Arsenictransfer chain along soil - sclerotium - stroma of Chinese cordyceps and the related health risk assessment

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Background. Chinese cordyceps (Lepidoptera: *Ophiocordyceps sinensis*)is a larval-fungus complex. Arsenic (As) concentration and distribution might differ during the stroma (ST) germinating process and ultimately vary between the sclerotium (SC) and the ST. As an important arsenic migration chain in the environment, the system from soil to Chinese cordyceps constitutes an exposure pathway to humans. In this study, the investigation on the As concentration in soil, SC and ST of Chinese cordyceps was carried out, and a risk assessment was conducted accordingly.

Methods. Soil and Chinese cordyceps samples were collected from the Tibetan Plateau in China. The samples were analyzed for the total As concentration and As species determination, which were conducted by inductively coupled plasma mass spectrometry (ICP-MS) and HPLC -ICP-MS, respectively.

Results. The concentration of total As in soil was much higher than that in SC and ST. The major As species was inorganic As in soil, among which iAs^{V} was the majority. In SC and ST, organic As was the predominant species, among which some unknown organic As was the majority. There are significant differences in the As distribution and As composition among soil environment, SC and ST. The risk assessment indicated that chronic daily ingestion in children and adults groups was higher than inhalation and dermal contact. The hazard index (*HI*) of non-carcinogenic risk and the cancer risk (*CR*) for human health were $HI \leq 1$ and $CR < 1 \times 10^{-4}$, respectively.

Conclusion. Chinese cordyceps possess highly efficient detoxifying characteristics and play a certain significant role in As transformation during completing its life process. Additionally, our study found the levels of As in soils from the Chinese cordyceps habits were higher than the soil background values of China, but the probabilities of health risks were within the acceptable levels for humans.

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ABSTRACT

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Keywords Chinese cordyceps, Arsenic, Soil, Health risk

INTRODUCTION

Chinese cordyceps (Fig. 1), known as a famous fungal, is a fungus-caterpillar complex distributed mainly in the Tibetan Plateau. The definite Latin name of this fungus was debated in recent decades. In this article, the fungus-caterpillar complex is represented by the term "Chinese cordyceps" (Dong et al., 2016). Chinese cordyceps goes through two stages to complete its life cycle, teleomorph and anamorph. In the summer, the ascospores erupt from mature stroma and form directly into conidia, or mycelium. In the autumn, conidia or mycelium in deeper soil can infect host (Zhang et al., 2012). Similarly, after incubating from the eggs scattered on the grassland, the host *Thitarodes* (Lepidoptera: *Hepialidae*) larvae infiltrate deep into the soil (Fig. 1A) and safely reside in the roots of their preferred substrates throughout the long-lasting larval stage (Fig. 1B) (Chen et al., 2009). After developing through four to five instars (approximately 2-3 years), the larvae may be infected by the fungus in the soil in June (Fig. 1D) (Zou et al., 2010). The larvae then become a fungal host, and gradually, their interior are filled with thread-like hyphae to form the caterpillar-shaped sclerotium (SC) (the so-called winter-worm, Fig. 1E) in the winter. The sclerotium germinate from the head of winter-worm in the coming spring when the frozen soil thaws, and grow into stroma (ST) (the so-called summer-grass, Fig. 1F) for approximately 2 months. Then, they mature and disperse millions of spores (Fig. 1C), initiating the next hostile takeover (Guo et al., 2017).

Chinese cordyceps has been used as traditional Chinese medicine for a long history. Its pharmaceutical functions, for example, antitumor, anti-inflammatory, antioxidant, anti-hyperglycemia, anti-apoptosis, immunoregulatory, and hepatoprotective have been reported in some research (Qi et al., 2014; Liu et al., 2015). However, the concentration of As (total As: 4.4-9.0 mg/kg) in Chinese cordyceps was at least three times greater than the reference value of 1 mg/kg (NHFPC, 2014), which was disclosed by the China Food and Drug Administration

(CFDA) (CFDA, 2016a), social concerns regarding functional food health have arisen. Immediately, the functional food promotion and publicity about Chinese cordyceps was suspended (CFDA, 2016b). These events have caused social panic and have badly affected the industrial chain of Chinese cordyceps (Wang et al., 2016).

