A study of arsenic speciation in soil, irrigation water and plant tissue: A case study of the broad bean plant, *Vicia Faba.*

Bashdar A. Sadee, Mike E. Foulkes and Steve J. Hill

School of Geography, Earth and Environmental Sciences, Plymouth University, Plymouth PL4 8AA, UK

Abstract:

Samples of soil, the broad bean plant, Vicia Faba and irrigation water were collected from the same agricultural site in Dokan, in the Kurdistan region of Iraq. Total arsenic and arsenic speciation were determined in all materials by ICP-MS and HPLC-ICP-MS, respectively. Available arsenic (11 %) was also determined within the soil, together with Cd, Cr, Cu, Ni, Zn, Fe and Mn. The concentrations of total arsenic were: soil (5.32 µg g-1), irrigation water (1.06 µg L-1), roots (2.065 µg g-1) and bean (0.133 µg g-1). Stems, leaves and pods were also measured. Inorganic As V dominated soil (90%) and root (78.3%) samples. However, organo-arsenic (MMA, 48.2% and DMA, 19.2%) was the more dominant species in the edible bean. The study provides an insight into the uptake, preferred disposal route, speciation changes and loss mechanism involved for arsenic with this food source.

Keywords: Arsenic speciation, broad bean, BCR-sequential extraction, ICP-MS.

1. Introduction

Arsenic (As) is considered a toxic element to humans, other animals and plants. As a consequence of contamination human health problems have occurred in various countries, often caused by high concentrations of As in the diet, originating from both drinking and irrigation waters, from crops (*via* soil), vegetables and meat products (Mayorga, Moyano, Anawar, & Garcia‐Sanchez, 2013). The detection of As in urine, faeces, skin, hair, nails and lungs has been used as indicators of potential As poisoning. Skin lesions are a later symptom of As toxicity, often a result of drinking

water contaminated with high levels of As (Jain & Ali, 2000). Both natural geological sources and anthropogenic activities, the latter such as the industrial production of pesticides, herbicides, wood preservatives and particularly mining, have increased As levels in ground water and soils leading to worldwide environmental concerns (Bhattacharya, Welch, Stollenwerk, McLaughlin, Bundschuh, & Panaullah, 2007).

The topic of elemental speciation is now a well-established area of research. Studying the chemical forms of elements help elucidate the mobility, biological availability distribution, and toxicity of the chemical element (Gupta *et al*., 2012; Ure & Davidson, 2002). The inorganic As species, arsenite (As ^{III}) and arsenate (As ^{V}) are classified as being carcinogenic (Mandal & Suzuki, 2002), and the organic As species monomethylarsonic acid (MMA) and dimethylarsinic acid, whilst less toxic than inorganic As, are still classified as being cancer promoters (Heitkemper, Vela, Stewart, & Westphal, 2001; Sanz, Muñoz-Olivas, & Cámara, 2005). The guideline level for As in drinking water is considered to be 10 µg L^{-1}(Commission Directive, 22.5.2003; Petrusevski, Sharma, Schippers, & Shordt, 2007; WHO, 2006). A maximum limit for As in food products has not yet been established by the EU. However, the maximum levels are established in national legislation in some member States. For example, the statutory limit of As in foods in the UK is 1 µg g^{-1} fresh weight (Warren, Alloway, Lepp, Singh, Bochereau, & Penny, 2003). The Food and Agriculture Organisation and World Health Organisation (FAO/WHO) has recommended a provisional tolerable weekly intake (PTWI) of not more than 15 µg inorganic As kg^{-1} body weight (WHO, 2011). However, the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM Panel) in its Scientific Opinion on Arsenic in Food decided that this PTWI value is no longer suitable. Therefore, based on the epidemiological studies they suggested a

benchmark dose lower confidence limit $BMDL_{01}$ between 0.3 and 8.0 µg kg^{-1}b. w. per day for an increased risk of cancer of the lung, skin, bladder and skin lesions (EFSA, 2014).

Plants can readily accumulate As, facilitating the transfer from soil to the food chain. The predominant inorganic As species in the oxic layer of soils is arsenate which has a similar chemical behaviour to phosphate. Therefore, As^{V} can enter plant species *via* the phosphate transporter system, after which the arsenate may be converted into arsenite in the plant biomass. The uptake and phytotoxicity of the As depends on the levels of phosphate in soil. In the soil particles at low levels of phosphate As can displace phosphate which increases the uptake and phytotoxicity, whereas high levels of phosphate compete with As at root surfaces which results in a decline in uptake and phytotoxicity (Mayorga, Moyano, Anawar, & Garcia‐Sanchez, 2013).

The field of As speciation has developed rapidly following the development of sensitive and species-specific detection methods. High-performance liquid chromatography (HPLC) coupled with inductively coupled plasma mass spectrometry (ICP-MS), is now the analytical technique of choice in many laboratories because of the selectivity and sensitivity for As species (B'hymer & Caruso, 2004).

The determination and distribution of the plant-available element and contents of soil is important in agricultural studies of plant nutrition and plant and animal element-deficiency disease. This includes both major nutrients such as nitrogen, phosphorus and potassium as well as the essential trace elements and the potentially toxic elements (Ure & Davidson, 2002). Heavy metal speciation together with the fractionation in the environmental media using sequential extraction techniques is of

major interest offering a realistic estimate of actual environmental impact. The various sequential extraction procedures used for sediments and soils (Zhang, Shan, & Li, 1998) were harmonized by the BCR (now the Standards, Measurements and Testing Programme; SM&T) which has developed a three stage, sequential extraction protocol (Cuong & Obbard, 2006). According to this extraction protocol metals can be divided into acid soluble/exchangeable, reducible and oxidisable fractions (Baig *et al.*, 2009).

The aims of this study were to investigate both the total As and As species in different parts of a common vegetable food crop, the legume broad bean *Vicia Faba.* This crop had been grown in Dokan south east Arbeel in the Kurdistan region of Iraq. Since the As levels in the plant reflect the local growing environment, soil in which the broad beans were cultivated together with the irrigation water were both included in the study. This was to understand how the local habitat may impact on the uptake, and help elucidate the accumulation and transformation of As in these plants which are an important food source.

2. Experimental

2.1 Chemicals and Reagents

All chemicals used in this study were of analytical grade and used without further purification. All solutions were prepared using Milli-Q water (18 MΩ cm) unless otherwise stated. Total Cd, Cr, Cu, Ni, Zn, Fe, Mn and As standards were prepared from the high purity stock solution 100 µg mL^{-1} in 5 %HNO_3 (CPI international, Peak performance, USA). Arsenic oxide (As^{III}) and the DMA were purchased from Sigma

(Gillingham, Dorset, UK), and MMA was purchased from Greyhound Chromatography and Allied Chemicals (Birkenhead, Merseyside, UK). The reagents, $Na_2HAsO_4.7H_2O$ (As ^{V}), In, Ir and Ammonium dihydrogen orthophosphate AnalaR standards were obtained from VWR International (MERCK), Lutterworth, Leicestershire, UK. Hydrogen peroxide 37% and nitric acid 70% were supplied by Merck, Poole, Dorset, UK. Orthophosphoric acid 86.75%, hydrochloric acid and L-ascorbic acid were purchased from Fisher Scientific (Loughborough, UK). Spinach GBW10015 certified reference material (Institute of Geophysical and Geochemical Exploration, Langfang, China) was purchased from LGC standards (Middlesex, UK). The certified reference material BCR 701 was obtained from Sigma-Aldrich (Gillingham Dorset, UK), while the loam soil (ERM-CC141) was purchased from European Commission (Gel, Belgium).

