Arsenic transfer chain in soil-sclerotium-stroma of Chinese cordyceps and its health risk assessment for human exposure via soil

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Background As an important environment migration chain of arsenic(As), the soil and Chinese cordyceps system constitutes an exposure pathway to humans.

Methods In this study, sclerotium (SC) and stroma (ST) in Chinese cordyceps and habitat soils were collected from the Qinghai-Tibet Plateau to determine the concentrations and species of arsenic through inductively coupled plasma mass spectrometry (ICP-MS).

Results The total concentrations of As ranged from 0.82 to 1.13 mg/kg dry weight in ST, from 4.64 to 5.68 mg/kg dry weight in SC, and from 13.03 to 16.31 mg/kg in soils. The major components were a cluster of unknown organic As compounds, accounting for 90.5% to 92% in SC and 74.8% to 76.8% in ST. The relative abundance of toxic inorganic As as a percentage of total As was as follows: soil (58.4%-63.1%) > ST (23%-25.2%) > SC (6%-7.3%). The ratio of inorganic to organic As in ST (0.30-0.34) was higher than that in SC (0.06-0.08). Results indicate that the uptake of As from soil in Chinese cordyceps was limited, and Chinese cordyceps possess highly efficient detoxifying characteristics. The levels of As in the tested soils were higher than the soil background values of China. However, the probabilities of health risks were found to be within the acceptable levels for humans, but children are more susceptible.

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Keywords: Chinese cordyceps; Arsenic; Soil; Health risk

Introduction

Chinese cordyceps (Fig. 1), a mysterious entomogenous fungus distributed on the Qinghai-Tibet Plateau, is popularly referred to as winter-worm-summer-grass (Dong Chong Xia Cao in

Chinese). The definite Latin name of this fungus has been debated in recent decades; however, according to the Jinhu declaration for *Cordyceps* industry development (Dong et al., 2016), both the teleomorph and anamorph stages have been approved as Chinese cordyceps. Thus, in this paper, we use Chinese cordyceps to refer to both its teleomorph and its anamorph stages. The "worm" is a caterpillar-shaped sclerotium (SC), and the "grass" is the stroma (ST) of the fungus. In the summer, the ascospores erupt from mature stroma, develop into infective conidia, and gradually infiltrate into deeper soil with the rainfall (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 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 is filled with thread-like hyphae to form the caterpillar-shaped SC (the so-called winter-worm, Fig. 1E) in the winter. The stroma germinates out of the head capsule in the coming spring when the frozen soil thaws, and grows for approximately 2 months. Then, they mature (the so-called summer-grass, Fig. 1F) and disperse millions of spores (Fig. 1C), initiating the next hostile takeover (Guo et al., 2017).

Figure 1 near here.

Chinese cordyceps has been utilized in China and adjacent countries for thousands of years as a rare functional food to promote health and to treat diverse chronic diseases. Its remarkable functions such as antitumor, anti-inflammatory, antioxidant, anti-hyperglycemia, anti-apoptosis, immunoregulatory, and hepatoprotective have been reported in modern pharmaceutical research (Qi et al., 2014; Liu et al., 2015). However, since the China Food and Drug Administration

(CFDA) revealed in 2016 that a total As content of 4.4-9.9 mg/kg (CFDA 2016a), at least three times greater than the reference value of 1 mg/kg for total As in functional foods (GB16740-2014) (National 2014), was detected in Chinese cordyceps, social concerns regarding human health have arisen. Immediately, on February 26, 2016, the CFDA ordered that all the pilot work on Chinese cordyceps as a functional food be discontinued (CFDA 2016b). These events have caused a great social panic and uproar in the functional food market and have seriously affected the Chinese cordyceps-dependent economic chain (Wang et al., 2016).

Arsenic, a common material in the ecosphere that can be transferred into organisms through the food chain, is the perceived by some as the most concerning hazardous material in the world due to its toxicity. The toxicity of As is dependent on its chemical form, with inorganic arsenic species (iAs), such as arsenite ($As^{Ⅲ}$) and arsenate ($As^{Ⅴ}$), being the most toxic, which are the majority species in the soil and water (Huang and Ke, 2004). When the iAs transfer into organisms along the food chain, iAs begin to be transformed into organic species in vitro. The initial metabolic products are monomethylarsonic acid (MMA) and dimethylarsenic acid (DMA), which are much less toxic than iAs to human beings, and their subsequent metabolic products, such as some other organic As complexes (arsenocholine, arsenobetaine, and various arsenosugars and arsenolipids), are mostly considered nontoxic (Hua and Carew, 2011; Styblo and Razo, 2000). Thus, these As transforming processes in organisms are generally considered as the detoxing process of iAs. Recently, several organic As species (oAs), discovered as intermediate metabolites (MMA^{III}, DMA^{III}, $DMMTA^{V}$) in organisms or discovered as hydrocarbons (AsHCs) in animal-sourced foods, were found to be highly toxic (Styblo and Razo, 2000; Meyer et al., 2014). In recent research (Guo et al., 2018), we found that although the total As were predominant (4.00 to 5.25 mg/kg), the iAs generally considered toxic were minor ($As^{Ⅲ}$:

