In vitro study of soil arsenic release by human gut microbiota and its intestinal absorption by Caco-2 cells

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HIGHLIGHTS

- Soil As release (3.7–581.2 mg kg⁻¹) was observed in the colon.
- 44.2–97.6% of arsenite generated was in the soil solid phase.
- Different degree of cellular absorption of soil As metabolites was observed.
- Arsenic in colon digests was transformed more quickly than that in soil solid phase.
- Gut microbiota can directly release soil arsenic, particularly As-bearing amorphous Fe/Al-oxides.

GRAPHICAL ABSTRACT

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ABSTRACT

Arsenic (As) speciation is essential in assessing health risks from As-contaminated soil. Release of soil-bound arsenic, As transformation by human gut microbiota, and the subsequent intestinal absorption of soil As metabolites were evaluated. A colon microbial community in a dynamic human gut model and the intestinal epithelial cell line Caco-2 were cultured. Arsenic speciation analysis and absorption of different As species were undertaken. In this study, soil As release (3.7–581.2 mg kg⁻¹) was observed in the colon. Arsenic in the colon digests was transformed more quickly than that in the soil solid phase. X-ray absorption near-edge spectroscopy (XANES) analysis showed that 44.2–97.6% of arsenite [As(III)] generated due to arsenate [As(V)] reduction was in the soil solid phase after the colon phase. We observed a high degree of cellular absorption of soil As metabolites, exhibiting that the intestinal absorption of monomethylarsonic acid and As(III) (33.6% and 30.2% resp.) was slightly higher than that of dimethylarsinic acid and As(V) (25.1% and 21.7% resp.). Our findings demonstrate that human gut microbiota can directly release soil-bound arsenic, particularly As-bearing amorphous Fe/Al-oxides. Determining As transformation and intestinal absorption simultaneously will result in an accurate risk assessment of human health with soil As exposures.

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1. Introduction

Arsenic (As), a ubiquitous toxic contaminant, is extensively distributed in the soil environment (Bhattacharya et al., 2007).

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Chronic exposure to soil As is associated with serious risks to human health because As is regarded as a class 1 carcinogen (Ljung et al., 2006). Inadvertent oral ingestion of As-contaminated soil and dust has been considered an important exposure pathway, especially through outdoor hand-to-mouth behavior by children (Bradham et al., 2011). Generally, most inorganic As (iAs) is rapidly absorbed in the small intestine, so As methylation by intestinal microorganisms is considered to contribute little to methylation *in vivo* (Vahter, 1983). However, soil/food-bound As can get into the colon, which is inhabited by an abundant microbial community. In an oral exposure, the metabolism of ingested soil As by gut microorganisms has been investigated with *in vivo* and *in vitro* experiments (SHIME model, Simulator of Human Intestinal Microbial Ecosystem) (Naranmandura et al., 2007a; Van de Wiele et al., 2010; Yin et al., 2015). Arsenic species detected in the colon, listed in order of decreasing toxicity, are monomethylarsonous acid (MMA^{III}), dimethylarsinous acid (DMA^{III}), arsenite [$\text{As}(\text{III})$], arsenate [$\text{As}(\text{V})$], monomethylmonothioarsonic acid (MMMTA^{V}), monomethylarsonic acid (MMA^{V}), and dimethylarsinic acid (DMA^{V}) (Naranmandura et al., 2007b; Van de Wiele et al., 2010). Arsenic transformation and speciation analysis must be considered an essential part of the risk assessment following soil As exposure.

