsenic species, such as monomethylarsonic acid
108 [MMA(V)], dimethylarsinic acid [DMA(V)] and monomethylmonothioarsonic acid
109 [MMMTA(V)], were also observed in the active human colon stage. Furthermore, arsenic
110 bioaccessibility varied in the colon phase among these methods (Yin et al., 2016). Sun et al.
111 (2012) also found highly toxic monomethylarsonous acid [MMA(III)] in colon digests of
112 arsenic-contaminated rice, resulting in a higher human health risk. These findings indicated
113 that various arsenic transformations have occurred in the digestive tract, especially with the
114 involvement of human gut microorganisms, which resulted in the complexity of human
115 health risk assessment. However, studies of the effects of human gut microbiota on arsenic
116 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 ($Na_2HAsO_4 \cdot 12H_2O$) 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 ºC (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 ºC, 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 ^{o}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 ^{o}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 $(\text{Arsenic})_{\text{filtrate}}$ 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), $(\text{Arsenic})_{\text{soil}}$ 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 [$CaCu(AsO_4)OH$], 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.

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

288 a methylated thioarsenical species [MMMTA(V)], were detected simultaneously, which
289 accounted for 17.8-41.5% of the total soluble arsenic in the colon phase (Figure 3c). Arsenic
290 speciation in the sterile colon phase was also analyzed to confirm the contribution of gut
291 microbiota to arsenic metabolism. Only inorganic arsenic, including As(III) and As(V), was
292 found, with As(V) being dominant (Figure 3d). The amount of As(V) accounted for 46.7%
293 and 76.8% of the total arsenicals in the active and sterile colons, respectively, suggesting the
294 significant reduction of As(V) by gut microbes. Previous studies demonstrated that As(III)
295 showed a lower affinity for sorption to iron oxides than that of As(V) (Cao et al., 2003; Dixit
296 and Hering, 2003), and the high proportion of As(III) in the active colon phase could
297 increase bioaccessibility (Yin et al., 2015). These results suggested that the presence of colon
298 microorganisms not only increased the bioaccessibility of arsenic but also had the potential
299 to actively metabolize inorganic arsenic into methylated arsenical and thioarsenical species,
300 as reported in previous studies (Laird et al., 2007; Van de Wiele et al., 2010; Yin et al.,
301 2015). The colon microbial suspensions were collected from the improved SHIME model for
302 16S rDNA extraction and then high throughput sequencing. The results showed that the
303 average abundances of the Enterobacteriaceae and Bacteroides genera were 38.0% and
304 22.3% respectively (Figure S4). Previous studies have shown that some species in the
305 Enterobacteriaceae and Bacteroides genera can methylate arsenic (Isokpehi et al., 2014; Li et
306 al., 2016; Yu et al., 2016). Thus, it is reasonable to hypothesize that human colon microbes
307 have a high level of arsenic methylation potential. Compared with the diverse arsenic
308 speciation in the colon phase, only inorganic arsenic was detected in the stomach and small
309 intestinal phases (Figure 3a, 3b), and the dominant form was As(V), which accounted for
310 77.6-87.4% and 73.9-77.1% in the stomach and small intestinal phases, respectively.

311 Overall, As(III) rather than As(V) was the substrate for arsenic methylation, thus, the
312 high reduction of As(V) in the active colon phases suggests the possibility of subsequent
313 arsenic methylation in our study. Although the detailed mechanism of arsenic methylation is
314 still a highly controversial topic, arsenic methylation has been proven to be catalyzed by the
315 enzyme As(III) *S*-adenosylmethionine (SAM) methyltransferase (named ArsM in microbes
316 and AS3MT in mammals) (Ajees and Rosen, 2015; Cai et al., 2018). It is also clear that the
317 products of the enzyme are all trivalent and that the pentavalent species are the result of
318 non-enzymatic oxidation in the air (Yang and Rosen, 2016). Thus, we proposed that
319 intracellular As(III) was methylated by ArsMs from gut microbes to MMA(III) and DMA(III)
320 and then oxidized to MMA(V) and DMA(V) by oxygen in the air during sample preparation
321 and measurement. At the same time, dissolved oxygen is naturally present in gut fluids, and
322 the oxidation process could also occur in the colon phase. This is further supported by the
323 fact that DMA(III) is more sensitive to oxygen than MMA(III), and only MMA(III) but no
324 DMA(III) was detected in the samples. Furthermore, because most intracellular As(III) is
325 bound to intracellular thiols or thiols in proteins to yield trivalent protein-bound arsenicals
326 under the exposure of glutathione (GSH), thiolation could be a competitive reaction to form
327 stable pentavalent protein-bound arsenicals under the exposure of H_2S, which can be further
328 hydrolyzed to MMMTA(V) and DMMTA(V) (Sergio et al., 2014). In this study, due to the
329 abundant presence of sulfate-reducing bacteria that produce H_2S in the human fecal and
330 colon microbiota, a considerable amount of MMMTA(V) was detected in the samples.
331 However, the reason why no DMMTA(V) was detected needs further study.

