https://doi.org/10.17221/10/2021-PSE

Arsenic accumulation, speciation and bioavailability in rice cultivated in arsanilic acid exposed soil

Wanling He, Xiaoli Li, Shu Guo, Longbang Yang, Dong Li*

College of Animal Science and Technology, Henan University of Science and Technology, Luoyang, Henan, P.R. China

**Corresponding author: hwling921@126.com*

Citation: He W.L., Li X.L., Guo S., Yang L.B., Li D. (2021): Arsenic accumulation, speciation and bioavailability in rice cultivated in arsanilic acid exposed soil. Plant Soil Environ., 67: 307–316.

Abstract: The present study used various amounts of P-arsanilic acid (AsA) in pot experiments to evaluate the effects of AsA on arsenic (As) accumulation, speciation and meanwhile using the *in vitro* digestion/Caco-2 cell model to evaluate the bioavailability of As in rice. The results indicated a linear relationship between As in rice and As in soil, and at 75 mg AsA/kg of soil, As content in rice exceeded the statutory permissible limit of 0.2 mg As/kg dry weight in China. Speciation studies indicated that inorganic As (Asi), dimethylarsinic acid (DMA), and monomethylarsonic acid (MMA) were the main As species in rice. Bioavailability of As experiment indicated that As uptake and transport amount by Caco-2 cells increased with increasing As accumulation in rice. In general, the content of AsA in soil reached or exceeded 75 mg/kg, which is not suitable for growing rice.

Keywords: arsenic acid; food chain; cytotoxicity; contamination; food safety

Arsenic (As) is a naturally-occurring metalloid element, which can be detected in environmental media due to a cultivar of natural and human processes. Long-term intake of high concentrations of inorganic As may cause skin, bladder, liver, kidney, and prostate and lung cancers (Huang et al. 2014, Cohen et al. 2016) and a number of non-carcinogenic diseases, such as diabetes, cardiovascular, reproductive, and neurological diseases (Maull et al. 2012, Naujokas et al. 2013). The cytotoxicity of As depends on its oxidation state and chemical structure (speciation). Although inorganic As and its metabolites are generally considered to be more important from a human health point of view, other organic As species have become the focus of current research.

P-arsanilic acid (AsA) has been widely used as an animal feed additive for promoting growth and preventing disease for broiler chickens and pigs (Zhang et al. 2014, Mangalgiri et al. 2015). The content of AsA in pig and chicken antibiotics is approximately 45 and 30 mg/kg, respectively (Straw et al. 2002). Yao et al. (2013) pointed out in a report that 25.4% of 146 animal feeds contained organic arsenic, with an average content of 21.2 mg/kg, in the form of AsA. About 1 million kg of AsA is consumed annually in the United States, and more was consumed in China and other developing countries (Wang et al. 2014, Fisher et al. 2015). Although both the West (USA, EU) and China no longer add arsenic to animal feed, there is, however, a legacy of As in soils from poultry manure containing As from past years. The previous study has shown that the arsenic content in pig manure and chicken litter is 89.3 and 21.6 mg/kg, respectively (Yao et al. 2006). Liu et al. (2015) established that AsA and $As^{(V)}$/$As^{(III)}$ were the main As species in the environmental matrixes (surface soils, sediments and surface water) of high-density pig farms in the Pearl River Delta (southern China). After the swine fever outbreak in Zhejiang Province in China in 2013, the impact of AsA residues in pig farm waste and agricultural soil added with swine manure have attracted considerable interest (Wang et al. 2014). In addition, when animal manure enters the environment and when untreated waste is stored in agricultural sites or used as organic fertiliser, AsA can

Supported by the National Natural Science Foundation of China, Grant No. U1504324.

https://doi.org/10.17221/10/2021-PSE

be broken down into more toxic metabolites (Wang et al. 2014). In the transformation process, inorganic arsenic enters the soil through the environment, and arsenic can be absorbed by vegetables and enter the food web and eventually transferred to the human body (Yao et al. 2010, Huang et al. 2014). Therefore, such a situation may lead to a huge risk of arsenic pollution, leading plants in the polluted areas to absorb and accumulate this element and endanger human health through food chains. However, the risk posed by As species in rice depends on the net content and the bioavailability of arsenic. Recently, a Caco-2 cell model has been employed to estimate the bioavailability of minerals in cereals as this model mimics the digestion of humans (He et al. 2008). Caco-2 cell monolayers constitute a well-established intestinal epithelial model (Ekmekcioglu 2002). The incorporation of Caco-2 cells grown on solid or microporous supports in *in vitro* digestion models, allowing mineral uptake and/or transport to be estimated, improves the systems used for bioavailability studies (Ekmekcioglu 2002).

Among various cereal crops, rice is one of the major routes of arsenic exposure to rice-dependent populations (Li et al. 2011) because rice is more efficient than other cereal crops in accumulating arsenic in shoots and grains (Williams et al. 2007). Even if grown in soils containing low arsenic levels, rice may contain higher concentrations of arsenic (Lu et al. 2009, Meharg et al. 2009). Recently study have confirmed that rice not only absorbs inorganic arsenic but also absorbs AsA, which is partially transformed into other arsenic species, including $As^{(III)}$, $As^{(V)}$, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Geng et al. 2017). However, although AsA contaminated poultry or swine litter is widely used as farm manure, its effect has received little attention. Thus, it is essential to investigate the transportation, transformation and toxicity of AsA in rice plants.

In this study, a pot experiment as well as a Caco-2 cell model was carried out to evaluate the effects of AsA contaminated soil on As accumulation, speciation and bioavailability in rice, and the ultimate goal of this study was to assess the risks of utilising AsA as the feed supplement and AsA-polluted manure as the fertiliser.

MATERIAL AND METHODS

Rice experiment protocol

Surface soil was acquired from a paddy field of the experimental farm at the Henan University of Science and Technology (34°6'30"N, 112°0'10"E) with organic carbon of 1.25%, cation exchange capacity (CEC) 167.3 $mmol_+/kg$, pH 7.52, available potassium, nitrogen and phosphorus 105.62, 89.34, 63.04 mg/kg, respectively, with the concentration of total As 14.21 mg/kg, which was lower than the upper limit of soil background value 15 mg/kg in China (Weng et al. 2000). The soil samples were collected, air dried, smashed with shovels and hammers, and then passed through 2 mm sieves and mixing wells. Pots (24.5 × 21 × 29 cm) were packed with 8 kg of soil, and arsanilic acid was dissolved in distilled water and sprayed on the soil in concentrations of 30, 75, 150, 225, and 300 mg AsA/kg of soil, respectively. No arsenic was added in the control group. There were six replicates per AsA treatment levels. Three months later, the arsenic content in the soil did not change much.

