Arsenic accumulation and speciation in strawberry plants exposed to inorganic arsenic enriched irrigation

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ABSTRACT

The accumulation and transformation of arsenic species have been studied in the context of hydroponic cultivation of strawberry plants. Cultivation experiments have been performed by adding inorganic arsenic at concentrations of 10, 100 and 1000 µg L^{-1} via root irrigation. The total arsenic content was determined by Hydride Generation-Atomic Fluorescence Spectrometry (HG-AFS). The accumulation was dependent on the concentration of arsenic added to the irrigation and the arsenic species. Arsenic (III) accumulated at higher rates than arsenic (V). A greater accumulation of arsenic was found in roots (0.44-4.10 mg kg^{-1}) than in stems (0.43-1.27 mg kg^{-1}) and fruits (0.22-0.30 mg kg^{-1}). The speciation results obtained by HPLC-HG-AFS analysis indicated that the addition of As(III) resulted in a partial methylation producing monomethyl arsenic (MMA) and dimethyl arsenic (DMA). After As(V) addition, only MMA was observed and this was accompanied with a notable reduction in the ratio of As(V) to As(III).

Keywords: strawberry plants; irrigation; arsenic; speciation; fruits; HPLC-HG-AFS

1. Introduction

Arsenic is regarded as a toxic and carcinogenic element (IARC, 2004). High concentrations of arsenic in drinking water, soil, crops, vegetables, animals and the food chain may cause human health problems (Nagajyoti, Lee, & Sreekanth, 2010; Zhao, McGrath & Meharg, 2010; Abdul, Jayasinghe, Chandana, Jayasumana, & De Silva, 2015). Following the overwhelming accumulation of evidence relating to the chronic toxicological effects of arsenic, the World Health Organization guideline now recommends a maximum of 10 μg L^{-1} of arsenic in drinking water (WHO, 2011a). Some legal regulations for arsenic in animal feed and food have also been introduced, and they are focused on inorganic arsenic defined as the sum of arsenite (As(III)) and arsenate (As(V)) (Petursdottir, Sloth, & Feldmann, 2015).

Food safety is of paramount public concern, as relatively high amounts of arsenic may be present in some foods (Sirot, Guérin, Volatier, & Leblanc, 2009). Elevated arsenic concentrations are naturally found in seafood (Taylor et al., 2017). This seems to be related to the relatively high concentration of arsenic (1.5-2 μg L^{-1}) in seawater, which is then ingested by marine life (Urgast, Adams, Raab, & Feldmann, 2010). In contrast, foods of terrestrial origin such as cereals, meats, vegetables, fruits and dairy products usually contain a lower arsenic concentration (<0.1 mg kg^{-1}) (Sigrist, Hilbe, Brusa, Campagnoli, & Beldomenico, 2016). In some cases, arsenic concentrations of up to 1.27 mg kg^{-1} and 2.20 mg kg^{-1} have been reported for fruits and vegetables, respectively (WHO, 2011b). The presence of arsenic in crops, vegetables, animals and food products has been recently reviewed (Upadhyay, Shukla, Yadav, & Srivastava, 2019). Since inorganic arsenic is the most toxic fraction of this metalloid, the Food and Agriculture Organization (FAO) and WHO initially recommended a provisional tolerable weekly intake (PTWI) of 15 μg of inorganic As kg^{-1} body weight (WHO, 2011a). However, in 2010 this value was no longer considered appropriate. Therefore, a benchmark

dose ($BMDL_{0.5}$) was introduced and it noted a 0.5% increased incidence of lung cancer with an exposure of 3.0 µg kg^{-1} body weight per day (WHO, 2011b). The inorganic arsenic fraction represents 28-100% of the total arsenic concentration in the edible tissues of vegetables (carrot, garlic, potato, and beetroot), depending on the species, growth stage and organ (Munoz et al., 2002).

Hence, there is a need for robust validated methods for selective extraction and determination of inorganic arsenic in food matrices, as indicated by international agencies (WHO, 2011b). In order to refine risk assessment of inorganic arsenic, it is also required to produce data regarding the different arsenic species in food commodities to support dietary exposure assessment and dose-response data for the possible health effects (EFSA, 2009). In this sense, there is a research gap relating to the development of low cost and simple analytical methods and techniques to reliably determine arsenic species and inorganic arsenic in foods.