4.1-6.0% and As^{V}: 1.3-3.2%), and low toxic oAs (MMA^{III} and DMA^{III}) were below the level of detection after examining the As speciation in Chinese cordyceps through HPLC-ICP-MS according to the Chinese standard of functional food (National, 2014). The largest proportion of As was composed of unknown organic As species, which were considered as a kind of arsenosugar according to a H_2O_2 test and chromatography (Guo et al., 2018).

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 the complex of the host larva (substrate) and mycelium of the fungus, while the stroma is purely composed of the fungus (Zhang et al., 2012). Thus, the As concentration and distribution might differ during the stroma germinating process and may ultimately, vary between the sclerotium and the stroma of the Chinese cordyceps. This paper aims to clarify the total As, As speciation ($As^{Ⅲ}$, $As^{Ⅴ}$, MMA, DMA, AsB), and distribution among the SC, ST, and habitat soil. Furthermore, a health risk assessment of arsenic in the soil for humans was estimated. Thus, the results fairly evaluate the bio-accumulated ability of As in Chinese cordyceps and provide a potential marker in discriminating the products of natural Chinese cordyceps. Furthermore, based on the arsenic concentration in soil collected from the Chinese cordyceps habitat, the health assessment provides basic data for the population living in the plateau areas.

Materials and Methods

Sample collection and preparation

Twenty Chinese cordyceps samples of similar size (approximately 0.3 g per sample) were collected at each site from the native habitats in Shergyla Mountain, Tibet. Site A, located at 29°36' N, 94°36' E; Site B, located at 29°35' N, 94°36' E; Site C, located at 35°14' N, 91°48' E.

126 And corresponding topsoil samples were collected. All samples were stored inside ice-cold cages
127 and delivered to the laboratory.
128 In the laboratory, all of the Chinese cordyceps were freeze-dried to reach a constant weight,
129 sectioned into SC and ST subsamples, and named as $SC_{A/B/C}$ and $ST_{A/B/C}$ according to sampling
130 sites. To gather enough material for subsequent analysis, each of the five SC subsamples was
131 combined into a batch sample, and each of the ten ST subsamples was combined (each stroma is
132 approximately 85% of the sclerotium weight). Therefore, the original twenty Chinese cordyceps
133 taken at each sampling site were organized into 4 batches of sclerotium samples and 2 batches of
134 stroma samples. Soils collected from the study area were named as A/B/C according to sampling
135 sites. All soil samples were freeze-dried for 48 h and then ground into powders with a grain size
136 of less than 150 mesh. The powdered samples were sealed in sealable plastic bags and stored at 4
137 °C until analysis.
138 **Sample digestion**
139 The digestion methods for the determination of the total As and As species in SC and ST
140 followed those of previous references (Guo et al., 2018). Briefly, for total As analysis, the
141 sample powder was digested with concentrated HNO_3 in a microwave digestion system, and for
142 As speciation analysis, the sample powder was diluted with 0.15 mol/L HNO_3 and incubated in a
143 90 °C water bath for 12 hours. All the digestion products were centrifuged, filtered, and stored at
144 4 °C before analysis.
145 The digestion method for total As analysis of soil samples was as follows. Approximately 1 g of
146 each powdered sample was mixed with 20 mL of concentrated nitric acid (HNO_3) and digested
147 in a microwave-assisted system with the following procedures: it was (1) heated to 120 °C in 5
148 min and held at 120 °C for 5 min; (2) heated to 150 °C in 5 min and held at 150 °C for 5 min; (3)

heated to 170 °C in 5 min and held at 170 °C for 5 min; (4) heated to 190 °C in 5 min and hold at 190 °C for 20 min; and (5) cooled to room temperature. For As species analysis, 0.2 g of each pre-dried sample was extracted with 10 mL 1 mol/L phosphoric acid (H_3PO_4) and 10 mL 0.5 mol/L ascorbic acid in a 50 mL polyethylene centrifuge tube and then incubated in a 95 °C water bath for 1 h. When it was cooled to room temperature, it was centrifuged for 15 min at the speed of 8000 rpm. Then, collected supernatant was passed through a sieve with a pore size of 0.22 μm and stored in sealable plastic bags at 4 °C until analysis.

Based on our previous studies (Guo et al., 2018), the As^{III} could not be separated from the other arsenic species. Therefore, As speciation analysis was conducted based on a comparison between the extracts that were digested according to the above, and 1 mL of H_2O_2 was added to extracts before analysis.