The seeds were treated using 1.5% (*v/v*) NaOCl, washed and imbibed in a thin layer of deionised water overnight. After germination, the seedlings were grown in a 14/10 h light/dark cycle at 30/25 °C (day/night) and 70–80% relative humidity using 300 $\mu mol/m^2/s$ light. Four healthy seedlings of 20 days old were then transplanted into individual pots. The water level was maintained at 2–3 cm above the soil surface. All experimental pots received P_2O_5 as superphosphate (0.42 g/pot) and K_2O as KCl (0.85 g/pot) prior to seedling transplantation. Each experimental pot was added with urea-N (0.56 g/pot; two-third as fertiliser and one-third as topdressing during tillering). Rice was harvested about 90 days following transplantation. Plants were harvested from each experimental pot, artificially threshed, rinsed with 0.01 mol/L HCl, and then with deionised water. After drying, the samples were shelled by an electric sheller (JLGJ-4.5, Taizhou Instrument Co. Ltd., Taizhou, China) and ball milled (Retsch MM301, Haan, Germany) and stored at –20 °C until analysis.

Reagents and solutions

Ultrapure water (18.2 MΩ·cm) was acquired by means of a Thermo Scientific Nanopure Water Purifier (Thermo, Waltham, USA). HPLC grade Methanol (J. T. Baker, Philipsburg, USA) was utilised. Pure (≥ 99.99%) of argon and nitrogen were purchased from Luoyang Feilier Specialty Gases Co., LTD (Luoyang, China). AsA (98% purity) was sourced from Wuhan XRD Chemical Co., LTD (Wuhan, China). The Asi, MMA and DMA standard stock solutions expressed by As concentration was issued by the Chinese National

https://doi.org/10.17221/10/2021-PSE

Material Center and stored in the dark at 4 °C. The working standards were obtained by diluting stepwise every day with ultrapure water.

Sample digestion and total As analysis

Total arsenic analysis was carried out based on GB/T 5009.11-2003, China. Both the polished and unpolished powdered rice samples homogenised *via* grinding were added (5.0 g) into a 50 mL digestion tube and allowed to sit in a fume cupboard overnight with 10 mL HNO_3 (80%) at room temperature for cool digestion. Further, it was heated on a heating block in steps from 100 °C to 180 °C until the solution becomes clear with 1–2 mL left. Subsequently, the solution was cooled and filled into 50 mL colorimetric tubes. The tubes were also treated with 2.5 mL hydrochloric acid (50%) solution and 2.5 mL thiourea (5%) and ascorbic acid (5%) mixed solution. The sample was diluted with deionised water to 50 mL, and total As was measured by an atomic fluorescence spectrometer (AFS9130, Jitian Instrument Co. Ltd., Beijing, China) after 30 min. Three blank samples and two standard references, GSB-5 (cabbage) and GSB-6 (spinach), were used. In AFS analysis, six water arsenic standard solutions in the range of 5–50 µg/L, and the correlation coefficient was greater than 0.9999, the coefficient of variation was 0.57~1.66%, the recoveries (%) ranged from 95.28% to 99.30%.

All glass containers for the determination of total arsenic were drenched in 20% HNO_3 (HNO_3:water = 4:1) for 24 h, rinsed with deionised water and dried. HPLC-ICP-MS was used to determine As species in polished and unpolished rice.

The method reported by Geng et al. (2017) has been used to extract different As species. Both the polished and unpolished rice (0.5 g) were added into digestion tubes, and 50% (*v:v*) aq. methanol (10 mL) was introduced. The mixture was assisted with an ultrasonic device for 30 min and centrifuged at 3 170 × *g* for 30 min. Following this, the supernatant was filtered by a 13 mm syringe filter (Membrana, Wuppertal, Germany) and stored at –20 °C.

The As species were determined by comparing the retention times with the standards. Quantitative analysis of Asi, MMA, DMA and AsA was based on the external curves obtained by corresponding standards.

Parameters of ICP-MS and HPLC

For the ICP-MS: RF incident power of 1.5 kW, the reflected power < 5 W, a concentric atomiser for high purity argon carrier gas at a flow rate of 1.12 L/min, the auxiliary gas flow rate of 1.0 L/min, plasma gas flow rate of 15 L/min solution capacity of 0.3 mL/min, in sample depth is 9.5 mm, the quality of the number of detected m/z = 75 (As), m/z = 35 (Cl), the residence time was 0.25 s (m/z = 75) and 0.01 s (m/z = 35).

For the HPLC: anion exchange column G1836-65002 (polymethacrylate alkanol, guaternary ammonium), mobile phase containing 0.2 mmol/L EDTA and 2 mmol/L phosphate buffer methanol system (v/v = 95/5), the flow rate was 0.8 mL/min, column temperature 25 °C, in sample volume 50 µL. Cation exchange column 1 shodex rspak NN614 (sulfonic acid), column 2G1836-65002, the mobile phase containing 4 mmol/L NH_4NO_3, 1.5 mmol/L 2,6-two carboxyterminal acid of 2 mmol/L HNO_3 aqueous solution of methanol (v/v = 95/5), the flow rate was 0.8 mL/min, column temperature was 50 °C, in a sample volume of 50 µL.

Determination of As bioavailability

Preparation of samples. Aliquots of 50 g of polished and/or unpolished rice were cooked for 15 min in water and then homogenised at maximum speed for 10 s with Braun 4142 blender. The homogenates were lyophilised before conducting the bioavailability of As experiment.