2.2 Instrumentation

The ICP-MS analysis was performed using an X Series 2 instrument (Thermo Scientific, Hemel Hempstead, UK). The operating conditions employed are described in Table 1. Collision cell technology was used to eliminate possible interferences. In and Ir were used as internal standards for all samples at a final concentration of 10 µg L^{-1}. The mass spectrometer was set to sample ion intensities (peak jumping option) at the anaylzed mass ($^{75}As^{+}$). The signal intensity was sampled at m/z 115 ($^{115}In^{+}$) and m/z 193 ($^{193}Ir^{+}$) used for internal standardization.

The HPLC instrumentation operating conditions are illustrated in Table 1. Chromatographic separations were carried out using a Jasco chromatographic pump (Japan) with a 250 x 4.6 mm column packed with 10 µm particle size Hamilton PRP-

X100 anion exchange resin. A 50 x 4.6 mm guard column packed with the same material was used to protect the analytical column. A Rheodyne 7152 injection valve (Rheodyne, Cotati, CA, USA) employing a six-way injection port was used. The interfacing between HPLC and ICP-MS was carried out using Teflon capillary tubing (0.5 mm i.d) which connects the column outlet directly with an inlet to the nebulizer. An Oakton pH meter (Eutech Instruments, Singapore) was used to take pH readings. The ICP-MS instrument was set to time-resolved data acquisition. Data for arsenic (m/z 75) were recorded using the peak jumping acquisition and displayed as mass-intensity-time plots as demonstrated in Fig. 1. The concentration of each arsenic species was calculated using peak area and compared with known concentration standard solutions for qualitative (using retention time and spiking) and quantitative measurements.

2.3 Sampling and Sample preparation

Broad beans, together with the various parts of the plant (root, stem, leaf, pod and bean), soil samples and irrigation water were collected from the same agricultural sites located in Dokan southeast of Arbeel-Iraq in April 2014. The broad bean samples were placed in sealed containers at the point of collection. These were then washed with Milli-Q water and stored in a cooled plastic box. All samples were frozen at -40 ºC for 12 hrs in a freezer and then placed in a freeze drier for 48 hrs at -40 ºC. All dried samples were ground using an agate pestle and mortar to a fine powder and then sieved using a nylon 180 µm sieve. The samples were then stored in brown glass bottles and placed in a desiccator in order to avoid exposure to light and moisture until required for analysis.

2.4 Total arsenic content of the soil.

Total As available in the sample soil and the residues from the BCR fractionation steps were digested using a modified version of the LGC aqua-regia protocol as follows;-

1- Only sub-samples of soil material that had passed through a 180 µm nylon mesh were subjected to acid extraction.

2- Each sample (approx. 0.50 g) was weighed accurately into a pre-cleaned beaker and then transferred to a large pre-cleaned long neck digestion tube.

3- Each beaker was then re-weighed so that an accurate weight of sample transferred to the tube could be obtained.

4- Concentrated hydrochloric acid (35%; 8 mL) and concentrated nitric acid (70%; 2 mL) was added and the sample allowed to stand at room temperature for at least one hour so that easily oxidised material could be destroyed.

5- After this process, the digestion tubes were placed in a Tecator digestion block (Digestion system 12, 1009 digester) and heated at 110 ˚ C for two hours.

6- After cooling, the extracts were transferred, quantitatively, without filtering to pre-cleaned 100 mL capacity volumetric flasks and diluted to volume with deionised water.

7- Three replicates of each sample were prepared together with three replicates of the CRM Loam soil (ERM-CC141), European Commission, Geel, Belgium) for each batch digestion. The certified reference material was digested in the same way, while a procedural blank was prepared for analysis, omitting the sample.

2.5 Water samples

The irrigation water samples were collected from the on-site farm well where the plant and soil samples were collected and stored in HDPE sealed tubes. The pH of the water samples was adjusted with nitric acid to 2.0 in order to preserve any As speciation and prevent interchanges between species. Total arsenic was measured using ICP-MS.

2.6 Total As determination in plant samples

A Mars X press microwave lab station (CEM, USA) with 100 mL closed Teflon vessels with Teflon covers was used for the acid digestion of plant samples. Freeze dried plant samples (0.25 g) were weighed in triplicate and placed into separate Teflon reaction vessels. Then 5 ml HNO_3 (70%) and 2 mL H_2O_2 (30%) were added and the vessels were sealed. All samples were then digested for 43 min total digestion time at 1600 W. In the first step of the digestion, the temperature increased up to 160 ºC over 15 min and stayed at this temperature for a further 5 min. In the second step of the digestion the temperature increased from 160 to 200 ºC in 8 min and stayed at this temperature for a further 15 min. After digestion the Teflon reaction vessels were allowed to stand at room temperature until cool. Once the digestion was completed the samples were transferred quantitatively into pre cleaned 25 mL capacity volumetric flasks and made up to volume with 2% (v/v) HNO_3, prepared using Milli-Q water. The samples and standards were spiked with internal standards of In and Ir to give a final concentration of 10 µg L^{-1}. In and Ir were used to correct for instrument drift (due to sample viscosity effects, mass transport, etc.) during the measurement period, using ICP-MS. The certified reference material

GBW10015 (spinach) was used to validate the total arsenic determination in different parts of the broad bean plant.

2.7 Phosphoric acid extraction for arsenic speciation in soil.

Soil samples, approx. 0.5 g were accurately weighted into a microwave vessel tube, and 25 mL of a mixture of 1.0 mol L^{-1} phosphoric acid and 0.1 mol L^{-1} ascorbic acid was then added to the tube. Ascorbic acid was added to inhibit any oxidation reaction of arsenic species (Garcia-Manyes, Jiménez, Padro, Rubio, & Rauret, 2002). Samples were then extracted in a Mars Xpress microwave lab station (CEM, USA). The vessels were subjected to microwave digestion procedures under controlled conditions at 80 ˚ C for 20 min (Lou, Liu, & Liu, 2014). After cooling, the samples were quantitatively transferred to a 50 mL capacity centrifuge tube. The same digestion procedure was repeated for all the samples. A reagent blank was prepared in similar fashion, but omitting the sample. The extracted samples were then centrifuged at 2000 rpm (Sanyo Centaur 2, UK) for a period of 20 minutes and the supernatant decanted into pre-cleaned 100 mL capacity volumetric flasks. The samples were then diluted to volume using Milli-Q water.

2.8 Extraction procedure for arsenic speciation in plant materials

Foster *et al.* (2007) used a procedure using 2% nitric acid to extract As species from marine plants and animal digestive tissues. They showed that dilute nitric acid is able to effectively remove inorganic As from tissue without oxidation of other major species in marine organism. Sun *et al.* (2009) also used nitric acid but at concentration of 1 % for extraction of As species. This latter method was adopted for this study. A sample (0.50 g) sample was accurately weighed into a 50 mL polypropylene digest tube and 20 mL of 1% of nitric acid added. The samples were

then left overnight. Samples were then extracted using a Mars Xpress microwave lab station (CEM, USA). In the first step of the extraction, the temperature increased to 55 ºC over 3 min and was then held at this temperature for 10 min. In the second step of the extraction the temperature was increased from 55 ºC to 75 ºC over 3 min and then held for 10 min. Finally the temperature for extraction was raised to 95 ºC and held at this temperature for 30 min. Samples were then cooled to room temperature and centrifuged at 3000 rpm for half an hour. The supernatant was filtered through a 0.45 µm filter (Millipore) and kept at 4 ºC until required for analysis. The GBW10015 (spinach) certified reference material was used to validate the extraction of arsenic species in different parts of the broad bean plant.