332 **3.3 Implication for the health risk assessment**

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

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

345 Furthermore, arsenic toxicity is one of the primary parameters for assessing the health
346 risks associated with arsenic exposure. The toxicity of arsenic is highly dependent on its
347 speciation (Bissen and Frimmel, 2003). Previous studies demonstrated that the LD_{50} of
348 MMA(III) was 12 times lower than that of As(III), which indicated that MMA(III) is a much
349 more toxicant and potent enzyme inhibitor than As(III) (Drobná et al., 2005; Petrick et al.,
350 2001). This is also reflected by a larger cellular uptake and accumulation of MMA(III)
351 compared to As(III) in human urothelial cells and rat hepatocytes (Drobná et al., 2005;
352 Styblo et al., 2000). Furthermore, the finding of MMMTA(V) formation by the human colon
353 microorganisms raises questions about its toxicological importance. Hinrichsen et al. (2015)
354 demonstrated that the cellular retention of MMMTA(V) was lower than that of DMA(V), but
355 the intestinal transport of MMMTA(V) was similar to that of As(V) in Caco-2 cell assays.
356 Although the absorption kinetics of MMMTA(V) across the epithelium is not well
357 characterized, there is evidence that some methylated thio-arsenicals elicit a more efficient
358 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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Notes

The authors declare no competing financial interest.