Arsenic determination of samples

The total As was measured using ICP-MS (Agilent 7800, Santa Clara, CA, USA). The operating parameters of the equipment were as follows: radio frequency (RF) power, 1550 W; carrier gas, 1.05 L/min; collision mode, helium (HE) flow 4.2 mL/min; plasma gas flow rate = 15 L/min; auxiliary gas flow rate = 0.1 L/min; and selected isotope = *m/z* 75 and quantified using an external calibration curve from As Ⅴ standards. Five As species ($As^{Ⅲ}$, $As^{Ⅴ}$, MMA, DMA, AsB) were separated by an Agilent 1260 HPLC system (Santa Clara, CA, USA), identified, and quantified using an external calibration curve from the mix standards (MMA Ⅴ, DMA Ⅴ, $As^{Ⅲ}$, $As^{Ⅴ}$, and AsB). The chromatograph was equipped with a standard autosampler, IonPac AG19 guard column (4×50 mm), and an IonPacAS19 separation column (4×250 mm). The HPLC conditions were as follows: a mobile phase of 10 mmol/L anhydrous sodium acetate, 3 mmol/L potassium nitrate, 10 mmol/L sodium dihydrogen phosphate, 0.2 mmol/L disodium

ethylenediaminetetraacetate buffer, and a flow rate at 1.0 mL/min. The separated As species were examined by ICP-MS (as described previously).

The analytical performances of the proposed ICP-MS method for total As content analysis and HPLC-ICP-MS method for As species analysis were validated by determining the linearity, limits of detection, limits of quantification, and agreements with the certified reference materials as shown in our previous study (Guo et al., 2018).

Health risk assessment of As in soil

There are three pathways of metal exposure to humans through the soil: oral ingestion, inhalation, and dermal contact. According to the model of human health evaluation by the United States Environment Protection Agency (USEPA) (USEPA, 1989), the estimated average daily doses (*ADD*, mg/(kg·d)) through the three exposure pathways are calculated separately as follows:

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

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

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

where the ADD_{ing}, ADD_{inh}, and ADD_{dermal} are the average daily doses through ingestion, inhalation and dermal contact, respectively, and C is the concentration of total As in soil (mg/kg). The values of exposure parameters for children and adults applied to equations 1-3 are given in Table S1 by referring to Chinese assessment guidelines for an environmental site (Ministry, 2014) and USEPA health risks assessment (USEPA, 2011).

The risk characterization of As was classified as carcinogenic by USEPA (USEPA, 2011), as well as non-carcinogenic for health risk. In order to evaluate the human health non-carcinogenic risk of As exposure from soil in detected sites, the hazard quotient (*HQ*) was applied:

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

198 $HI = \sum HQ_i$ (5)

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

204 Carcinogenic risk (*CR*) was calculated as follows:

205 $CR = ADD_{ing/inh/dermal} \times SF$ (6)

206 $CR_T = \sum CR$ (7)

207 where *SF* is the carcinogenic risks slope factor of As; the values through ingestion, inhalation,
208 and dermal contact were: 1.5, 4.3×10^{-3}, and 1.5, respectively (USEPA, 2011; Ministry, 2014;
209 USEPA, 2013; Liu et al., 2008). CR_T is the sum of *CR* for the three pathways. The probability of
210 cancer risk for consumers over a lifetime is characterized by *CR* with an acceptable range of
211 1.0×10^{-6} to 1.0×10^{-4}. A value of $CR < 1.0\times10^{-6}$ indicates no obvious concern for cancer risk,
212 whereas $CR > 1.0\times10^{-4}$ is likely to be harmful to human beings.

213

214 ## Results

215 ### Total arsenic concentration

216 The concentrations of total As in the SC and ST are reported in Table 1. The level of total As in
217 the SC was between 4.64 and 5.68 mg/kg of dry mass due to their absolute advantage in weight
218 proportion (approximately 85%). The level of total As in the ST was much lower than that in the
219 SC and ranged from 0.82 to 1.13 mg/kg of dry mass, close to the reference value of 1 mg/kg for
220 total As in functional foods (GB16740-2014) (National, 2014). The ratio of the total As