***In vitro* digestion of samples.** The preparation of digestive juice and the *in vitro* digestion process was slightly modified according to a reported method (He et al. 2008, Wei et al. 2012). In short, a part of cooked samples (5 g) was added to a 15 mL mixture, which contained 140 mmol/L NaCl and 5 mmol/L KCl. In gastric digestion, the pH was brought to 2 using HCl, 0.5 mL of pepsin was added and incubated for 2 h at 37 °C. In intestinal digestion, the pH was brought to 5 using 1 mol/L $NaHCO_3$, and 2.5 mL of the pancreaticobiliary mixture (37.5 mL of 0.1 mol/L $NaHCO_3$ containing 0.075 g of trypsinase and 0.45 g of bile extract) was added and incubated at 37 °C for 2 h. Then, the sample was cooled for 10 min to stop intestinal digestion and pH was brought to 7.4 using 0.5 mol/L NaOH. In order to inhibit the activity of protease, the intestinal digestive juice was heated for 4 min at 100 °C, then cooled and centrifuged for 1 h at 3 500 *g* at 4 °C. Supernatants were used for the bioavailability of As experiment. Total As the content of *in vitro* digestion solution was determined

https://doi.org/10.17221/10/2021-PSE

through Agilent 7500a ICP-MS (Agilent Technologies, Santa Clara, USA).

Preparations of Caco-2 monolayers. The cells were purchased from the Institute of Biochemistry and Cell Biology (China) and were all between 20 and 43 generations. They were cultured with 5 mL of Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, 4 mL/L antibiotics, 25 mg/L amphotericin B, and 100 µmol/L non-essential amino acids (GIBCO, Grand Island, USA). Experiments were conducted with cells reseeded on polyester membrane filter cell culture inserts (0.4 µm pores, 4.7 cm^2 growth area, corning, New York, USA) inside six-well transwell cell culture chambers at a cell density of approximately 2.5×10^5 per insert. The upper and bottom chambers were fulfilled with a 1.5 mL and 2.5 mL culture medium, respectively. The medium was replaced every two days in the first two weeks and once a day in the last seven days, then the As bioavailability experiment was studied on day 21. Cells were incubated with 5% CO_2 and 95% air at 37 °C. Caco-2 cells differentiated on insert membrane after 21 days post-confluence and developed a tight junction monolayer, which was evaluated by transepithelial electrical resistance (TEER) measurement with a Millipore Millicell-ERS instrument according to a technique described by MacCallum et al. (2005). Transwell without Caco-2 cells was treated as TEER blank. The monolayer displayed adequate TEER values of 560–590 Ωcm^2.

As uptake (retention and transport) by Caco-2 cells. Retention and transport experiments were studied with cells grown on filters 21 days after seeding. In brief, prior to the experiment, the cells in each well were washed two times by HBSS, the upper chamber was added with 1.5 mL digestive juice *in vitro*, and the lower chamber was added with 2.5 mL HBSS (Wei et al. 2012). The cells were incubated for 2 h at 37 °C in 5% CO_2 with 95% relative humidity and then harvested for analysis.

At the end of As bioavailability experiment, the digested solution covering the cells was removed, and incubation solution (in the bottom chamber) was harvested, and total arsenic was analysed in order to evaluate transepithelial transport. Meanwhile, cell surfaces of the monolayers were also harvested, and the total arsenic was analysed in order to evaluate arsenic retention. Arsenic retention and transport percentages were calculated with respect to the initial quantity of As added to the Caco-2 cell cultures. Total As content was measured by ICP-MS.

Statistical analysis

Statistical difference analysis was performed using One-way ANOVA (SPSS 18.0, Chicago, USA). Means were considered to be a significant difference if P values were < 0.05.

RESULTS

Total As concentrations in unpolished and polished rice

The As concentrations in unpolished and polished rice are shown in Figure 1A. Results indicated that increasing the quantity of AsA significantly augmented total As concentrations both in unpolished ($P < 0.05$) and polished rice ($P < 0.05$). When added in excess of 75 mg/kg of AsA, total As levels in unpolished rice exceeded 1.0 mg As/kg dry weight (DW) in rice grain. The total As concentration in unpolished rice of all treatment groups were 0.19 ± 0.01, 0.46 ± 0.04, 1.05 ± 0.08, 1.19 ± 0.07, 1.44 ± 0.07, and 1.89 ± 0.06 mg As/kg DW, respectively. In contrast, the total As content in polished rice of all treatment groups were 0.06 ± 0.00, 0.12 ± 0.00, 0.21 ± 0.00, 0.27 ± 0.01, 0.32 ± 0.02, and 0.42 ± 0.03 mg As/kg DW, respectively.

Arsenic speciation in unpolished and polished rice

Arsenic speciation in unpolished and polished rice is shown in Figure 1. It can be seen that all treatment groups contained inorganic As, MMA, DMA and other As species except for MMA in the control group for unpolished rice and MMA in the control group and 30 mg-AsA treatment for polished rice.

As seen in Figure 1B, for unpolished rice, a significant ($P < 0.05$) variation was observed in inorganic As concentration between the control and other treatment groups. Inorganic As concentration in the 75 mg-AsA treatment was highest and reached 0.72 mg As/kg DW, which was significantly higher ($P < 0.05$) than that grown in 30, 150 and 225 mg-AsA treatments, while no significant variations ($P > 0.05$) were identified among groups added with 150, 225 and 300 mg-AsA. For polished rice, inorganic As concentration in the 300 mg-AsA treatment was highest and reached 0.116 mg As/kg DW, which was greater ($P < 0.05$) than that of the control group and 30 mg-AsA treatment, meanwhile, no significant differences ($P > 0.05$) were identified among other treatments.