Arsenic, an environmental contaminant that could disperse and enter humans through the food chain, is considered to be the most concerning hazardous material in the world due to its toxicity (Styblo et al., 2000). Arsenic is found to be the toxic effect depending on its species. Inorganic arsenic (iAs) is the carcinogenic to people, like trivalent iAs (arsenite, $As^{Ⅲ}$) and pentavalent iAs (arsenate, $iAs^{Ⅴ}$), which are widely present in the soil and water (Huang and Ke, 2004). When iAs transfer into organisms along the food chain, it would be transformed into organic arsenic species (oAs) by the organisms. Monomethylarsonic acid (MMA^{V}) and dimethylarsinic acid (DMA^{V}) are the major metabolic products of iAs, which have lower toxicity than iAs. For the next process, the subsequent metabolites, such as organic As compounds (arsenocholine, arsenobetaine (AsB), various arsenolipids and arsenosugars), are mostly considered nontoxic (Hua et al., 2011; Styblo et al., 2000). Thus, these As transforming processes in organisms are generally considered as the detoxing process of iAs. Moreover, some trivalent metabolites, monomethylarsonous acid (MMA^{III}), dimethylarsenic acid (DMA^{III}) in animals and human cells or arsenic-containing hydrocarbons, such as $C_{17}H_{37}AsO$, $C_{19}H_{41}AsO$ and $C_{23}H_{37}AsO$ in seafoods, have been confirmed to exert cytotoxicity (Arroyo-Abad et al., 2010; Meyer et al., 2014). In our previous work (Guo et al., 2018b), it found that unknown organic As species (oAsU), which were considered as arsenosugars, comprised a large proportion of total As in Chinese cordyceps.

Unlike other mushrooms of which only the stroma or fruiting body is consumed (Larsen et al., 1998; Kuehnelt et al., 1997), Chinese cordyceps is a larval-fungus complex, and the sclerotium is

105 the complex of the host larva (substrate) and mycelium of the fungus, while the stroma is purely
106 composed of the fungus (Zhang et al., 2012). In this context, this study is designed to determine
107 the arsenic species and distribution along soil habitat - sclerotium - stroma. Since the Qinghai-
108 Tibet Plateau has higher environmental background value of As than that in other regions in
109 China, this study also aims to investigate the risk assessment of As in the soils around Chinese
110 cordyceps habitat.

111 ## MATERIALS AND METHODS

112 **Sample collection and preparation**

113 Three sites from the endemic areas in Shergyla Mountain, Tibet, were selected for this study.
114 Site A, located at 29°36' N, 94°36' E; Site B, located at 29°35' N, 94°36' E; Site C, located at
115 35°14' N, 91°48' E. During mid-July of 2017, we took fifteen soil samples in 10-20 cm topsoil
116 and twenty Chinese cordyceps samples about 0.3 g each from each sampling site. The samples
117 were kept in the icebox and carried back to the laboratory.

118 In the laboratory, these selected samples were dehydrated by freeze drying process. The
119 Chinese cordyceps samples were devided into two subsamples: SC and ST. Considering ST was
120 light and thin compared with SC, each of the ten ST subsamples were combined to form a batch
121 sample and each of the five SC subsamples were combined to form a batch sample. Therefore,
122 the twenty Chinese cordyceps samples collected from each sampling spot were divided into 4
123 batches of sclerotium samples and 2 batches of stroma samples and named as $SC_{A/B/C}$ and
124 $ST_{A/B/C}$ according to sampling sites. Soil samples were prepared by ground up into powders with
125 a grain size of less than 150 mesh. Every five powdered soil samples were combined into one
126 batch and named as A/B/C according to sampling sites.

127 **Sample digestion**

For total As analysis in SC and ST, 0.1g of each pre-dried sample was digested with concentrated nitric acid (16mol/L) in the high-temperature and microwave-assisted method. The digestion methods followed our previous reference (Guo et al., 2018b). To determine the As speciation, 1 g of the sample powder was digested with 20 mL 0.15 mol/L HNO_3 at 90 °C using water bath for 12 hours (Guo et al., 2018b). When cooled to room temperature, all of the digested product was centrifuged for 15 min at the speed of 7104 g. Then, the collected supernatant was filtered through a sieve having a mesh aperture of 0.22 μm and kept in cold storage until analysis.

To determine the total As concentration in soil, approximately 0.1 g of each powdered sample was blended with a mixed solution of hydrochloric acid at 12mol/L and concentrated nitric acid at 16mol/L with the volume ratio of 3:1. The digestion procedures were performed referring to the standard method HJ 803-2016 (MEPP 2016). Different As species were extracted by reference to the Thomas (1997). Briefly, 10 mL 1 mol/L phosphoric acid (H_3PO_4) was added into 0.2 g pre-dried sample, processed and cooled under the microwave condition. The extract was filtered and diluted with distilled water, prepared for analysis.