The topic of elemental speciation is a well-established area of research. Studying the chemical forms of the elements helps to elucidate their mobility, biological availability, distribution and toxicity (Ure & Davidson, 2002; Gupta, Nayak, Agarwal, Dobhal, Uniyal, & Singh, 2012). Inorganic arsenic compounds are generally considered more toxic than the organic compounds (Mattusch, Wennrich, Schmidt, Reisser, & Fresenius, 2000). The toxicity of arsenic species follows the order As(III) > As(V) > MMA (monomethyl-arsenic) > DMA (dimethyl-arsenic). There are reports of transformation of arsenic species in plant systems (Kuehnelt, Lintschinger, & Goessler, 2000). Therefore, the quantification of arsenic species in vegetables is of great importance are important for investigation of their accumulation and metabolism in the leaves, roots, shoots, grains or fruits of edible plants grown in different conditions (Zhao, McGrath, & Meharg, 2010).

Speciation analysis in vegetables is achieved by extraction of arsenic species followed by analytical techniques that involve liquid chromatography coupled to atomic spectroscopy and/or mass spectrometry (Zhao, Li, Xu, Luo, & Ma, 2015; Sons, Lee, Kim, Lee, & Nam, 2019). As an alternative to liquid chromatography, total inorganic arsenic can be determined by selective arsine generation with atomic absorption or atomic fluorescence spectrometry as under the conditions of high acidity DMA does not form a hydride. This approach however relies on the criteria that no MMA is present in the sample which is often the case for most food samples. (Chen, Corns, Stockwell & Huang, 2014; Cerveira, Pozebon, Poméu de Moraes, & Silva de Fraga, 2015).

Most of the studies on arsenic speciation in food plants are focused on rice and its products (flour), which is due to its importance as a major food. Rice cultivation is sometimes affected by arsenic polluted irrigation (Abedin, Cresser, Meharg, Feldmann, & Cotter-Howells, 2002; Farías, Londonio, Quintero, Befani, Soro, & Smichowski, 2015; Dos Santos, Pozebon, Cerveira, & de Moraes, 2017). Arsenic speciation dynamics in rice cultivation have been recently reviewed, highlighting the reduction of As(V) to highly mobile As(III) during flooding, as well as the roles of microorganisms in methylation reactions (Kumarathilaka, Seneweera, Meharg, & Bundschuh, 2018). Other horticultural vegetables studied in relation to arsenic exposure are mustard (Jedynak, & Kowalska, 2011), bean (Sadee, Foulkes, & Hill, 2016), carrot (Mayorga, Moyano, Anawar, & García-Sánchez, 2013; Bergqvist, Herbert, Persson, & Greger, 2014), spinach (Shahid et al., 2017) and lettuce (Ma, Yang, Kong, & Wang, 2017).

There is scarce information available regarding the accumulation of arsenic in strawberry plants (*Fragaria ananassa* Duch), a cultivation with worldwide importance. Strawberry cultivation is of particular relevance in Spain, the leading strawberry producer in Europe.

Approximately 90% of the Spanish production is concentrated in the province of Huelva (SW Mainland Spain), which occupies a surface of ca. 7000 Ha with an estimated annual production of $3.6x10^5$ tonnes in 2016 (MAPAMA, 2011; FAO, 2018). The presence of arsenic in some areas of the aquifer near the farming areas (Kohfahl, Sánchez-Rodas Navarro, Mendoza, Vadillo, & Giménez-Forcada, 2016) motivates the study of the response of strawberry plants to possible arsenic exposure. Also, there is no information available about the arsenic uptake rate into different parts of the strawberry plants, as well as a lack of knowledge on species transformation within the fruits.

The purpose of this work is to study the accumulation of arsenic in strawberry plants irrigated with arsenic enriched water (arsenite and arsenate), with consideration of roots, stems and fruits. The possible transformation of inorganic arsenic into less toxic methylated species (MMA, DMA) in the fruits has been studied. Speciation analysis of arsenic with liquid chromatography coupled to atomic fluorescence spectrometry has been performed. This information will allow us to know whether the inorganic arsenic accumulated in the fruits represents a health risk.