2.9 Sequential extraction method for As in soil

The extraction method employed was adopted from the modified BCR sequential extraction (three-step) procedure for the determination of extractable trace metal contents (Rauret *et al.*, 2000)**.** The sequential extraction was performed using the steps described below:

-Step 1 (acid-soluble fraction – bound to carbonates)

For each sample, three 1.0 g dry soil replicates were placed into separate 50 mL polypropylene centrifuge tubes. Acetic acid (40 mL, 0.11 $molL^{-1}$) was then added to each of the tubes which were subsequently shaken for 16 h at a room temperature of 22 ± 5 ºC. The extract was then separated from the solid phase by centrifugation at 3000 rpm for 20 min. The supernatant liquid was decanted into a 50 mL polypropylene centrifuge tube and stored in a refrigerator at 4 ºC prior to measurement. The residue was washed with 10 mL of de-ionized water and then

shaken again for 15 min prior to being centrifuged for 20 min at 3000 rpm. The supernatant was decanted and removed carefully to avoid loss of the solid residue.

-Step 2 (reducible fraction – bound to Fe and Mn oxides)

Hydroxylamine hydrochloride (40 mL, 0.5 mol L^{-1}) adjusted to a pH of around 1.5 by adding of 2 mol L^{-1} HNO_3 was added to the centrifuge tube containing the residue from step 1. Again, the tube was shaken for 16 h at 22 ± 5 ºC at a speed of 30 ± 10 rpm. The extract was separated from the solid phase by centrifugation and decantation as described for Step 1 and again stored at 4 ºC. The solid residue was washed as in Step 1 before proceeding to Step 3.

-Step 3 (oxidizable fraction – bound to organic matter and sulfides)

Hydrogen peroxide (10 mL of 8.8 mol L^{-1} H_2O_2 (pH of 2)) was added carefully, in small aliquots, to the residue from Step 2 in the centrifuge tube. The vessel was loosely covered with its cap and left for 1 h at room temperature for 1 h with occasional shaking. The tube was then placed in a water bath for 1 h at 85 ± 2ºC with occasional shaking during the first 30 min. The volume was then reduced to around 2 – 3 mL by further heating of the uncovered tube. Another 10 mL of 8.8 molL^{-1} H_2O_2 (pH 2) was added. Again, the covered tube was heated to 85 ± 2 ºC and the digestion left for 1 h so that the volume in the uncovered tube was reduced almost to dryness. After cooling, 50 mL of 1.0 mol L^{-1} ammonium acetate (adjusted to pH 2 by adding concentrated HNO_3) was added to the residue and the tube was shaken for 16 h at room temperature (overnight). The extract was separated from the solid phase by centrifugation and decantation as described above and stored at 4ºC.

3. Results and discussion

3.1Results of CRM analyses for total arsenic

To validate the applied procedures, total As was determined in different certified reference materials for the different sample matrices. The analytical validity for arsenic was based on the use of CRMs, GBW 10015 spinach, loam soil (ERM-CC141) and BCR 701-lake sediment. These reference materials were selected to represent plant and soil samples. Good recoveries were found for total As in the spinach and loam soil, with values of 108 and 98 % of the certificate value respectively. The results for total arsenic content in all CRMs are shown in Table 2.

3.2 Total arsenic determinations

i) Soil sample

The different concentrations of As in different parts of the broad bean plant clearly has implications regarding the potential toxicity of As in this food crop if grown in areas with available As in the soil. To investigate the As uptake by broad beans further, the total concentration of As was also measured in soils collected from the same points as the plants. The modified LGC extraction protocol was applied to measure the total As content (aqua regia extractable content) in the soil and the concentration of total aqua regia extractable As in the soil samples was determined to be 5.32 µg g^{-1}(Table 3).

ii) Irrigation water

The total concentration of As in the irrigation water was 1.06 µg L^{-1} (Table 3) which is significantly below the recommended limiting value for As in drinking water by the WHO (10 µg L^{-1}) (Chen, Smith, Winefordner, Tu, Kertulis, & Ma, 2004; Commission Directive, 22.5.2003). Since the concentration of As in water is very low and lies near

the limit of detection for species measured using HPLC-ICP-MS, speciation on the water was not performed. However, at this level, irrigation water was not felt to be significant As to the growing plant but would be a facilitator at the root surface and soil interface, assisting arsenic mobility.

iii) Plant material

The concentration of total As in the different parts of the broad bean plant (root, stem, leaf, pod and bean) ranged from 0.133-2.065 µg g^{-1} (Table 3).The majority of the As in the investigated plants was located in the roots, with a mean value for the triplicate measurements of 2.065 ± 0.033 µg g^{-1} dry weight (mean ± SD), while the lowest concentration of As was found in the bean (0.133 µg g^{-1}). The concentration of As in the bean of the broad bean plant found in this study was higher than that reported by Xu *et al*. (1985) and Munoz *et al.* (2002) but lower than the result of Bundschuh *et al.* (2012) whose values ranged from 2.3-2.9 µg g^{-1}. To validate the applied procedure, arsenic was determined in the certified reference material GBW10015 (spinach) which demonstrated full recovery.

3.2 Arsenic speciation analysis

i) Soil sample

Loam soil was used as a CRM to validate the phosphoric acid extraction procedure of As species from the crop soil that the broad bean plant was cultivated in. The results showed good extraction efficiency (95.6%) of total As in extracts compared with the certified value and 97.81% with respect to the aqua-regia extractable As content in loam soil, Table 4. The As extraction efficiency from the soil was 99.62%. The major species found in the soil was As^{V} (4.584 µg g^{-1}) with smaller quantities of

As ^{III} (0.564 µg g^{-1}) present. The stability of the As species (As ^{III} and As ^{V}) under the applied extraction conditions was also measured by spiking those As species standards into the loam soil reference material. The results demonstrated good stability for both species after the extraction with a variability of only 2% for both species when considering the analytical uncertainty. This high stability may be attributed to adding ascorbic acid to the extractant media to prevent oxidation of As species (Giral, Zagury, Deschênes, & Blouin, 2010).

ii) Plant sample

Inorganic species of As are generally considered to be the most toxic forms to humans (Jain & Ali, 2000). The inorganic species of As tend to predominate in plants (Jedynak, Kowalska, Harasimowicz, & Golimowski, 2009; Jedynak, Kowalska, Kossykowska, & Golimowski, 2010) including a number of different crops and vegetables (Signes-Pastor*et al.*, 2008;Smith, Juhasz, & Weber, 2009). These workers found that the same species predominated both in the roots and the stems (Jedynak, Kowalska, Kossykowska, & Golimowski, 2010). A wide range of organisms are able to metabolise As from its inorganic to organic forms, and this may be shown to occur in plants (Meharg & Hartley-Whitaker, 2002). Arsenobetaine and arseno-sugars have been measured in some terrestrial plants and this may indicate metabolism of arsenic in plants (Kuehnelt, Lintschinger, & Goessler, 2000). It has been reported that some algae are able to metabolise arsenic. Simple methylated arsenic species such as MMA and DMA can be produced by algae through reduction of As ^{V} to As ^{III} by a methylation process. Whereas, arsenoriboses, specifically OH-ribose, PO_4-ribose and OSO_3-ribosehave been formed through the glycosidation process (Thomson, Maher, & Foster, 2007). Although external methylated species of arsenic were not found in surrounding soil or water, arsenic

species such as MMA, DMA and tetramethylarsonium ions have been found in a number of plants (Koch, Wang, Ollson, Cullen, & Reimer, 2000). The presence of these As species in plants indicates that the methylation process may occur within plants.