Arsenic determination of sample

The total As was measured by ICP-MS (Agilent 7800, Santa Clara, CA, USA). The separation of As species ($iAs^{Ⅲ}$, $iAs^{Ⅴ}$, MMA, DMA and AsB) were conducted by HPLC (Agilent 1260, Santa Clara, CA, USA) and the separated As species were determined by ICP-MS. Based on our previous study (Guo et al., 2018b), the iAs^{III} could not be separated from the other arsenic species. To determine the level of iAs^{III}, 1 mL H_2O_2 was added into the extraction to fully oxidize the iAs^{III} to $iAs^{Ⅴ}$ and the arsenic species were analyzed before (Figs. 2B to 2C, 2F) and

after (Figs. 2D to 2E, 2G) addition to H_2O_2. Accordingly, the iAs^{III} was calculated by subtracting the level of iAs^{V} before addition to H_2O_2 from the level of iAs^{V} after addition to H_2O_2.

Each sample was performed in triplicate. The concentrations of total As and As species were quantified using calibration curves, which made with standard samples (National Institute of Metrology, Beijing, China).

The analysis precision was tested by the blank reagent and Chinese national standard substances of green Chinese onion: GBW10049 (GSB-27), and of yellow croaker: GBW08573. Wide linear responses ranged in 0.5 to 500 μg/L for the total As determination and ranged in 0.2 to 300 μg/L for the As species determination were obtained, and the correlation coefficients were greater than 0.9997 (Table S1). The relative standard deviations (RSD) was less than 10 % (Table S2) and the recoveries of these certified reference materials were within the acceptable range (Table S3).

Health risk assessment of As in soil

Arsenic has been documented as chemical carcinogen by USEPA (USEPA, 2011), as well as non-carcinogens for human. These arsenic can be migrated to plants and enter the human body through oral ingestion in food chain. Meanwhile, inhalation of soil / dust and dermal contact are also pathways of metal exposure to humans. Therefore, to comprehensively assess the hazards form the arsenic exposure, all of these three exposure pathways are taken in consideration.

According to the model of human health evaluation by the United States Environment Protection Agency (USEPA) (USEPA, 1989), the average daily doses (*ADD*, mg/(kg·d)) through the three exposure pathways (ingestion: ADD_{ing}; inhalation: ADD_{inh}; dermal contact: ADD_{dermal}) were calculated separately as follows:

$$ADD_{ing} = C \times \frac{IngR \times EF \times ED}{BW \times AT} \times 10^{-6} \quad (1)$$

$$ADD_{inh} = C \times \frac{InhR \times EF \times ED}{PEF \times BW \times AT} \quad (2)$$

$$ADD_{dermal} = C \times \frac{SA \times AF \times ABF \times EF \times ED}{PEF \times BW \times AT} \times 10^{-6} \quad (3)$$

where C (mg/kg) is the concentration of total As in soil, EF (days/year) is the exposure frequency, ED (years) is the exposure duration, BW (kg) is the body weight, AT (days) is the average time, PEF (m^3/kg) is the particular emission factor, SA (cm^2) is the surface area of exposed skin, AF (mg/cm^2) is the skin adherence factor, ABF is the absorption factor, $IngR$ (mg/d) and $InhR$ (m^3/days) is the ingestion rate and inhalation rate, respectively. The parameter values for children and adults are shown in Table S4 by referring to Chinese assessment guidelines for an environmental site (MEPP, 2014) and U.S. exposure factors handbook (USEPA, 2011).

The hazard quotient (HQ) was calculated separately as follows:

$$HQ = \frac{ADD}{RfD} \quad (4)$$

$$HI = \sum HQ_i \quad (5)$$

where RfD is the non-carcinogenic reference dose for As (mg/(kg·d)); the values through ingestion, inhalation, and dermal contact is: 3.0×10^{-4}, 1.5×10^{-5}, and 3.0×10^{-4}, respectively (MEPP, 2014; USEPA, 2013; Liu et al., 2008). HI is the total exposure hazard index. If HQ or $HI < 1$, there is no concern for non-carcinogenic effects, whereas potential non-carcinogenic risks may occur in cases where HQ or $HI > 1$.

Carcinogenic risk (CR) was calculated as follows:

$$CR = ADD_{ing/inh/dermal} \times SF \quad (6)$$

$$CR_T = \sum CR \quad (7)$$

where SF is the slope factor of As and the values through ingestion, inhalation, and dermal contact is: 1.5, 4.3×10^{-3}, and 1.5, respectively (USEPA, 2011; MEPP, 2014; USEPA, 2013; Liu et al., 2008). CR_T is the sum of CR for the three pathways. The probability of cancer risk for

humans over a lifetime is characterized by *CR* with an acceptable range from 1.0×10^{-6} to 1.0×10^{-4}. If $CR < 1.0\times10^{-6}$, it suggests no significant effect on human beings, whereas $CR > 1.0\times10^{-4}$ is likely to be harmful to human beings.