2. Materials and methods

2.1. Reagents

Standard solutions of 1000 mg L^{-1} of As(III), DMA, MMA and As(V) species were prepared using sodium arsenite ($NaAsO_2$) (Panreac), dimethylarsinic acid ($C_2H_7AsO_2$) (Sigma Aldrich), sodium methylarsonate ($CH_3AsO(ONa)_2 \cdot 6H_2O$) (Carlo Erba) and sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$) (Panreac), respectively. Intermediate solutions of 1 mg L^{-1} were prepared daily and employed to obtain the calibration solutions with concentrations in the 1-10 µg L^{-1} range. The mobile phase for liquid chromatography consisted of aqueous solutions of K_2HPO_4

(Merck) and KH_2PO_4 (Panreac). In addition, the generation of hydrides was carried out using aqueous solutions of HCl and $NaBH_4$ (Panreac). All solutions were prepared with ultrapure Milli-Q (Millipore) water. NIST SRM 1568b (rice flour) and LGC7162 (strawberry leaves) reference materials were employed for validation of total arsenic determination by acid digestion. NIST SRM 1568b (with inorganic As, DMA and MMA certified content) was also employed for arsenic speciation analysis.

2.2. Methodology for arsenic speciation and total arsenic analysis

Speciation determination of the fruits was carried out by coupling liquid chromatography, hydride generation and atomic fluorescence spectrometry (HPLC-HG-AFS). For the separation of the arsenic species, an anion exchange column (Hamilton PRPX-100 150 mm x 4.1 mm, 10 µm) was used. The mobile phase was 20 mmol L^{-1} KH_2PO_4/K_2HPO_4 (pH 5.8, flow rate 0.8 mL min^{-1}), pumped with a Shimadzu PU-2080 Plus pump. The order of elution was As(III), DMA, MMA and As(V), and separation was accomplished in less than 10 min. After separation of the arsenical species, they were converted into the corresponding hydrides by adding solutions of 4 mol L^{-1} HCl and 1.5 % (w/v) $NaBH_4$ (stabilized in 1% (w/v) NaOH), both at a flow rate of 1 mL min^{-1}. A PS Analytical Millennium Excalibur 10.055 detector equipped with a Photron Superlamp was employed for AFS detection of the arsines. Data were recorded using Clarity Chromatography Software 2.6.2.226 from DataApex. A chromatogram of the arsenic species obtained by HPLC-HG-AFS is shown in the Supplementary Material (see Fig. S1). The concentration range to obtain the linear calibration graphs for each individual arsenic species was 1-10 µg L^{-1}. These instrumental settings have been previously reported for arsenic speciation studies in water and biological samples (Sánchez-Rodas, Geiszinger, Gómez-Ariza, & Krancesconi, 2002; Kohfahl, Sánchez-Rodas Navarro, Mendoza, Vadillo, & Giménez-

151 Forcada, 2016). The determination of the total arsenic content of the samples (roots, stems and
152 fruits) was achieved after acid digestion and analysis with HG-AFS.
153
154 *2.3. Strawberry cultivation and addition of arsenic via root irrigation*
155 The strawberry plants were cultivated at an experimental plot of Adesva (Technological
156 Center of the Agribusiness) located in Lepe (Huelva, Spain). Hydroponic cultivation of the
157 plants was performed using commercially available coconut fibre as a substrate. Cultivation
158 started in October, and the addition of inorganic arsenic irrigation was conducted via irrigation
159 for one month (May), once the fruition had started.
160
161 Six experiments were performed on the addition of arsenic species to the strawberry
162 cultivation via root irrigation (Table 1). They corresponded to the addition of either As(III) or
163 As(V) to the irrigation water at concentrations of 10, 100 or 1000 μg L^{-1}. For each experiment,
164 two sacks of substrate were used, and each sack contained eight strawberry plants. An aliquot
165 of 50 mL of each arsenic solution was added manually to each strawberry plant daily, using
166 plastic syringes. A cultivation blank sample without arsenic addition was also considered.
167
168 *2.4. Sampling and sample treatment*
169 The sampling of the fruits was carried out once per week during May. A composite
170 sample of ca. 60-70 g corresponding to 4 ripe strawberries was obtained from each of the six
171 arsenic cultivation experiments and the blank sample. Strawberries were randomly picked from
172 different plants. The amount of fruit collected every sampling day was between 420-490 g. The
173 strawberries were frozen and lyophilized for ca. 96 hours (Telstar model Cryodos-80). The
174 lyophilized strawberries were crushed and homogenized. A total of 24 arsenic enriched fruit
175 composite samples were obtained during the four week sampling period for the 6 addition

cultivation experiments, plus 4 composite samples for the culture blank in the same period. At the end of the four week sampling period, plants were uprooted. Roots and stems were lyophilized, crushed and homogenized in a similar way as the fruits. Arsenic concentrations in the three parts of the plants were referred to the dry weight of the samples after lyophilization.