The different As species concentration found in this study are presented in Table 4.The extraction efficiencies ranged between 85% and 98%. Arsenic accumulation was found to be greater in the roots compared with other parts of the broad bean plant. The root As concentration was 2.065 µg g^{-1} which is more than fifteen times higher than the concentration of As in the bean itself. Studies performed by other workers have shown the same behaviour in *Vicia Faba* with the majority of As absorbed, and stored in the roots (Austruy *et al.*, 2013). The concentration of total As decreased gradually from the root to the bean of the broad bean plant. The leaf is an exception where As was found to accumulate at higher concentrations than in the stem and pod. This reduction in the total amount of As may reflect the eliminating metabolic pathway of As in broad bean plants.

Inorganic As (As^{III} and As^{V}) were the major species found in the root, stem, leaf and pod of the broad bean plant, while the organic As species (MMA and DMA) were the major species in the bean of the broad bean plant (Fig. 2). The species As^{III}, As^{V} and MMA were detected in all parts of the broad bean plant, whilst DMA was found in all organs of broad bean except in the leaf which was below the limit of detection. In the root of the plant, inorganic As (As^{III} and As^{V}) represented the predominant species (94 %) compared with the organic As species which was the minor arsenic species. As^{V} was found to be the major species (78%) with smaller amounts of As^{III} (16%). While DMA and MMA comprised of only 2% and 3% of total As concentrations in the root, respectively.

In the stem of the plant 51 % of total As was found to be its inorganic forms (As^{III} and As^{V}) with As^{V} being predominant, representing alone 33% of the total As in the stem of the broad bean plants in comparison with other As species. In the leaf As^{V} was found to be the major species of As accounting for 56% of the total concentration of As, but As^{III} was also present as a minor species. DMA was below the limit of detection.

In the pod of the broad bean, 35% of total As in the pod was As^{V} while MMA was a minor species with 12% of the total As concentration. The results showed that DMA was higher than As^{III} and MMA in the same part of the broad bean plant. In contrast, in the bean the organic As species were the main As species and MMA was found to be 49% of the total As concentration whilst As^{III} (7%) represented the lowest concentration of the As species. The highest levels of both As^{V} and As^{III} in this study were found in the root, while interestingly the lowest levels of both species were found in the bean of the plant. The highest concentration of MMA was found in the leaf (0.101 µg g^{-1}), with the same species giving the lowest value in the pod. In contrast the lowest value of DMA was found in the leaf and the highest value (0.07 µg g^{-1}) in the pod of the investigated plant.

The statutory limit set for As content in fruit, crops and vegetables is 1.0 µg g^{-1} (fresh weight) (Carbonell-Barrachina, Burlo, Lopez, & Martínez-Sánchez, 1999), therefore the As concentrations in edible parts of the broad bean (pod and bean) in this study were below this maximum limit. However, it is noted that both stems and leaves from the plant may form part of the diet of cattle and this other-animal food source may therefore allow arsenic to enter the human food chain.

3.3 Plant ‘available’ arsenic

To assess plant available arsenic in soils a standard BCR protocol was applied. Because the value of As has not been certified in BCR 701(CRM used to validate the sequential extraction procedure in soil) for use with a sequential extraction procedure (three steps), the value of other metals (including Cd, Cr, Cu, Ni and Zn) were measured and compared in order to validate the applied procedure. However, Alvarez *et al.* (2012) have measured the As concentration in BCR 701 using the sequential extraction procedure and they performed just steps 1 and 2. The concentrations of As in steps 1 and 2 were 2.1 ± 0.1 µg As g^{-1} (mean ± SD) and 17.6 ± 0.4 µg As g^{-1} (mean ± SD), respectively. These results are comparable to Alvarez et al’s study who found the concentrations of arsenic in step one was 2.1 ± 0.1 µg As g^{-1} (mean ± SD) and in step two was 21.2 ± 1.1 µg As g^{-1} (mean ± SD). A standard sediment reference material BCR-701, (European Community Bureau of Reference) was analysed using 3 replicates. The extractable contents and recoveries in each step of the standard reference material used are presented in Table 5. The recoveries of all metals from step1, except for Zn (80%) and Cd (81%) were higher than 90%, ranging from 90 to 113%. The recoveries of all metals from step 2 apart from Cd (72%) were between 90 % and 108%. For step 3, the recoveries of all metals except for Cu (72%) ranged from 91 % to 96%. It is noted that the total element contents is not an adequate means to assess the mobility of trace elements, their availability and eco-toxicity to plants whereas the determination of specific chemical forms or types of binding are useful tools for this purpose (De Gregori, Fuentes, Olivares, & Pinochet, 2004). Although a phosphoric acid procedure was applied to extract the arsenic species in the studied Dokan soil, with inorganic As ^{V} being a major species, this is not an indication that the total arsenic

contents are mobile and/or that the broad bean plant can take up all of the As species. The maximum amount of As which could potentially be mobilised in the soil under study (pseudo-total content) was 5.32 µg g^{-1}. Overall only some 17 % of As in the analysed Dokan soil was mobilized using the BCR procedure in contrast to the 91.4 % of arsenic from BCR 701 that was mobilised using the same BCR sequential extraction, across all three stages. In this work, it was observed that 11 % As (0.6µgg^{-1}) was present in an easily acid-soluble extractable form. It is well-documented that results obtained with the reagent from this step may provide good correlation with plant uptake (Gleyzes, Tellier, & Astruc, 2002). The 'reducible fraction' extracted from the soil under study gave 4 % As (0.22 µgg^{-1}), which is considered mostly bound to the structure of primary and secondary minerals. In comparison, the BCR-701 material released 74 % of its As with this fraction. The arsenic associated with the 'organic materials and sulfides' fraction in the Dokan soil studied was only 2 % (0.1 µg g^{-1}). A soluble trace amount bound to this phase can be released by the degradation of organic matter under oxidizing conditions. The released organic fraction in the oxidisible fraction is not considered to be plant available (bioavailable) because being associated with stable humic substances there is only a slow release of small quantities of metals (Filgueiras, Lavilla, & Bendicho, 2002). Arsenic in the studied soil was found to be bound mainly within the residual fraction (83%, 4.27 µg g^{-1}). Table 5 also places on record the extraction efficiencies for the major metallic species Cd, Cr, Cu, Ni, Zn, Fe and Mn present in the Dokan soil using the same 3 step BCR sequential extraction procedure. The profiles of availability are particularly different to the BCR lake CRM but demonstrate that, of the total level measured, elements like Cd, Cu, Ni, and Zn are more readily

available in step 1 and 2; the acid soluble/plant available fraction and the reducible fraction. The ‘oxidisable-bound to organic matter’ etc. fraction contributed less.