221 concentration in the ST to that in the SC was found to be in the range of 0.18-0.20 and indicated
222 the Chinese cordyceps may not be an arsenic-accumulating fungus.
223 The concentrations of total As in the soil samples were distributed between 13.03 and 16.31
224 mg/kg (Table 1). Compared to the soil background values of China (Wei and Zheng, 1991), the
225 levels of total As in all of the tested samples were higher. Similarly, compared to the
226 geochemical baseline value of chemical elements in urban soil in China (Cheng et al., 2014), the
227 levels of total As in all of the tested samples were higher than the baseline value. Meanwhile, the
228 level of As in site A and site C exceeded the upper limits of geochemical baseline values, falling
229 within the areas with a high baseline.
230 **Arsenic species**
231 All SC and ST samples were analyzed using HPLC-ICP-MS to determine the content of different
232 As species (the chromatograms are shown in Fig. 2). The results are reported in Table 1. Under
233 the H_2O_2 treatment, most of the As in the large peak area was not oxidized to As^V (Figs. 2D and
234 2E), which proved that the major overlapped peak was not the toxic As^{III} but various unknown
235 organic As species (oAsU, with a retention time from 3.2 to 4 min). However, it was not possible
236 to evaluate these percentages due to the lack of appropriate standards.
237 In the SC, organic As species were predominant, and the percentage of total As content ranged
238 from 92.7% to 94%. Among the organic As species, the major components (representing 90.5%
239 to 92% of the total As) were oAsU. AsB was also detected as a minority compound in these
240 samples, ranging from 2% to 2.3%. The two potential toxic organic As species, DMA and MMA,
241 were found to be negligible in the SC samples. The relative abundance of inorganic As as a
242 percentage of total As ranged from 6% to 7.3%. The ratios of inorganic to organic As and As^V to
243 As^{III} ranged from 0.06 to 0.08 and 0.32 to 0.39, respectively. In the ST, organic As, while

relatively less abundant as a percentage of total As when compared with those in SC, was still the majority species, ranging from 75% to 77% of the total As. Almost all the organic As species were a cluster of unknown compounds, except for trace amounts of AsB and DMA found in ST_B. The relative abundance of inorganic As as a percentage of total As ranged from 23% to 25.2%. The ratios of inorganic to organic As and As^V to As^{III} ranged from 0.30 to 0.34 and 1.71 to 2.71, respectively. To present the results more intuitively, histogram graphs are shown in Figure 3. Results indicate that during the germination and development of the stroma, inorganic As was preferably transferred from the original substrate.

The concentrations for different As species in soil samples are presented in Table 1 (the chromatograms are shown in Figs. 2F and 2G). As shown in our previous study (Guo et al., 2018), the concentrations of the total As in different extraction methods determined by HPLC-ICP-MS and ICP-MS were quantitively consistent with the Chinese cordyceps samples. However, unextractable arsenic (uAs) in soil samples resulted from subtracting between total As and concentrations of different As species determined by HPLC-ICP-MS (Fig. 3A). The uAs might originate from insoluble secondary mineral, which formed with arsenic and other metals by coprecipitation (Liu et al., 2018). As expected, inorganic As is the predominant species in the soil, significantly more abundant as a percentage of total As compared with that in SC and ST. Of these inorganic As species, the major component (representing 58.4% to 63.1% of the of the total As) was the powerful toxic As^V. In all of the tested samples, organic As species were the minority, and the percentage of total As content ranged from 36.9% to 41.6%. Among the organic As species, the major components (representing 14.4% to 34% of the total As) were an unknown compound, and AsB and DMA were also detected as minority compounds in these

266 samples. The results show that the original As in plants taken up from the soil was the toxic
267 inorganic As.
268 Table 1 near here.
269 Figure 2 near here.
270 Figure 3 near here.
271 **Hazard assessment of the soil**
272 The calculated average daily doses (*ADD*) for non-carcinogens and carcinogens through different
273 exposure pathways decreased in the following order: $ADD_{ing} > ADD_{inh} > ADD_{derm}$, and ADD_{ing} w
274 as far greater than the other two (Tables 2 and 3). The value of the dose for children through
275 ADD_{total} was higher than for adults. The results of human health risk assessment of As in these
276 soils showed that the potential non-carcinogenic significant risk is negligible since the *HI* was
277 less than 1 (Table 4). The cancer risks (*CR*) for human health were at an acceptable level (total
278 $CR < 1 \times 10^{-4}$) (Table 4). Ingestion, for adults and children, is the most common exposure
279 pathway, which was consistent with exposure assessment. It revealed that non-carcinogenic and
280 carcinogenic risks in humans were associated with the exposure pathway, with children, in
281 general, more vulnerable than adults.
282 Table 2-4 near here.
283

284 ## Discussion

285 **Arsenic transfer chain during Chinese cordyceps formation**
286 As a special organism produced in the Qinghai-Tibetan soil ecosystem (Li et al., 2008), Chinese
287 cordyceps is both a consumer and a de-composer in the food-chain. The As concentration in
288 Chinese cordyceps is the outcome of complicated synergies of the soil (its living environment