The SHIME model, a thorough *in vitro* human gastrointestinal simulator, demonstrated that human gut microbiota have high microbial metabolic potency toward soil As (Van de Wiele et al., 2010; Yin et al., 2015). Clearly, human gut microbiota can actively metabolize As through reduction, methylation, and thiolation. Yin et al. (2015) reported the As speciation of residual soils in the colon phase by X-ray absorption near-edge structure (XANES) and found the distribution of $\text{As}(\text{III})$ and $\text{As}(\text{V})$ in the colon digest and soil solid phase, illustrating that large amounts of $\text{As}(\text{III})$ (8.5–13.7% of total As) were adsorbed onto the soil solid phase. Nevertheless, with consideration to absorption in small intestine, confirming whether human gut microbiota in the colon have the potency to induce As release directly from soils and investigating the conceivable mechanism are urgently needed. If confirmed, we can *in vitro* explore soil As metabolism by human gut microbiota and these data can result in more accurate risk assessment associated with oral exposure to As-contaminated soils.

The development of *in vitro* research utilizing Caco-2 cell cultures, which simulates the intestinal cell retention and transport process, can offer a more reliable approximation to *in vivo* conditions in evaluating bioavailability at the intestinal level (Ekmekcioglu, 2002). The intestinal epithelial cell line Caco-2 of human colon carcinoma origin has been successfully used to estimate the bioavailability of some elements (e.g., cadmium, lead, selenium, mercury, arsenic) (Fu and Cui, 2013; Moreda-Pineiro et al., 2011). Researchers have examined cellular retention and intestinal transport with Caco-2 cells from the standard solutions of different As species [e.g., $\text{As}(\text{V})$, $\text{As}(\text{III})$, MMA^{III} , MMA^{V} , DMA^{III} , DMA^{V} , MMMTA^{V} , dimethylmonothioarsenate (DMMTA^{V})] (Calatayud et al., 2012c; Hinrichsen et al., 2015; Laparra et al., 2005b). These previous data suggest the different methylated arsenicals have a more efficient cellular absorption capacity than iAs, but the pentavalent methylated As, $\text{MMA}(\text{V})$ and $\text{DMA}(\text{V})$ have a lower absorption than iAs (Calatayud et al., 2010; Juhasz et al., 2006). There are some factors affecting intestinal transport of these As species, including pH, phosphate or other parameters (Calatayud et al., 2010). Combination of the *in vitro* gastrointestinal methods and Caco-2 cells has been used to simulate digestion and absorption processes of different As species from foods (Alava et al., 2015; Moreda-Pineiro et al., 2011). However, there are no data regarding As intestinal absorption using Caco-2 cells after *in vitro* digestion (stomach and small intestine) and the subsequent As transformation by human colon microbiota from As-contaminated

soils; accordingly, it was essential and urgent to estimate the absorption of soil As metabolites with different degrees of toxicity.

Therefore, in the present study, the PBET method (gastric and small intestinal phases), combined with the SHIME (colon phase) and Caco-2 cells (intestinal absorption) was used to investigate (1) As speciation and distribution between the colon digest and the soil solid phase, (2) whether and to what extent soil-bound As in the colon can be released by human gut microbiota, and (3) the degree of intestinal absorption of different As species metabolised by human gut microbiota based on Caco-2 cells.

2. Materials and methods

2.1. Arsenic-contaminated soils

In this study, four soils collected from mining and farmland locations in China were used, with As concentrations ranging from 15.5 to 3225.6 mg kg^{-1} . All soil samples were air-dried and sieved to a particle size fraction of $<250 \mu\text{m}$ for *in vitro* studies, which is most likely to stick to the hands of exposed humans (Kelly et al., 2002). Soil physicochemical properties were measured on triplicate samples as described previously (Yin et al., 2015) and listed in Table S1.

Arsenic speciation in soils was examined using As K-edge XANES, which was performed on beamline 1W2B at the Beijing Synchrotron Radiation Facility (BSRF), China. The steps and reference materials have been described in our previous literature (Yin et al., 2015). Data analysis was conducted using Athena (Demeter 0.9.20). Every sample was normalized, and linear combination fitting (LCF) was used to identify As species from reference materials.

Sequential soil As fractionation was determined by Kim et al. (2014), and the following operationally defined As forms were (F1) non-specifically adsorbed, (F2) specifically adsorbed, (F3) amorphous Fe and Al oxides, (F4) crystalline Fe and Al oxides, and (F5) residual phase. Samples ($0.45 \mu\text{m}$ filter) were analyzed for soluble As by inductively coupled plasma mass spectrometry (ICP-MS, 7500a; Agilent, Palo Alto, CA, USA).