It is interesting to note that, while the readily extractable As from the Dokan soil was only nearly 0.6 µg g^{-1}; (~11 % of the 5.32 µg g^{-1} aqua regia total), the levels of As encountered with the root system were nearly four times this available quantity on a mass-for-mass basis. Despite this almost preconcentrating effect at the root system the plants strategy to deal with the arsenic-loading is suggested in the distribution and speciation of arsenic within the ‘disposable’ plant tissue; with totals showing that leaf > pod > stem and with protection of the bean and a restriction at the root.

One way of presenting and interpreting the findings of this survey may be seen in the Schematic diagram figure 3. Here, the distribution of total arsenic and arsenic species from soil, through root, stem, leaf, pod and finally bean is shown both in concentration terms and fractional contribution for each compartment. It is noted that

i) the inorganic As ^{V} content decreases from the root to the bean both in absolute concentration and as a fraction.

ii) the Arsenic ^{III} content as a fraction remains approximately constant except for that in the bean. It decreases in absolute concentration throughout and finally as a fraction.

iii) the organo-arsenic content in the plant compartments is virtually constant and suggests it is transported from the root system but is not a later major metabolite of the plant, i.e. possibly made around the root system and then absorbed. The exclusion of inorganic arsenic to the bean results in an ‘organic-to-inorganic’ ratio of 2:1.

iv) the route taken to the leaf for As is selective showing increased absolute concentration and fractional content suggesting this is a means of disposing of inorganic As (As ^{III} and As ^{V}). The organo-arsenic content of the leaf, however, is approximately the same as other compartments in absolute concentration terms and lower as a fraction.

3.4 Conclusion

Chemical speciation of As in food is important as the toxicity varies greatly between different species. This study provides useful information for a better understanding of the uptake and distribution of As species in different parts of the broad bean plant (root, stem, leaf, pod and bean) and its coping strategy for this less desirable element. In addition, this study includes the measurement impact that available As from the soil it is grown in has on the plants. The root system of broad bean plants accumulates the highest level of total As compared with the other compartments from the same plant. The As speciation in different 'compartments' of the broad bean was dominated by inorganic forms (50.85-94.31%) with the exception of the bean itself, where organic As (DMA and MMA) was predominant accounting for 68 % of the total As found in the bean. The major As species found in the root, stem, leaf and pod was inorganic As ^{V} while in the bean it was MMA. In addition, the highest concentrations of both inorganic As ^{V} and As ^{III} in the different parts of the broad bean plant were found in the root. However, the highest concentration of DMA was found in the pod while the highest level of MMA was found in the leaf.

Acknowledgment

A special thanks to The Ministry of Higher Education and Scientific Research in the Kurdistan region of Iraq for financing the PhD studentship.

Referencies

Álvarez, M. A., & Carrillo, G. (2012). Simultaneous determination of arsenic, cadmium, copper, chromium, nickel, lead and thallium in total digested sediment samples and available fractions by electrothermal atomization atomic absorption spectroscopy (ET AAS). *Talanta, 97*(0), 505-512.

Austruy, A., Wanat, N., Moussard, C., Vernay, P., Joussein, E., Ledoigt, G., & Hitmi, A. (2013). Physiological impacts of soil pollution and arsenic uptake in three plant species: Agrostis capillaris, Solanum nigrum and Vicia faba. *Ecotoxicology and Environmental Safety, 90*, 28-34.

B'hymer, C., & Caruso, J. (2004). Arsenic and its speciation analysis using high-performance liquid chromatography and inductively coupled plasma mass spectrometry. *Journal of Chromatography A, 1045*(1), 1-13.

Baig, J. A., Kazi, T. G., Arain, M. B., Shah, A. Q., Sarfraz, R. A., Afridi, H. I., Kandhro, G. A., Jamali, M. K., & Khan, S. (2009). Arsenic fractionation in sediments of different origins using BCR sequential and single extraction methods. *Journal of Hazardous Materials, 167*(1–3), 745-751.

Bhattacharya, P., Welch, A. H., Stollenwerk, K. G., McLaughlin, M. J., Bundschuh, J., & Panaullah, G. (2007). Arsenic in the environment: biology and chemistry. *Science of The Total Environment, 379*(2), 109-120.

Bundschuh, J., Nath, B., Bhattacharya, P., Liu, C.-W., Armienta, M. A., Moreno López, M. V., Lopez, D. L., Jean, J.-S., Cornejo, L., Lauer Macedo, L. F., & Filho, A. T. (2012).

Arsenic in the human food chain: the Latin American perspective. *Science of The Total Environment, 429*(0), 92-106.

Carbonell-Barrachina, A., Burlo, F., Lopez, E., & Martínez-Sánchez, F. (1999). Arsenic toxicity and accumulation in radish as affected by arsenic chemical speciation. *Journal of Environmental Science & Health Part B, 34*(4), 661-679.

Chen, R., Smith, B. W., Winefordner, J. D., Tu, M. S., Kertulis, G., & Ma, L. Q. (2004). Arsenic speciation in Chinese brake fern by ion-pair high-performance liquid chromatography–inductively coupled plasma mass spectroscopy. *Analytica Chimica Acta, 504*(2), 199-207.

Cuong, D. T., & Obbard, J. P. (2006). Metal speciation in coastal marine sediments from Singapore using a modified BCR-sequential extraction procedure. *Applied Geochemistry, 21*(8), 1335-1346.

De Gregori, I., Fuentes, E., Olivares, D., & Pinochet, H. (2004). Extractable copper, arsenic and antimony by EDTA solution from agricultural Chilean soils and its transfer to alfalfa plants (Medicago sativa L.). *Journal of Environmental Monitoring, 6*(1), 38-47.

Commission Directive (22.5.2003). *Official Journal of the European Union, L 126/34, EN,* .

EFSA. (2014). Dietary exposure to inorganic arsenic in the European population.*EFSA Journal 2014;12(3):3597, 68 pp. doi:10.2903/j.efsa.2014.3597*.

Filgueiras, A. V., Lavilla, I., & Bendicho, C. (2002). Chemical sequential extraction for metal partitioning in environmental solid samples. *Journal of Environmental Monitoring, 4*(6), 823-857.

Foster, S., Maher, W., Krikowa, F., & Apte, S. (2007). A microwave-assisted sequential extraction of water and dilute acid soluble arsenic species from marine plant and animal tissues. *Talanta, 71*(2), 537-549.

Garcia-Manyes, S., Jiménez, G., Padro, A., Rubio, R., & Rauret, G. (2002). Arsenic speciation in contaminated soils. *Talanta, 58*(1), 97-109.

Giral, M., Zagury, G. J., Deschênes, L., & Blouin, J.-P. (2010). Comparison of four extraction procedures to assess arsenate and arsenite species in contaminated soils. *Environmental Pollution, 158*(5), 1890-1898.

Gleyzes, C., Tellier, S., & Astruc, M. (2002). Fractionation studies of trace elements in contaminated soils and sediments: a review of sequential extraction procedures. *TrAC Trends in Analytical Chemistry, 21*(6), 451-467.

