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Total arsenic and arsenic speciation in indigenous food stuffs

by

Bashdar Abuzed Sadee

A thesis submitted to Plymouth University
in partial fulfilment for the degree of

Doctor of philosophy

School of Geography, Earth and Environmental Sciences
Faculty of Science and Engineering

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Work submitted for this research degree at Plymouth University has not formed part of any other degree either at Plymouth University or at another establishment.

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Total arsenic and arsenic speciation in indigenous food stuffs

Bashdar Sadee

Abstract

The properties of an element are highly dependent on its chemical form, it's called elemental speciation. This study evaluates the arsenic species found in a range of food stuffs together with growing environments and toxicity issues. Total arsenic concentrations in fish tissue and vegetable crops were determined by ICP-MS following microwave-assisted acid digestion using nitric acid/hydrogen peroxide, trypsin and cellulase enzymatic extraction procedures. The extracted arsenic species were then quantified using HPLC-ICP-MS. A dilute nitric acid (1 % (v/v)) digestion procedure was also used to extract arsenic species from rice and the different parts (root, skin, stem, leaf and grain) of a range of plant crops. The study was extended to include the aqua-regia extractable arsenic content of the soils collected from the area where the plants had been cultivated in the Kurdistan region of Iraq. Irrigation water was also investigated, but found to contain low levels of arsenic.

An anion-exchange HPLC-ICP-MS method was developed, using sulphate and phosphate, for the separation and quantification of AsB, MMA, DMA, $InAs^{III}$ and $InAs^{V}$. The results obtained for fish samples were in the range of 3.53-98.80 μg g^{-1} (dry weight) with non-toxic AsB being the predominant species. The $InAs^{V}$ concentration was in the range of 0.1-1.19 μg g^{-1} for all fish species except for the John Dory which was below the limit of detection (0.027 μg g^{-1}).

Total arsenic, arsenic species, and total multi-elements (including Ag, Al, B, Ba, Be, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Si, Ti, V and Zn) were determined in rice samples from Kurdistan, Iraq and other regions of geographical origin. The transport of arsenic from the soil and irrigation water into roots, stem, leaf and subsequently into the grain or bean of the plants is important when assessing the potential health risks from food crops. For the soil sample, $InAs^{V}$ was found to be the major species with smaller quantities of $InAs^{III}$. After applying a full BCR sequential extraction procedure to the soils, it was found that 7.87 - 21.14 % of the total arsenic was present in an easily acid-soluble extractable form.

Finally, a novel method was developed to measure total arsenic and arsenic species associated with vegetative DNA. In rice plant, it was found that $InAs^{V}$ incorporated within the DNA molecule in which it could replace phosphate. It was also found that the concentration of $InAs^{V}$ associated with DNA molecule decreased with decreasing total arsenic in the rice plant from the root to the leaf.

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I would like to express my deepest gratitude and sincerest respect to my supervisors Prof Steve Hill and Dr Mike Foulkes for their countless advice, encouragement and motivation throughout this project. This project could not have been completed without the guidance and support of my supervisor team.

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Chapter one

Introduction

1. Arsenic species in food

Elemental speciation is well established as an important discipline in analytical chemistry. Arsenic (As) is a ubiquitous element in the environment having been introduced *via* both natural and anthropogenic routes[1]. It can be found in the atmosphere, the pedosphere, the hydrosphere and the biosphere. In addition to the biological mechanisms, including microbiological processes, physico-chemical processes such as oxidation-reduction, precipitation/solubilisation, and adsorption/desorption determine the biogeochemical behaviour of arsenic [2]. Routine determination of the arsenic content of a sample can be achieved by measurement of the total arsenic using a quantitative procedure[3]. Although arsenic has the reputation of being a toxic element, it also well established that its toxicity critically depends on the chemical form in which it exists and that inorganic species, arsenite ($InAs^{III}$) and arsenate ($InAs^{V}$), are classified as more toxic than organo arsenic compounds[4]. The oxidation state of organic arsenic forms also changes the toxicity, so that trivalent methylated forms are likely to be more toxic than previously thought[5]. Arsenobetaine (AsB) is the major arsenic species in fish and other seafood, and arsenocholine (AsC) is considered as a precursor of AsB, which is the end product of marine arsenic metabolism[6]. These are not considered toxic compounds[7]. Other arsenicals such as monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), are less toxic than inorganic arsenic[4], and together with trimethylarsine oxide are often found in marine organisms, together with many arsenosugars and arsenic containing lipids in the case of marine algae and seaweed [8, 9].

The accumulation of arsenic by plants and fauna of marine origin is relatively high compared to that in other food sources[10, 11], therefore, many arsenic speciation studies have focused on these types of food. Even though the majority of ingested arsenic (75%) is contributed by fish and shellfish, it generally represents only a small percentage (2%) of the daily dietary intake [12]. Seaweeds used in human foods have a total arsenic content of between 0.031-149 μg g^{-1} and inorganic arsenic between <0.014 to 117 μg g^{-1} [13]. In fish, the arsenic contents vary according to the species of fish concerned; average concentrations vary between 5 and 100 μg g^{-1} [11], although conger and dogfish may contain elevated values of 100 to 250 μg As g^{-1}. In flat fish the values vary between 10 to 60 μg g^{-1} [14]. Nevertheless it has been confirmed that these elevated concentrations in seafood cause little risk to health, since 80-90 % of arsenic is in the organic form (AsB, AsC, arsenosugars, and arsenolipids) [7] . Rattanachongkiat *et al.* [15] in their study of arsenic speciation in sardines demonstrated that among 95% of arsenic extracted (5.8 μg g^{-1} dry weight), 77% was AsB, 17 % DMA and 6% inorganic arsenic.

Because of its widespread nature, arsenic exists in all natural waters and concentrations of arsenic between <0.5 μg L^{-1} and more than 5000 μg L^{-1} have been reported. The World Health Organisation (WHO) recommended threshold value for arsenic in drinking water is 10 μg L^{-1} [16]. However, freshwater usually contains less than 10 μg L^{-1} and frequently less than 1.0 μg L^{-1} of arsenic. In some cases, much higher concentrations in groundwater have been monitored. In such areas, often more than 10% of wells are affected (sometimes up to 90%), with arsenic levels exceeding 50 μg L^{-1}. It has been reported that some countries such as Argentina, Chile, Mexico, China, and Hungary and more recently in West Bengal (India), Bangladesh and Vietnam have high levels of arsenic in ground water [17]. The inorganic arsenic species, $InAs^{III}$ and $InAs^{V}$, are the predominant species found in water[18-20], although the concentration of each

species varies. A study of thermal waters in New Zealand for example [21], found concentrations up to 8.5 mg As L^{-1} with the trivalent arsenic form being the dominant species and contributing up to 90 % of total arsenic. The concentration of arsenic in seawater is less than 2 µg L^{-1}. Baseline concentrations of arsenic in unpolluted surface water and groundwater typically range between 1-10 µg L^{-1} [21]. The weathering and dissolution of arsenic-bearing rocks, minerals and ores also lead to occurrence of arsenic in water [22], and the arsenic cycle through the groundwater compartment has an important impact on human toxicology [23]. It has been concluded by the International Agency for Research on Cancer [24] that there is sufficient evidence in humans to suggest that arsenic in drinking-water causes cancers of the urinary bladder, lung and skin. According to a study that has been conducted in West Bengal, 94 % of those people exposed to high levels of arsenic in drinking water had leukomelanosis and hyperkeratosis and they can lead to skin cancer [22.]

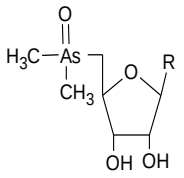
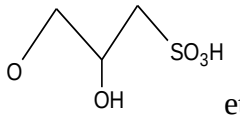
1.1 Chemistry of arsenic

Arsenic is a metalloid which ranks 20th in natural abundance and 12th in the human body[25]. It has been used as a medicine, and it has also been utilized in various fields such as electronics, agriculture, livestock, metallurgy, industry[21], pesticides[26], and fertilizers[27]. More than 245 minerals contain arsenic, the most important arsenic bearing minerals are orpiment (As_2S_3), realgar (AsS), mispickel (FeAsS), loelling-ite ($FeAs_2$), niccolite (NiAs), cobaltite (CoAsS), tennantite ($Cu_{12}As_4S_{13}$), and enargite (Cu_3AsS_4) [28]. The origins of high arsenic concentrations in the environment are through volcanic eruption and other natural processes, and human activities such as the disposal of industrial waste chemicals, the smelting of arsenic bearing minerals, the burning of fossil fuels, and the application of arsenic compounds in many products over the past hundred years [29]. Mining operations contribute high level of arsenic and other heavy metals which are mobilized in the soil and then accumulated in the food chain via

plants[30-32]. Arsenic exists in four oxidation states, +V (arsenate), +III (arsenite), 0 (arsenic), and –III (arsine and arsenide). The most common species in nature are the two highest oxidation states, while the two lowest are rare [33]. Apart from arsenite, arsenate and their methylated derivatives, there are also other compounds such as "fish arsenic" (AsB and AsC), and arsenosugars; all of which are compounds of environmental interest. Figure 1.1 shows the molecular formulae of some common arsenic compounds.

Arsenous acid (arsenite) $InAs^{III}$	$As(OH)_3$
Arsenic acid (arsenate) $InAs^{V}$	$AsO(OH)_3$
Monomethylarsonic acid MMA(V)	$CH_3AsO(OH)_2$
Dimethylarsinic acid DMA(V)	$(CH_3)_2AsO(OH)$
Trimethylarsine oxide TMAO [As^{V}]	$(CH_3)_3AsO$
Arsenobetaine AsB [As^{V}]	$(CH_3)_3As^{+}CH_2COO^{-}$
Arsenocholine AsC [As V]	$(CH_3)_3As^{+}CH_2CH_2OH$
Trimethylarsine TMA [As III]	$(CH_3)_3As$

Arsenosugars AsRbF:

Arsenosugars  where R = $OCH_2CH(OH)CH_2R$ or  etc.

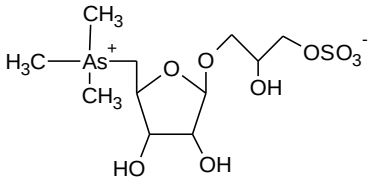
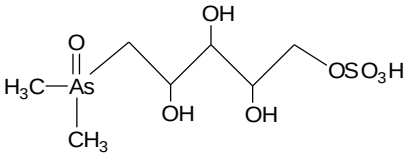
And  and derivatives 

Figure 1.1 Examples of some common arsenic species.

1.2 Toxicity and health hazards

Toxicity of arsenic in humans depends on chemical speciation and the oxidation state of the arsenic [34, 35]. It is considered that the toxicity of arsenic increases in the order of AsB; arsenosugar, dimethylarsinic acid; monomethylarsonic acid, arsenate and arsenite [36]. To humans, trivalent arsenic is about 60 times more toxic than the oxidized pentavalent state, because the $InAs^{III}$ can react with sulfydryl groups, whereas the arsenate does not [37]. Inorganic arsenic compounds are about 100 times more toxic than organic arsenic compounds (DMA and MMA) [38]. The 50 % lethal dose (LD_{50}) values in rat for some arsenical species are illustrated in Table 1.1. It can be seen from the table that $InAs^{III}$ is more toxic by a factor of between 200 and 300 times than AsC and trimethylarsine oxide, respectively while trimethylated compounds are virtually non-toxic [34, 39].

Table 1.1 Lethal dose LD_{50} values of arsenic species in rat [34, 39, 40].

Arsenic species	Dose (μg g^{-1})
Arsine	3.0
$InAs^{III}$	14.0
$InAs^{V}$	20.0
TMA^{+}	890
MMA	700-1800
DMA	700-2600
AsB	>10,000
AsC	6500

As discussed previously, the toxicity of arsenic compounds depends on the chemical form and its oxidation state. A change in the oxidation state or in the attached organic group will critically influence the chemical characteristics of individual elements. The toxic inorganic forms of arsenic including the oxy-anions arsenite $InAs^{III}$ and arsenate $InAs^{V}$ have been identified as carcinogens [41]. Almost 50 % of ingested arsenic in the human body is expelled in the urine, whereas small quantities are also excreted through the faeces, skin, hair, nails and lungs. Detection of arsenic in urine, faeces, skin, hair, nails and lungs was utilized as an indicator of the arsenic hazards to the population. Skin lesions are a late symptom of arsenic toxicity, and are a result of drinking water contaminated by arsenic. Various diseases such as conjunctivitis, hyperkeratosis, hyperpigmentation, some diseases of cardiovascular and nervous systems, skin cancer, gangrene, leucomelonisis, non-pitting swelling, hepatomegaly and splenomegaly may occur because of long term exposure to arsenic contaminated water [42]. In the advanced stages of arsenic toxicity, effects on the lungs, uterus, genitourinary tract and other parts of the body have been detected. Moreover, high arsenic concentration in the drinking water also leads to the number of stillbirths and spontaneous abortions increasing significantly [43].

Acute and sub-acute arsenic toxicity have been investigated for a long time. Ingestion of contaminated food or drink may cause acute arsenic poisoning which requires quick medical attention. The main symptoms of this type of toxicity include burning and dryness of the mouth and throat, dysphasia, colicky abdominal pain, projectile vomiting, profuse diarrhoea, and hematuria. Muscular cramps, facial oedema and cardiac abnormalities, as well as shock can develop quickly because of dehydration[42].

Sub-acute arsenic toxicity results in problems with the respiratory, gastro-intestinal, cardio-vascular, nervous and haematopoietic systems. Loss of appetite, nausea and

some vomiting, dry throat, shooting pains, diarrhoea, nervous weakness, tingling of the hands and feet, jaundice and erythema are symptoms of this kind of poisoning[44].

Long-term exposure leads to hair loss, brittle and loose nails, eczema, darkened skin exfoliation and horny nodules forming on the palms and soles[42]. There is substantial evidence indicates that arsenic in drinking water can lead to diseases such as skin cancer and several internal cancers, especially lung, bladder and kidney cancers[45].

1.3 Biomethylation of arsenic

Biomethylation is defined as the formation of both volatile and non-volatile methylated compounds of metals and metalloids. The major volatile methylated arsenic compounds formed by biomethylation have the structure of $(CH_3)_nAsH_{3-n}$; for n=1, 2 and 3, the products are mono-, di- and trimethylarsine, respectively. Methylarsonate and dimethylarsinate are the major non-volatile compounds [46]. Biomethylation of arsenic happens not only in a wide range of microorganisms but also in algae, plants, animals, and humans. Owing to having a diverse range of organioarsenical compounds which have complex natural product chemistry, mono-, di- and trimethylarsenic species are formed as a result of biodegradation of these compounds. Fredrick Challenger and his associates at University of Leeds identified volatile arsenical compounds, for instance, trimethylarsine (Me_3As) in the 1930s. A mechanism of biomethylation of arsenic by fungi was proposed by Challenger in the mid1940s. Since then, significant advances in research has developed in this area of study [47].