2.2. Dynamic SHIME

The *in vitro* colon microbial community utilized in the experiments was cultured and maintained in a modified SHIME described previously (Van de Wiele et al., 2010). SHIME consisted of five compartments simulating the stomach, small intestine, ascending colon, transverse colon and descending colon. Generally, fresh fecal microorganisms were obtained from one volunteer (Sun et al., 2012; Van de Wiele et al., 2010), a 28-year-old Chinese male volunteer with no history of antibiotic treatment in the six months before this study. Briefly, the different colon compartments were inoculated with fresh fecal microorganisms. Feed solution detailed in Yin et al. (2016) was added three times per day to provide digested nutrition for the colon microorganisms. After 3–4 weeks of adaptation, stable microbial communities were obtained in the colon compartments. The distal colon microbial fermentation activity (short chain fatty acid production and ammonium production) and community composition are consistent with that of previous SHIME runs and an *in vivo* situation (Molly et al., 1994; Yin et al., 2015).

2.3. Estimation of bioaccessible As

The As bioaccessibility and speciation from four soils were investigated using a modified PBET method (Ruby et al., 1996; Tang et al., 2006) for the gastric and small intestinal phases combined with SHIME for the colon phase. Briefly, soils (0.3 g), in triplicate

were added to polypropylene conical centrifuge tubes (50 mL) with the gastric and small intestinal solutions (30 mL) at a soil/solution (s/s) ratio of 1:100. Following the small intestinal phase, we made a comparison of the absence/presence of small intestinal digests in the colon phase. The intestinal digest was removed and the colon solution was added to the residual soils at a ratio (s/s) of 1:100; this was the no intestinal digest-colon phase (NI-colon phase). In the alternate process, these digests were transferred into 100 mL anaerobic serum bottles with 30 mL of colon solution from the descending colon compartment of the dynamic SHIME system at a ratio (s/s) of 1:200; this was the colon phase. Subsequently, 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 18 h.

2.4. Caco-2 cell culture

The Caco-2 cell line was purchased from the Institute of Basic Sciences of the Chinese Academy of Medical Sciences School of Basic Medicine of the Peking Union Medical College. A detailed description is consulted in [Fu and Cui \(2013\)](#). In brief, cells (passage number 25–30) were routinely grown in 25 cm² flasks in Dulbecco's modified Eagle's medium (DMEM, Hyclone, USA) supplemented with 20% (v/v) fetal bovine serum (FBS, Hyclone), 1% (v/v) nonessential amino acids (NEAA) and 1% antibiotics (complete medium). The cell lines were incubated at 37 °C, 95% air and 5% CO₂ in a humidified atmosphere, and the medium was replaced every 2–3 d. When the cell monolayer reached a confluence of approximately 80%, the cells were subcultured with a solution of 0.25% trypsin-EDTA (ethylene diamine tetraacetic acid). To establish the Caco-2 intestinal model, cells were seeded at a density of 5×10^4 cells cm² on a transwell plate (Millipore 24-well Millicell Hangcell Culture Insert, 0.4 µm pore size membrane, PET; Corning Incorporated, NY, USA). The media was replaced every other day for 21 d to obtain confluent, differentiated cell monolayers ([Dhuique-Mayer et al., 2007](#)). The cellular monolayer integrity was checked by measuring trans-epithelial electrical resistance (TEER) with a volt-ohm meter equipped with a chopstick electrode (Millicell ERS-2, Millipore, USA).