https://doi.org/10.17221/10/2021-PSE

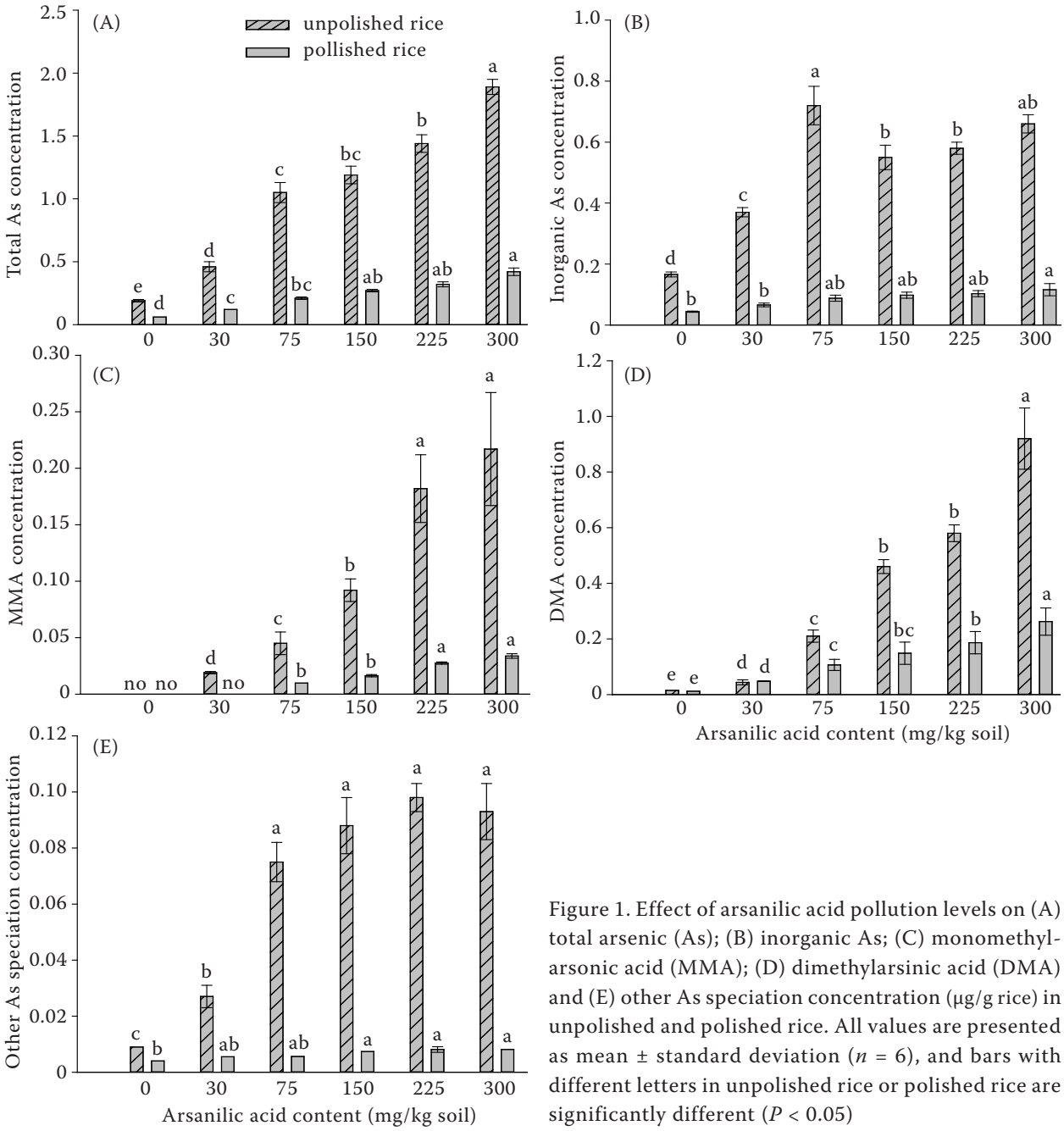

Figure 1. Effect of arsanilic acid pollution levels on (A) total arsenic (As); (B) inorganic As; (C) monomethylarsonic acid (MMA); (D) dimethylarsinic acid (DMA) and (E) other As speciation concentration (μg/g rice) in unpolished and polished rice. All values are presented as mean ± standard deviation (n = 6), and bars with different letters in unpolished rice or polished rice are significantly different (P < 0.05)

As seen in Figure 1C, MMA was not detected in unpolished rice in the control group, whereas the highest MMA concentration in unpolished rice was acquired in 300 mg-AsA treatment, which was significantly higher (P < 0.05) than that in 30, 75 and 150 mg-AsA treatments. However, no significant differences (P > 0.05) were identified between 225 mg and 300 mg-AsA treatments. For polished rice, no MMA was observed both in control and 30 mg-AsA treatment, MMA concentrations in 225 mg and 300 mg-AsA treatments were considerably more (P < 0.05) than that in 75 mg and 150 mg-AsA treatment. Nevertheless, no significant variation (P > 0.05) was seen either between 225 mg and 300 mg, or between 75 mg and 150 mg-AsA treatments.

As seen in Figure 1D, DMA concentrations both in unpolished and polished rice displayed wide variations among the six treatments. Increasing the amount of AsA considerably increased the DMA content both in unpolished and polished rice. DMA concentrations both in unpolished and polished rice reached highest in 300 mg-AsA treatment, which was signifi-

https://doi.org/10.17221/10/2021-PSE

cantly greater ($P < 0.05$) than that in 30, 75, 150 and 225 mg-AsA treatments. For DMA concentrations in polished rice, no noteworthy difference ($P > 0.05$) was identified between the level of 75 and 150, 150 and 225 mg-AsA treatments, respectively. For DMA concentrations in unpolished rice, no noteworthy variation ($P > 0.05$) was detected between 150 and 225 mg-AsA treatments.

Other arsenic species were also detected both in unpolished and polished rice (Figure 1E). Other As speciation concentrations in unpolished rice in 75, 150, 225, and 300 mg-AsA treatments were considerably higher ($P < 0.05$) than that in control and the 30 mg-AsA treatment, however, no significant differences ($P > 0.05$) were detected among 75, 150, 225 and 300 mg-AsA treatments. Meanwhile, for polished rice, no significant differences ($P > 0.05$) were identified among all the treatment groups.

The proportion of various As speciation in unpolished and polished rice

The proportion of various As speciation in unpolished rice is shown in Figure 2A. It can be seen that the dominant As species were inorganic-As, DMA, MMA and other As species, respectively. The proportion of inorganic As decreased with the increase of the added level of AsA. In contrast, the proportion of DMA and MMA increased with the increase of AsA. In general, when the level of AsA added in soil increased from 30 to 300 mg/kg, the proportion of inorganic As decreased from 80.43% to 34.92%, whereas the proportion of DMA increased from 9.5% to 48.68%, and the proportion of MMA increased from 4.13% to 11.48%. Meanwhile, the proportion of other As speciation varied with the AsA addition level and was relatively lower when compared with inorganic As and DMA, which accounted for between 4.92% to 7.39% from different addition levels of AsA. No MMA was detected in unpolished rice in the control group.