Biomethylation of arsenic was once recognized as a detoxification route but this changed after finding trivalent methylation of metabolites, MMA (III) and DMA (III) in human urine [48, 49]. Numerous studies revealed that these arsenic metabolites are more toxic than inorganic arsenic or any of the pentavalent intermediates [50, 51]. It is now

considered that the methylation of inorganic arsenic may not be a detoxification mechanism but it could be an activation process[52].

1.3.1 Microbial Biomethylation of arsenic

As stated above, a chemical proof was provided by Challenger that certain fungi, including *Scopulariopsis brevicaulis,* were capable of methylating inorganic arsenic [53, 54]. An arsenic biomethylation pathway is proposed in Figure 1.2 which shows a series of steps; the methylation starts from alternating reduction of pentavalent arsenate to trivalent and oxidative methylation to a pentavalent state. The trivalent arsenic species, including those methylated, are more toxic than the pentavalent arsenicals [5]. Several studies have extended the number of fungi known to be capable of arsenic biomethylation[55].

Methylation of inorganic arsenic by bacteria has been studied extensively in methanogenic bacteria. Bacterial volatilization of arsenic was reported by McBride and Wolfe [56] , which demonstrated that dimethylarsine was formed by *Methanobacterium bryantii*. Since then several volatile methylated arsenical compounds have been detected in pure anaerobic cultures and anaerobic ecosystems. For example, it has been shown that *Methanobacterium formicium* was an effective producer for the formation of mono-, di- and trimethylarsine (i.e., gaseous $MeAsH_2$, Me_2AsH and Me_3As) from inorganic arsenic, this including sulfate-reducing bacteria, including *Desulfovibrio vulgaris* and *D. gigas*; and a *peptolytic bacterium, Clostridium collagenovorans* [57].

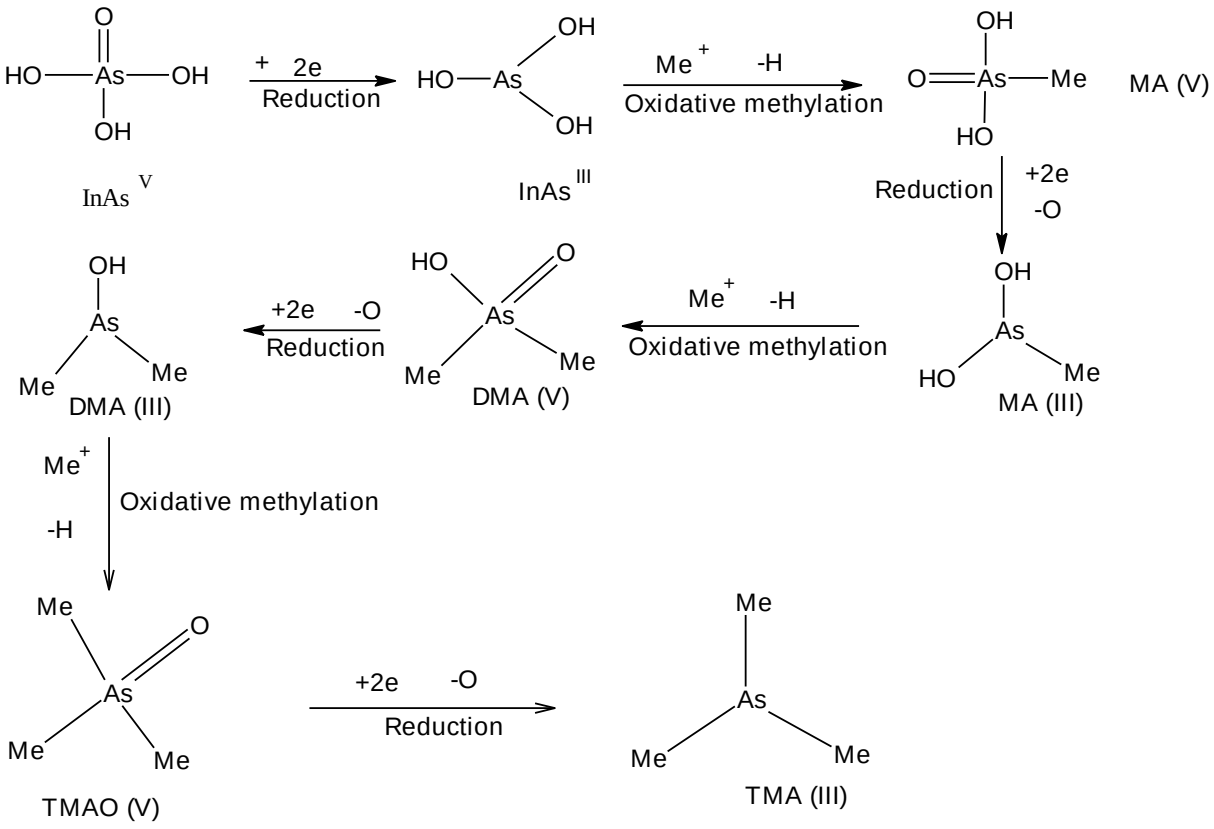


Figure 1.2 Microbial Biomethylation of arsenic.

1.3.2 Methylation of arsenic by plants

Meharg *et al.* have explained how arsenic is metabolised in terrestrial plant tissues [58] . These papers may be classified into two categories. The first category includes the extraction of arsenic species from plant tissues without regard to whether these forms are being chelated or not. A range of arsenic species have been reported in terrestrial plants that have also been found in other organisms, for instance, methylated arsenic species, AsB and arsenosugars. The second category includes works that assume chelation. These studies suggest that a complex is formed in some plant tissues between phytochelatins (PCs) and $InAs^{III}$ [58]. Arsenic is available to plants mainly as $InAs^{V}$ which is taken up *via* the phosphate transport mechanism and is reduced in the plant to $InAs^{III}$ [59]. The inorganic species of arsenic tend to predominate in plants [60, 61] including crops

and vegetables [62, 63]. These studies also found that the same species predominated both in the roots and the stems of the same plant [60]. A several organisms are able to metabolise arsenic from its inorganic to organic forms, and this may be shown to occur in plants [58]. AsB and arseno-sugars have been determined in some terrestrial plants and this may indicate metabolism of arsenic in plants [64]. 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 $InAs^{V}$ to $InAs^{III}$ by a methylation process. Whereas, arsenoriboses, specifically OH-ribose, PO_4^--ribose and OSO_3^--ribose have been formed through the glycosidation process [65]. 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 [66]. The presence of these arsenic species in plants indicates that the methylation process may occur within plants.

1.4 Arsenic in the Environment

Monitoring arsenic's toxicity has become a priority as a result of historical uses of arsenic in pharmaceutical products and industry. More recently, arsenic exposures in natural sources such as food, water and soil have provided a focus for the study of arsenic toxicology. Thus a key area of research in public health comes from understanding the environment.

1.4.1. Arsenic in air

Particulate inorganic $InAs^{III}$ and $InAs^{V}$ represent the major arsenic constituents of air compared with organoarsenicals. Arsenic vapour is almost entirely present as As_4O_6 which derives from the combustion of arsenic-bearing sulphides in coal. Trace amounts of arsine (which may be released by micro-organisms in soils) and methylarsines also occur. As well as being potential inhalation hazards, there is also the probability of

contamination of surface soils, sediments, organisms, and water near attachment points[67]. The European Commission stated that levels of arsenic in air range from 0-1 ng m^{-3} in remote areas, 0.2-1.5 ng m^{-3} in rural areas, 0.5-3 ng m^{-3} in urban areas and up to almost 50 ng m^{-3} in the vicinity of industrial sites[68].

1.4.2 Arsenic in soil

The soil guideline and health criteria for arsenic are 12 and 20 µg g^{-1} for resident soil and 500 µg g^{-1} for commercial or industrial soil [69]. It has been found that the natural concentration of arsenic in soils globally ranges from 0.01 to over 600 µg g^{-1}, with an average of 2-20 µg g^{-1}. According to research by Hughes *et al.,* [70] the natural concentration of arsenic in soil was in the range of less than 1 to 97 µg g^{-1} in areas with no anthropogenic sources of arsenic. Inorganic arsenical compounds dominated over organic arsenic species in the soil [71]. In soils, pentavalent arsenic predominates because of oxidation of trivalent arsenicals [67].

There are a number of ways which may lead to exposure to arsenic in soil. The most significant exposure pathway for soil is known to be incidental ingestion. However, dietary and drinking water sources contribute by far the highest overall background exposure to arsenic [72].

Arsenic's bioavailability, which is defined as the amount of arsenic absorbed into the body compared with the total ingested arsenic, evaluates the potential digestion of arsenic from the soil. It is well known that the absorption of arsenic by the body occurs for water dissolved arsenic to a greater extent than the ingested arsenic in soil or other solid media [72].

1.4.3 Arsenic in plants

A several arsenic species has been detected in plant tissues that have been grown on both arsenic-contaminated and uncontaminated sites. These include inorganic arsenite and arsenate, methylated arsenic species, AsB and arseno-sugars [58]. Inorganic arsenic species ($InAs^{III}$ and $InAs^{V}$) are the predominant in terrestrial plants while in marine organism organic arsenic species are the dominant species [73, 74]. Arsenic species can also be toxic for the majority of terrestrial plants [61]. When plants are exposed to arsenic, they can response in a number of different ways. Some plants are considered to be arsenic resistant and are able to assimilate high level of arsenic even when they are growing on arsenate contaminated soil [75]. Arsenic resistance mechanisms include reduced arsenic uptake, which is conducted through the suppression of phosphate/arsenic uptake system in the resistance plant [58] . *Andropogon scoparius* and *Agrostis castellana* are examples of arsenic resistance plants. Plant species which are not resistance to arsenic suffer will show symptoms ranging from inhibition root growth to death.

Some other plants are classified as arsenic hyperaccumulation plants and are able to tolerance high levels of arsenic and compartmentalize it with different parts of the plant. The Brake fern, *Pteris vittata L.*, is able to hyperaccumulate arsenic in its shoots with concentrations reaching levels ∽100 fold higher than soil concentration[76]. Singh *et al.* categorized fern into three groups based on the arsenic accumulation in the fronds: High accumulator (>500 µg g^{-1}), medium accumulator (>250-500 µg g^{-1}) and low accumulator (< 250 µg g^{-1}) [77].

1.4.4 Arsenic in water

Ground water arsenic contamination has already been identified in 20 countries around the world. The most affected countries, in order severity, are Bangladesh, West Bengal

India, Inner Mongolia (China) and Taiwan[78]. Millions of people in arsenic-contaminated ground water areas drink water with arsenic concentration ≥ 50 μg L^{-1} [17, 78]. WHO has recommended the concentration of arsenic in drinking water is 10 μg L^{-1} [79]. It has been estimated by the U.S. Geological Survey that the median ground water concentration is

1 μg L^{-1} or less, although some groundwater aquifers, especially in the western United States, can contain much higher levels. For instance, the median levels of arsenic measured in Nevada were 8 μg L^{-1}, while in Western Nevada and central California in United States levels of naturally occurring arsenic as high as 100 μg L^{-1} have been recorded in drinking water [80]. In eastern New England, the concentration of arsenic in ground water is higher than 10 μg L^{-1}. The drinking Water Inspectorate in the UK have set a limit of 50 μg L^{-1} of arsenic in drinking water [81]. Bedrock aquifers often produce private wells from which drinking water unsuitable for consumption can be extracted, although arsenic contamination from drinking water extracted from unconsolidated aquifers is rare. 30 % of water from wells in meta-sedimentary bedrock units aquifers, particularly in Maine and New Hampshire in the USA, can contain arsenic concentrations of greater than 10 μg L^{-1} [82]. Virtually all of the arsenic found in water is in the inorganic forms arsenite and arsenate which are trivalent and pentavalent, respectively because they are stable [70]. Some examples are summarized in Table 1.2.

Table 1.2 Arsenic concentrations in ground water in different countries.

Location	Sampling period	Arsenic source	Concentration µg L^{-1}	Reference
Laos PDR	2008	Tube-well water	<0.05-278	[83]
Kandal, Cambodia	Not mentioned	Aquifer, wells	15-1300	[84]
		Shallow wells	0-1000	
South Vietnam	2007	-	<1.0-850	[85]
West Bengal, India	2000	Hand tube well	21-176	[86]
		Shallow tube well on agriculture land	40-182	
Michigan, USA	1997	Shallow groundwater	0.5-278	[87]
Baseline, UK	Not mentioned	Groundwater	<0.5-10	[17]
Southwest, England	Not mentioned	Groundwater (mining area)	<1.0-80	[88]
Southern Thailand	Not mentioned	Shallow groundwater (mining contaminated)	1.25-5114	[89]

1.5 Arsenic in the diet

Today, inorganic arsenic is not intentionally used as a preservative added to food as it was in the late 1800s and early 1900s [90]. It is, however, well known that the diet contains inorganic and organic arsenic [70, 90]. The WHO has established a provisional maximum tolerable daily intake (PMTDI) of 2.1 µg inorganic As kg^{-1} day^{-1} body weight to cover risks from both water and food, although these guidelines are not for a specific food [91]. However, the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM Panel) in its Scientific Opinion on Arsenic in Food agreed that this provisional tolerable weekly intake (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} body weight per day for an increased risk of cancer of the lung, skin, bladder and skin lesions [92].