289 and the alternative food of the host larvae), plants (a favorite food of the host larvae), host larvae,
290 and Chinese cordyceps. The total As abundance in Chinese cordyceps habitat soil was 13.03-
291 16.31 mg/kg (average value: 14.67mg/kg), a value higher than the average abundance on Earth.
292 Furthermore, the proportion of inorganic As was largest. To adapt to the toxic circumstances, the
293 original organisms in this soil ecosphere have developed detoxifying strategies to survive.
294 Although the As transfer chain and corresponding metabolism from the habitat soil to Chinese
295 cordyceps have not been investigated previously, with reference to previous research on other
296 plants (Zhao et al., 2009; Lomax et al., 2012), animals (Healy et al., 1998), and fungi (Gonzálvez
297 et al., 2009; Soeroes et al., 2005; Chang et al., 2019), the complicated delivery and
298 transformation of As can be briefly inferred as follows. First, through the passive absorption of
299 the plants' roots, the original As in the soil is transported and isolated into the plant vacuoles to
300 avoid its toxic effects. During this process, the As keeps its original speciation because the plants
301 appear to be unable to methylate inorganic As to organic As species due to the lack of genes
302 responsible for As methylation (Zhao et al., 2009; Lomax et al., 2012). The host Thitarodes
303 larvae, which use the plants' tender roots as their preferred food for approximately 3 years (Fig.
304 1B), first reduce the uptake of As^{V} reduced to As^{III} by its reductase and then methylate As^{III} to
305 low toxic MMA or DMA via methylationase. Subsequently, MMA and DMA are detoxified into
306 nontoxic organic As compounds. Chinese cordyceps infects Thitarodes larvae and utilizes them
307 as a substrate, simultaneously playing certain roles in As transformation during its mycelium
308 growth and caterpillar-shaped SC development. Two months later, the As is rearranged during
309 ST germination and growth (Fig. 1F). Therefore, the critical detoxification processes that
310 changed inorganic As into organic As are conducted by the larvae and Chinese cordyceps

because the animals and fungus are known to contain methylationase (Healy et al., 1998; Zhong et al., 2016; Zhang et al., 2017).

In this study, the total As concentration in SC was approximately 6 mg/kg (the organic As proportions were over 90%, in which the initial organic products of arsenate detoxification such as MMA and DMA were not detected). The significant contrasts in the As concentration and speciation between the SC and soil environment proved the highly efficient detoxifying mechanisms of the larva-fungi union because they reduced two-thirds of the total As and even the low-toxic organic arsenics (MMA and DMA), which were completely detoxified into other forms. However, based on the As concentration of SC, the detailed contribution of the Chinese cordyceps could not be fairly assessed because SC was the complex of the residual tissue of the host larvae and condensed Chinese cordyceps mycelium. Thus, we investigated the As distribution in ST, which is composed only of the Chinese cordyceps mycelium without any interference from the host tissue. The results showed that compared with the As distribution in SC, the total As content in ST was only about one-fifth of that in SC (Fig. 3A), and the ratios of inorganic to organic As and As^{V} to As^{III} in ST were several times that found in SC (Fig. 3B and C). The plummet of the total As from SC to ST, as shown with the black dashed line in Figure 3A, provided strong evidence that during germination and the development of the stroma, Chinese cordyceps absorbs nutrients in addition to As, but it could filter out up to about 80% As to prevent future toxicity to offspring (ascospores). The increase in the ratios of inorganic to organic As and As^{V} to As^{III} from SC to ST, as shown with the red dashed line in Figure. 3B and C indicates that during the stroma detoxification process, inorganic As might more easily penetrate the stroma. Based on the As transfaction from SC to ST, it could be inferred that

Chinese cordyceps is not an As-accumulating fungus such as *Laccaria amethystea* (Larsen et al., 1998) and *Collybia butyracea* (Kuehnelt et al., 1997), but an As-diluting fungus.

Hazard assessment of the soil

Higher concentrations of As in soil were found in study areas other than the As-level divisions of China because arsenic mainly comes from a parent material. Due to physical weathering being in effective in the Qinghai-Tibet Plateau, there was little element released or leached from rocks during weathering and pedogenesis (Li et al., 2008). This result of arsenic species in skeleton soil was consistent with data from calcareous soils (Xie et al., 2006) and indicated the similarity of arsenic species from the different soils. A previous study reported that the level of As in Lhasa was 20 mg/kg, which was higher than in our tested sites (Cheng et al., 2014). The elevated As concentration in Lhasa might be attributed to transported pollutants in addition to the local background values of the soil.