2.5. Absorption of As species by Caco-2 cells

To assess the absorption of bioaccessible As after the colon phase across the gut epithelium, this study was undertaken using standard As aqueous solutions and colon digests by an *in vitro* Caco-2 cell line model. For As absorption through Caco-2 cells, the colon digests were heated by immersion in a water bath for 5 min, 90 °C to denature enzymes used for digestion, and then filtrated with a 0.2 µm Supor membrane (PN 4612, Pall Corporation, MI, USA). The samples were diluted 1:3 with DMEM. The apical side of the cell monolayers received 0.5 mL of the dilution, and the basolateral side received 0.5 mL of FBS-free medium. After 4 h of incubation at 37 °C, 5% CO₂, and 95% relative humidity, the media from the apical chamber was harvested. TEER values (>600 Ohms cm²) were measured to control cell monolayer integrity during the experiments.

All samples passed through 0.45 µm filter were subsequently stored at –80 °C until analysis. The total arsenic was quantified using ICP-MS. Arsenic speciation was determined by high-performance liquid chromatography (HPLC) coupled with ICP-MS. 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 standards [As(III), As(V), DMA^V, MMA^V and MMTA^V] were

included as quantified by external calibration curves with peak areas. MMTA^V was synthesized by this method ([Alava et al., 2012](#); [Rubin et al., 2014](#)). The sum of all chromatographic As species detected in the filtrate (0.45 µm) of the colon digests was used to determine As bioaccessibility. In addition, the chromatographic recovery was validated previously ([Yin et al., 2015](#); [Zhu et al., 2008](#)).

3. Results and discussion

3.1. Arsenic speciation in the colon digests and arsenic release from soils by human colon microbiota

The As bioaccessibility values in the small intestinal and colon phases were highly variable among four contaminated soils ([Table 1](#) and [Table S1](#)). We observed that As bioaccessibility in the colon phase was 1.7–5.8 times that of the small intestinal phase. Significantly, a large amount of soil As (3.7–581.2 mg kg^{–1} with mean value 212.0 mg kg^{–1}) was released in the colon phase by human gut microbiota ([Table 1](#)). Through the control test with the sterile colon solution for soils 1–3, a certain degree of As dissolution (17.7–146.4 mg kg^{–1}) from soils was observed, while the values were lower than As release in the NI-colon phase. Accordingly, the bioaccessible As concentration in the colon phase was 5.7–664.6 mg kg^{–1} with mean value 267.4 mg kg^{–1} ([Fig. S1](#)). Arsenic bioaccessibility in the colon phase (20.6–36.8%) was 1.1–2.0 times that of the NI-colon phase. Subsequently, a high degree of transformation by human gut microbiota was observed, including As reduction (7.4–29.9%), and methylation (0.7–44.8%). The presence of DMA^V, MMA^V, and MMTA^V was detected as methylarsenicals with different extents of As methylation in the colon phase ([Table 1](#)). The methylation percentage in soil 4 was higher with 44.8% and 42.1%, respectively, in the NI-colon and colon digests. The NI-colon and colon digests of soils 1–3 displayed a methylation percentage of 0.7–13.5% and 3.3–17.4% respectively. Additionally, a significant amount of As(III) (6.4–105.9 mg kg^{–1}) was observed and inorganic As was predominantly present in the colon phase.

Similar to previous studies, our findings showed that human colon microbiota have the capacity for presystemic As metabolism ([Van de Wiele et al., 2010](#); [Yin et al., 2015](#)). Importantly, the difference in the metabolic activity and ability in the absence/presence of the small intestinal digests, illustrated that human gut microbiota gave priority to transform dissolved As. In this study, plenty of As(III) was observed in the colon digests and soil solid phase. The As(V) reduction process can directly occur in the solid phase; As(V) in the digests was reduced to As(III), and then a part of these As(III) generated was adsorbed onto the soil solid phase. As [Zhu et al. \(2014\)](#) summarized the biotransformation between different As species, As(III) is primarily methylated into MMA^V, and then the MMA^V is transformed into DMA^V during the process of As metabolism. On the other hand, MMA^V can be an intermediate in the production of MMTA^V (with unknown toxicity) by microbial thiolation; the sulfide source can be H₂S, which sulfate-reducing bacteria (SBR) from the human gut tract have the ability to produce ([Rubin et al., 2014](#)). In addition, DMA^V can be converted into other methylated thioarsenates, such as trimethylarsine sulfide, in animal gastrointestinal environments ([Kubachka et al., 2009](#); [Kuroda et al., 2004](#)). MMA^V can be reduced to the highly toxic MMA^{III}, which indicates that As methylation will not lead to detoxification ([Van de Wiele et al., 2010](#)). Finally, S-adenosylmethionine is known to transfer its methyl group to As(III) ([Zhu et al., 2014](#)), but the understanding of methyl group donors is limited. We need to further elucidate the mechanism of As methylation in human gut tract.