For extraction of the arsenic species from the fruits, 200 mg of lyophilized strawberries was weighed and 10 mL of Milli-Q water was added. The extraction was aided by heating in a microwave oven at 90 W for 4 min. The extracts were centrifuged at 5300 rpm for 5 min. The supernatant was submitted to a clean-up step using a C18 cartridge. The eluate was filtered with a 0.45 μm PVDF syringe filter. Finally, the extracts were analyzed by HPLC-HG-AFS to determine the four arsenic species.

Total arsenic determination in fruits, stems and roots was performed after acid digestion. A portion of 100 mg of homogenized dry sample was weighed in a Teflon digestion vessel and mineralized with 2 mL of 65% HNO_3 and 2 mL of H_2O_2 at 180 °C by heating on a hot plate. The residue was dissolved in 2% HNO_3 to a final volume of 10 mL. Subsequently, the total arsenic content was determined with HG-AFS.

Parametric statistical test (Student's t-test) was applied to the arsenic accumulation data in the strawberry plants, using software package STATISTICA 7.0 (Statsoft). An α-value of 0.05 was adopted as the critical level for statistical testing giving a 95% confidence level.

3. Results and Discussion

3.1 Validation of analytical procedures for total arsenic and arsenic speciation.

The total arsenic content of the strawberry samples was determined by acid digestion followed by HG-AFS analysis. The validation of this analytical procedure was conducted by analyzing reference materials. The total arsenic concentrations obtained for NIST 1568b rice flour and LGC7162 strawberry leaves (0.276 ± 0.010 mg kg^{-1} and 0.25 ± 0.02 mg kg^{-1}, respectively) did not significantly differ from the analytically derived certified values (0.285 ± 0.014 and 0.28 ± 0.07 mg kg^{-1}, respectively).

The arsenic speciation analysis of the samples was performed by aqueous extraction aided by microwave radiation and HPLC-HG-AFS analysis. The validation of the speciation procedure was performed by analyzing NIST 1568b rice flour. The concentration obtained for inorganic arsenic (sum of As(III) and As(V)) was 0.091±0.001 mg kg^{-1} and did not differ from the analytically derived certified value (0.092±0.001 mg kg^{-1}). In addition, the experimental values for DMA (0.181±0.001 mg kg^{-1}) and MMA (0.0118±0.0012 mg kg^{-1}) were similar to the certified concentrations (0.180±0.012 mg kg^{-1} for DMA, 0.0116±0.0035 mg kg^{-1} for MMA). To confirm the effective extraction of the arsenic species from the strawberry fruits samples, an spiking experiment was conducted by duplicate additions of 125 µL of a 0.5 mg L^{-1} solution of the four arsenic species (in methanol) to 0.2 g of a strawberry sample. After evaporation of the solvent, the samples were extracted with water, filtered, submitted to C18 cartridge clean up and analyzed by HPLC-HG-AFS. The results indicated quantitative recoveries for all the arsenic species: 95±5% As(III), 102±5% DMA, 99±3% MMA and 99±2% As(V).

3.2. Accumulation of arsenic in roots, stems and fruits of strawberry plants

The results of the cultivation experiments indicated that the accumulation of arsenic in the strawberry plants was dependent on the level of exposure and the particular arsenic species

added to the irrigation. The roots of the strawberry plants were found to have the highest amount of accumulated arsenic. The concentrations of arsenic in the roots increased progressively as the concentrations of As(III) and As(V) introduced to the irrigation increased (Table 1). In the cultivation experiments with low and medium arsenic concentrations (10 and 100 μg L^{-1}), the accumulation trend was similar for both As(III) and As(V). In those cases, the arsenic concentration in the roots increased from 0.27 to 0.46 mg kg^{-1} for As(III) exposure, and from 0.27 to 0.39 mg kg^{-1} for As(V) exposure. However, when the plants were exposed to high arsenic concentrations (1000 μg L^{-1}) the accumulation in the roots was ten-fold higher for As(III) than for As(V) (4.1 mg kg^{-1} vs. 0.44 mg kg^{-1}). The different accumulation rates of As(III) and As(V) can be attributed to the different mechanisms of uptake for each arsenic species in the plants. It has been reported that As(III) is efficiently taken up through the silicon transport pathway, probably by sharing nodulin26-like intrinsic proteins, whereas As(V) is analogous to phosphate and competes for the same transport in the roots (Nagajyoti, Lee, & Sreekanth, 2010; Zhao, McGrath, & Meharg, 2010; Wang, Peng, Tan, Ma, & Rathinsabathi, 2015).