Compared to other potentially toxic elements in soil, As, in general, can cause damage to humans through long exposure (Wei et al., 2016; Tsuda and Babazono, 2010). There has always been a focus on the As transported from the heavy pollution in the soil for health assessment, but from endogenic arsenic, there has been a lack of attention. Estimating the non-carcinogenic and incremental lifetime cancer risks associated with exposure to As in detected soils indicated that there were no significant risks for humans. In the surrounding soil of a coking plant heavily polluted by As, the cancer risk due to ingestion exposure was higher than the acceptable level for children, and the ADD_{ing} was far greater than in our study (Chen et al., 2019). However, even small amounts of As in soil transported into plants' roots and accumulated in vegetables (Zeng et al., 2008) can be a threat to children's health through the food chain (Li et al., 2018; Manzoor, 2018). Therefore, the risk assessment posed by As might be associated with exposure dose as

356 well as its toxicity. However, there is great uncertainty in risk assessment of As in that there are
357 different toxicity and health effect, caused by concentrations, species, and absorption in the
358 body. Therefore, determination of the potential risk of the As in the food chain should be carried
359 out at various levels to provide strong evidence for our inference.
360

361 Conclusions

362 This result clarifies that arsenic migrates along the environmental soil-sclerotium-stroma chain.
363 The levels of As and species were different among habitat soil, SC, and ST. The results indicate
364 that Chinese cordyceps was not as much an As-accumulating fungus as traditionally believed.
365 Chinese cordyceps could filter out the majority of As to prevent future toxicity to offspring;
366 during the stroma detoxification process, inorganic As might more easily penetrate the stroma. In
367 addition, the health risk assessment of As found that the carcinogenic risk and non-carcinogenic
368 risk in the study areas were acceptable. Risks in humans were influenced by the exposure
369 pathway, with children generally more vulnerable than adults.
370

371 Declaration of interest statement

372 The authors report no conflict of interest for this article.
373

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Figure 1

Life history of Chinese cordyceps.

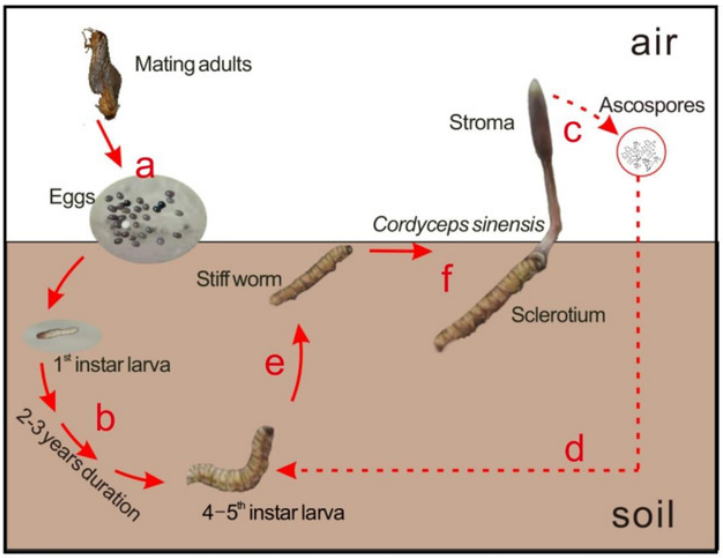

Figure 1: Life history of Chinese cordyceps.

(A) The eggs of the host insect scattered on the grassland incubate. (B) The host larvae safely reside in the soil throughout the long-lasting larval stage. (C) The ascospores erupt from mature fruiting bodies of Chinese cordyceps. (D) The 4-5th instar larvae may be infected by infective conidia of the Chinese cordyceps fungus in the soil. (E) The caterpillar-shaped sclerotium (winter-worm) formed. (F) The stroma germinates out of the head capsule and the mature Chinese cordyceps (summer-grass-winter-worm) formed.

Figure 2(on next page)

Chromatograms obtained in quantification by HPLC-ICP-MS.