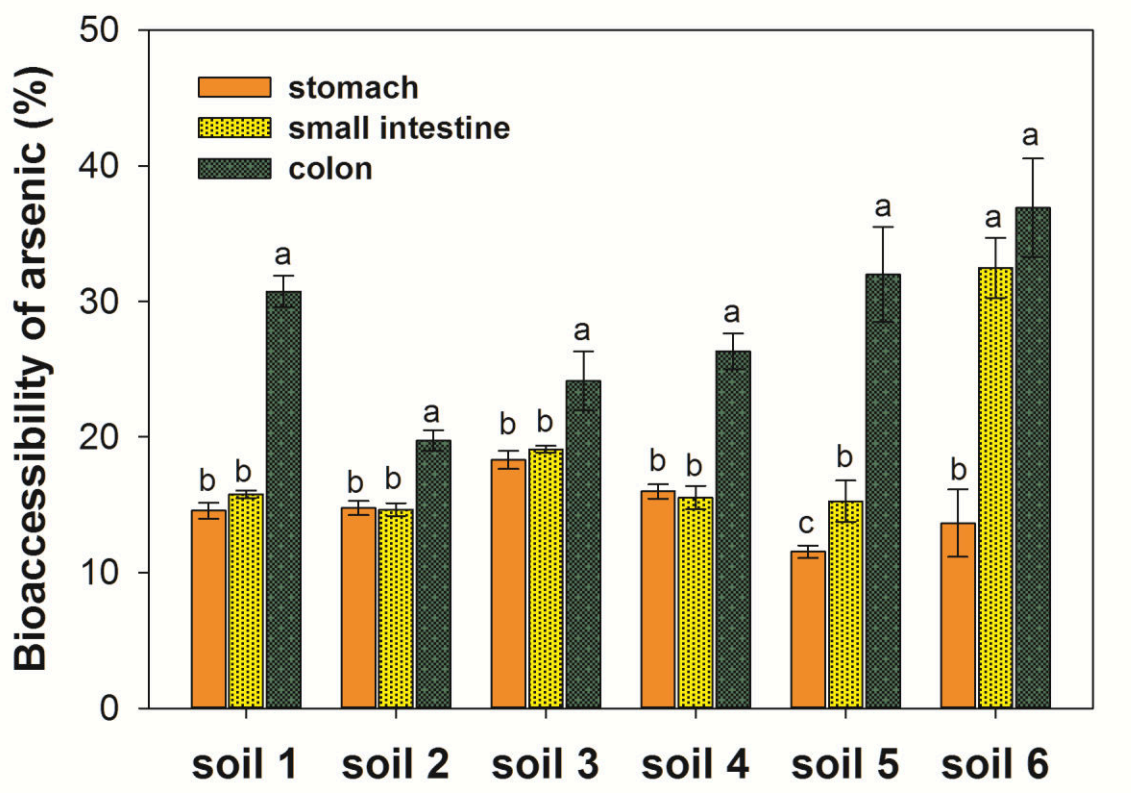
Figure captions

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 ± 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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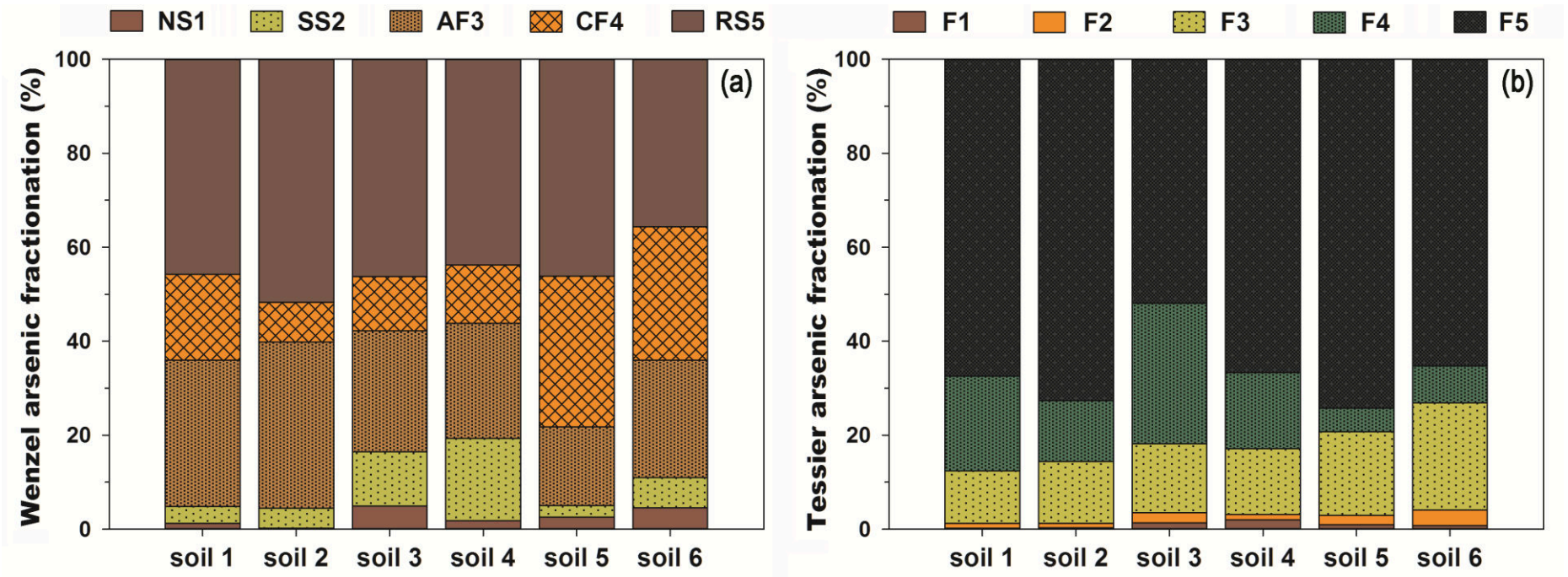
403