Estimates of inorganic arsenic in diet are varied. In the UK , according to a survey by Rose [93] it was suggested that the amount of inorganic arsenic consumed by adult is

0.03-0.09 pg kg^{-1} body weight day^{-1}. In the United States, it was estimated that the average adult intake is 3.2 µg day^{-1}, with a range of 1-20 µg day^{-1} [94]. While in children (1 to 6 years age), the dietary intake of inorganic arsenic was estimated to have a mean of 3.2 µg day^{-1}, with a range of 1.6 to 6.2 µg day^{-1} [95]. Recently, a higher intake level has been estimated by the European Food Safety Authority (EFSA) [70]. However, simplifying assumptions which are related to the ratio of inorganic arsenic to total arsenic in food are used to determine these estimates [70]. It has been reported by EFSA that the national arsenic exposure from food and water across 19 European countries utilizing lower bound and upper bound concentrations have been measured to be in the range 0.13-0.56 µg kg^{-1} body weight [96]. It has also been shown in some reports that some of our foodstuffs are contaminated with arsenic. Most foodstuffs contain organic arsenic compounds and a total concentration of less than 1 µg g^{-1} [71]. Seafood is the main source of arsenic in diet [97]. Rice can contain a relatively high amount of arsenic. Rice provides 70 % of energy of daily food intake of over half of the world's population especially in Asian developing countries[98]. Rice can accumulate arsenic typically about 0.100-0.400 µg g^{-1} [11]. Raber *et al.* [99] demonstrated that inorganic arsenic and total arsenic of 10 rice sample were 0.025-0.171 µg g^{-1} and 0.036-0.218 µg g^{-1}, respectively. When the diet is not rice-based wheat will be the major contributor to the consumption of inorganic As. It has been found the total arsenic concentration in wheat samples ranged between 0.0086-0.166 µg g^{-1} dry weight and 91-95% of the arsenic was found to be exist in inorganic, while the rest was mainly DMA[99, 100]. Table 1.3 shows total arsenic and inorganic arsenic concentrations in 20 different food stuffs in the UK[93].

1.6 Toxicity in food

The most toxic arsenic species in food are inorganic arsenic, $InAs^{III}$ and $InAs^{V}$, followed by organic arsenic such as MMA(V), DMA (V) and (TMAO) which are considered less toxic. However, some organic arsenic species are found in food such as

AsC, AsB and arsenosugers which are estimated harmless. Trivalent methylated species such as MMA (III) and DMA (III) have been detected in the human urine [48]. These methylated arsenicals are more toxic than inorganic forms[101, 102] but they have not been found in any foodstuffs, possibly due to lack of a suitable extraction method.

Table 1.3 Concentration (µg g^{-1}) of inorganic and total arsenic in the 20 food groups of the 2006 UK Total Diet Study[93].

Food group	Inorganic arsenic µg g^{-1}	Total arsenic µg g^{-1}
Bread	<0.01	< 0.005
Miscellaneous cereal	0.012	0.018
Carcase meat	<0.01	0.006
Offal	<0.01	0.008
Meat products	<0.01	0.005
Poultry	<0.01	0.022
Fish	0.015	3.99
Oils and fats	<0.01	< 0.005
Eggs	<0.01	< 0.003
Sugars and preserves	<0.01	0.005
Green vegetable	<0.01	0.004
Potatoes	<0.01	0.005
Other vegetables	<0.01	0.005
Canned vegetables	<0.01	0.005
Fresh fruit	<0.01	0.001
Fruit products	<0.01	0.001
Beverages	<0.01	0.003
Milk	<0.01	<0.001
Dairy produce	<0.01	< 0.003
Nuts	<0.01	0.007

1.7 Aims of the study

The aims of this work are:

1. To develop a simple, yet effective, instrumental system for the separation of toxic from non-toxic arsenic species. This may be achieved by coupling an affective separation technique such as HPLC to separate the arsenic species with ICP-MS for detection.

2. To develop an extraction procedure to extract arsenic species from real samples whilst maintaining the integrity of the sample's speciation from its collection and storage, through its extraction and finally to its measurement.

3. To use the developed analytical approach for the determination of arsenic species in fish samples those have been purchased in the local markets.

4.To develop a suitable analytical procedure for the determination of total arsenic and arsenic species in a range of vegetables, cereals, rice and some root crops.

5. To apply the developed methodology for vegetables and rice to a range of important local food stuffs collected in Kurdistan region of Iraq.

6. To apply HPLC-ICP-MS for the estimation of arsenicals in different parts of selected plants to include roots, straw, leaves and grain as well as the soils in which the plants are cultivated, in order to elucidate the migration pathways for arsenic in the selected plants.

7. To enhance our theoretical understanding of the mechanisms involved by investigation inorganic and organic arsenic species within cellular DNA of selected foods.

Chapter two

Techniques for total arsenic determination and arsenic speciation in food and water

Although a wide range of techniques exists for the chemical analysis of food, metals and metalloids such as arsenic tend to be measured using atomic spectrometry. This chapter discusses the techniques available to determine both total arsenic and individual arsenic species in food and water.

2.1 Total arsenic determination in food samples

Analytical methods comprise two main stages: sample preparation and instrumental techniques. For the determination of arsenic in food, the sample preparation is usually carried out via a mineralization step and a wide range of instrumental techniques are used such as atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS). Although it is well understood that information on the total element concentration is insufficient when assessing the toxicity, total levels are a useful indicator, particularly for food on non-marine origin.

2.2. Methods to speciate arsenic in food samples

2.2.1 Definition of speciation and its impact

The topic of elemental speciation is now a major focus of research. The chemical species are specific forms of an element, defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure [103]. Studying chemical forms of elements is very important because the distribution, mobility, biological availability and toxicity of chemical elements critically depend on their chemical species not just on their concentrations [77, 104].

Florence [105] defined speciation as the determination of the different physico-chemical species of the element, which together make up its concentration in a sample. The elements in the environment may have different oxidation states and form various species. The different species of the same element may have different chemical and toxicological properties. Hence, determination of the total concentration of an element is insufficient knowledge in terms of the actual physico-chemical forms of the element necessary for understanding its toxicity, biotransformation, etc, and quantification of individual species is necessary to understand toxicity and biotransformation of elements in environmental and biological systems.

Speciation is generally accomplished in three steps: sample preparation, species separation, and detection. Quantitative determination of each of the forms of an element independently, without interferences from the other forms, is a fundamental requirement. In this respect, providing the desired information whilst maintaining the original sample integrity is the ultimate target of an ideal element speciation method. The combination of analytical techniques and methodologies, such as spectroscopic, chromatographic, and electrochemical analysis are involved in the elemental speciation in the absence of such a method.

2.2.2 Sampling and sampling pre-treatment for speciation

Maintaining the concentration and chemical structure of the original species during the sample preparation and extraction steps are critical requirements for obtaining information on accurate arsenic speciation [106]. During these procedures problems may result from losses during sampling, unrepresentative samples [107], contamination, inter conversion between species, inefficient extraction of the analyte, and the possibility of precipitation and wall effects from the sample container [108-110]. The possible risk of a redox interconversion of inorganic arsenic forms to other species can be minimized

using microwave-assisted extraction [109]. Microorganisms can participate in a range of element transformations including a change in valence (i.e. oxidation/reduction) or chemical form (i.e. solid, liquid and gas) [111]. It is well-known that many microorganisms (bacteria, fungi and yeast) have ability of biomethylate arsenic and both volatile (e.g., methylarsines) and nonvolatile (e.g., methylarsonic acid and dimethylarsinic acid) compounds are formed [46]. Biological samples should be kept at low temperatures as bacteria can degrade the integrity of the sample. Drying is often used for the stabilization of samples particularly freeze-drying or lyophilisation which tend to reduce determinant loss [112].

2.2.3 Extraction

Sample extraction is one of the crucial steps in the analysis of food samples. It is important to avoid chemical transformation of the species during the extraction process, and to ensure the full extraction of each species. Extraction procedures employ a range of approaches including solid-liquid extraction[113] liquid-liquid extraction[110], solid phase extraction (SPE) [114] and solid phase microextraction (SPME) [115]. Solid sample preparation generally includes milling, grinding, freeze drying or sieving following by some forms of extraction. Enhanced techniques such as soxhlet[116], sonication[117], pressurized liquid extraction (PLE)[118], microwave-assisted extraction (MWA)[119] and supercritical fluid extraction (SFE) [120] have also been utilized for the determination of arsenic in food, although as discussed below, some of these approaches may be problematic for some matrices.

2.2.3.1 Solvents

Numerous extraction methods have been utilized for total, total inorganic and full arsenic speciation [121]. The extraction is most often achieved via water, methanol, methanol-water solvent systems or infrequently acetonitrile-water [122, 123], and sequential

extractions are common. Some food stuffs have also been treated with enzymes; α-amylase has been used with freeze-dried apple samples. The cellulose in freeze-dried apple samples is broken down by α-amylase and extraction yields of arsenic species are improved; this treatment may be followed by extraction with acetonitrile-water [124, 125]. A trypsin digestion procedure may be performed on fish samples, and AsB is not decomposed by this process [126]. McKiernan *et al.* [127] used a sequential extraction to extract arsenic species from fish tissue; fats and lipids were removed from the mixture using acetone and then the arsenic species extracted by water-methanol 150:150 (v/v). A summary of research papers focusing on extraction methods for arsenic species in food is shown in Table 2.1.

Table 2.1 Extraction procedures for determining arsenical species in food.

Extraction solution	Shaking/mixing	Sonication	MW-assisted heating	Sub/ supercritical fluid	PLE	Soxhlet
Water	10, 128, 129, 130, 131, 132, 133, 74, 134, 135	130, 136, 125, 10, 133	10, 130, 133, 137-139	133, 140	132, 141, 40	10, 133
Methanol	10, 142	10, 143, 144	10, 143		141, 40	10, 143, 133
Methanol/ water mixture	10, 128, 130, 131, 132, 128, 133, 145	10, 130, 125, 141, 18, 143, 133, 145	10, 146, 147, 130, 148, 18, 119, 133, 147, 149, 150	151	132, 152, 141, 40, 153-155	10, 143
Ionic extractants	128, 131, 141, 131, 133	130, 125, 18, 133, 156, 157,158	159, 160, 130, 119, 130, 133, 156	129		
Enzymes	125, 141, 15, 161,162	18, 163, 134		164		
Others	10, 145, 165, 166	10, 18, 65, 157, 167-169	10, 119, 170, 171	129, 133, 172-175	154, 176-178	10

2.2.3.2 Extraction systems

Common extraction methods including mixing/shaking, sonication, microwave-assisted heating and accelerated solvent extraction are presented in Table 2.1. The preservation of the organoarsenic species is the main requirement of a successful extraction procedure prior to speciation analysis. Thermal and microwave heating have been used for arsenic speciation analysis, following optimization of the microwave conditions. The direct energy of the microwave can be managed using the programming options (controlled power, time, temperature, and/or pressure) of modern commercial instruments. Arsenic species have been removed from fish using microwave-assisted extraction [123], and $InAs^{III}$ and $InAs^{V}$ have been quantified from plant material by using microwave-assisted extraction [179]. Another enhanced extraction techniques is pressurized liquid extraction or accelerated solvent extraction. Here the applied temperature, and raised pressure, maintain the solvent below its boiling point, to facilitate safe and rapid extraction [180]. Most instrumental systems can be programmed at various temperature and heating/static times for the solvent within the sample cell. Supercritical fluid extraction has also been used to extract arsenic species from different food matrices [73].

Ultrasound probe sonication can be used to aid the removal of the determinant from the sample matrix. A standard ultrasonic bath operating at a frequency of 40 kHz may often be used to extract the analytes from solids faster than by using classical methods[120, 181]. Insoluble arsenic fractions such protein bound arsenic and/or lipid arsenic have traditionally been little researched due to the absence of a suitable analytical methods and difficulties of a total recovery of species[120]. These drawbacks have been tackled by combining enzymatic treatment with ultrasonic probe sonication in more recent studies[163].

Supercritical fluid extraction (SFE) has some favourable characteristics which make it attractive as an extraction technique, including the low viscosity and diffusion coefficients[121]. However, it has not found widespread use for speciation studies due to is low extraction efficiency for highly polar or ionic compounds[122]. The addition of complexing agents and/ or modifiers may partly address these problems and enhance extraction efficiencies[182].

Pressurized liquid extraction (PLE) is another automated approach which can provide fast extractions using low solvent volumes and avoiding filtration [183, 184]. This method has been reported for arsenic speciation in marine biological materials including mussels and fish samples[124]. However, PLE is not without its problems for speciation studies since dispersion of the sample in an inert medium is a fundamental step. When this dispersal is not homogenous a large reduction in extraction efficiency will be observed[154].

Microwave assisted extraction (MAE) is a viable replacement to conventional techniques for many matrices, offering acceptable and reproducible efficiencies, together with a reduction in extraction times, low solvent volumes, and the opportunity of fast and multiple extraction[183],[126] This approach has found widespread application in speciation studies for arsenic. Optimisation of MAE is straight forward because of the low number of parameters involved, such as choice of solvent, solvent volume, temperature, extraction time, power and matrix characteristic[183].

2.2.4 Methods of separation

Liquid chromatography (LC) is a method often used for arsenic speciation in food. It provides separation of both inorganic and organic forms of arsenic. The coupling of ICP-MS, ICP-AES and HG-AAS with liquid chromatography has also been widely used for arsenic speciation, since LC offers good separation of many arsenic species using a

simple interface for real time measurement [185, 186]. Arsenical species have been separated using several techniques including anion-exchange HPLC with either isocratic or gradient-step elution or cation-exchange HPLC with isocratic elution. Ion-pair HPLC has also been utilized[187]. Since there is sometimes a requirement for the separation of anions and cations of arsenic in a single run, column-switching systems, which involve a combination of anion-exchange and reversed-phase separation, have been developed [187,188, 189]. The coupling of gas chromatography (GC) with ICP-MS has also been used[190]. Speciation analysis of organometallic compounds in complex environmental and industrial samples have been achieved by combination of capillary GC with ICP-MS to utilise the high resolving power of GC and the sensitivity and specificity of ICP-MS [191]. Using GC speciation can be an attractive technique because of the lack of condensed mobile phase although there is often the need for derivatisation of the determinant prior to analysis [192].

In recent years, the number of reports on the use of capillary electrophoresis (CE) has continued to grow. CE is an attractive technique for elemental speciation since it has several unique characteristic in comparison with GC or HPLC methods i.e. high resolving power, rapid, effectual separations, minimal reagent consumption and the probability of separation with only minor disturbances of the existing equilibrium between different species [193]. A wide range of inorganic and organic arsenic species can be separated by this technique [194]. Several element-selective detector have been coupled with CE including both ICP-AES and ICP-MS [195,196] . Yang *et al.* [197] have analysed seafood using capillary electrophoresis-inductively coupled plasma mass spectrometry. $InAs^{III}$, $InAs^{V}$, MMA and DMA have been separated and determined in dried *Mya arenaria I* and shrimp within 10 min. CE has also been coupled to ICP-MS to quantify the arsenic species AsB, $InAs^{III}$, $InAs^{V}$, DMA, MMA in fish[198].

Micro-scale separation has become a popular technique due to the improved separation efficiency, reduced analysis time and reduction in sample consumption[12, 199]. Micro-bore and narrow-bore have been coupled with ICP-MS as a result of their compatibility with ionisation sources of MS [12]. Narrow-bore-HPLC column coupled with ICP-MS has been used by Wangkarn and Pergantis [200] to analyse wines. Arsenite at trace levels was found to be the only arsenic species in the analysed wines.