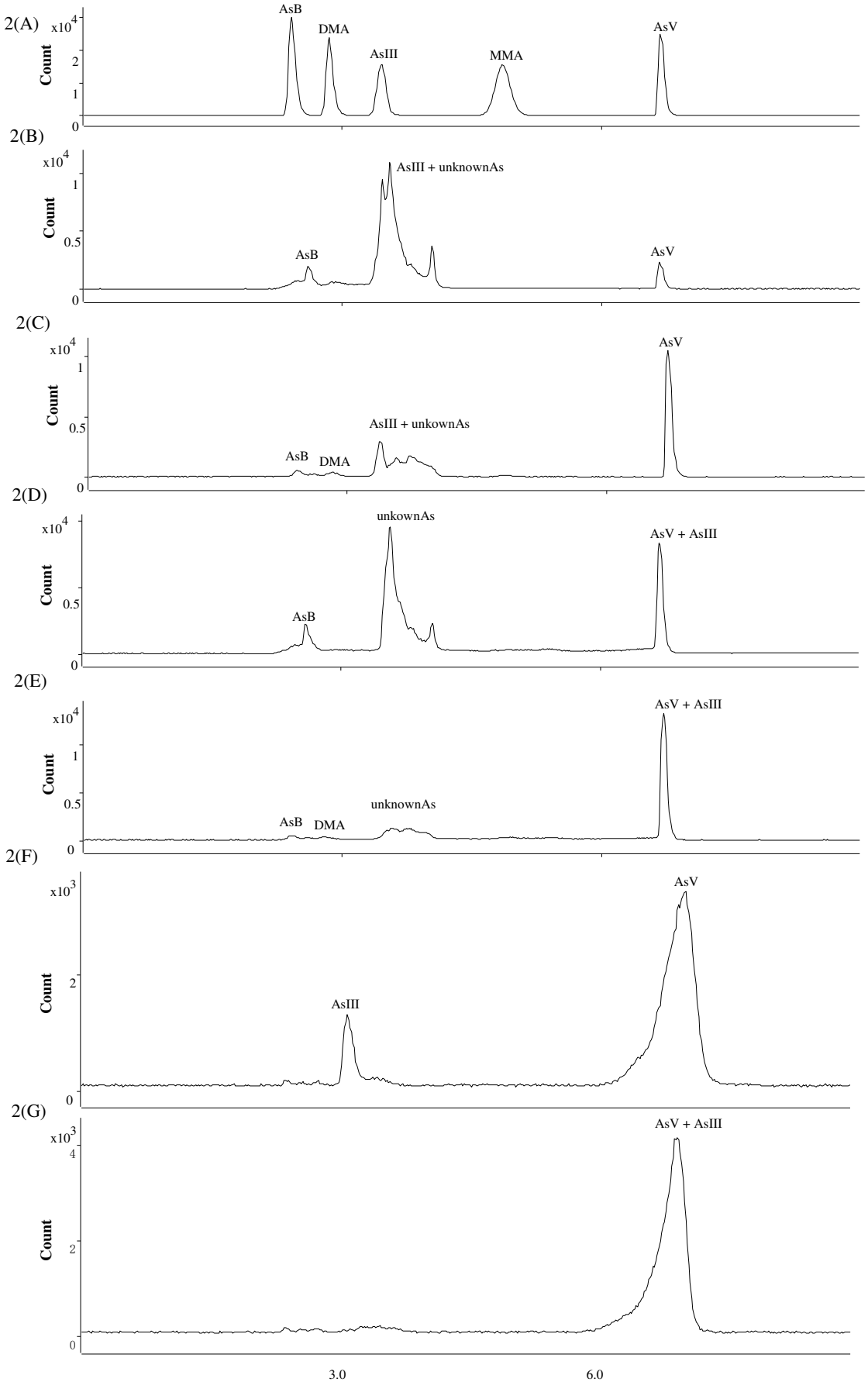
2(A)
AsB
DMA
AsIII
MMA
AsV
Count
2(B)
AsIII + unknownAs
AsB
AsV
2(C)
AsV
AsIII + unkownAs
AsB
DMA
2(D)
unkownAs
AsV + AsIII
AsB
2(E)
AsV + AsIII
unknownAs
AsB
DMA
2(F)
AsV
AsIII
2(G)
AsV + AsIII
3.0
6.0

Figure 2. Chromatograms obtained in quantification by HPLC-ICP-MS.

(A) The mix standards of AsB, DMA, As^{III}, MMA and As^{V}, at 10 ppb of each arsenic species. (B) and (C) This was extracts of SC and ST collected from site B, respectively. The As^{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 As^{III} was transformed into As^{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).

Figure 3(on next page)

The abundances of As species in soli, SC, and ST. (mg/kg).