Gupta, V. K., Nayak, A., Agarwal, S., Dobhal, R., Uniyal, D. P., Singh, P., Sharma, B., Tyagi, S., & Singh, R. (2012). Arsenic speciation analysis and remediation techniques in drinking water. *Desalination and Water Treatment, 40*(1-3), 231-243.

Heitkemper, D. T., Vela, N. P., Stewart, K. R., & Westphal, C. S. (2001). Determination of total and speciated arsenic in rice by ion chromatography and inductively coupled plasma mass spectrometry. *Journal of Analytical Atomic Spectrometry, 16*(4), 299-306.

Jain, C. K., & Ali, I. (2000). Arsenic: occurrence, toxicity and speciation techniques. *Water Research, 34*(17), 4304-4312.

Jedynak, L., Kowalska, J., Harasimowicz, J., & Golimowski, J. (2009). Speciation analysis of arsenic in terrestrial plants from arsenic contaminated area. *Science of The Total Environment, 407*(2), 945-952.

Jedynak, L., Kowalska, J., Kossykowska, M., & Golimowski, J. (2010). Studies on the uptake of different arsenic forms and the influence of sample pretreatment on arsenic speciation in White mustard (< i> Sinapis alba</i>). *Microchemical Journal, 94*(2), 125-129.

Koch, I., Wang, L., Ollson, C. A., Cullen, W. R., & Reimer, K. J. (2000). The predominance of inorganic arsenic species in plants from Yellowknife, Northwest Territories, Canada. *Environmental Science &Technology, 34*(1), 22-26.

Kuehnelt, D., Lintschinger, J., & Goessler, W. (2000). Arsenic compounds in terrestrial organisms. IV. Green plants and lichens from an old arsenic smelter site in Austria. *Applied Organometallic Chemistry, 14*(8), 411-420.

Lou, C., Liu, W., & Liu, X. (2014). Quantitative analysis of arsenic speciation in guano and ornithogenic sediments using microwave-assisted extraction followed by high-performance liquid chromatography coupled to hydride generation atomic fluorescence spectrometry. *Journal of Chromatography B, 969*(0), 29-34.

Mandal, B. K., & Suzuki, K. T. (2002). Arsenic round the world: a review. *Talanta, 58*(1), 201-235.

Mayorga, P., Moyano, A., Anawar, H. M., & Garcia‐Sanchez, A. (2013). Uptake and Accumulation of Arsenic in Different Organs of Carrot Irrigated with As‐Rich Water. *CLEAN–Soil, Air, Water, 41*(6), 587-592.

Meharg, A. A., & Hartley-Whitaker, J. (2002). Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. *New Phytologist, 154*(1), 29-43.

Muñoz, O., Diaz, O. P., Leyton, I., Nuñez, N., Devesa, V., Súñer, M. A., Vélez, D., & Montoro, R. (2002). Vegetables collected in the cultivated Andean area of northern Chile: total and inorganic arsenic contents in raw vegetables. *Journal of Agricultural and Food Chemistry, 50*(3), 642-647.

Petrusevski, B., Sharma, S., Schippers, J. C., & Shordt, K. (2007). Arsenic in drinking water. *Delft: IRC International Water and Sanitation Centre, 17*(1).

Rauret, G., López-Sánchez, J.-F., Sahuquillo, A., Barahona, E., Lachica, M., Ure, A., Davidson, C., Gomez, A., Lück, D., & Bacon, J. (2000). Application of a modified BCR sequential extraction (three-step) procedure for the determination of extractable trace metal contents in a sewage sludge amended soil reference material (CRM 483), complemented by a three-year stability study of acetic acid and EDTA extractable metal content. *Journal of Environmental Monitoring, 2*(3), 228-233.

Sanz, E., Muñoz-Olivas, R., & Cámara, C. (2005). A rapid and novel alternative to conventional sample treatment for arsenic speciation in rice using enzymatic ultrasonic probe. *Analytica Chimica Acta, 535*(1–2), 227-235.

Signes-Pastor, A., Mitra, K., Sarkhel, S., Hobbes, M., Burló, F., De Groot, W., & Carbonell-Barrachina, 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*(20), 9469-9474.

Smith, E., Juhasz, A., & Weber, J. (2009). Arsenic uptake and speciation in vegetables grown under greenhouse conditions. *Environmental Geochemistry and Health, 31*(1), 125-132.

Sun, G.-X., Williams, P. N., Zhu, Y.-G., Deacon, C., Carey, A.-M., Raab, A., Feldmann, J., & Meharg, A. A. (2009). Survey of arsenic and its speciation in rice products such as breakfast cereals, rice crackers and Japanese rice condiments. *Environment International, 35*(3), 473-475.

Thomson, D., Maher, W., & Foster, S. (2007). Arsenic and selected elements in inter‐tidal and estuarine marine algae, south‐east coast, NSW, Australia. *Applied Organometallic Chemistry, 21*(6), 396-411.

Ure, A., & Davidson, C. (2002). Chemical speciation in soils and related materials by selective chemical extraction. *Chemical Speciation in the Environment,* (pp 265-300), Second Edition.

Warren, G., Alloway, B., Lepp, N., Singh, B., Bochereau, F., & Penny, C. (2003). Field trials to assess the uptake of arsenic by vegetables from contaminated soils and soil remediation with iron oxides. *Science of The Total Environment, 311*(1), 19-33.

WHO. (2006). *Guidelines for drinking-water quality: First addendum to third edition, Recommendations* (Vol. 1): World Health Organization.

WHO. (2011). *Guidelines for Drinking-water Quality* (Fourth ed.). Geneva, Switzerland

Xu, J., & Thornton, I. (1985). Arsenic in garden soils and vegetable crops in Cornwall, England: implications for human health. *Environmental Geochemistry and Health, 7*(4), 131-133.

Zhang, T., Shan, X., & Li, F. (1998). Comparison of two sequential extraction procedures for speciation analysis of metals in soils and plant availability. *Communications in Soil Science & Plant Analysis, 29*(7-8), 1023-1034.

Arsenical route system	Compartment	Total As in compartment shown µg g^{-1}	As^{III} µg g^{-1}	As^{V} µg g^{-1}	Total inorganic As µg g^{-1}	DMA µg g^{-1}	MMA µg g^{-1}	Total organic arsenic µg g^{-1}
Protected	Bean	0.133	0.009 (7 %)	0.024 (21 %)	0.033 (28 %)	0.022 (19 %)	0.055 (49 %)	0.077 (68 %)
Disposable	Pod	0.258	0.049 (21 %)	0.082 (35 %)	0.131 (56 %)	0.070 (30 %)	0.027 (12 %)	0.097 (42 %)
Disposable	Leaf	0.489	0.091 (22%)	0.232 (56 %)	0.323 (78 %)	<LOD	0.101 (24 %)	0.101 (24 %)
Conduit	Stem	0.212	0.035 (18 %)	0.062 (33 %)	0.097 (51 %)	0.050 (26 %)	0.044 (23 %)	0.099 (49 %)
Restricted system	Root	2.07	0.324 (16 %)	1.585 (78 %)	1.909 (94 %)	0.041 (2 %)	0.068 (3 %)	0.109 (5 %)
Available fraction		0.6 (11 %)						
	Soil	5.32	0.564 (10 %)	4.584 (90 %)	5.148 (100 %)	<LOD	<LOD	<LOD

Fig. 1 Schematic diagram of the distribution of total arsenic, the variesAs species and possible metabolite routes for different parts of the broad bean plant and its soil.