The proportion of various As speciation in polished rice is shown in Figure 2B. For polished rice, the proportion of inorganic As also decreased with the increase of AsA, whereas the proportion of DMA and MMA increased with the increase of AsA. In general, when the AsA level added in soil increased from 30 mg/kg to 300 mg/kg, the proportion of inorganic As decreased from 55% to 27.62%, whereas the proportion of DMA increased from 40% to 62.43%, and the proportion of MMA increased from 4.67% to 8.59%. The proportion of other As speciation was small and also decreased, and no MMA was detected in control and 30 mg-AsA treatments.

As bioavailability in Caco-2 cells

Total arsenic in bioaccessible fraction added to Caco-2 cells and percentages of total uptake (retention + transport) are shown in Table 1. For unpolished rice, the total uptake arsenic content varies from 17.31 to 151.81 ng; for polished rice, the total uptake arsenic content varies from 10.34 to 33.07 ng. For total uptake as percentages calculated with respect to the amount added, it is seen that total cellular uptake varies from 11.76% to 17.75% for

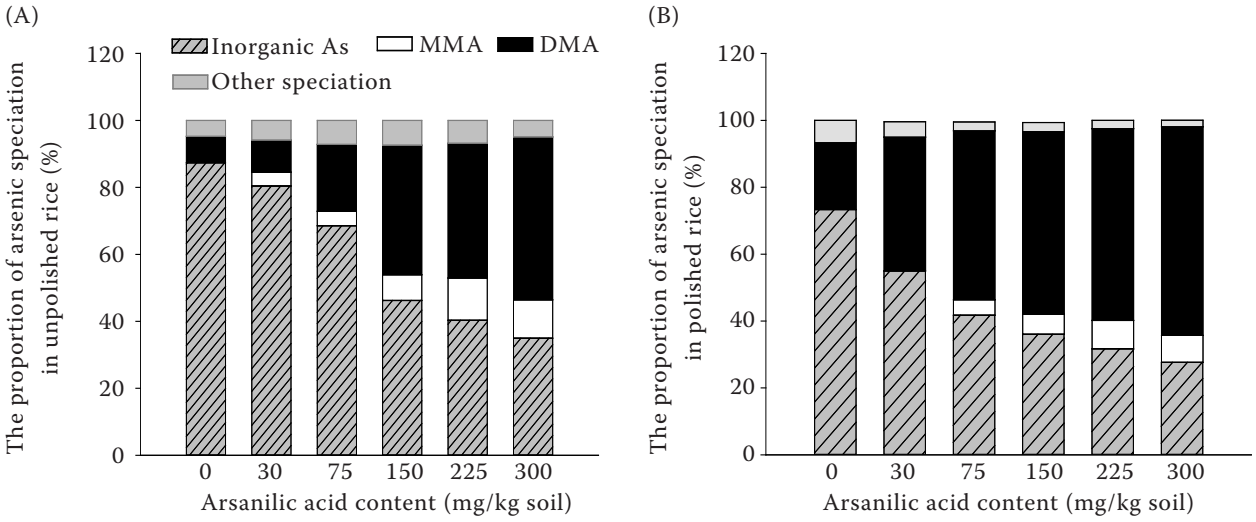

Figure 2. Effect of arsanilic acid pollution levels on the proportion of various arsenic (As) speciation in unpolished rice (A) and polished rice (B). All values are presented as mean ± standard deviation ($n = 6$)

https://doi.org/10.17221/10/2021-PSE

Table 1. Arsenic (As) bioavailability from rice grain treated with different arsanilic acid levels using the *in vitro* digestion/Caco-2 cell model[a]

Arsanic acid levels (mg/kg)	Total As in digestive juice (ng)[b]		Uptake As (ng)[c]		Uptake rate (%)[d]	
	unpolished rice	polished rice	unpolished rice	polished rice	unpolished rice	polished rice
0	97.50 ± 2.62[f]	45.20 ± 1.33[e]	17.31 ± 0.71[d]	10.34 ± 0.31[d]	17.75	22.88
30	277.15 ± 5.85[e]	84.31 ± 3.39[d]	42.79 ± 2.21[c]	18.88 ± 0.67[c]	15.44	22.39
75	565.50 ± 5.11[d]	107.30 ± 2.32[c]	86.27 ± 2.71[b]	20.53 ± 0.89[c]	15.26	19.13
150	755.58 ± 7.27[c]	142.39 ± 3.58[b]	97.93 ± 3.51[b]	25.22 ± 0.38[b]	12.96	17.71
225	992.67 ± 12.08[b]	178.75 ± 3.41[a]	126.39 ± 7.36[a]	32.55 ± 0.91[a]	12.73	18.21
300	1290.60 ± 7.71[a]	190.12 ± 4.42[a]	151.81 ± 5.83[a]	33.07 ± 1.29[a]	11.76	17.39

[a]All values are presented as mean ± standard deviation (n = 6), and bars with different letters in unpolished rice or polished rice are significantly different ($P < 0.05$); [b]Total As content in the aliquot (1.5 mL) of bioaccessible fraction added to cell cultures; [c]Uptake As evaluated as (retention As in cell monolayer + transport As in basal medium); [d]Uptake rate evaluated as [(retention + transport)/total arsenic content added to cell culture] × 100

unpolished rice, whereas from 17.39% to 22.88% for polished rice.