404 Figure 2



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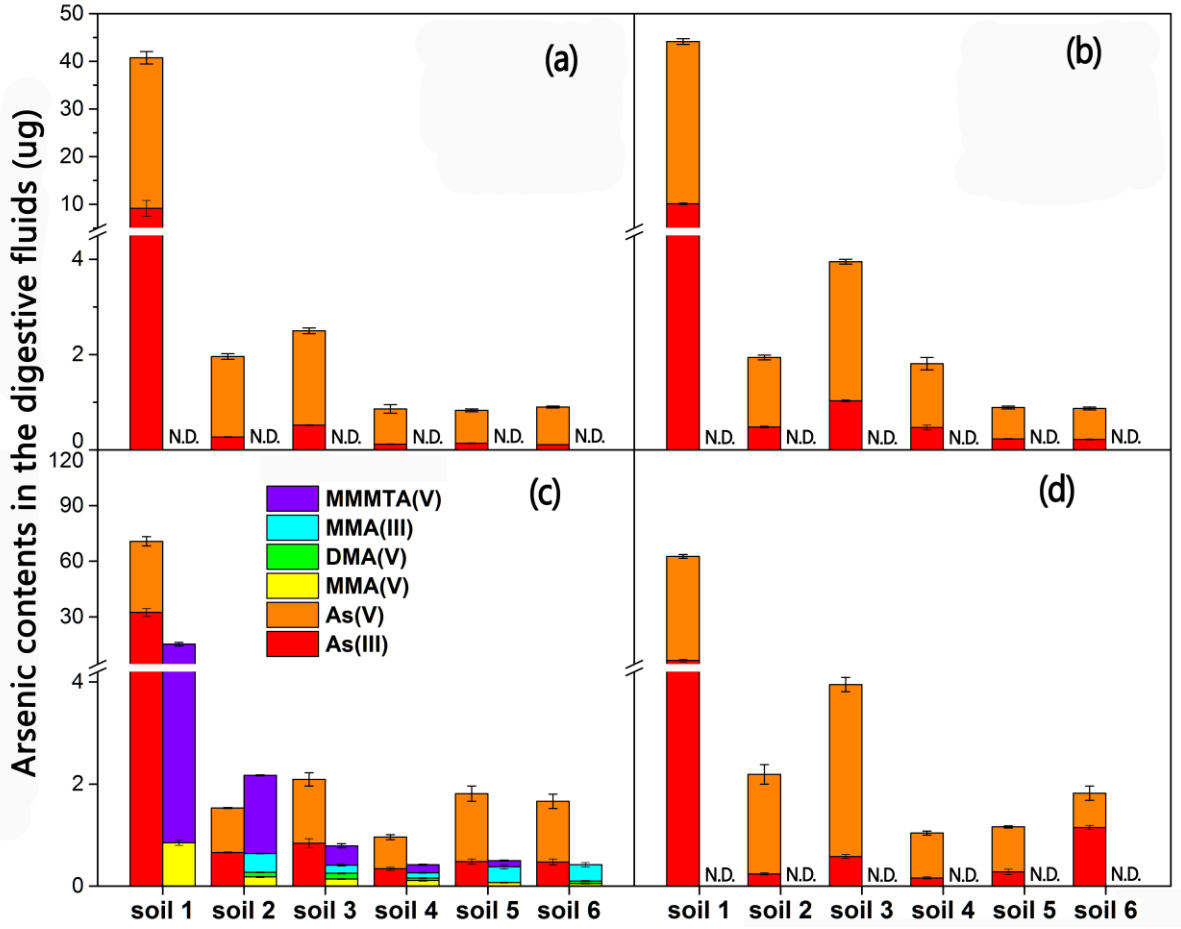
407 Figure 3



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409

Tables

Table 1 Physicochemical properties of the soils (values are represented as averages ± 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±0.08	1.83±0.02	133.52±23.15	1.69±0.04	36.92±1.94	777.25±20.51	22.48±1.28	28.33±2.84	2.85±0.17
soil 2	Longyan, Fujian	mining land	5.25±0.02	3.34±0.09	74.97±5.26	0.71±0.03	30.73±1.73	36.92±1.13	12.41±0.17	20.65±1.23	3.22±0.10
soil 3	Suzhou, Jiangsu	chemical land	6.76±0.06	16.79±0.13	372.23±45.99	6.32±0.23	34.22±0.93	33.09±0.72	17.17±0.65	28.05±1.09	0.28±0.01
soil 4	Suzhou, Jiangsu	chemical land	5.13±0.06	3.32±0.17	335.75±53.59	9.03±0.10	52.32±2.00	14.64±0.95	14.97±0.70	21.69±1.24	0.23±0.01
soil 5	Chongqing	battery plant	7.60±0.06	2.10±0.08	10.24±4.03	7.43±0.17	28.06±0.55	20.06±1.57	22.19±1.02	23.06±1.68	0.40±0.02
soil 6	Chongqing	battery plant	7.60±0.02	1.53±0.05	16.93±1.61	8.77±0.12	30.45±3.75	15.68±0.73	19.72±0.23	22.90±0.81	0.39±0.01

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

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