Separation with off line detection depends on the chemical or physical separation of the element of interest. Particular arsenic species are separated selectively before determination as arsenic; for instance, formation of $AsCl_3$ (reasonably volatile, non-polar) from arsenite which is ultimately separated from other organoarsenicals by distillation or solvent partitioning. Off line detection methods have been applied to the separation and determination of inorganic arsenic ($InAs^{III}$ and $InAs^{V}$) and organic arsenic (MMA and DMA) in fish (skate, hake, albacore, blue fin tuna and blue whiting)[201,202, 203] , plant extracts[204] and raw vegetables [205].

Organoarsenical compounds have also been quantified by HPLC-MS with LODs 30 $ngmL^{-1}$ approaching those of HPLC-ICP-MS. HPLC-MS and HPLC-MS-MS are most often used to characterize arsenicals, such as AsB, AsC, arsenosugars in biota like algae[206], oyster[207] and clams [208]. Different chromatographic conditions have been used for arsenic speciation in various matrices (Table 2.2).

2.3 Methods of detection

2.3.1 X-ray spectroscopic techniques

X-ray spectroscopic methods are being increasingly used for arsenic speciation analysis. They are most often used for geological samples [209, 210] but can also be used for arsenic-

rich biological samples [211, 212]. The possibility of conducting speciation analysis on solid environmental samples without the need for extraction of the elements species has been investigated and a number of X-ray spectroscopic techniques have been used to measure total arsenic and arsenic speciation in different solid environmental and biological samples. However these techniques may have limited application for food analysis, due to the relatively poor detection limits and problems from the high intensity of the X-ray beam modifying the samples [213, 214]. X-ray sorption near edge spectrometry (XANES) and X-ray fine structure (EXAFS) have been used for arsenic speciation in biological environmental samples [215, 216], *Daphnia pulex*[217], plant material [218, 219] , seaweed [220] and rice grain [221].

2.3.2 Mass spectrometry

MS is the most frequently applied method for identifying and elucidating unknown compounds in foods following speciation. Ionization of the compounds can be achived by techniques such as ionspray, electrospray, atmospheric pressure chemical ionization (APCI), electron ionization (EI), and fast atom bombardment. Because most arsenic compounds are not volatile, some form of derivatization is require before GC separation. Many arsenic speciation methods are based on conversion of arsenic into the corresponding methylarsine by sodium borohydride, although methyl thioglycolate has been used to derivatise methylarsenic to produce lipophilic species[222]. Methyl thioglycolate has been used to derivatize MMA, DMA and inorganic arsenic for extraction into cyclohexane prior to chromatographic separation.

Mercaptanes/dimercaptanes or thioglycolic acid methyl esters have also been used to derivatize phenylarsinc compounds before injecting into the GC-MS[223], GC-ICP-MS has been successfully used to detect a range of arsenic-containing hydrocarbons in commercial fish oils[224] and seafood[176].

2.3.3 Detection by AAS, AFS and AES

In atomic spectrometry, an excitation source is required to atomise or ionise the determinant of interest. The advantage of these techniques is their inherent sensitive and element specific detection. Electrothermal atomic absorption spectroscopy (ETAAS) has previously found preference over flame AAS for arsenic studies since the sensitivity is greater by a factor of 10-100 times [225]. Both fraction collection and on-line coupling of HPLC with ETAAS have been reported offering detection limit in the range of a few nanogram [201, 226-230].

Due to its low detection limit and high selectivity, hydride generation atomic absorption spectroscopy (HG-AAS) was traditionally one of the most widely used methods for arsenic speciation [39, 231-233], particularly for reducible arsenic compounds such as $InAs^{III}$, $InAs^{V}$, MMA and DMA. The volatile arsenic species is produced using either by zinc/hydrochloric acid or sodium borohydride/acid mixtures and the volatile arsenic species produced are transported to the detection system with argon gas. By forming arsine gas, the determinant is easily and efficiently separated from its sample matrices and transported to the detection system, sometimes via a cryogenic pre-concentration step to obtain better detection limits. However, a number of organo arsenicals, for instance AsB and AsC, cannot be detected by this method since they are not able to produce volatile hydrides. In this case, the separation of these species prior to HG-AAS is required followed by conversion of the individual arsenic species via photolysis or chemical destruction [3]. As a result of incorporating these techniques, AsB and AsC may be determined using hydride generation, although controllable reaction conditions and the reduction of certain interfering elements may be required[234]. Total arsenic in seafood has been determined by HG-AAS after performing a dry-ashing to the sample[235]. A summary of publications employing HG-AAS and HPLC coupled with HG-AAS is presented in Table 2.2.

Coupling atomic fluorescence spectrometry (AFS) with HPLC is now a well-established and useful technique for arsenic speciation. AFS can rival ICP-MS regarding performance criteria such as detection limits, reproducibility, repeatability, and sensitivity for arsenic. AFS also offers low purchase and running cost, shorter warm up times prior to analysis and easy handling [236]. HPLC-(UV)-HG-AFS has been applied to arsenic speciation for the both lobster hepatopancreas-NRCC-TORT1 reference material and several environmental samples with the detection limits ranging from 0.1 to 0.3 μgL^{-1} [236].

Finally, atomic emission spectroscopy may be used as an alternative technique for arsenic speciation. Chausseau *et al.* [237] concluded that HPLC-ICP-AES is a reliable technique for arsenic speciation when very low limit of detections are not required; they reported detection limits better than 10 $\mu g\ L^{-1}$ for $InAs^{III}$, DMA and 20 $\mu g\ L^{-1}$ for $InAs^{V}$. The technique can also be used in conjunction with HG, although it should be remembered that not all arsenic species may be determined using this approach.

2.3.4 Detection by ICP-MS

The merits of ICP-MS are well documented [238], and this approach is now the method of choice in most laboratories for arsenic determination. The main advantages that the ICP-MS has over the other techniques are its low detection limits, 1-10 pg mL range for quadrupole instruments, large linear dynamic range, rapid, multi-element capability for many elements and potential to use isotopic studies (although not arsenic) [239]. Despite all of these advantages there are some limitations using ICP-MS for arsenic speciation. The use of ICP-MS alone does not provide direct molecular information and it is impossible to identify individual arsenic species without some form of prior separation usually by HPLC. A summary of works employing HPLC coupled with ICP-MS is

presented in Table 2.2. A schematic diagram of an ICP-MS instrument is shown in Figure 2.1 [240].

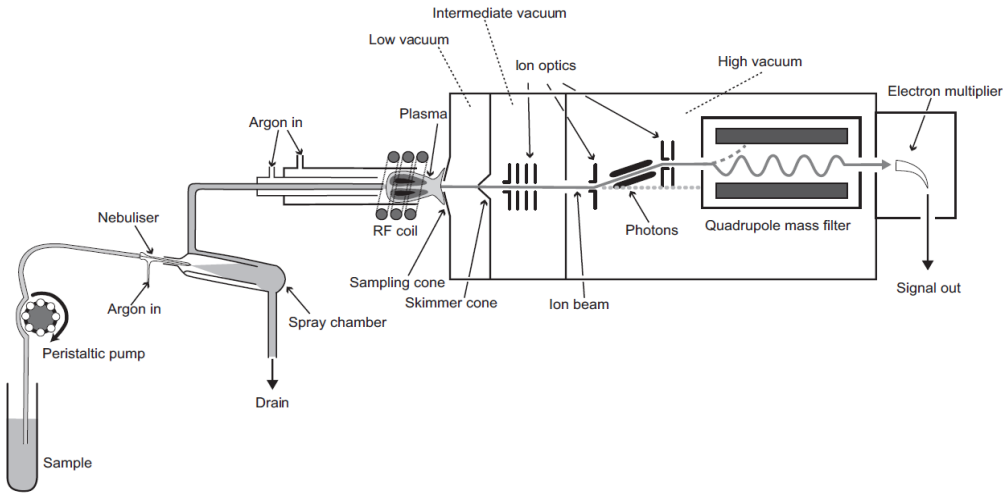


Figure 2.1 schematic diagram of the major components of ICP-MS instrument [240].

Interferences can be a problem in ICP-MS, particularly when there is an isobaric overlap due to polyatomic ions formed by combination of two or more atoms. The most significance polyatomic ions are formed from the most abundant isotopes of argon, atmospheric gases, and the solvents or acids used during sample preparation [241]. A major polyatomic interference for arsenic [arsenic is monoisotope m/z 75] is $^{40}Ar^{35}Cl$. Incomplete dissociation, or recombination in cooler plasma regions may lead to the formation of refractory oxides, especially in the boundary layer around the sampler cone [242]. Some examples of common interference in ICP-MS instrumentation are:

$^{40}Ar^{16}O^{+}$ in the determination of $^{56}Fe^{+}$

$^{38}ArH^{+}$ in the determination of $^{39}K^{+}$

$^{40}Ar^{+}$ in the determination of $^{40}Ca^{+}$

$^{40}Ar^{40}Ar+$ in the determination of $^{80}Se_{+}$

$^{40}Ar^{35}Cl^{+}$ in the determination of $^{75}As^{+}$

$^{40}Ar^{12}C^{+}$ in the determination of $^{52}Cr^{+}$

$^{35}Cl^{16}O^{+}$ in the determination of $^{51}V^{+}$

Interference problems can be attenuated in ICP-MS by several methods. Since, any isobaric overlap may be corrected by calculating the relative contribution of the interfering analyte, polyatomic interferences can be tackled via mathematical correction [243]. However, mathematical correction leads to error especially when multiple steps are applied. Another method is adding another gas such as nitrogen, oxygen, air, helium, and hydrogen to the argon plasma, which can minimise inherent polyatomic interferences. Addition of nitrogen gas to an argon plasma has been found very effective because of increasing signal and decreasing argon and O-based interferences [244]. Nitrogen-argon plasma, are more energetic and hotter than argon plasmas alone because of the higher thermal conductivity of nitrogen causing more efficient energy transfer within the plasma. Addition of N_2 gas to the plasma, for instance, leads to the formation of new interferences such as N^+, N_2^+, NO^+ and ArN^+, as well as ClN^+ if the sample matrix has significance concentrations of Cl. A consequence this may lead to an increase in background peaks [245]. Addition of this gas contributes to an increase in overall background as a result of increased photon emission in the interface. An alternative method to minimising polyatomic interferences is operating under cool plasma conditions and higher nebulizer gas flow rates.

Finally, a more recent approach has been used for interference reduction based on the collision or reaction cell to promote selected reactions. The layout of a typical collision reaction cell is shown in Figure 2.2. In the collision/reaction cell the ion beams enter a cell filled with a specifically selected gas. This is positioned prior to the analyser quadrupole. After removing the interfering species from an analyte via interaction with the gas, the analyte emerges to enter the mass spectrometer to be measured [240]. Cells with quadrupole ion guides are usually called reaction cells since the identification and rejection of unwanted reaction products from reactive gasses can be done before mass separation and ion detection [246]. Hexapole and octapole ion guides are generally known

as collision cells, operating under lower gas pressure than reaction cells and under non-thermal conditions, where ions have higher kinetic energy [247].

For arsenic, a reduction in the $^{40}Ar^{35}Cl^{+}$ interference can be achieved using a collision reaction cell including gases such as H_2, O_2, NH_3, CH_4, NO, CO_2 and C_2H_4 [248-250].

Sector field (SF)-ICP-MS is perhaps the ultimate choice for elemental speciation studies due to its sensitivity and ability to resolve isobaric overlaps [251]. Some examples of arsenic speciation studies using this technique include arsenic speciation in xylem sap of cucumber [252], freshwater fish [253] and fish sample [253].

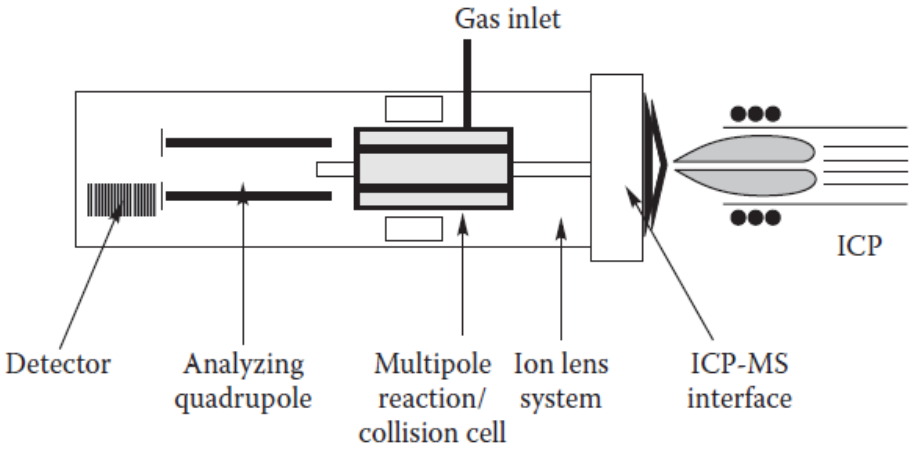


Figure 2.2 Layout of typical collision/reaction cell instrument [254].

Suppression/ enhancement effects and signal drift are categorized as non-spectroscopic interference. There are several possible factors which lead to suppression or enhancement effects like changing in sample transport to plasma, ionisation in the plasma and transmission of the ion beam. Suppression can result from samples with a

high level of total dissolved solids [240]. Matrix matching calibration solutions to samples, dilution, or by chemically separating determinants from the matrix before analysis can reduce most matrix effects[255, 256]. Non-spectral interferences can be corrected by using an internal standard with mass number close to that of determinant element(s) [257]. The high level of total dissolved solids may cause drift in signal through deposition of material on the sampler and skimmer cones, and on the ion lenses. Either internal standards or external drift correction procedures can correct this kind of interference [258].

2.3.5 Carbon enhancement of the arsenic signal

Signal enhancement is a well-known phenomenon in inductively plasma mass spectrometry. The addition of carbon to the argon plasma of an ICP–MS causes an increase in the proportion of arsenic atoms that are ionised by the charge transfer effect. This increases the observed counts per second for the arsenic signal at m/z 75 [259-261]. Traditionally this has been achieved through the addition of organic solvents to the sample matrix [261]or to the mobile phase[262] to improve sensitivity. Signal enhancement can also be obtained by addition of aqueous solutions of volatile carbon compounds (acetone, methanol, and acetic acid) directly into the thermostatic spray chamber [263].