DISCUSSION

Arsenic accumulation in unpolished and polished rice

Rice cultivated in arsenic-contaminated soils accumulates high amounts of As (Abedin et al. 2002a, b). Thus, arsenic uptake by the plant is critical in transferring this toxic element to the food chain, thus posing a potential threat to human health (Meharg and Rahman 2003). Rahman et al. (2008) reported that when grown on soil treated with 40 mg As/kg soil, the highest As concentrations in rice grains of plants were observed and the As value was 0.5 ± 0.02 mg/kg. He et al. (2012) established that total As contents of 31 rice samples (60% of which were planted in the US) were between 0.09 ± 0.004 and 0.85 ± 0.03 mg/kg, with an average of 0.27 ± 0.161 mg/kg. Juskelis et al. (2013) reported that the average concentrations of total As and inorganic-As (Asi) in infant rice cereal were 0.174 and 0.101 mg/kg, respectively. Das et al. (2004) found that the average concentration of arsenic in rice was 0.136 ± 0.08 mg/kg. Abedin et al. (2002a) indicated that the total As concentration value in rice grain ranged from 0.15 to 0.24 mg/kg when irrigated by As-contaminated groundwater. The arsenic species of 260 rice samples from Guangdong province were investigated by Lin et al. (2015); the results showed that the total concentration of As species ranged from non-detect to 0.226 mg/kg, with an average of 0.057 mg/kg. This current study indicated that the total As and Asi contents of unpolished rice grains were 1.05 ± 0.08 and 0.72 ± 0.063 mg/kg after applying 75 mg of arsanilic acid per kg soil, which were higher than observed in the studies mentioned above. The total As content in unpolished rice in the current study surpassed the legal limit of 1.0 mg/kg in Australia (National Food Authority 1993) and was higher than the 0.15 mg/kg limit in China (GB2762-2005). It is speculated that the high concentration of total As and Asi obtained in this experiment might be related to the AsA level added to the soil. Therefore, the present study indicated that soil composed of 75 mg/kg AsA or more was seriously polluted and was not suitable for rice cultivation.

At the 37th session of the Commission, the Codex Committee adopted a maximum content of inorganic arsenic in polished rice of 0.2 mg/kg (Codex Alimentarius Commission 2014). In July 2014, the World Health Organisation (WHO) also recommended that the content of inorganic As in white rice should not exceed 0.2 mg/kg and that in brown rice should not exceed 0.4 mg/kg (Sohn 2014). In the current study, the concentration of Asi in polished rice ranged from 0.044 to 0.116 mg/kg, which did not surpass the 0.2 mg/kg limit.

Arsenic speciation in unpolished and polished rice

The forms of As accumulated in rice are very important for assessing As levels and exposure risk assess-

https://doi.org/10.17221/10/2021-PSE

ment because the toxicity of arsenic in any foodstuff is closely related to its chemical form. Several studies have revealed inorganic As species as the principal As compounds in grains (Signes-Pastor et al. 2008, Cubadda et al. 2010). The research groups of Meharg et al. (2009) and Zavala et al. (2008) reported $As^{(III)}$, $As^{(V)}$, MMA and DMA as main As forms. Qu et al. (2015) identified $As^{(III)}$, DMA and $As^{(V)}$ as the principal species in rice. In the current study, the dominant As species both in unpolished and polished rice were Asi, MMA and DMA, which is consistent with the results of previous studies. It was shown that most of the AsA absorbed by rice from soil was degraded into inorganic arsenic or converted to methylated species. Previous studies have shown that organic As species and their degradation compounds could be accumulated in water, spinach and turnips (Yao et al. 2009, 2010). Geng et al. (2017) speculated that AsA was initially converted into inorganic-As and organic As species in the soil, which were subsequently absorbed from the soil by rice roots, partially retained and transferred to other plant parts. As a result, the present study confirmed this possibility by detecting inorganic-As, MMA and DMA in rice grains. However, AsA was not transported to grains but transformed into other As species eventually.

A survey of As speciation in rice grains by Williams et al. (2005) showed that inorganic As accounted for 64% to 81% of As in rice grains from Europe, Bangladesh, and India, 42% in American and most of the remaining As speciation was DMA. Smith et al. (2008) found that most As in rice grain was in the form of DMA, accounting for between 85% and 94% of overall As a recovery, and $As^{(III)}$ comprised the rest of the As species; whereas $As^{(V)}$ was not detected. Rahman et al. (2014) established that inorganic-As was chiefly found in Asian rice (86–99%), while DMA was the main source of overall As in Australian-grown rice (18–26%). In the current study, the concentration of the dominant As species in rice grain varied widely with the AsA level added to the soil. For unpolished rice, the concentrations of the major As species were in the order Asi (with the proportion ranged from 40.28% to 80.43%) > DMA (with the proportion from 8.69% to 40.28%) > MMA (with the proportion from 4.35% to 12.5%) when the AsA level in soil was less than 225 mg/kg when the AsA level exceeded 225 mg/kg, the concentration order of major As species were DMA (with the proportion of 48.68%) > Asi (with the proportion of 34.92%) > MMA (with the proportion of 11.64%). For polished rice, the concentration order of major As species were also DMA (with the proportion ranged from 52.38~61.90%) > Asi (with the proportion from 28.57% to 42.86%) > MMA (with the proportion from 4.76% to 9.38%) when the AsA level in soil exceeded 30 mg/kg. Lin et al. (2015) reported that the contents of the As species in 260 rice samples from Guangdong province were $As^{(III)}$ > $As^{(V)}$ > DMA > MMA. The discrepancy results in our study may be related to both the additive AsA and the added level because most articles reported used inorganic-As as an additive, and the added level in our study also varied widely. It has been shown that arsenic methylate (especially DMA) migrates more easily to aerial parts of rice than inorganic-As (Raab et al. 2007). Zhao et al. (2009) also reported that although overall plant accumulation and in plant production of DMA is low, the export of DMA to grain is highly efficient. This may be the reason why higher concentrations of DMA were obtained in rice grains from higher addition levels of AsA when compared with the concentrations of Asi in our study.

Bioavailability of As in rice samples

The toxic effect of As on human health depends on the level of dietary intake. However, for a better understanding of the implications of rice consumption for the assessment of arsenic-related health risks, the effect of cooking on inorganic arsenic contents and its bioavailability (i.e., the fraction of absorbed arsenic that reaches the systemic circulation) is an aspect to be taken into account. Juhasz et al. (2006), by means of an *in vivo* model, established that the bioavailability of As in rice was widely dependent on its chemical form; inorganic As has high bioavailability (89.4 ± 9%), whereas the bioavailability of DMA was low (33.1 ± 3.2%). In the current study, the Caco-2 cell model was employed to evaluate the bioavailability of As in rice grains, which has been proved to be a valuable and accurate tool to assess trace element bioavailability in cereal food (Lung'aho et al. 2011, Wei et al. 2012). The present study demonstrated that intracellular As concentration in Caco-2 cells increased steadily with increasing As accumulation both in polished and unpolished rice; meanwhile, As uptake rate by Caco-2 cells varied from 17.39% to 22.88% for polished rice, the results were consistent with the study of Laparra et al. (2005), who examined the bioavailability of As in rice cooked in As-contaminated water using simulated *in vitro*

https://doi.org/10.17221/10/2021-PSE

gastro-intestinal digestion and Caco-2 cells, and found that As uptake by Caco-2 cells varied from 3.9% to 17.8%. However, the present study only measured the bioavailability of total As in polished and unpolished rice grains. Further studies are needed to detect the bioavailability of different As species in rice grains.