Statistical analysis

Data were analyzed in Microsoft Excel 2013 (Microsoft, Redmond, WA, USA) and SPSS 13.0 (IBM, Chicago, IL, USA). The levels of As are calculated as the means ± standard deviations (SD).Wilcoxon and Kruskal-Wallis tests were used to check the significance in the concentrations of total As and As species among different samples. Significant difference was considered as $p < 0.05$.

RESULTS

Total arsenic concentration

The concentrations of total As in soil of the sampling spots are presented in Table 1. The results indicated the total concentration of As was the highest in site A (16.31 mg/kg) and was the lowest in site B (13.03 mg/kg). Compared with the soil background values in China (Wei et al., 1991), the mean concentrations of total As in A, B and C were 1.5, 1.2 and 1.4 times higher, respectively.

The concentration of total As in SC and ST are reported in Table 1. The mean concentration of total As in SC from the study area was between 4.64 and 5.68 mg/kg. By comparing the reference value of total As in functional foods (NHFPC, 2014), it was observed that total As in SC was about 5 times higher. The mean level of total As in ST ranged from 0.82 to 1.13 mg/kg, close to the reference value (NHFPC, 2014).

The concentration of total As decreased as follows: soil > SC > ST ($p < 0.01$, Wilcoxon and Kruskal-Wallis tests, Table S5).

Arsenic species

The concentrations of different As species in soil samples are given in Table 1 (the chromatograms are shown in Figs. 2F to 2G). The results showed that inorganic As were abundant in site A, B and C, in which iAs^{V} was significantly higher than iAs^{III} (Table S6). By comparing with inorganic As, the concentration of organic As was significantly lower (Table S6). Besides, small amounts of AsB can be detected in organic As.

The concentrations of different As species in SC and ST samples are presented in Table 1 (the chromatograms are shown in Fig. 2). Under the H_2O_2 treatment, most of the As species in the large peak area were not oxidized to iAs^{V} (Figs. 2D to 2E), which proved that the major overlapped peak was not the toxic iAs^{III} but various unknown organic As species (oAsU). However, it was not possible to evaluate their definite compounds and structures due to the lack of appropriate standards.

In the SC samples, inorganic As were in the minority, in which iAs^{III} was significantly higher than iAs^{V} (Table S6). By comparing with inorganic As, the concentration of organic As was significantly higher. Among these organic As, oAsU was abundant, while DMA and MMA were almost negligible. In the ST samples, inorganic As were also in the minority, while iAs^{V} was significantly higher than iAs^{III} (Table S6). By comparing with inorganic As, the concentration of organic As was significantly higher (Table S6). Among the detected organic As species, oAsU was the predominant species, only leaving AsB and DMA detected in minor amounts in some samples.

Hazard assessment of the soil

The calculated average daily doses (*ADD*) for non-carcinogens and carcinogens are summarized in Tables 2 to 3. Overall, the *ADD* through different exposure pathways decreased in the

following order: $ADD_{ing} > ADD_{inh} > ADD_{derm}$, indicating that ingestion is the major exposure path way. Meanwhile, children are more vulnerable than adults because of the higher *ADD*. The results of human health risk assessment of As in soil suggested that the potential non-carcinogenic significant risk was negligible since the *HI* was less than 1 (Table 4). The cancer risks (*CR*) for human health were at an acceptable level (total $CR < 1 \times 10^{-4}$) (Table 4).

DISCUSSION

Arsenic transfer chain during Chinese cordyceps formation process

As a special organism growing in the Tibetan Plateau (Li et al., 2008), Chinese cordyceps is both a consumer and a de-composer in the food-chain. Our study revealed the total As abundance in Chinese cordyceps habitat soil was higher than the average abundance on Earth. Furthermore, inorganic As accounted for the majority of total As in soil. To adapt to the toxic circumstances, the original organisms in this soil ecosphere have developed detoxifying strategies to survive. Although the As transfer chain and corresponding metabolism from the habitat soil to Chinese cordyceps have not been investigated previously, with reference to previous research on other plants (Zhao et al., 2009; Lomax et al., 2012), animals (Healy et al., 1998), and fungi (Gonzálvez et al., 2009; Soeroes et al., 2005; Chang et al., 2019), the complicated delivery and transformation of As can be briefly inferred as follows. First, through the passive absorption from plants' roots, the original As in the soil is transported and isolated into the plant vacuoles to avoid its toxic effects. During this process, the inorganic As keeps its original speciation because plants cell can not regulate the methylation of As due to lack of methyltransferase (Zhao et al., 2009; Lomax et al., 2012). The host *Thitarodes* larvae, which take the plants' tender roots as their preferred food for about 2-3 years (Fig. 1B), first reduce the ingested iAs^{V} to iAs^{III} by their

reductase and then methylate $iAs^{Ⅲ}$ to low toxic MMA or DMA via methylationase. Subsequently, MMA and DMA are detoxified into other nontoxic As compounds. Notably, fungus also contain methylationase for the methylation (Tang et al., 2016; Zhang et al., 2017). As expected, the results of the present study revealed that both the As concentration and speciation were significantly different between soil environment and SC. It can be inferred that the larva-fungi union has the highly efficient detoxifying mechanisms through which the inorganic As ingested by *Thitarodes* larvae had been turned into organic As.