The accumulation in stems followed a similar trend as in roots (Table 1). Overall, the concentration of arsenic found in stems was significantly lower than in the roots (t-test, $p<0.002$) for both As(III) and As(V) exposure, with the exception of As(V) at 1000 μg L^{-1} (t-test, $p>0.7$). Once more, as expected, the highest accumulation was observed for 1000 μg L^{-1} arsenic exposure. The accumulation was again higher for As(III) (1.27 mg kg^{-1}) than for As(V) (0.43 mg kg^{-1}). The concentration ratio As_{roots}/As_{stems} was in most cases higher than 1.0, indicating a preferential accumulation in the roots rather than in the stems. When exposed to the highest arsenic concentration, the ratio As_{roots}/As_{stems} was 3.2 for As(III) exposure, whereas a lower ratio As_{roots}/As_{stems} =1.0 was obtained for As(V) exposure. The preferential

accumulation in the roots has also been found in other edible plants, such as broad bean plants (Sadee, Foulkes, & Hill, 2016). However, when other edible plants, such as carrots, are exposed to arsenic-rich water, the accumulation is higher in the leaves than the roots (Mayorga, Moyano, Anawar, & García-Sanchez, 2013).

The accumulation of arsenic in the fruits was lower than it was in the roots and stems. Of the three levels of arsenic exposure, accumulation in the fruits occurred only with the highest level of arsenic (1000 μg L^{-1}) exposure. The results in Table 2 correspond to the total arsenic concentrations of the samples after acid digestion and analysis with HG-AFS. A progressive accumulation of arsenic was observed throughout the four weeks of the studied period for both species. As it happened in roots and stems, the accumulation was greater for As(III) than for As(V) exposure. This preferential accumulation of As(III) compared to As(V) was significantly higher after the 3^{rd} and 4^{th} weeks of exposure (t-test, $p<0.04$ and $p<0.03$, respectively), but not during the 1^{st} and the 2^{nd} weeks (t-test, $p>0.7$ and $p>0.18$, respectively). The concentration of arsenic in the fruits increased from 0.06 to 0.30 mg kg^{-1} when As(III) is applied, and from 0.04 to 0.22 mg kg^{-1} for As(V).

The different accumulation rate of arsenic in fruits, stems and roots can be due to a detoxification mechanism, similarly as proposed for other plants. For carrots, it has been indicated that arsenate absorbed by roots is reduced to arsenite and transported to leaves via chelating agents (e.g. phytochelatins, GSH), and thus the toxicity of arsenic is reduced. This translocation generally can explain the amount of arsenic in different plant organs. (Mayorga, Moyano, Anawar, & García-Sanchez, 2013). The location of the As-complexes within plant tissue is not clear known, although it is known that these complexes are stable in vacuoles

under acidic conditions. The transporters for uptake in leaf cells are assumed to be similar as those in roots (Zhao, McGrath, & Meharg, 2010).

3.3. Analysis of speciation of arsenic in the strawberry fruits

Speciation analysis of arsenic for As(III), As(V), MMA and DMA was successfully carried out in the fruits after water extraction of the samples aided by microwave radiation and analysis by HPLC-HG-AFS. Speciation analysis in the fruits was only possible in the experiments corresponding to exposure to 1000 μg L^{-1} of As(III) and As(V). No accumulation in the fruits was found for exposure at 10 or 100 μg L^{-1} arsenic. The results of speciation analysis of the fruits of these two experiments during the four weeks of study are shown in Table 3. Speciation analysis was also attempted in the roots and stems, but no arsenic species were detected in the aqueous extracts. This difficulty in effectively extracting arsenic species with water or water/methanol mixtures from these parts of terrestrial plants been previously reported, reporting extraction efficiencies as low as 3% (Ruiz-Chancho, López-Sánchez, Schmeisser, Goessler, Francesconi, & Rubio, 2008).