In conclusion, the current study demonstrated that the average As content in rice was linearly related to As level in growing soils, and the contents of overall and inorganic As in unpolished rice exceeded the statutory limits in plants. Bioavailability of As study indicated that the uptake of As by Caco-2 cells is augmented with increasing As accumulation levels in rice. Growing rice on arsenic-contaminated soil could pose a potential health hazard to the baby population in the west as unpolished rice is viewed as more "healthy" and is widely used in baby food. Therefore, it is very necessary to consider adverse health effects on humans caused by eating arsenic-contaminated rice, especially unpolished rice. It is suggested that rice should not be planted in soils containing 75 mg AsA/kg or more since the As content in rice planted on them exceeds the statutory permissible limit of China.

REFERENCES

Abedin Md.J., Cresser M.S., Meharg A.A., Feldmann J., Cotter-Howells J. (2002a): Arsenic accumulation and metabolism in rice (*Oryza sativa* L.). Environmental Science and Technology, 36: 962–968.

Abedin Md.J., Cotter-Howells J., Meharg A.A. (2002b): Arsenic uptake and accumulation in rice (*Oryza sativa* L.) irrigated with contaminated water. Plant and Soil, 240: 311–319.

Cohen S.M., Chowdhury A., Arnold L.L. (2016): Inorganic arsenic: a non-genotoxic carcinogen. Journal of Environmental Sciences, 49: 28–37.

Cubadda F., Ciardullo S., D´Amato M., Raggi A., Aureli F., Carcea M. (2010): Arsenic contamination of the environment-food chain: a survey on wheat as a test plant to investigate phytoavailable arsenic in Italian agricultural soils and as a source of inorganic arsenic in the diet. Journal of Agricultural and Food Chemistry, 58: 10176–10183.

Das H.K., Mitra A.K., Sengupta P.K., Hossain A., Islam F., Rabbani G.H. (2004): Arsenic concentrations in rice, vegetables, and fish in Bangladesh: a preliminary study. Environment International, 30: 383–387.

Ekmekcioglu C. (2002): A physiological approach for preparing and conducting intestinal bioavailability studies using experimental systems. Food Chemistry, 76: 225–230.

Fisher D.J., Yonkos L.T., Staver K.W. (2015): Environmental concerns of roxarsone in broiler poultry feed and litter in Maryland, USA. Environmental Science and Technology, 49: 1999–2012.

Geng A.J., Wang X., Wu L.S., Wang F.H., Chen Y., Yang H., Zhang Z., Zhao X.L. (2017): Arsenic accumulation and speciation in rice grown in arsanilic acid-elevated paddy soil. Ecotoxicology and Environmental Safety, 137: 172–178.

He W.L., Feng Y., Li X.L., Yang X.E. (2008): Comparison of iron uptake from reduced iron powder and $FeSO_4$ using the Caco-2 cell model: effects of ascorbic acid, phytic acid, and pH. Journal of Agricultural and Food Chemistry, 56: 2637–2642.

He Y., Pedigo C.E., Lam B., Cheng Z.Q., Zheng Y. (2012): Bioaccessibility of arsenic in various types of rice in an *in vitro* gastrointestinal fluid system. Journal of Environmental Science and Health, Part B, 47: 74–80.

Huang L., Yao L., He Z., Zhou C., Li G., Yang B., Deng X. (2014): Roxarsone and its metabolites in chicken manure significantly enhance the uptake of As species by IARC (International Agency for Research on Cancer) 2012. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 100C: Arsenic, Metals, Fibres, and Dusts. Lyon, International Agency for Research on Cancer.

Juhasz A.L., Smith E., Weber J., Rees M., Rofe A., Kuchel T., Sansom L., Naidu R. (2006): *In vivo* assessment of arsenic bioavailability in rice and its significance for human health risk assessment. Environmental Health Perspectives, 114: 1826–1831.

Juskelis R., Li W.X., Nelson J., Jack C.C. (2013): Arsenic speciation in rice cereals for infants. Journal of Agricultural and Food Chemistry, 61: 10670–10676.

Laparra J.M., Vélez D., Barberá R., Farré R., Montoro R. (2005): Bioavailability of inorganic arsenic in cooked rice: practical aspects for human health risk assessments. Journal of Agricultural and Food Chemistry, 53: 8829–8833.

Li G., Sun G.X., Williams P.N., Nunes L., Zhu Y.G. (2011): Inorganic arsenic in Chinese food and its cancer risk. Environment International, 37: 1219–1225.

Lin K., Lu S.Y., Wang J., Yang Y.Y. (2015): The arsenic contamination of rice in Guangdong Province, the most economically dynamic provinces of China: arsenic speciation and its potential health risk. Environmental Geochemistry and Health, 37: 353–361.

Liu X.P., Zhang W.F., Hu Y., Hu E., Xie X., Wang L., Cheng H. (2015): Arsenic pollution of agricultural soils by concentrated animal feeding operations (CAFOs). Chemosphere, 119: 273–281.

Lu Y., Adomako E.E., Solaiman A.R.M., Islam M.R., Deacon C., Williams P.N., Rahman G.K.M.M., Meharg A.A. (2009): Baseline soil variation is a major factor in arsenic accumulation in Bengal Delta paddy rice. Environmental Science and Technology, 43: 1724–1729.

Lung'aho M.G., Mwaniki A.M., Szalma S.J., Hart J.J., Rutzke M.A., Kochian L.V., Glahn R.P., Hoekenga O.A. (2011): Genetic and physiological analysis of iron biofortification in maize kernels. PlosOne, 6: e20429.

MacCallum A., Hardy S.P., Everest P.H. (2005): *Campylobacter jejuni* inhibits the absorptive transport functions of Caco-2 cells and disrupts cellular tight junctions. Microbiology, 151: 2451–2458.

https://doi.org/10.17221/10/2021-PSE

Mangalgiri K.P., Adak A., Blaney L. (2015): Organoarsenicals in poultry litter: detection, fate, and toxicity. Environment International, 75: 68–80.