Considering that SC was the complex of host larvae and mycelium, it was not possible to fairly evaluate the effect of Chinese cordyceps on As transformation only based on changes in the SC. Thus, we concentrated on the concentration and distribution of As across the ST, which grew only from the Chinese cordyceps without any interference from the host tissue. Here we found that the level of total As from SC to ST has been reduced greatly. And the level of iAs^{III} was significantly higher than that of iAs^{V} in the SC, but the reverse occurred in the ST. The results provided strong evidence that although the host larvae ingested large amounts of toxic iAs^{III} from the soil due to iAs^{III} solubility (Andrahennadi and Pickering, 2008), Chinese cordyceps could turn substantial parts into low toxic iAs^{V} to prevent toxicity to offspring (ascospores in ST).

Till now, the cultivation of wild Chinese cordyceps, which occurred from wild *Thitarods* in the wild habitat soil, has not been succeeded due to many unanswered questions on the mechanism of occurrence. Artificial cultivation in the laboratory was based on the cultivated *Thitarods* fed with prepared feed with low As background, and its life history (6 months) was much shorter than wild Chinese cordyceps (2-3 years). Our previous study (Guo et al., 2018a) comparatively studied the total As and As species in wild Chinese cordyceps and cultivated Chinese cordyceps.

In laboratory, the cultivated Chinese cordyceps was bred under artificial circumstance with trace As in place of the high concentrations of As on the Tibetan Plateau. The results showed that As concentration in cultivated Chinese cordyceps was much lower than that in wild Chinese cordyceps. The finding provided important evidence that for wild Chinese cordyceps, species and level of As were affected by the comprehensive function of soils, host larvae and Chinese cordyceps fungus. Based on the previous study and the results of this experiment, it could be inferred that unlike *Laccaria amethystea* (Larsen et al., 1998) and *Collybia butyracea* (Kuehnelt et al., 1997) which can accumulate As, Chinese cordyceps is a fungus with ability to reduce As.

Arsenic concentration in soil and health risk assessment

In this study, the total As concentration in soil samples measured by ICP-MS was much higher than the sum of the five As species measured by HPLC-ICP-MS. The difference between the two was unextracted arsenic ores (Liu et al., 2018). Therefore, inorganic arsenic was the predominant forms in soil and we took the concentration of total As to assess the potential risk posed by soil arsenic. A previous study reported that the As level of the soil sample in Lhasa was higher than that in our tested sites (Cheng et al., 2014) and the elevated As concentration might be related to transportation pollutants in addition to the local background values.

Arsenic can exist in almost all environmental media, especially in the soil and be accumulated in plants and eventually sneak into the body through the food chain (Wei et al., 2016; Tsuda and Babazono, 1992). As for the Tibetan Plateau we studied, animal husbandry and dairy industry have long occupied the important position on the local economy. Arsenic can pose most health risks through soil - plants - food - human pathways. Based on our results, although As geological background value was higher than that in China, there were no seriously threat to human health. It is worth noting that children were generally more vulnerable than adults, which is consistent

with many other studies (Chen et al., 2019; Li et al., 2018). However, due to the toxicity of As species differs, further studies should be focus on the potential risk caused by toxic As species rather than the total As.

CONCLUSIONS

This study found that the distribution and species of As were different among habitat soil, SC, and ST, suggesting that Chinese cordyceps was not an As-accumulating fungus as traditionally believed. In addition, the process of arsenic degrading and translocation occurred in the above three was explained. Overall, this study can provide new insight into the detoxification mechanism of Chinese cordyceps under high As stress and would be beneficial to the revival of Chinese cordyceps - dependent industrial chain. The risk assessment found that there was little risk for humans caused by As in the high geological background area of Qinghai-Tibet Plateau. In order to provide more evidence, the determination of potential risk caused by different arsenic species should be carried out in the further study.

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Competing Interests

The authors declare there are no competing interests.

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Figure 1

Life cycle of Chinese cordyceps.