Table 2.2 Arsenic in food and natural water.

Matrix	Species	Technique	Separation conditions	Time of separation minute	Amount of sample µL	Detection limits ng mL^{-1}	References
Rice	$InAs^{III}$, $InAs^{V}$, DMA and MMA	HPLC-ICP-MS	PEEK PRP-X100 anion exchange column; mobile phase, 20 mM ammonium phosphate buffer, pH 4.5, 40 °C	-	40	Not Given	159
Rice	$InAs^{III}$, $InAs^{V}$, DMA	HPLC-ICP-MS	Waters IC-Pak Anion HR column; mobile phase, 10 mM $(NH_4)_2CO_3$, pH 10. Dionex AS7 & AG7 column; mobile phase, 12.5 mM HNO_3, pH 1.8. Hamilton PRP-X100 column; mobile phase, 10 mM $NH_4H_2PO_4$,10 mM NH_4NO_3, pH 6.3.	-	25	$InAs^{III}$: 0.10 $InAs^{V}$: 0.10 DMA: 0.13	141
Rice	$InAs^{III}$, $InAs^{V}$, DMA and MMA	HPLC-ICP-MS	PRP-X100 anion-exchange column (Hamilton); mobile phase, 20 mM $NH_4H_2PO_4$, pH 5.6, 40 ºC.	10	20	$InAs^{III}$: 1.3 $InAs^{V}$: 1.3, DMA: 1.3 MMA: 1.3	264
Rice	$InAs^{III}$, $InAs^{V}$, DMA, MMA	HPLC-ICP-MS	Column X-Select (Charged Surface Hybrid; CSH) C18; mobile phase, 7.5 mM tetrabutylammonium hydroxide, 10 mM ammonium phosphate monobasic, 5% methanol, pH 8.25.	9	25	$InAs^{III}$: 0.1 $InAs^{V}$: 0.2 DMA: 0.1 MMA: 0.2	162
Rice, straw	AsB, $InAs^{III}$, DMA, MMA, $InAs^{V}$	HPLC-ICP-MS	Hamilton PRP-X100 anion exchange column; mobile phase, 10 mM HPO_4^{-2} / $H_2PO_4^{-}$, 2% (v/v) methanol, pH 8.5.	11	100	AsB: 0.0136 $InAs^{III}$: 0.0196 DMA: 0.0127 MMA: 0.0143 $InAs^{V}$: 0.0194	265
Rice	$InAs^{III}$, MMA, DMA $InAs^{V}$	HPLC-HG-AAS	PRP-X100 analytical and guard anion-exchange column (Hamilton, Reno, NV, USA); mobile phase, 10 mM HPO_4^{-2}/H_2PO^{-4}, pH 6.0.	-	-	$InAs^{III}$: 0.015 MMA0.06 DMA: 0.06 $InAs^{V}$: 0.06	163
Rice	$InAs^{III}$, $InAs^{V}$, MMA, DMA	HPLC-HG-AFS	Hamilton PRP-X 100 anion-exchange column (250 mm × 4.1 mm I.D. 10 µm); mobile phase, 15 mM phosphate buffer, pH 6.	-	-	Not Given	266
Plant	$InAs^{III}$, $InAs^{V}$ DMA, MA and TMAO	HPLC-ICP-MS	Cation exchange: ZORBAX 300-SCX column; mobile phase, 20 mM pyridine, pH2.6. Anion exchange: PRP-X100 column; mobile phase, 20 mM $NH_4H_2PO_4$, pH 6. Anion exchange: PRP-X100 column; mobile phase, 20 mM NH_4HHCO_3, pH 10.3.	7-12	20	Not Given	267

Table 2.2 Continued

Matrix	Species	Technique	Separation conditions	Time of separation minute	Amount of sample µL	Detection limits ng mL^{-1}	References
Plant	$InAs^{III}$, $InAs^{V}$, DMA and MMA	HPLC-ICP-MS	Hamilton PRP-X100 anion-exchange column; mobile phase, 30 and 100 mM TRIS acetate buffer, pH 7.	13	200	Not Given	268
White mustard	$InAs^{III}$, $InAs^{V}$, DMA and MMA	HPLC-ICP-MS	Anion exchange column PRP-X100; mobile phase, 0.01M Na_2HPO_4 (80%), 0.01 M NaH_2PO_4 (20%), pH 6.	–	100	Not Given	60
Carrots	$InAs^{III}$, $InAs^{V}$, MMA, DMA, AsB	HPLC-ICP-MS	Column, Waters IC-Pak Anion HR; mobile phase, 10 mM ammonium carbonate, pH 10.	7	20	$InAs^{III}$: 0.15, $InAs^{V}$: 0.11, MMA: 0.13, DMA: 0.24, AsB: 0.14	40
Fruit and vegetable	$InAs^{III}$, $InAs^{V}$, DMA and MMA	HPLC-ICP-MS	PRP -X100 anion exchange column; mobile phase, ammonia phosphate buffer (6.6 mM ammonium dihydro-phosphate, 6.6 mM ammonium nitrate), pH 6.2.	-	100	Not Given	160
Apple	$InAs^{III}$, DMA, MMA, $InAs^{V}$	HPLC-ICP-MS	Hamilton PRP-X100 anion exchange column with mobile phase A: 12.5 mM $(NH_4)_2CO_3$; pH 8.5: mobile phase B: 50 mM $(NH_4)_2CO_3$	30	200	$InAs^{III}$: 0.089, DMA: 0.034, MMA: 0.063, $InAs^{V}$: 0.19	269
Xerocom-us badius	$InAs^{III}$, $InAs^{V}$, and DMA	HPLC-HG-AAS	A-First analytical system:Column Supelco LC SAX-1; mobile phase, phosphate buffer (50 mM Na_2HPO_4 and 5 mM $KH_2PO_4.2H_2O$). B-Second analytical system: Column, Zorbax SAX, mobile phase, phosphate buffer (100 mM Na_2HPO_4 and 10 mM $KH_2PO_4.2H_2O$).	-	-	Not Given	270
Plant (bean, rice, hot pepper)	$InAs^{III}$, $InAs^{V}$, and DMA.	HPLC-HG-AFS	Hamilton PRP-X100 anion-exchange column; mobile phase, 5 mM ammonium phosphate buffers, pH 4.7 for 4.1 min; 30 mM at pH 8.0 for 6.0 min; 5 mM at pH 4.7 again for 10 min, in order to equilibrate the column before the following analysis)	21	100	$InAs^{III}$: 1.5, DMA: 2.4, MMA: 2.1, $InAs^{V}$: 1.8	130
Feed additive	$InAs^{III}$, $InAs^{V}$, DMA ,MMA, Roxarsone (ROX) and p-arsanilic acid (ASA).	HPLC-ICP-MS	PRP-X100 anion exchange chromatographic column (Hamilton, USA); ZORBAX Eclipse XDB-C18 chromatographic column (Agilent, USA); mobile phase, A: H_2O; B: 50 mM $(NH_4)_2HPO_4$, pH 6.0.	20	15-25	$InAs^{III}$:0.04,$InAs^{V}$:0.15, DMA:0.24, MMA:0.36, ROX:0.5, ASA:0.092	271

Table 2.2 Continued

Matrix	Species	Technique	Separation conditions	Time of separation minute	Amount of sample µL	Detection limit ng mL^{-1}	Reference
Algae and freshwater plant	glycerol-arsenosugar (gly-sug), $InAs^{III}$, $InAs^{V}$, DMA and MMA	HPLC-ICP-MS	PRP-X100 (Hamilton, USA) column; mobile phase, 20 mM $NH_4H_2PO_4$, and Zorbax SCX300 (Agilent, Germany) column; mobile phase, 20 mM pyridine.	10	20	$InAs^{III}$: 2 $InAs^{V}$: 8 MMA: 5 DMA:3 gly-sug:15	135
Seaweed	AsB, $InAs^{III}$, $InAs^{V}$, DMA , Ribose-OH, Ribose-PO_4, Ribose-SO_3	HPLC-ICP-MS	Anion-exchange Hamilton PRP-X100 anion-exchange; mobile phase, 20 mM NH_4HCO_3, pH 9.0, 1% MeOH.	25	50	Not Given	157
Clams and Seaweed	$InAs^{III}$ $InAs^{V}$	HPLC-HG-AAS	Hamilton PRP-X100 anion exchange column; mobile phase, 20 mM ammonium phosphate pH 6.	_	-	Not Given	98
Porphyra	$InAs^{III}$, $InAs^{V}$, MMA, DMA and AsB	HPLC–(UV)–HG–AFS	Hamilton PRP-X100 anion exchange column; mobile phase, 3 mM $(NH_4)_2HPO_4$, pH 8.7.	-	-	$InAs^{III}$: 2.7 $InAs^{V}$: 8.3 MMA: 2.1 DMA: 1.8 AsB: 2.1	272
Ground water	$InAs^{III}$, $InAs^{V}$, DMA and MMA	HPLC-ICP-MS	Strong cation exchange (SCX); strong anion exchange (SAX) cartridge; mobile phase, 1 M HNO_3 for DMA, and 5 mL of 80 mM acetic acid , 5 mL of 1 M HNO_3.	-	-	$InAs^{III}$: 0.12 $InAs^{V}$: 0.02 MMA: 0.02 DMA: 0.03	273
Water	AsB, $InAs^{III}$, $InAs^{V}$, MMA and DMA.	HPLC-ICP-MS	Column, Dionex AS7 anion-exchange; mobile phase, A: 2.5 mM $NH_4H_2PO_4$, pH 10.0, B: 50 mM $NH_4H_2PO_4$.	30	20	AsB: 0.024 $InAs^{III}$: 0.017 $InAs^{V}$: 0.026 MA: 0.026 DMA: 0.023	274
Fresh water and seawater	AsB, $InAs^{III}$, DMA, MMA and $InAs^{V}$	HPLC-HG-AAS	Anion exchange column (Hamilton, Reno, NV, USA); mobile phase, 25 mM phosphate, pH 5.8.	-	-	AsB: 0.3 $InAs^{III}$: 0.08 DMA: 0.1 MMA: 0.1 $InAs^{V}$: 0.3	236
Fresh water	$InAs^{III}$, MMA, DMA $InAs^{V}$	HPLC-HG-AAS	Anionic column (Hamilton PRP-X100), mobile phase (17 mM $H_2PO_4^-/HPO_4$, pH 6.0)	-	-	$InAs^{III}$: 0.1 $InAs^{V}$: 0.6 MMA: 0.3 DMA: 0.2	275
Ground water	$InAs^{III}$ and $InAs^{V}$	HPLC-HG-AAS	Anion-exchange column Supelco LC-SAX1 and thermostatted by column oven (CTO-10ASvp); mobile phase phosphate buffer (50 mM Na_2HPO_4, 5 mM, KH_2PO_4; pH 5.4).	-	-	$InAs^{III}$ 7.8 $InAs^{V}$ 12.0	276
Fresh water	$InAs^{III}$, MMA, DMA $InAs^{V}$	HPLC-HG-AFS	Hamilton PRP-X100 anion exchange column; mobile phase A; $NH_4H_2PO_4/(NH_4)_2HPO_4$)5 mM, pH 4.8, mobile phase B:$NH_4H_2PO_4/(NH_4)_2HPO_4$) 30 mM, pH 8.0	20	100	$InAs^{III}$: 0.05 $InAs^{V}$: 0.06 MMA: 0.07 DMA: 0.05	277

Table 2.2 Continued

Matrix	Species	Technique	Separation conditions	Time of separation minute	Amount of sample µL	Detection limit ng mL^{-1}	Reference
Algae, fish tissue and Shellfish	Inorganic arsenic, DMA, AsB, Arseniosugar PO_4, Arseninosugar OH, Arsinosugar SO3,	HPLC-ICP-MS	Cation exchange Dionex Ionpac CS-10 column; mobile phase, 5 mM pyridinium, pH 2. Anion exchange Hamilton PRP-X100 column; mobile phase, 20 m M NH_4HCO_3, PH 10.3.	–	50	Not Given	34
Fish and sediment	AsB, AsC, DMA, MMA, $InAs^{III}$ and $InAs^{V}$.	HPLC-ICP-MS	Hamilton PRPX-100 column; mobile phase A, 10 mM $NH_4H_2PO_4$- $(NH_4)_2HPO_4$, 2% CH_3CN, pH 6.5; mobile phase B, 100 Mm $(NH_4)_2HPO_4$, pH 7.95.	10	20	AsC: 0.5, AsB: 0.5 $InAs^{III}$: 0.5, DMA: 1.0 MMA: 1.0 $InAs^{V}$: 1.5	278
Fish, mussel	AsB, AsC, DMA, MMA, $InAs^{III}$ and $InAs^{V}$.	HPLC-ICP-MS	Column, Hamilton PRP-1; mobile phase, 0.5 mM tetrabutylammon-iumphosphate–4mM phosphate buffer, pH 9.	9	20	AsC: 9, AsB: 6 $InAs^{III}$: 6, $InAs^{V}$: 25 MMA: 22, DMA: 10	279
Dogfish	AsB, DMA, MMA, $InAs^{III}$ and $InAs^{V}$	HPLC-ICP-MS	Anion-pairing column, 10-µm PRP-1; mobile phase, 0.5 mM tetrabutylammonium hydroxide,5% methanol, pH 7. Anion-exchange column, PRPX-100 (Hamilton); mobile phase, 8 mM phosphate buffer, pH 7; cation-pairing column PRP-1 (Hamilton); mobile phase, 5% methanol, 2.5% acetic acid and 50mM sodium dodecylsulphate, pH 2.5.	9	200	AsB: 5.0 $InAs^{III}$:1.0	280
Fish tissues	AsB, $InAs^{III}$, DMA, MMA and $InAs^{V}$	HPLC-ICP-MS	Metrosep™ Anion Dual 3 column; mobile phase, A: 5 mM NH_4NO_3: B: 50 mM NH_4NO_3, 2 %(v/v) methanol, pH8.7	12	100	AsB: 22, $InAs^{III}$: 15 DMA: 16, MMA: 14 $InAs^{V}$: 17	281
Dorm 2, fish	AsB, DMA, MMA, $InAs^{III}$ and $InAs^{V}$	HPLC-ICP-MS	Hamilton PRP-X100 column; mobile Phase, A: 15 mM $(NH_4)_2CO_3$, 2% MeOH, pH 9: B: 50 mM $(NH_4)_2CO_3$, 2% MeOH, pH9.	22	200	AsB: 0.003, $InAs^{III}$: 0.01, DMA: 0.004, MMA: 0.003	282
Fish, molluscs and crustaceans	AsB, $InAs^{III}$, DMA, MMA and $InAs^{V}$	HPLC-ICP-MS	A Hamilton PRPX-100 column, mobile phase, A: 60 mM ammonium carbonate, pH9: B:H_2O	15	60	Not Given	137
Fish tissue, DORM-2	AsB, DMA, MMA, $InAs^{III}$ and $InAs^{V}$	HPLC-ICP-MS	Dionex Ionpac AS4A4 column; mobile Phase, A: 0.4 mM HNO_3, pH 3.4: B: 50 mM HNO_3, pH 1.3.	–	100	AsB: 0.042, $InAs^{III}$: 0.066, $InAs^{V}$: 0.045, MMA: 0.059, DMA: 0.044	283