Table 1
Arsenic bioaccessibility, transformation, and release in the colon phase.

Sample	As bioaccessibility (%)	Soil solid phase ^a		As methylation ^b		As(V) reduction ^c			As(V) transformation ^d (mg kg ⁻¹)		As(V) release ^e (mg kg ⁻¹)	
		As(III)		As(V)				Solid		Aqueous		
		(mg kg ⁻¹)	(%)	(%)	(mg kg ⁻¹)	(%)	(mg kg ⁻¹)	(%)	(%)	(%)		
In the colon phase In the NI-colon phase (no intestinal digest-colon phase)												
Soil 1	16.2 ± 1.2	28.3	8.7	91.3	7.1	13.5	38.3	11.8	74.0	26.0	45.4	52.3
Soil 2	30.0 ± 0.2	105.3	12.5	87.5	3.2	1.3	148.5	17.6	44.2	55.8	151.7	253.1
Soil 3	18.0 ± 1.7	341.4	10.6	89.4	4.0	0.7	238.2	7.4	90.5	9.5	242.2	581.2
Soil 4	23.7 ± 0.1				1.7		44.8					3.7
In the colon phase												
Soil 1	32.8 ± 2.2	36.6	11.3	88.7	18.5	17.4	43.0	13.3	85.1	14.9	61.5	66.9
Soil 2	34.8 ± 0.1	185.9	22.0	78.0	15.1	5.1	252.2	29.9	58.0	42.0	267.3	183.9
Soil 3	20.6 ± 1.5	391.8	12.1	87.9	21.9	3.3	272.6	8.5	97.6	2.4	294.5	549.2
Soil 4	36.8 ± 1.6				2.4		42.1					5.7

^a As speciation after the colon phase measured by XANES, and soil 4 with lower As concentration not included.

^b Calculated by dividing the sum of MMA^V, DMA^V, and MMTA^V by As concentration in the colon digests.

^c As(III) concentration and percentage (of total soil As) due to microbial As(V) reduction; the percentage of As(III) generated in the colon digest and the soil solid phase.

^d The sum of As methylation and As(V) reduction.

^e Soil As(V) release in the colon phase excluding As concentration in the small intestinal digests.

3.2. Arsenic speciation in the soil solid phase and soil As fractions

The important part of this study consisted of As speciation analysis in the soil solid phase by XANES (Table S2 and Fig. S2). Subsequently, summarizing As speciation changes in the digest and soil solid phase, we comprehensively calculated two important values of As(V) reduction and As(V) transformation by human gut microbiota taking into account both the NI-colon and colon phases in soils 1–3 (Table 1, Table S2, and Fig. S1). To keep the reliability and accuracy of data, soil 4 with lower As concentration was not measured. In consideration of both digests and soil solid phases, As(III) concentrations generated by microbial reduction in the NI-colon phase were 38.3–238.2 mg kg⁻¹, lower than that in the colon phase. Importantly, 44.2–97.6% of the generated As(III) was attached to the soil solid phase. In comparison to the origin soils exceeding 95% As(V), the amount of As(III) on the soil solid phase of the colon phases increased by 28.3–266.0 mg kg⁻¹. In addition, it is important to note that there is particularly high As(V) transformation in soil 3, which illustrates that microbial metabolism may have a threshold value. Furthermore, the transformed As(V) concentrations [which included As(III) due to microbial reduction and organic As by methylation and thiolation] reached 61.5–294.5 mg kg⁻¹, which were higher than corresponding values in the NI-colon phase. The result demonstrated that As in the colon digests was transformed more quickly than that in the soil solid