This figure was modified according to our previous study (Guo et al., 2018b). (A) The eggs came out of the insect host and started hatching. (B) The host larvae lived in the soil throughout the long-lasting larval stage. (C) The ascospores were germinated and released from the perithecia. (D) The ascospores infected the 4-5th instar larvae under the ground. (E) The caterpillar filled with threadlike hyphae and formed the sclerotium. (F) The fungus grew out from the head and formed the stroma, Chinese cordyceps finally formed.

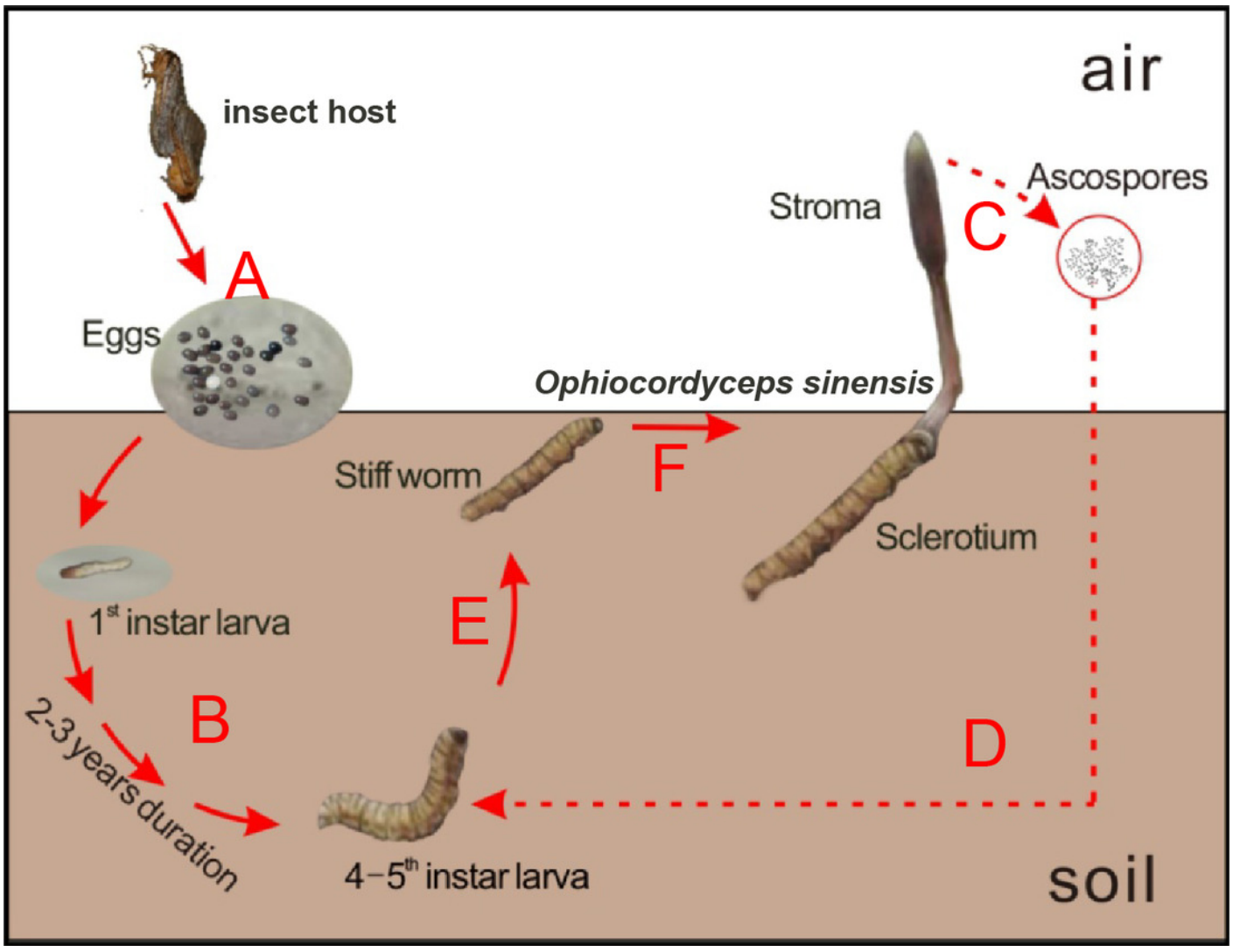

Figure 2

Chromatograms obtained in quantification by HPLC-ICP-MS.

(A) The mix standards of AsB, DMA, iAs^{III}, MMA and iAs^{V}, at 50 ppb of each arsenic species. (B) and (C) This was extracts of SC and ST collected from site B, respectively. The iAs^{III} and the other unknown peaks were overlapped. (D) and (E) This was oxidation products of the extracts of SC (B) and ST (C), the iAs^{III} was transformed into iAs^{V} when the extracts was added with H_2O_2. (F) This was extract of soil sample collected from site B. (G) This was oxidation products of the extracts of soil sample (F).