Table 2.2 Continued

Matrix	Species	Technique	Separation conditions	Time of separation minute	Amount of sample µL	Detection limit ng mL^{-1}	Reference
Fish and oyster	AsB, AsC, $InAs^{III}$, $InAs^{V}$, DMA, MMA	CE-ICP-MS	15 mM Tris solution containing 15 mM SDS (pH 9.0) was used as the electrophoretic buffer and the applied voltage was set at 122 kV.	0.2	0.02	0.3-0.5	284
Fish, crustacean	AsB, $InAs^{III}$, $InAs^{V}$, DMA, MMA	HPLC-ICP-MS	Hamilton PRP-X100 anion exchange column; mobile phase, A: 5.0 mM Na_2SO_4, pH 10-10.5; B: 50 mM Na_2SO_4, pH 10-10.5 (fish and crustacean). Hailton PRP-X100 anion exchange column; mobile phase, A: H_3PO_4, pH 7.5: B: 50 mM, pH 6 (Sediment).	15	100	Not Given	15
Marine organisms	Arsenosugar glycerol, arsenosugar phosphate, arsenosugar sulfonate and arsenosugar sulfate	HPLC-ICP-MS	ZirChrom-SAX column; mobile phase, 1 mM $NH_4H_2PO_4$, pH 5.6; Hypercarb (Thermo Electron Corporation, Runcorn UK) column; mobile phase, 13.8mM nitric acid, 2% (v/v) MeOH, pH 8.	20	20	1.5-2.0	285
Seafood	$InAs^{III}$, MMA, DMA, $InAs^{V}$, AsB, AC, TMA^{+} and TMAO	HPLC-ICP-MS	An IonPac AG4 guard column and an IonPac AS4A analytical column (both from Dionex Corpn, USA); mobile phase, A: 0.4 mM HNO_3, pH 3.3; B: 50 mM HNO_3, pH1.3.	15	100	$InAs^{III}$: 0.03, MMA: 0.05 DMA: 0.05 $InAs^{V}$: 1.6 AsB: 0.08 AsC: 0.14 TMA^{+}: 0.09 TMAO: 0.13	286
Seafood	AsB, AsC, $InAs^{III}$, DMA, MMA and $InAs^{V}$	HPLC-ICP-MS	IonPac AS7 anion exchange column; mobile phase, A: 1.0 mM HNO_3, 1 % (v/v) methanol, pH 2.9: B: 80 mM HNO_3, 1% (v/v), pH 1.3.	9.5	50	AsB: 8.5 AsC: 6.7 $InAs^{III}$: 5.4 MA:10.7 MMA: 10.8 $InAs^{V}$: 6.2	108
oyster tissue	DMA, MMA, $InAs^{V}$, oxo-arsenosugars:O-PO_4,S-Gly and S-PO_4.	HPLC-ICP-MS	Hamilton PRP-X100 column; mobile phase, A: 20 mM phosphate buffer, pH 5.6; B: 20 mM phosphate, pH 5.6, MeOH50 % (v/v), 40 ºC.	25	10	Not Given	269

Table 2.2 Continued

Matrix	Species	Technique	Separation conditions	Time of separation minute	Amount of sample µL	Detection limit ng mL^{-1}	Reference
Shrimp	AsB, DMA, $InAs^{III}$, $InAs^{V}$, OXO-As-$SugPO_4$, Thio-As-$SugPO_4$.	HPLC-ICP-MS	Hamilton PRP-X100 anion exchange column; mobile phase, 20 mM $NH_4H_2PO_4$, pH 6, 40 ºC. Cation exchange Supelcosil LC-SCX column, mobile phase, 20 mM pyridine at pH 2, 40 ºC. Reverse phase chromatography using a Shisheido Capcell PAK C18 MGII; mobile phase, 10-mM sodium 1-butansulfonate, 4-mM tetramethylammonium hydroxide, 4-mM malonic acid, 0.5% MeOH, pH 3.	19	–	Not Given	287
Bivalve mollucks	AsB, $InAs^{III}$, MMA, DMA, $InAs^{V}$, p-arsanilic acid (p-ASA)	HPLC-ICP-MS	Hamilton PRP-X100 column; mobile phase, A: 20 mM $(NH_4)_2HPO_4$, pH 6.0; B: 20 mM $(NH_4)_2CO_3$, pH 8.5.	15	200	Not Given	149
Edible periwinkles	TMA^{+}, AsB, MMA, glycerol arsenosugar and inorganic As	HPLC-ICP-MS	Hamilton PRP-X100 anion exchange column; gradient mobile phase, A: 4 mM NH_4NO_3; B: 60 mM NH_4NO_3, pH 8.65. Hamilton PRP-X200 cation-exchange column; mobile phase, 20 mM pyridine (C_5H_5N)/pH 2.7, formic acid (CH_2O_2).	8	-	Not Given	185
Biological tissues (certified material TORT-1 and fresh bivalve tissues)	AsB, $InAs^{III}$, MMA, DMA $InAs^{V}$	HPLC-HG-AAS	Column, Hamilton PRP X-100 strong anionic exchange column;mobile phase, phosphate buffers (10 mM and 100 mM at pH 5.8).	-	-	$InAs^{III}$: 1.1 DMA : 2.0 MMA: 1.9 $InAs^{V}$: 3.9	288
Biota sample	AsB, $InAs^{III}$, DMA, MMA and $InAs^{V}$	HPLC-HG-AAS	Anion exchange column (Hamilton, Reno, NV, USA); mobile phase, 25 mM phosphate, pH 5.8.	-	-	AsB: 0.3, $InAs^{III}$: 0.08 DMA: 0.1 MMA: 0.1 $InAs^{V}$: 0.3	143
Marine organism	$InAs^{III}$, $InAs^{V}$, MMA, DMA and AsB	HPLC–(UV)–HG–AFS	Hamilton PRP X-100 (25 cm×4.1 mm) column; mobile phase, 25 mM phosphate buffer, pH 5.8.	-	-	$InAs^{III}$: $InAs^{V}$: MMA: DMA: AsB=0.3	288
Canned cod liver tissue	Triethylarsine (Et_3As) , triphenylarsine (Ph_3As)	GC-ICP-MS	Column: HP-5MS (30 mm × 0.25 mm × 0.25 µm), carrier gas: He 2 mL/min,GC program; A: 40 °C, 10 °C /min to 60 °C, 30 °C/ min to 250 °C, 40 °C/ min to 280 °C B: 50 °C, 1 min, 50 °C/ min to 180 °C, 3 °C/ min to 220 °C 1 min, 15 °C/ min to 270 °C 8 min	20	-	Et_3As: 0.00005 Ph_3As: 0.00013	289

2.4 Conclusion

Arsenic species can be found in both plant derived and marine food stuffs. Arsenic exists in food as $InAs^{III}$ and $InAs^{V}$, organic arsenic (such as MMA, DMA) and tetramethylarsonium ion, AsC, TMAO, and arsenosugers. Sources such as fish and seafood are well known to contain relatively high concentration of AsB which is not toxic, whereas cereals for example rice, and drinking water may contain inorganic arsenic which may present a risk to health. This review of the literature suggests that appropriate analytical techniques now exists to determine the most common arsenic species in food and waters to ensure that current health guidelines are met.

Chapter 3

Determination of total arsenic and arsenic speciation in fish using HPLC-ICP-MS.

3.1 Introduction

It is well-known that the toxicity of heavy metals depends strongly on their chemical forms, therefore the quantitative determination of individual species has been of increasing interest. The speciation of arsenic is of particular importance because of the high levels found in fish and the differences in toxicity with oxidation state [42].

To date, over fifty arsenic species have been identified in marine organisms [11]. Organic arsenic forms such as AsB and different arsenosugars are the most common forms in marine products, whereas in food of terrestrial origin, inorganic arsenic and organic arsenic (DMA and MMA) are the predominant forms [189].

The extraction technique of choice for a particular application reflects both the matrix and the species to be determined. Trypsin has been used for extracting arsenic species from fish and crustaceans with an extraction efficiency of between 82-102 % [15]. Reyes *etal.*[281] used a microwave-assisted enzymatic extraction using a pronase E/lipase mixture to extract arsenic species from shark with extraction efficiency of 101 ± 0.01 % and from commercial fish (marlin) 63 ± 1%. Pepsin has been used as an extractant agent with pressurized liquid extraction to speciate arsenic forms in a range of different fish with an extraction efficiency of 95 to 103 %.

The analytical methods for speciation of arsenic in non-food and food samples generally involve the use of separation techniques coupled with a sensitive atomic detector. HPLC has been successfully coupled to ICP-OES [290] and ICP-MS [291]. Other atomic detectors, AAS [290] and AFS [292,293] and AES [294] have also been coupled with HPLC. Coupling of

HPLC and ICP-MS is of interest as it offers the efficient separation and detection of arsenic species [295, 296]. ICP-MS has advantages over other atomic detectors due to its multi-element characteristics, speed of analysis, detection limits and isotopic capability [254]. The aim of work described in this chapter was to develop a sensitive HPLC-ICP-MS method for use with the selective extraction techniques to determine arsenic species in both fish samples and appropriate reference material.

3.2 Experimental

3.2.1 Instrumentation

ICP-MS analysis was performed using an X Series 2 instrument (Thermo Scientific, Hemel Hempstead, UK). The operating conditions are described in Table 3.1. The common polyatomic interference $^{40}Ar^{35}Cl^{+}$ for As^{+} at mass-to-charge ratio (m/z) 75 may arise from the combination of chlorine introduced via the sample matrix and argon from the plasma. Collision cell technology was used to eliminate possible interferences. Caesium, indium and iridium 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 ($^{115}In^{+}$), m/z 193 ($^{193}Ir^{+}$) and m/z 133 ($^{133}Cs^{+}$) used for internal standardization.

Table 3.1 ICP-MS operating conditions used for the determination of total arsenic in sample digests and extracts.

ICP-MS	X Series 2	
	Peristaltic pump speed/ mL min^{-1}	1.2
	Nebulizer type	V-groove
	Spray chamber	Sturman-Masters
	Radio frequency power	
	Forward power/W	1400
Gas flows/L min^{-1}	Coolant	13
	Auxiliary	0.75
	Nebulizer	1.0
H_2 addition	Collision gas flow (mL min^{-1}) 7 % H_2 in He	3.6
(m/z)	As	75
	In	115
	Ir	193
	Cs	132
Dwell time (ms)	ICP-MS	10
	HPLC-ICP-MS	100

The HPLC instrumentation operating conditions are illustrated in Table 3.2. Chromatographic separations were carried out using a Jasco chromatographic pump (Tokyo, Japan) with a 250 X 4.1 mm column packed with 10 µm particle sizes Hamilton PRP-X100 anion exchange resin. A 50 X 4.1 mm guard column packed with the same material was used to protect the column. A Rheodyne 7152 injection valve (Rheodyne, Cotati, CA, USA), a six-way injection valve, was used for column loading and connected to the column. The interfacing between HPLC and ICP-MS was carried

out using a 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. A MSE centrifuge (Kent, England) was used to centrifuge the samples. The ICP-MS was set to time-resolved data acquisition. Data for arsenic (m/z 75) were recording using the peak jumping acquisition and displayed as mass-intensity-time plots. The concentrations of each arsenic species were calculated using peak areas and were compared with standard solutions.

Table 3.2 HPLC speciation and operating conditions used for arsenic speciation .

Parameters	Experimental conditions
Column dimensions	250 X 4.1 mm
Guard column dimensions	50 X 4.1 mm
Packing material	Hamilton resin PRP-X100, 10 µm particle size
Eluent flow rate	1.1-1.2 mL min^{-1}
Sample loop	20 µL
Competitive counter ion	Sulphate (Na_2SO_4)
Mobile phases	6.5 mM Na_2SO_4, 5 % CH_3OH, pH 10.2

3.2.2 Chemicals and Reagents

All commercial chemicals were of analytical grade and used without further purification. All solutions were prepared with Milli-Q (18 MΩ cm) unless otherwise stated. Total arsenic standards were prepared from a high purity stock solution 100 µg mL^{-1} in 5 % HNO_3 (CPI international, USA). Arsenic oxide ($InAs^{III}$) was purchased from Aldrich (Milwaukee, USA). The DMA and AsB were purchased from Sigma (Gillingham, Dorset, UK), and MMA was purchased from Chem Service (West Chester, Pennsylvania, USA). $Na_2HAsO_4.7H_2O$ ($InAs^{V}$), Cs, In, Ir, sodium chloride, polyvinyl

pyrrolidone (PVP), iso amyl alcohol and ammonium dihydrogen orthophosphate AnalaR standards were obtained from VWR International (MERCK, Lutterworth, Leicestershire, UK). Hydrogen peroxide 37 % and nitric acid 70 % (Merck, Poole, Dorset, UK) were used. GBW10015 spinach certified reference material (Institute of Geophysical and Geochemical Exploration, Langfang, China) was purchased from LGC standards (Middlesex, UK). Trypsin (from bovine pancreas: 11800 units mg^{-1}) was purchased from Sigma (Sigma, Gillingham, Dorset, UK). The CRM DORM 3 fish protein was obtained from National Research Council, Ottawa, Canada. CTAB, chloroform, EDTA and HCl were purchased from Fisher (Loughborough, Leicestershire, UK). Ethanol was obtained from Rathburn (Walkerburn, Scotland). Glassware and plastic centrifuge tubes were pre-cleaned by soaking for two days in 5 % Decon 90 (Merck) in Milli Q water followed by soaking for two days in 2 % v/v nitric acid made up with Milli Q water and then rinsed with Milli Q water prior to use.