Table 1 ICP-MS and HPLC operating conditions used for the determination of total arsenic and arsenic speciation in sample digests and extracts.

ICP-MS	X Series 2 (Thermo Scientific)	
	Peristaltic pump speed ml min^{-1}	1.1
	Nebulizer type	V-groove
	Spray chamber	Sturman-Masters
	Radio frequency power (W)	1350
Gas flows/L min^{-1}	Coolant	13
	Auxiliary	0.75
	Nebulizer	1.0
H_2 addition	Gas flow (mL. min^{-1}) 7 % H_2 in He	3.6
(m/z)	As	75
	In	115
	Ir	193
Dwell time (ms)	ICP-MS	10
	HPLC-ICP-MS	100
HPLC conditions for 1 % HNO_3 extracts		
Parameters	Experimental conditions	
Column dimension	250 x 4.6 mm	
Guard column dimension	50 x 4.6 mm	
Packing material	Hamilton resin PRP-X100, 10 µm particle size	
Eluent flow rate	1.1 mL min^{-1}	
Sample loop	20 µL	
Competitive counter ion	Ammonium dihydrogen phosphate ($NH_4H_2PO_4$)	
Mobile phases	20mM $NH_4H_2PO_4$, pH 6.0	

Table 2 Certified reference material analyses for total arsenic; all experimental values are given in µg g^{-1}, mean ± standard deviation (n=3)

CRM	Sample type	Certified value (Arsenic)	Experimental value obtained	Extraction efficiency %
Loam soil (ERM-CC141)	Soil	*7.5 ± 1.4	*7.33 ± 0.42	98
GBW10015 Spinach	Plant	0.230 ± 0.03	0.249 ± 0.008	108

*Aqua regia extractable content

Table 3 The concentration of As in crop soil, plant samples (µg As g^{-1} dry mass basis), and in irrigation water (µg As L^{-1}) mean ± SD (n=3)

Matrix	Total As (µg As g^{-1})
Crop soil	5.32 ± 0.420
Broad bean –root	2.065 ± 0.032
Broad bean –stem	0.212 ± 0.006
Broad bean –leaf	0.489 ±0.04
Broad bean –pod	0.258 ± 0.017
Broad bean –bean	0.133 ± 0.009
Water	1.06 ± 0.08 µgL^{-1}

Table 4 Results of analysis for arsenic speciation in the broad bean plant and soil dry weight (n=3)

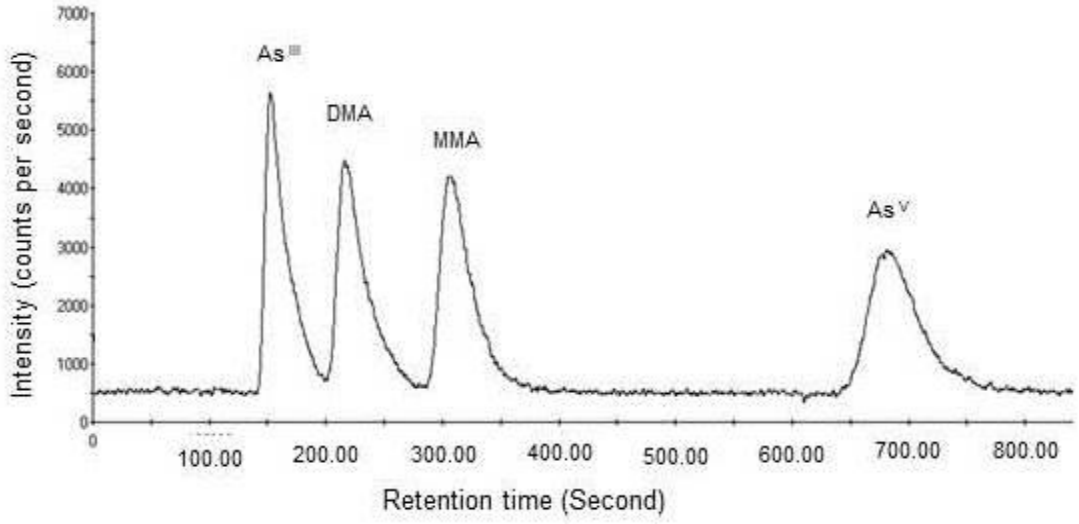
Vegetable broad bean	Microwave assist digestion µg g^{-1}± SD	As^{III} µg g^{-1} ± SD	DMA µg g^{-1} ± SD	MMA µg g^{-1} ± SD	As^{V} µg g^{-1} ± SD	Total arsenic in residue µg g^{-1} ± SD	Total arsenic in extracts µg g^{-1} ± SD	Efficiency of total species extraction %
Root	2.065 ± 0.033	0.324 ± 0.014	0.041 ± 0.007	0.068 ± 0.004	1.585 ± 0.087	0.041 ± 0.001	2.024 ± 0.175	98
Stem	0.212 ± 0.006	0.035 ± 0.003	0.050 ± 0.004	0.044 ± 0.003	0.062 ± 0.005	0.030 ± 0.001	0.191 ± 0.008	90
Leaf	0.489 ± 0.04	0.091 ± 0.006	<LOD	0.101 ± 0.001	0.232 ± 0.011	0.072 ± 0.005	0.415 ± 0.001	85
Pod	0.258 ± 0.017	0.049 ± 0.004	0.070 ± 0.006	0.027 ± 0.002	0.082 ± 0.002	0.032 ± 0.001	0.232 ± 0.006	90
Bean	0.133 ± 0.009	0.009 ± 0.001	0.022 ± 0.003	0.055 ± 0.003	0.024 ± 0.002	0.016 ± 0.001	0.114 ± 0.011	86
Spinach GBW10015	0.249± 0.008	0.107 ± 0.008	<LOD	0.034 ± 0.002	0.113 ± 0.011	<LOD	0.264 ± 0.018	106

Soil	Aqua regia (pseudo total) µg g^{-1} ± SD	As^{III} µg g^{-1} ± SD	DMA µg g^{-1} ± SD	MMA µg g^{-1} ± SD	As^{V} µg g^{-1} ± SD	Total arsenic in residue µg g^{-1} ± SD	Total arsenic in extracts (using phosphate) µg kg^{-1} ± SD	Efficiency of extraction %
Studied soil	5.32 ± 0.420	0.564 ± 0.001	<LOD	<LOD	4.584 ± 0.220	<LOD	5.300 ± 0.30	99.62
Loam soil ERM-CC141	7.33 ± 0.420	1.186 ± 0.003	<LOD	0.087 ± 0.010	5.873± 0.030	<LOD	7.170 ± 0.32	97.81
BCR 701	23.77± 1.840	8.450 ± 0.340	<LOD	<LOD	17.170 ± 0.110	<LOD	26.00 ± 0.69	109