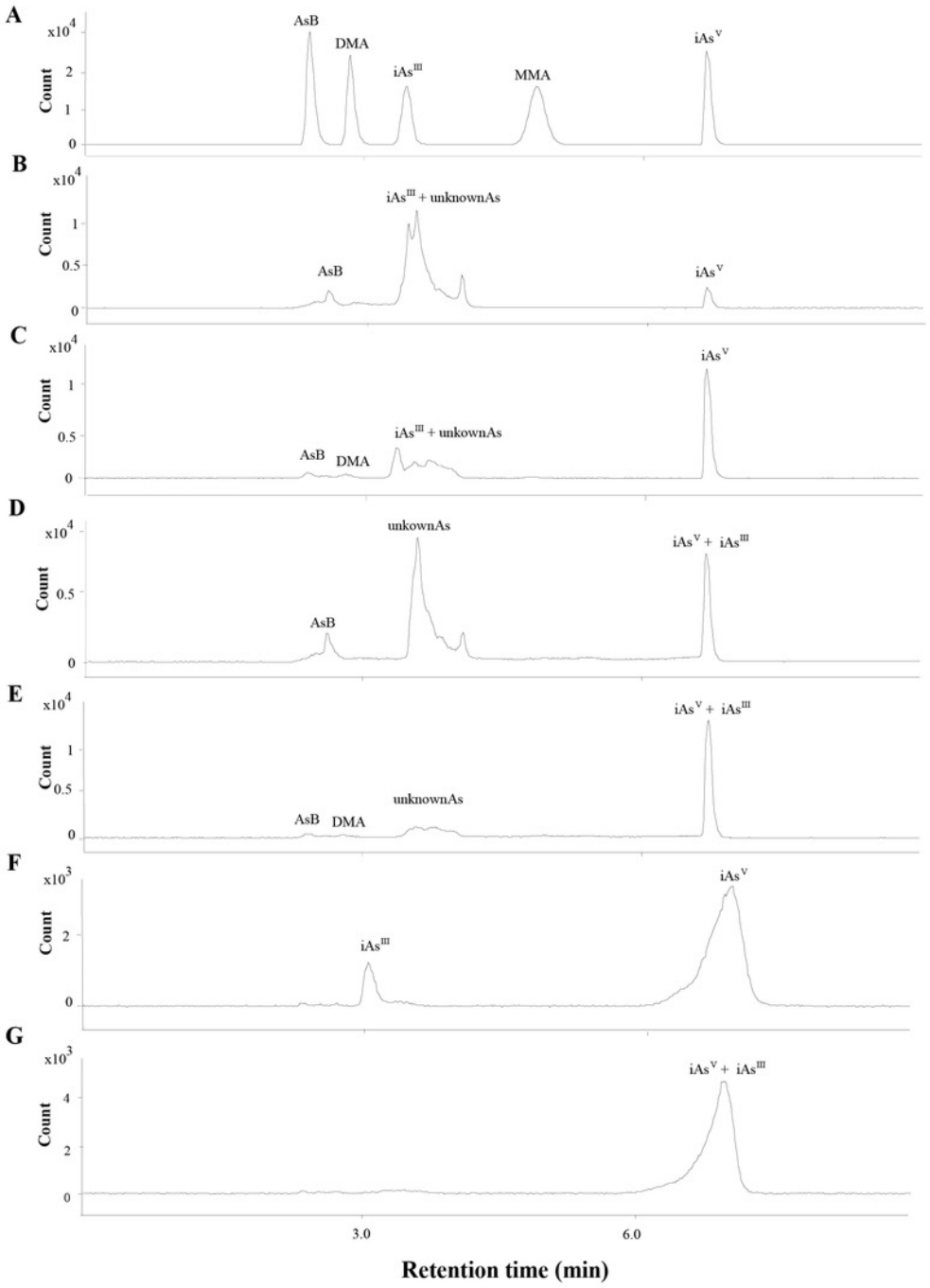
A
AsB
DMA
iAs^{III}
MMA
iAs^{V}
x10^4
Count
B
iAs^{III} + unknownAs
AsB
iAs^{V}
C
iAs^{V}
iAs^{III} + unkownAs
AsB
DMA
D
unkownAs
iAs^{V} + iAs^{III}
AsB
E
iAs^{V} + iAs^{III}
unknownAs
AsB
DMA
F
x10^3
iAs^{V}
iAs^{III}
G
iAs^{V} + iAs^{III}
3.0
6.0
Retention time (min)

Table 1 (on next page)

Concentrations[a] of arsenic speciation (mg/kg) (n=15 for A, B, C, n=20 for SC and ST).