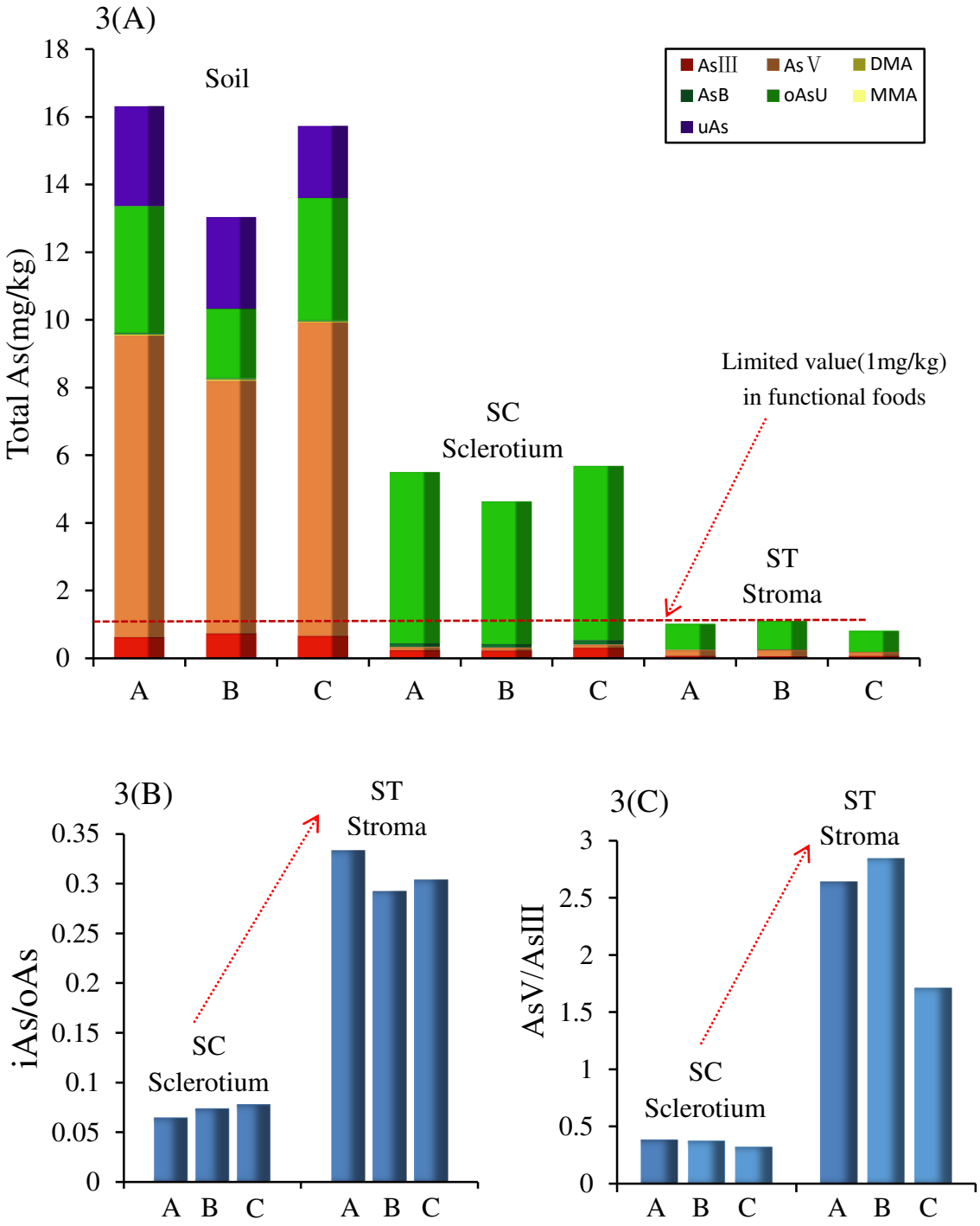

Figure 3. The abundances of As species in soli, SC, and ST (mg/kg).

(A) This was the concentration of As speciation. The most toxic iAs (AsIII and AsV) were shown in red and orange sections, the low toxic As (MMA and DMA) were in light and dark orange sections, the non-toxic As (AsB and oAsU) were in green sections and the unextractable arsenic were in purple sections (uAs). (B) This

was the variation of iAs/oAs ratio from SC to stroma ST. (C) This was the variation of AsV/AsIII ratio from sclerotium SC to stroma ST.

Table 1(on next page)

Concentrations[a] of arsenic speciation (mg/kg).

Table 1:

Concentrations[a] of arsenic speciation (mg/kg).

Sample	Dry Weight (g)	AsB[b]	DMA	MMA	oAsU	AsⅢ	AsⅤ	iAs	tAs
SC_A	3.2	0.11±0.01	nd[c]	nd	5.06±0.6	0.24±0.02	0.09±0.01	0.33±0.02	5.50±0.58
SC_B	3.0	0.10±0.01	nd	nd	4.22±0.55	0.23±0.02	0.09 ±0.00	0.32±0.02	4.64±0.54
SC_C	2.5	0.13±0.02	nd	nd	5.14±0.42	0.31±0.01	0.1±0.01	0.41±0.01	5.68±0.44
ST_A	1.1	nd	nd	nd	0.77	0.07	0.19	0.26	1.03
ST_B	1.1	0.01	0.01	nd	0.85	0.07	0.19	0.26	1.13
ST_C	1.0	nd	nd	nd	0.63	0.07	0.12	0.19	0.82
A	0.3	0.04±0.01	0.03±0.02	nd	3.75±0.25	0.62±0.15	8.91±0.35	9.54±0.21	16.31±1.82
B	0.2	0.03±0.01	0.04±0.01	nd	2.06±0.26	0.74 ±0.07	7.46±0.25	8.20±0.18	13.03±1.00
C	0.2	0.03±0.01	0.04±0.03	nd	2.88±0.29	0.66±0.03	9.26±0.39	9.92 ±0.37	15.72±1.57

[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, AsⅢ, AsⅤ, iAs, and tAs were the abbreviation of arsenobetaine, dimethylarsenic acid, monomethylarsonic acid, unknown organic arsenic, arsenite, arsenate, inorganic arsenic (total) and total arsenic, respectively; [C] not detected.

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Table 2(on next page)

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

Table 2:

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

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

Table 3(on next page)

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

Table 3:

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

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

Table 4(on next page)

Index of carcinogenic risk and non-carcinogenic risk.

Table 4:

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