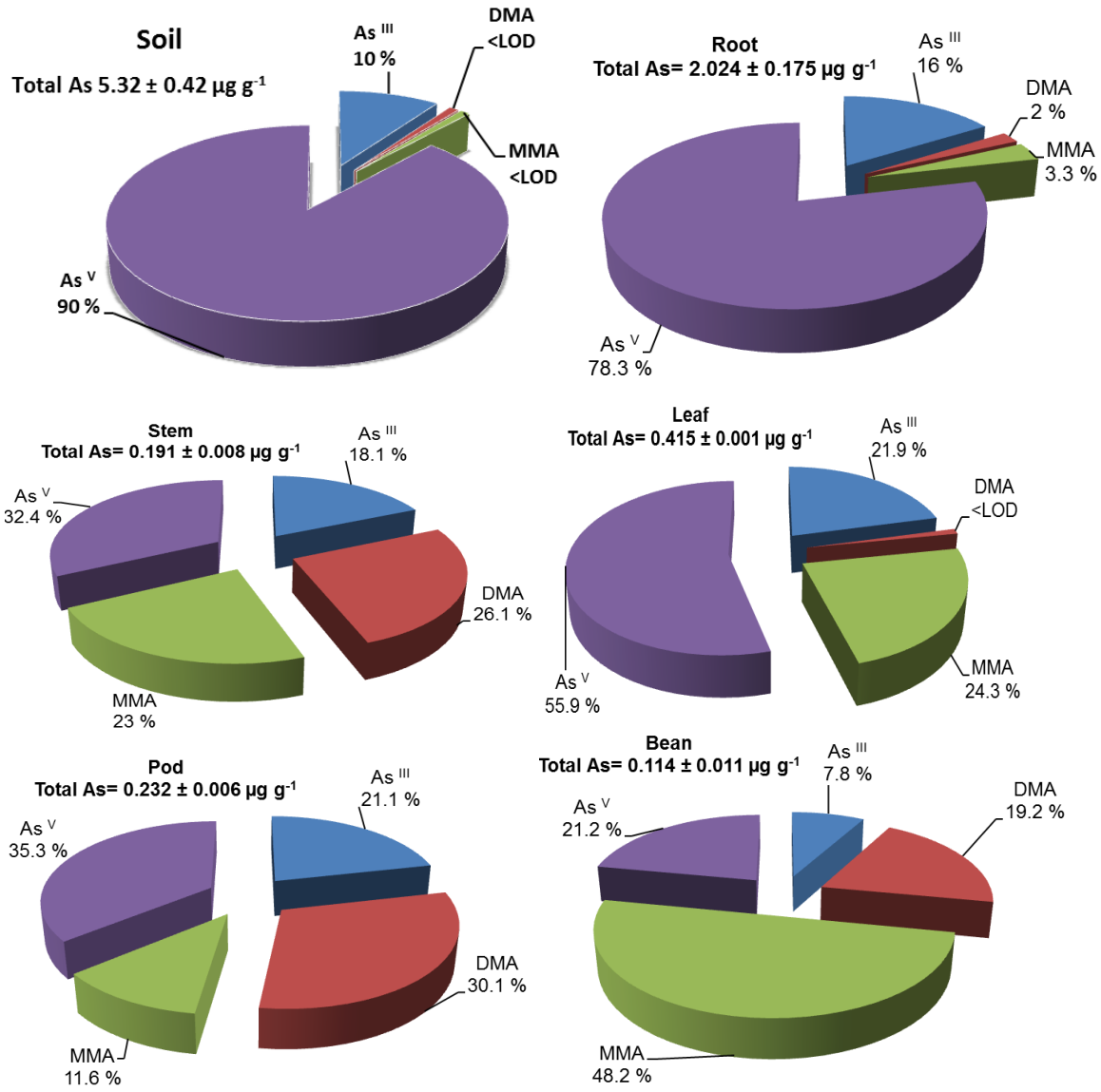
Table 5 Results of analysis of CRM (BCR 701-lake sediment) and soil under study using BCR-sequential extraction; mean± standard deviation (n=3)

Step of extraction	Element	Measured value(BCR-701-Lake sediment) µg g^{-1}	Certified value (BCR-701-Lake sediment) µg g^{-1}	Recovery from BCR %	Dokan Soil (this study) µgg^{-1}	% of total in Dokan soil based upon aqua-regia digest
Step 1						
	Cd	5.95 ± 0.11	7.34 ± 0.35	81	0.19 ± 0.005	63
	Cr	2.57 ± 0.22	2.26 ± 0.16	113	0.6 ± 0.01	1.1
	Cu	53.9 ± 3.85	49.3 ±1.7	109	6 ± 0.6	17
	Ni	13.89 ± 3.6	15.4 ± 0.9	91	22.5 ± 0.46	35
	Zn	165 ± 5	205 ± 6	80	2.6 ± 0.21	3.6
	Fe	140 ± 3	Not certified	-	2.89 ± 0.26	0.02
	Mn	166 ± 0.7	Not certified	-	161 ± 4.7	47
	As	2.1 ± 0.1	Not certified	-	0.6 ± 0.03	11
	As % of total	8.4	-	-	11	-
Step 2						
	Cd	2.72 ± 0.2	3.77 ± 0.28	72	0.088 ± 0.006	29
	Cr	49.4 ± 0.67	45.7 ± 2	108	2.15 ± 0.01	4
	Cu	112 ± 1.4	124 ± 3	90	24.3 ± 0.13	69
	Ni	26.3 ± 0.31	26.6 ± 1.3	99	11.44 ± 0.18	18
	Zn	103 ± 0.77	114 ± 5	90	30 ± 0.2	41
	Fe	6990 ± 46	Not certified	-	986 ±28.3	7.2
	Mn	121.6 ± 2.1	Not certified	-	144 ± 7	42
	As	17.6 ± 0.4	Not certified	-	0.22 ± 0.01	4
	As % of total	74	-	-	4	-
Step 3						
	Cd	0.26 ± 0.03	0.27 ± 0.06	96	0.007 ± 0.001	2.3
	Cr	136 ± 14	143 ± 7	95	5.65 ± 0.11	11
	Cu	39.5 ± 1	55 ± 4	72	0.54 ± 0.04	1.5
	Ni	14.6 ± 1.9	15.3 ± 9	95	10.45 ± 0.23	16
	Zn	42 ± 2	46 ± 4	91	0.7 ± 0.05	1
	Fe	944.4 ± 75.8	Not certified	-	5.67 ± 0.12	0.04
	Mn	19.3 ± 0.35	Not certified	-	14.49 ± 0.31	4.2
	As	2.2 ± 0.17	Not certified	-	0.1 ± 0.003	2
	As % of total	9	-		2	-
Aqua regia extractable						
	Cd	13 ± 0.16	11.7 ± 1 [a]	111	0.3 ± 0.03	-
	Cr	227.3 ± 2.5	272 ± 20 [a]	84	52.5 ± 5.1	-
	Cu	281 ± 1.8	275 ± 0.013 [a]	102	35.1 ± 2.8	-
	Ni	86 ± 1	103 ± 4[a]	83	65.1 ± 6.4	-
	Zn	410 ± 3	454 ± 19[a]	90	72.4 ± 1.7	-
	Fe	18597 ± 221	Not certified	-	13540 ± 900	-
	Mn	486 ± 5	Not certified	-	343 ± 6.7	-
	As	23.8 ± 1.8	Not certified	-	5.32 ± 0.42	-

[a] Indicative value



Supplementary Fig. 1 The speciation of arsenic using HPLC-ICP-MS. As^{III}, DMA, MMA and As^{V} 50 µg L^{-1}. The operating conditions are shown in Tables 1.



Supplementary Fig. 2 Arsenic in soil; root, stem, leaf, bean and pod of the broad bean plant.