Sample	organic arsenic					inorganic arsenic			tAs
	AsB[b]	DMA	MMA	oAsU	total	$As^{Ⅲ}$	$As^{Ⅴ}$	total	
A	0.04±0.01	0.03±0.02	nd	3.75±0.25	3.82±0.26	0.62±0.15	8.91±0.35	9.54±0.21	16.31±1.82
B	0.03±0.01	0.04±0.01	nd	2.06±0.26	2.13±0.24	0.74 ±0.07	7.46±0.25	8.20±0.18	13.03±1.00
C	0.03±0.01	0.04±0.03	nd	2.88±0.29	2.95±1.01	0.66±0.03	9.26±0.39	9.92 ±0.37	15.72±1.57
SC_A	0.11±0.01	nd[c]	nd	5.06±0.6	5.17±0.6	0.24±0.02	0.09±0.01	0.33±0.02	5.50±0.58
SC_B	0.10±0.01	nd	nd	4.22±0.55	4.32±0.55	0.23±0.02	0.09 ±0.00	0.32±0.02	4.64±0.54
SC_C	0.13±0.02	nd	nd	5.14±0.42	5.27±0.43	0.31±0.01	0.1±0.01	0.41±0.01	5.68±0.44
ST_A	nd	nd	nd	0.77	0.77	0.07	0.19	0.26	1.03
ST_B	0.01	0.01	nd	0.85	0.87	0.07	0.19	0.26	1.13
ST_C	nd	nd	nd	0.63	0.63	0.07	0.12	0.19	0.82

Note:

[a]Concentrations were presented in $SC_{A/B/C}$ and soil (A/B/C) as mean ± standard deviation, concentrations were presented in $ST_{A/B/C}$ as the average value; [b]AsB, DMA, MMA, oAsU, $As^{Ⅲ}$, $As^{Ⅴ}$ and tAs were the abbreviation of arsenobetaine, dimethylarsenic acid, monomethylarsonic acid, unknown organic arsenic, arsenite, arsenate, and total arsenic, respectively; [C] not detected.

Table 2(on next page)

Non-carcinogenic average daily exposure doses of As in soil (mg/kg·d).

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Site	ADD_{ing}		ADD_{inh}		ADD_{dermal}		ADD_{total}	
	Adults	Children	Adults	Children	Adults	Children	Adults	Children
A	2.8 E-05	2.0 E-04	3.2 E-09	5.6 E-09	7.0 E-10	3.2 E-10	2.8 E-05	2.0 E-04
B	2.2 E-05	1.6 E-04	2.5 E-09	4.5 E-09	5.6 E-10	2.5 E-10	2.2 E-05	1.6 E-04
C	2.7 E-05	1.9 E-04	3.1 E-09	5.4 E-09	6.7 E-10	3.0 E-10	2.7 E-05	1.9 E-04

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Table 3(on next page)

Carcinogenic average daily exposure doses of As in soil (mg/kg·d).

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Site	ADD_{ing}		ADD_{inh}		ADD_{dermal}		ADD_{total}	
	Adults	Children	Adults	Children	Adults	Children	Adults	Children
A	9.6 E-06	1.7 E-05	1.1 E-09	4.8 E-10	2.4 E-10	2.7 E-11	9.6 E-06	1.7 E-05
B	7.7 E-06	1.3 E-05	1.7 E-09	3.8 E-10	1.9 E-10	2.2 E-11	7.7 E-06	1.3 E-05
C	9.2 E-06	1.6 E-05	1.1 E-09	4.6 E-10	2.3 E-10	2.6 E-11	9.2 E-06	1.6 E-05

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Table 4(on next page)

Index of carcinogenic risk and non-carcinogenic risk.

Site	Groups	HQ_{ing}	HQ_{inh}	HQ_{dermal}	HI	CR_{ing}	CR_{inh}	CR_{dermal}	CR_T
A	Adults	0.09	2.59E-05	2.32E-06	0.09	1.4E-05	4.69E-12	3.58E-10	1.4E-05
	Children	0.66	4.54E-05	1.05E-06	0.66	2.5E-05	2.06E-12	4.05E-11	2.5E-05
B	Adults	0.07	2.07E-05	1.85E-06	0.07	1.1E-05	3.74E-12	2.86E-10	1.1E-05
	Children	0.52	3.63E-05	8.38E-07	0.52	2.0E-05	1.65E-12	3.23E-11	2.0E-05
C	Adults	0.09	2.49E-05	2.23E-06	0.09	1.4E-05	4.52E-12	3.45E-10	1.4E-05
	Children	0.63	4.38E-05	1.01E-06	0.63	2.4E-05	1.99E-12	3.90E-11	2.4E-05