In the experiment corresponding to the addition of 1000 μg L^{-1} of As(III), no arsenic species were detected in the first week, as their concentrations were below 0.01 mg kg^{-1}. As(III) was detected in the second week of study and its concentration increased until the fourth week (0.23 mg kg^{-1}). This increase was significant during the 2^{nd} and 3^{rd} week (t-Student, $p < 0.03$), but not the 4^{th} week (t-Student, $p > 0.2$). As(III) was partially converted into DMA and MMA and oxidized to As(V) at the end of the study period. Fig. S2 in the Supplementary Materials shows a chromatogram obtained by HPLC-HG-AFS corresponding to the analysis of arsenic speciation in the fruit in the fourth week of the study. The presence of methylated arsenic species in plants is usually explained by a pathway involving S-adenosylmethyltransferase that

act on the methyl donor S-adenosyl-L-methionine (SAM) and As(III) as initial substrate (Zhao, McGrath, & Meharg, 2010). In addition, the methylation of inorganic arsenic to DMA in some plants (e.g., rice) can be the result of rhizosphere associated bacteria (Arao, Kawasaki, Baba, & Matsumoto, 2011).

With respect to the addition of 1000 μg L^{-1} As(V) to the irrigation, the results showed a lower accumulation of inorganic arsenic as well as a lower transformation rate to methylated species. It was observed that As(V) was strongly reduced to As(III). This pattern has also been described in rice plants, indicating that arsenate is readily reduced to arsenite, which can later be detoxified by complexation with thio-rich peptides such as phitochelatines (Zhao, McGrath, & Meharg, 2010). The latter methylated species were detected in the fruit from the second week and increased until the fourth week (0.14 mg kg^{-1}). Arsenic (V) was only detected in the fourth week at a concentration of 0.03 mg kg^{-1}. In the case of methylated species, DMA was not detected throughout the study period while MMA was detected in the last two weeks of the experiment.

The speciation results indicated that the sum of methylated DMA and MMA represent 21% of the total arsenic content for As(III) exposure, the remaining 79% corresponding to inorganic arsenic. The percentage of methylated species was found to be a 5% (corresponding to MMA) for As(V) exposure. Other studies performed with rice grains found similar percentages for rice cultivation in Asia (up to 78% inorganic arsenic), whereas crops from Europe and USA showed an increasing trend of DMA in relation to total arsenic (Wang, Peng, Tan, Ma, & Rathinsabathi, 2015). The methylation in the fruit was lower than in other plants, such as the edible bean, in which DMA (19%) and MMA (48%) are the dominant species (Sadee, Foulkes, & Hill, 2016).

The sum values of the inorganic species after four weeks of 1000 µg L^{-1} exposure (Table 3) are 0.25 mg kg^{-1} and 0.17 mg kg^{-1} (with respect to dry weight) for irrigation with As(III) and As(V), respectively. The water content of the fruits was 89%, calculated by weighing them before and after lyophilization. Considering this water content, the arsenic concentration in the fresh fruits corresponded to 27.5 µg As kg^{-1} for irrigation with spiked As(III) and 11.2 µg As kg^{-1} for irrigation with spiked As(V). These results indicated that the arsenic content of the fruits does not represent a health hazard. It would be necessary to consume ca. 6.0-8.8 kg of strawberries daily to reach the limit established by WHO for arsenic of 3.0 µg As kg^{-1} body weight per day (WHO, 2011b), considering an average body weight of 55 kg. The presented results showed that even under a high exposure of the plants to As(III) or As(V), the inorganic arsenic content of the fruits may not represent a serious health risk in the case of human consumption.

Conclusions

This study provides information based on experimental data to better understand the accumulation and transformation of arsenic species in different parts of the strawberry plants (root, stem and fruits) cultivated on a hydroponic substrate. The cultivation experiments performed with arsenic exposure via root irrigation indicated a greater uptake for As(III) than for As(V). The accumulation depended on the concentration of arsenic species added to the water and the time of exposure. The maximum accumulation was higher for roots than for stems and fruits. The arsenic speciation analysis of the fruits revealed that inorganic arsenic underwent partial methylation. Additionally, a marked reduction of As(V) to As(III) was observed in the fruits when As(V) was applied to the plants. Overall, the inorganic arsenic accumulated in the fruits did not represent a health risk in the case of human consumption.

Acknowledgements

This work has been financially supported by project FQM 752 of the Andalusian Autonomous Government (Consejería de Economía, Innovación, Ciencia y Empleo).

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