phase. From the concentrations of different As fractions (Table S3), combined with our previous results (Yin et al., 2015), linear correlation analysis showed that there is a significant correlation between soil As(V) release and transformation in the colon phase (including bioaccessible As in the small intestinal phase) and the sum of soil F1, F2, F3 fractions (slope = 0.53; y-intercept = 91.3; $r^2 = 0.96$; $n = 7$; $P < 0.01$), as well as the sum of soil F1, F2, F3, and F4 fractions (slope = 0.43; y-intercept = 67.8; $r^2 = 0.96$; $n = 7$; $P < 0.01$) (Fig. 1). Soil As(V) release and transformation in the NI-colon phase was significantly correlated with the sum of soil F1, F2, F3 fractions ($r^2 = 0.97$; $n = 3$) (Fig. S3). Interestingly, soil 4 with low As concentration, had a certain As release (3.7–5.7 mg kg⁻¹), which illustrated that human gut microbiota can metabolize certain soil As fractions.

The present study demonstrates that human colon microbiota have the potency to release directly soil-bound As, particularly As-bearing amorphous Fe/Al-oxides. Generally, most or all of dissolved iAs as the predominant species from soil As exposure is absorbed in small intestine (Vahter, 1983; Yin et al., 2015). Therefore, significant soil-bound As (the fraction of As that did not release into the small intestine from soils) can end up in the colon with the abundant microbial community. Identifying As speciation and distribution between the digests and soil solid phase of colon phase, we observed soil-bound As release and transformation by human gut microbiota when, hypothetically, all of the As was absorbed in the

Fig. 1. Correlation between soil As(V) release and transformation (mg kg⁻¹) in the colon phase and sequential extraction of soil F1, F2, F3 As (A, slope = 0.53; y-intercept = 91.3; $r^2 = 0.96$; $n = 7$; $P < 0.01$), and F1, F2, F3, F4 As (B, slope = 0.43; y-intercept = 67.8; $r^2 = 0.96$; $n = 7$; $P < 0.01$) in 7 soils. (F1, non-specifically adsorbed; F2, specifically adsorbed; F3, amorphous Fe and Al oxides; F4, crystalline Fe and Al oxides).

small intestine. The important result was soil As release ($3.7\text{--}581.2\text{ mg kg}^{-1}$) and a certain amount of As(III) ($28.3\text{--}215.6\text{ mg kg}^{-1}$) on the soil solid phase. Accordingly, it once more illustrated the importance of XANES investigation of As speciation in the soil solid phase (Yin et al., 2015). During *in vitro* experiments, the mobility of As in soils is mainly controlled by adsorption/desorption processes involving metal oxides. It is reported that As mobilization was enhanced by As(V) reduction from As-bearing minerals in the presence of As(V)-reducing bacteria (Smeaton et al., 2012; Smith et al., 2014). As detailed in 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 capacity of As is weaker due to the reductive dissolution of Fe(III) oxides in the presence of Fe(III)-reducing bacteria; consequently, in the NI-colon phase with dissolved Fe concentrations (Fig. S4), the dissolution of Fe oxides can cause As release from residual soils after the small intestinal phase. However, in the opposite case, the formation of secondary Fe-minerals during Fe(III) reductive dissolution can sequester a number of As. In the colon phase, an increase of As(III) on the soil solid phase can result from the sequestration of secondary Fe-minerals in consideration of lower Fe concentrations compared to that in the small intestinal phase (Fig. S4). Therefore, As mobilization should be the result of concurrent processes including reductive dissolution of Fe(III) oxides, liberation and biotransformation of As(III/V), and

sequestration of As on Fe minerals. As mentioned, As(III) oxidation and As(V) sequestration by Mn oxides can also play a small, but influential role in As speciation and mobilization (Ying et al., 2011).