Maull E.A., Ahsan H., Edwards J., Longnecker M.P., Navas-Acien A., Pi J.B., Silbergeld E.K., Styblo M., Tseng C.H., Thayer K.A., Loomis D. (2012): Evaluation of the association between arsenic and diabetes: a national toxicology program workshop review. Environmental Health Perspectives, 120: 1658–1670.

Meharg A.A., Rahman Md.M. (2003): Arsenic contamination of Bangladesh paddy field soils: implications for rice contribution to arsenic consumption. Environmental Science and Technology, 37: 229–234.

Meharg A.A., Williams P.N., Adomako E., Lawgali Y.Y., Deacon C., Villada A., Cambell R.C.J., Sun G.X., Zhu Y.G., Feldmann J., Raab A., Zhao F.J., Islam R., Hossain S., Yanai J.T. (2009): Geographical variation in total and inorganic arsenic content of polished (white) rice. Environmental Science and Technology, 43: 1612–1617.

National Food Authority (1993): Australian Food Standard Code. Camberra, Australian Government Publication Service.

Naujokas M.F., Anderson B., Ahsan H., Aposhian H.V., Graziano J.H., Thompson C., Suk W.A. (2013): The broad scope of health effects from chronic arsenic exposure: update on a worlwide public health problem. Environmental Health Perspectives, 121: 295–302.

Qu H.O., Mudalige T.K., Linder S.W. (2015): Arsenic speciation in rice by capillary electrophoresis/inductively coupled plasma mass spectrometry: enzyme-assisted water-phase microwave digestion. Journal of Agricultural and Food Chemistry, 63: 3153–3160.

Raab A., Williams P.N., Meharg A., Feldmann J. (2007): Uptake and translocation of inorganic and methylated arsenic species by plants. Environmental Chemistry Letters, 4: 197–203.

Rahman M.A., Hasegawa H., Rahman M.M., Miah M.A.M., Tasmin A. (2008): Arsenic accumulation in rice (*Oryza sativa* L.): human exposure through food chain. Ecotoxicology and Environmental Safety, 69: 317–324.

Rahman M.A., Rahman M.M., Reichman S.M., Lim R.P., Naidu R. (2014): Arsenic speciation in Australian-grown and imported rice on sale in Australia: implications for human health risk. Journal of Agricultural and Food Chemistry, 62: 6016–6024.

Signes-Pastor A.J., Mitra K., Sarkhel S., Hobbes M., Burló F., de Groot W.T., Carbonell-Barrachina A.A. (2008): Arsenic speciation in food and estimation of the dietary intake of inorganic arsenic in a rural village of west Bengal, India. Journal of Agricultural and Food Chemistry, 56: 9469–9474.

Smith E., Juhasz A.L., Weber J., Naidu R. (2008): Arsenic uptake and speciation in rice plants grown under greenhouse conditions with arsenic contaminated irrigation water. The Science of the Total Environment, 392: 277–283.

Sohn E. (2014): Contamination: the toxic side of rice. Nature, 514: S62–63.

Straw B., Dewey C., Kober J., Henry S.C. (2002): Factors associated with death due to hemorrhagic bowel syndrome in two large commercial swine farms. Journal of Swine Health and Production, 10: 75–79.

Wang H.L., Hu Z.H., Tong Z.L., Xu Q., Wang W., Yuan S.J. (2014): Effect of arsanilic acid on anaerobic methanogenic process: kinetics, inhibition and biotransformation analysis. Biochemical Engineering Journal, 91: 179–185.

Wei Y.Y., Shohag M.J.I., Yang X., Zhang Y.B. (2012): Effects of foliar iron application on iron concentration in polished rice grain and its bioavailability. Journal of Agricultural and Food Chemistry, 60: 11433–11439.

Weng H.X., Zhang H.Y., Zou L.J., Zhang X.M., Liu G.S. (2000): Natural existence of arsenic in soil of China and its cause of formation. Journal of Zhejiang University (Engineering Science Edition), 34: 88–92. (In Chinese)

Williams P.N., Price A.H., Raab A., Hossain S.A., Feldmann J., Meharg A.A. (2005): Variation in arsenic speciation and concentration in paddy rice related to dietary exposure. Environmental Science and Technology, 39: 5531–5540.

Williams P.N., Villada A., Deacon C., Raab A., Figuerola J., Green A.J., Feldmann J., Meharg A.A. (2007): Greatly enhanced arsenic shoot assimilation in rice leads to elevated grain levels compared to wheat and barley. Environmental Science and Technology, 41: 6854–6859.

Yao L.X., Huang L.X., He Z.H., Zhou C.M., Li G.L. (2013): Occurrence of arsenic impurities in organoarsenics and animal feeds. Journal of Agricultural and Food Chemistry, 61: 320–324.

Yao L.X., Li G.L., Dang Z. (2006): Major chemical components of poultry and livestock manures under intensive breeding. Journal of Applied Ecology, 17: 1989–1992.

Yao L.X., Li G.L., Dang Z., He Z.H., Zhou C.M., Yang B.M. (2009): Arsenic speciation in turnip as affected by application of chicken manure bearing roxarsone and its metabolites. Plant and Soil, 316: 117–124.

Yao L.X., Li G.L., Dang Z., Yang B.M., He Z.H., Zhou C.M. (2010): Uptake and transport of roxarsone and its metabolites in water spinach as affected by phosphate supply. Environmental Toxicology and Chemistry, 29: 947–951.

Zavala Y.J., Gerads R., Gürleyük H., Duxbury J.M. (2008): Arsenic in rice: II. Arsenic speciation in USA grain and implications for human health. Environmental Science and Technology, 42: 3861–3866.

Zhang F.F., Wang W., Yuan S.J., Hu Z.H. (2014): Biodegradation and speciation of roxarsone in an anaerobic granular sludge system and its impacts. Journal of Hazardous Materials, 279: 562–568.

Zhao F.J., Ma J.F., Meharg A.A., McGrath S.P. (2009): Arsenic uptake and metabolism in plants. New Phytologist, 181: 777–794.

Received: January 10, 2021
Accepted: March 31, 2021
Published online: April 29, 2021