3.3 Determination of total arsenic and arsenic species in fish samples

3.3.1 Sample preparation

The different fish samples (ling, gurnard, grey mullet, pollock, dover sole, john dory, megrim, flounder, dab, sand sole, brill, lemon sole, halibut) were purchased from Plymouth Fish Market and then returned as quickly as possible to the laboratory. The bones, scales, heads and tails were separated from the tissues and the tissues of fish samples were washed with Milli-Q water for analysis. All samples were then frozen at - 40 ºC for 12 h in a freezer and then placed in a freeze drier for 48 h at - 40 ºC. The freeze-dried samples were then ground using an agate pestle and mortar to a fine powder and then sieved using a nylon sieve 250 µm. The samples were then stored in brown bottles and placed in a desiccator in order to avoid exposure to light and moisture until required for analysis.

3.3.2 Sample digestion procedures

For the determination of the total element concentrations it is essential to utilize a sample decomposition technique that will ensure that the determinants of interest remain in solution. The determinant must be stable and any chemicals used must not cause instrumental interferences that may increase the limits of detection, particularly in cases where trace or ultra-trace elemental levels are expected. The practice of microwave digestion has been comprehensively reviewed [297] for the dissolution of biological matrices. It has been revealed that the three primary components of carbohydrates, proteins and lipids completely decompose in nitric acid ($\geq$ 2 M) at temperature of between 145-165 ºC [298]. Nitric acid (70 % - azeotopic) has a boiling point of 122 ºC and in order to adjust the oxidizing potential of HNO_3, by means of elevating the temperature, closed vessel microwave conditions are used [298]. The overall decomposition process is further assisted by addition of hydrogen peroxide as the oxidizing power of HNO_3 increases. Once complete digestion has been achieved, the elements of interest remain in solution and can be determined by the chosen method of detection. The digestion for total arsenic in the fish tissue samples employed nitric acid and hydrogen peroxide.

Acidic microwave digestion can modify species form. Where speciation analysis is to be performed digestion procedures that retain the chemical form of the compound must be employed. The choice of suitable enzyme for the sample matrix where the cell contents can be released into solution without modifying arsenic species. Enzymatic digestions are widely reported in the literature with the effective extraction of the arsenic species under consideration [15, 108, 126, 194, 299]. Optimum conditions of pH and temperature must be employed, as enzyme activity is sensitive to these parameters.

3.3.2.1 Nitric acid digestion for the measurement of total arsenic

Total arsenic in biological and food samples may be determined successfully using the combination of microwave digestion and ICP-MS [291, 300]. Vessels were pre-cleaned by soaking for one day in 5 % Decon 90 (Merck) in Milli Q water followed by soaking for one day in 3% v/v nitric acid made up with Milli Q water, rinsed with Ultra-pure water and then dried by put them in oven for one day prior to use. A Mars Xpress microwave lab station (CEM, USA) with 100 mL closed Teflon vessels with Teflon covers was used for the acid digestion of samples. Freeze dried 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 then sealed. All samples were digested for 43 min at 1600 W. In the first step of the digestion, the temperature was increased up to 160 ºC in 15 min and then held at this temperature for a further 5 min. In the second step of the digestion the temperature was increased from 160 to 200 ºC in 8 min and then held 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 volumetric flasks and made up to volume with 2 % (v/v) HNO_3, prepared with Milli Q water. The samples and standards were spiked with the internal standards (In and Ir) to give the final concentration of 10 µg L^{-1}.

3.3.2.2 Extraction procedures for the speciation of arsenic in fish sample

It is well-documented that trypsin can degrade protein and transform protein to its simpler components such as peptides and amino acids. The extraction procedure used here is based on the work of Rattanachongkiat *et al.* [15]. This method breaks down dietary protein molecules to their component peptides and amino acids. Trypsin works effectively in a slightly alkaline environment, approximately pH 8 at 37 ºC [126]. The arsenical species in the fish samples and CRM DORM-3 fish protein were extracted

using trypsin enzyme in 0.1 M NH_4HCO_3. 0.25 g of freeze-dried sample and 0.1 g trypsin were accurately weighed into a potter homogeniser and 0.1 M NH_4HCO_3 (10 mL) were added and homogenized with the sample then transferred to a plastic centrifuge tube. Another 10 mL of 0.1 M NH_4HCO_3 was added to rinse the homogenizing tube and then transferred to the same centrifuge tube. The tubes were left in a shaking water bath at 37 ºC for 12 h prior to the samples being centrifuged at 4000 rpm for 20 min. The extract was quantitatively transferred into a 25 mL volumetric flask and spiked with Cs internal standard solution to give final concentration of 10 µg mL^{-1}. The samples were diluted to volume with 0.1 M NH_4HCO_3. The total arsenic in the residual solid was measured using ICP-MS after extraction of the total arsenic using microwave assisted-acid digestion. The extract was kept in darkness at 4.0 ºC for no longer than 1 week.

3.3.3 Determination of the mass balance for arsenic in the analysis of biological samples

To obtain data for mass balance calculations, the sum of species in the extracts together with the total element concentration in any residues must be accounted for. In general, the sum of concentrations of arsenic species (AsB, MMA, DMA and $InAs^V$) combined with any arsenic, in the residual solids from enzyme extraction, should be equal to the concentration of total arsenic using microwave assisted acid digetion. A summary of this mass balance approach is presented in Figure 3.1.

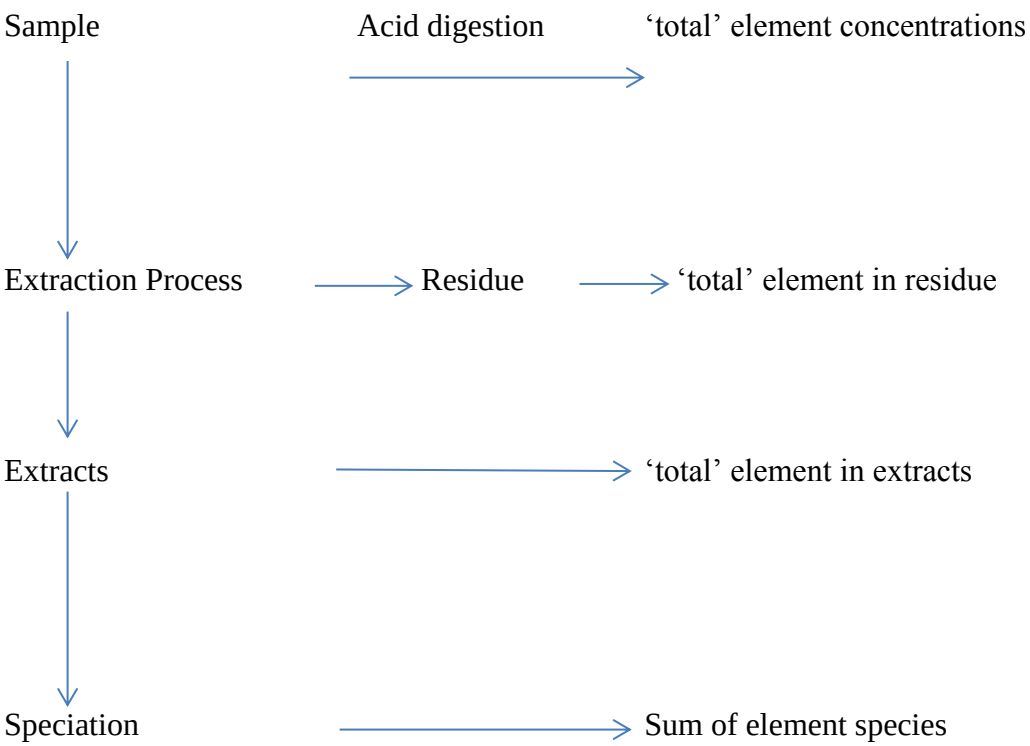


Figure 3.1 Flow diagram for arsenic mass balance in biological, rice and vegetable samples.

3.3.4 Optimisation of chromatographic conditions

The polarity of organic and inorganic compounds makes them amenable to both ion-exchange and reversed-phase separations. There are generally 5 arsenic species to be analysed in this study: $InAs^{III}$, $InAs^{V}$, DMA, MMA and AsB. Nevertheless, a number of methods for arsenic speciation in biological samples or water samples have been applied only to inorganic arsenic estimations [234, 301]. Other methods include an additional determination of DMA, MMA and AsB [108,302, 303]. Recently chromatographic speciation methods applied to biological media allow the determination of more arsenic species, including the tetramethylarsonium ion and the arsenosugars [36, 293].

Liquid chromatographic separation of arsenic compounds is influenced by the physico-chemical properties of the principal species; inorganic species and methylated forms are

weak polar acids, AsB is a weak acid with a permanent positive charge on the arsenic atom with a primary alcohol function and a very low polarity in comparisons with the other solutes. It is therefore difficult to select the chromatographic conditions (column, pH, and composition of the mobile phase) without compromise whatever the separation mode (ion exchange, ion pairs) because of these properties of arsenic species [304].

The chromatographic separation of arsenic species was performed using a high capacity anion-exchange based column with sulfate mobile phase. The main factors that affect the separation of arsenic species using HPLC are the values of pKa of the species, buffering capacity and ionic strength of the mobile phase. Table 3.3 shows that arsenic species (AsB, $InAs^{V}$, MMA and DMA) have very different acidity constants, which are used to provide a basis for this work.

Factors such as pH, ionic strength, and the temperature can be varied to improve the separation [305]. The degree of ionization of species is pH dependent. At pH 10.2 the arsenate is present in the form of $HAsO_4^{2-}$. This species has a doubly negative charge and, therefore, shows greater affinity for the anionic stationary phase than the other arsenic species under investigation therefore it would be expected to elute last. AsB elutes with the solvent front at pH 10.2 because it, being a quaternary arsonium compound, exists as a zwitterion at this pH. Arsenite, which presents in the form $H_2AsO_3^-$, and DMA at this pH will exist as a singly charged anionic species and also elute early due to a limited affinity for stationary phase through changes in determinant species mobility.

Table 3.3 pKa values for inorganic and organic arsenic [306, 307 308].

Arsenic compound		pK_a value
Arsenious acid ($InAs^{III}$)	$H_3AsO_3 \longrightarrow H_2AsO_3^-$	9.22
Arsenic acid ($InAs^{V}$)	$H_3AsO_4 \longrightarrow H_{(3-n)}AsO_4^{n-}$	2.20, 6.97,11.53
MMA	$CH_3AsO(OH)_2$	3.6, 8.2
DMA	$(CH_3)_2AsO(OH)$	1.28, 6.2
AsB	$(CH_3)_3As^+CH_2CO_2^-$	2.2

n=1, 2 or 3.

3.3.5 Certified reference materials

The analyst can use CRMs to validate a method and for demonstrating the accuracy of a determination. A CRM can be defined as a reference material, accompanied by a certificate, where one or more property values are certified by procedures, which establish traceability to an accurate realisation of the unit in which the property values are expressed, and where each certified values is accompanied by uncertainty at a stated level of confidence. An analyst can compare his result to a certified value using CRMs [12]. The use of CRM materials has been reviewed extensively with respect of quality control, method validation, interlaboratory testing, control charting and evaluation of analytical results using a matrix-matched CRM [309]. Several arsenic-containing CRMs have been developed, but most of them are certified for the total-element concentration. Species specific CRM materials are now crucial as a result of the increasing used for species specific measurement [12]. Amongst the CRMs available for arsenic are BCR627 (Tuna fish), BCR 710 (oyster tissue), DORM-2 (dog fish muscle), and SRM 1640 (natural water)[310]. Species specific materials include TORT-3 (lobster) and several from the National Metrology Institute of Japan (MNIJ), including CRM 7405 (seaweed) and CRM 7503a (rice flour).

3.4 Total arsenic and arsenic speciation in fish

Fish and marine-based products are the major source of arsenic in the human diet. AsB was the first organoarsenic compound detected in marine animals (in lobster in1977) [311]. However, its biosynthetic pathway is still unknown [11]. The vast majority of total arsenic in marine animals such as in most finfish and shellfish is represented by AsB not inorganic arsenic [312].

3.5 Development of HPLC-ICP-MS for fish samples

The ionic character of different arsenic species at particular pH and oxidation conditions indicate that the best separation may be achieved using anion-exchange chromatography and this was used throughout. Various parameters were considered: column length, type and particle size of anion-exchange resin, pH of mobile phase, concentration of mobile phase and the type of counter ion.

Figure 3.2 demonstrates the elution pattern of the four arsenic species under investigation using the conditions derived experimentally from the Hamilton PRP-X100 column. Baseline separation was achieved successfully and the analysis complete in 850 seconds using the chromatographic conditions illustrated in Table 3.2.

Mobile phase Na_2SO_4 eluted AsB and DMA in the early stage of separation and then MMA and $InAs^V$ were eluted with increasing separation time.

The optimum conditions were: the Hamilton PRP-X100 resin (250 X 4.1 mm) dimension with 10 µm particle size and guard column (10 µm), with a mobile phase 6.5 mM Na_2SO_4 containing 5 % (v/v) methanol, pH 10.2, and a mobile phase flow rate 1.2 mL min^{-1}.

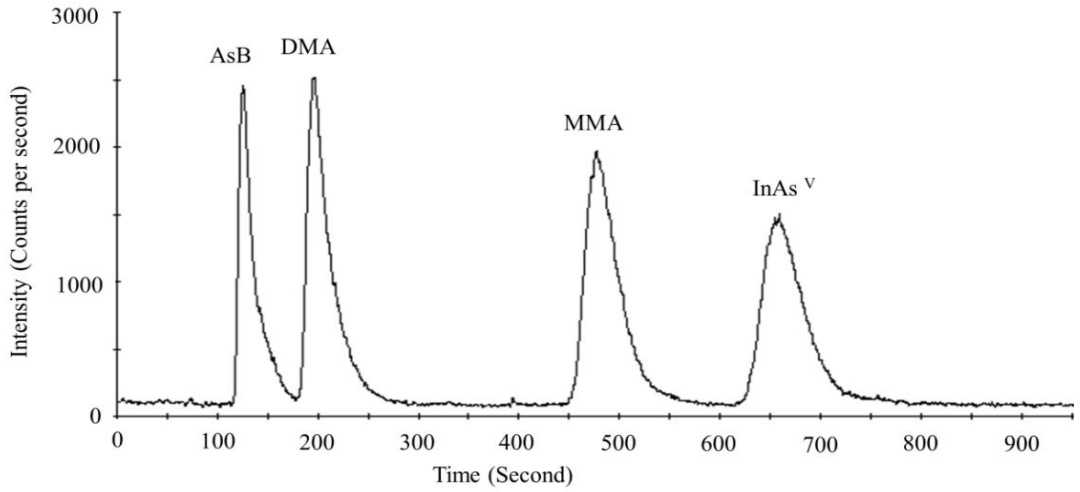


Figure 3.2 Chromatogram of four arsenic standards in aqueous solution. AsB, DMA and MMA and $InAs^{V}$ 50 µg L^{-1} As, employing a Hamilton PRP-X100 anion-exchange HPLC column using the conditions described in Table 3.2.