3.3. Intestinal absorption of soil As metabolites

Another important observation was the high degree of intestinal absorption using Caco-2 cells of As metabolites [As(V), As(III), MMA^V, DMA^V] by human colon microbiota from As-contaminated soils. Through exposure to four As standards (Table S4), intestinal absorption of As(III) (33.7%) clearly exceeded that of all other As species, and the transport of the pentavalent organoarsenic compounds (MMA^V and DMA^V) through the Caco-2 cell monolayer is low (Calatayud et al., 2010; Hinrichsen et al., 2015). For soil As metabolites, the intestinal absorption of different As species was variable (Fig. 2). The absorption of MMA^V, DMA^V, As(III), and As(V) was 33.6%, 25.1%, 30.2%, and 21.7%, respectively, and that of total As species from the colon digests reached 27.0%, which was higher than that of the NI-colon digests. Soil 2 had the highest intestinal absorption (49.3%) of total As metabolites from the colon digests. The inorganic As has a higher absorption concentration than that of MMA^V and DMA^V, which is similar to other studies (Calatayud et al., 2010; Juhasz et al., 2006). Synchronously, the colon digests of soils 1 and 2 displayed a higher absorption of 41.6% with an additional methylated thioarsenate (MMMTA^V). Regarding the thiolated As species, MMMTA^V was least concerned. In accordance with previous literature, MMMTA^V with comparatively less toxicity displays a

Fig. 2. Absorption of soil As metabolites by Caco-2 cell culture incubated for 4 h: As(III), DMA^V, MMA^V, As(V), MMMTA^V, and sum of different As species. Data are presented as the mean $\pm$ standard deviation ($n = 3$).

high intestinal absorption (Naranmandura et al., 2007b; Hinrichsen et al., 2015). These studies emphasize the necessity for considering As metabolism by human gut microbiota in the colon as a relevant process when assessing As toxicokinetics.

The intestinal absorption of food As metabolites with more methylated arsenicals is notably different (Alava et al., 2015; Laparra et al., 2005a), which can result from the difference in As metabolism by human gut microbiota between foods and soils. The complex soil background (e.g., organic matter, phosphate, and metal ion concentrations) can cause the variability in As absorption, which needs further exploration. The difference in As absorption depended on the various As species, and corresponding transporters, including glucose transporters (GLUT and SGLT), organic anion transporting polypeptides (OATPs), aquaporins (AQPs), and phosphate transporters (NaPi and PiT) (Calatayud et al., 2012a). Hence, in the complex environment from soil As exposure, these comprehensive factors can control the As absorption process. In human intestinal transit, As transformation and intestinal absorption are synchronous. Taking into account As absorption, it needs to be determined whether some As can be redesorbed from the soil solid phase, which can inevitably increase the human health risk. Furthermore, mammalian or human cells including Caco-2 cells have the ability to metabolize iAs or DMA^{III} (Calatayud et al., 2012b; Naranmandura and Suzuki, 2008). Arsenic transformation concurrently by human colon microbiota and intestinal epithelial cell lines needs to be further investigated.

4. Conclusions

The present study shows that human gut microbiota has the potency to release a large amount of soil-bound As, particularly As-bearing amorphous Fe/Al-oxides in the colon phase. The metabolic activity of As in the colon digests by human gut microbiota was faster than that in the soil solid phase. In addition, we are the first to report the different intestinal absorption of soil As metabolites [As(III), As(V), methylated arsenicals (MMA^V and DMA^V) and thioarsenicals (MMMTA^V)] across the gut epithelium using Caco-2 cells. The complicated background of the soil matrix results in the different absorption between As standards and soil As metabolites. Therefore, (i) the absorption mechanism of different As species under the complex background, and (ii) the toxicity of methylated arsenicals and thioarsenicals need further study. In this context, gut microbial transformation and intestinal absorption must be crucial component to incorporate when predicting bioavailability and evaluating the health risks from oral soil As exposure.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.chemosphere.2016.10.091>

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