3.5.1 Detection limit

The detection limits of arsenic species under study are shown in Table 3.4. Detection limits were determined by 3 times standard deviation of the sample blank using HPLC-ICP-MS under the conditions shown in Table 3.2.

Table 3.4 Detection limits (3 x standard deviation of sample blank) determined during analysis for AsB, DMA, MMA and $InAs^{V}$ in fish tissue using HPLC-ICP-MS.

Arsenic species	Detection limit µg g^{-1}
AsB	0.015
DMA	0.022
MMA	0.034
$InAs^{V}$	0.027

3.5.2 Efficiency of the collision cell

The collision cell was connected with the ICP-MS and mixture of gases (7 % H_2 in He) was passed through it. As mentioned previously, arsenic suffers from major spectral interferences for example $^{40}Ar^{35}Cl^{+}$ generated by ions derived from the plasma gas, matrix component, or the solvent/acid used in sample preparation. The collision cell was used to remove a possible interference including polyatomic interferences such as polyatomic $^{40}Ar^{35}Cl^{+}$. Different concentrations of hydrochloric acid (1.0, 10 and 50 μgmL^{-1}) were injected to the system and the response for apparent arsenic 75 (m/z) was monitored. The results are presented in Table 3.5. By increasing the concentration of HCl the response for apparent of arsenic 75 (m/z) increased.

Table 3.5 Effect of chlorine interference on the arsenic 75 (m/z) response when using ICP-MS (µg As L^{-1} ± SD).

Concentration of HCl µg mL^{-1}	Apparent 'concentration' of As 75 µg L^{-1}
1	0.144 ± 0.01
10	0.32 ± 0.01
50	0.61 ± 0.03

3.5.3 Results of the analysis of CRMs.

In order to validate the method development the CRM DORM-3 (fish protein) was used. The digestion technique described in section 3.3.2.1 was applied to determine 'total nitric acid available' arsenic. The results obtained are shown in Table 3.6. Digestion efficiency was 101 %. The obtained value was in good agreement with the certified value of DORM-3.

Table 3.6 Certified Reference Material analysis for total arsenic; mean ± SD (n=3).

Materials	Characteristic	Certified value µg g^{-1}	Concentration obtained µg g^{-1}	Digestion efficiency %
DORM-3	Fish protein	6.88 ± 0.3	6.94 ± 0.36	101

Statistical agreement with the certified values for the CRM can be evaluated by comparing the difference between the found and certified values, Δm, and the expanded uncertainty of Δm, UΔ, which was estimated by combining the standard uncertainties for the found and certified values. If $\Delta_m \leq U_\Delta$ then the two values are in agreement[313]. Where Δ_m and U_Δ can be calculated as following:

Δ_m: $\left| C_m - C_{CRM} \right|$

Δ_m : absolute difference between mean measured value and certified value

C_m: mean measured value

C_{CRM}: certified value

The uncertainty of Δ_m is U_Δ, which is calculated from the uncertainty of the certified value and uncertainty of the measurement result as following

$$u_\Delta = \sqrt{u^2_m + u^2_{CRM}}$$

$U_\Delta := 2 \cdot u_\Delta$

U_Δ: expanded uncertainty of difference between result and certified value

u_Δ: combined uncertainty of result and certified value (=uncertainty Δ_m)

u_m: uncertainty of the measurement

u_{CRM}: uncertainty of the certified value

The standard uncertainty, u_{CRM}, of the certified value is calculated by dividing the stated expanded uncertainty by the coverage factor (k=2).

In DORM-3 fish protein there was not significant difference between the measurement result and certified value because U_Δ (0.78 µg g^{-1}) was >Δ_m (0.06 µg g^{-1}).

3.6 Results and discussion from analysis of 'real' fish samples

Fish samples were purchased from Plymouth Fish Market in May 2013 for the determination of total arsenic. Classical extraction methods using water [177], a mixture of water/methanol [291] or methanol/water followed by extraction of arsenic species from the residue with 2% nitric acid [185] have been used to extract arsenic species from marine species with variable extraction efficiencies. Water was used to extract AsB, AsC, $InAs^{III}$, DMA, MMA, $InAs^{V}$ from lobster tissue and TORT-2 with a total extraction efficiency for arsenic of between 67.6 - 81.3 % [133]. Li *et al.*[34] used methanol water to extract arsenical species in fish and shellfish with an extraction efficiency of between 61-91 %, While Gomez-Ariza *el al.* [236] achieved extraction efficiencies 76-96 % for arsenic species in seafood using a methanol extraction and 91- 95 % using a water-methanol extraction agent.

The values obtained for total arsenic in fish are presented in Table 3.7 using procedure described previously in section 3.3.2.1. Arsenic species in food of marine origin are often found with relatively high concentrations. The fish tissues under investigation (ling, gurnard, grey mullet, pollock, john dory, dover sole, lemon sole, sand sole, brill, dabs, megrim, halibut and flounders) have a total arsenic content of between 3.53 to

98.8 µg As g^{-1}, on dry weight basis. The highest concentration of arsenic was found in halibut (98.8 µg As g^{-1}) while the lowest value was found in grey mullet (3.52µg As g^{1}). These values are in line with previous studies [11].

The use of enzymes as an extraction agent for arsenic species may offer some advantage due to the selective hydrolysis of the major components within the cell. Thus, the mass of material could be decreased significantly, requiring less sample dilution and offering the ability to determine arsenic species which are not extracted with conventional techniques (water or water/methanol) [124]. Trypsin was therefore selected to extract arsenic species found in the tissues of various fish.

The trypsin extracted the arsenic species effectively from the fish samples and reference material DORM-3. A chromatogram obtained from the sand sole sample using anionic-exchange HPLC-ICP-MS is shown in Figure 3.3.

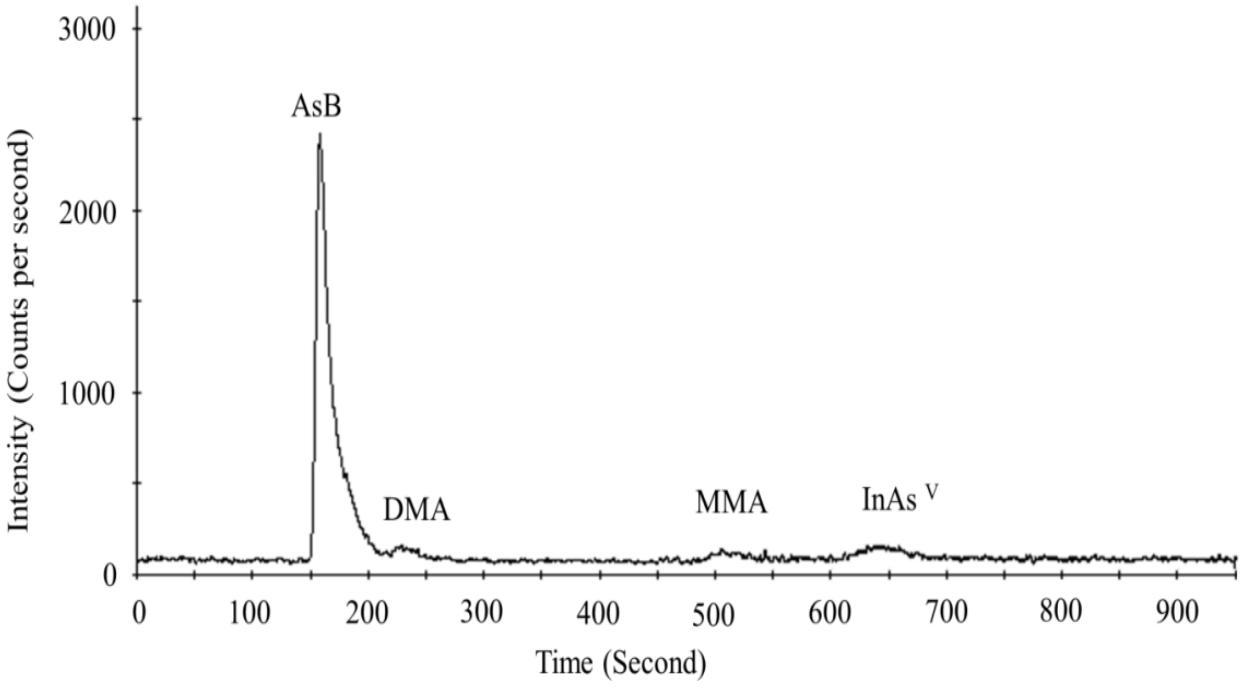


Figure 3.3 Chromatogram of sand sole using anionic-exchange HPLC-ICP-MS, using conditions described in Table 3.1 and 3.2.

Table 3.7 Results of analyses for arsenic concentration in the fish samples (dry weight); all values are given in µg g^{-1} of arsenic, mean ± SD (n=3).

Fish	Microwave assist digestion	AsB	DMA	MMA	$InAs^V$	Total arsenic in residue	Total arsenic in extract	Efficiency of extraction%
Ling	19.44 ± 0.24	17.99 ± 1.5	<0.022	0.18 ± 0.01	0.42 ± 0.01	0.42 ± 0.01	18.60 ± 1.58	96
Gurnard	12.21 ± 0.62	11.98 ± 0.18	<0.022	0.53 ± 0.01	0.19 ± 0.01	<0.009	12.49 ± 0.95	102
Grey mullet	3.53 ± 0.19	3.41 ± 0.15	0.46 ± 0.02	0.32 ± 0.02	0.60 ± 0.03	<0.009	3.73*	106
Pollock	25 ± 1.04	23 ± 0.59	<0.022	0.61 ± 0.04	0.22 ± 0.01	0.29 ± 0.01	22.51 ± 1.97	90
Dover sole	51.32 ± 2.28	51.18 ± 4.77	0.1 ± 0.01	0.58 ± 0.07	0.30 ± 0.01	0.42 ± 0.01	49.53 ± 2.89	97
John dory	3.61 ± 0.21	3.60 ± 0.12	0.25 ± 0.02	<0.034	<0.027	0.35 ± 0.002	3.27 ± 0.21	91
Megrim	27.69 ± 2.63	26.47 ± 1.44	<0.022	0.27 ± 0.03	0.55 ± 0.02	0.03 ± 0.002	28.10 ± 0.33	101
Flounder	27.10 ± 1.50	25.64 ± 1.92	0.16 ± 0.01	0.57 ± 0.02	0.90 ± 0.11	0.11 ± 0.007	26.01 ± 1.75	96
Dab	53.10 ± 3.75	51.20 ± 2.27	<0.022	0.30 ± 0.02	0.74 ± 0.02	<0.009	50.77 ± 0.73	96
Sand sole	33.23 ± 2.4	29.37 ± 2.91	<0.022	0.63 ± 0.01	1.09 ± 0.03	0.50 ± 0.04	34.83 ± 1.71	105
Brill	15.25 ± 1.32	13.07 ± 0.69	<0.022	0.61 ± 0.02	0.40 ± 0.02	0.10 ± 0.01	15.60 ± 0.69	102
Lemon sole	75.00 ± 3.62	74.09 ± 3.57	0.13 ± 0.01	0.24 ± 0.02	0.5 ± 0.04	0.28 ± 0.04	75.43*	101
Halibut	98.80 ± 6.92	97.74 ± 5.20	<0.022	0.40 ± 0.03	0.64 ± 0.04	0.89 ± 0.1	98.39 ± 2.37	100
DORM-3	6.94 ± 0.36	5.29 ± 0.51	1.06 ± 0.04	0.38 ± 0.02	0.17 ± 0.01	0.02 ± 0.001	7.18 ± 0.32	103

*Value based on the standard addition method

The analytical results obtained in this study using trypsin gave good extraction efficiencies ranging from 90 to 106 % for all arsenic species. As reported in the literature, AsB was the most widespread and abundant of the organoarsenic compounds found in the fish samples. The values ranged between 3.41 to 97.74 μg g^{-1}, dry weight. The values for DMA varied according to the species of fish concerned with average concentrations between 0.1 to 0.46 μg g^{-1} for dover sole, john dory, grey mullet, flounder and lemon sole. Other species of fish gave values below the detection limit (0.021 μg g^{-1}). $InAs^{V}$ was found in the range of 0.19 to 1.09 μg g^{-1} except john dory which was under limit of detection (0.027 μg g^{-1}). Nevertheless, MMA was found at trace levels in these fish with values were between 0.18 and 0.61 μg g^{-1}. John dory was an exception being below the limit of detection (0.034 μg g^{-1}). Matrix matching standard addition was performed on extracts of grey mullet and lemon sole using trypsin, and as a consequence the extraction efficiency was improved for both fish samples.

The flatfish examined in this study i.e. dover sole, megrim, flounder, dabs, sand sole, brill, lemon sole and halibut were found to contain highest level of arsenic, again with the exception of john dory which contained a relatively low concentration of arsenic. These fish are bottom feeders and are highly carnivorous feeding on the bivalves and molluscs which filter feed on the sea beds. The arsenic concentration in some of the fish was high, for instance, Lemon sole in excess of 75 μg g^{-1}. However these values are not unprecedented. Simon *et al.* [126] have reported the concentrations of arsenic in Lemon sole which were collected in the Plymouth Fish Market in the range of 149.6-172.9 μg g^{-1} and Luten *et al.* [314] found arsenic concentration at high level between 150-173 μg g^{-1}. In contrast, Baeyens *et al.* [315] have found values of 39.70 μg g^{-1}. The results for this study for ling, sand sole, dover sole, and brill, 19.44, 33.23, 51.32 and 27.69 μg g^{-1} respectively, are lower than that obtained by Baeyens *et al.* [315] for the same species,

which were 13.6, 54.8, 57.2 and 38.8 µg g^{-1}, respectively. The concentration of total arsenic obtained for dab, 53.10 µg g^{-1} was higher than that achieved by Baeyens *et al.* (40.8 µg g^{-1}) [315] but lower than found in the study by Sirot *et al.* (87.2 µg g^{-1} dry weight (21.8 µg g^{-1} wet weight)) [316]. The results from this study for John dory, ling and grey mullet (3.61 and 19.44 µg g^{-1} and 3.53 µg g^{-1}) are similar to the results obtained for the same fish (4.48 and 18.8 µg g^{-1} and range of 5.33-8 µg g^{-1}) by Sirot *et al.* [316] and Usero *et al.* [317]. However, the concentration of total arsenic in gurnard was almost half of total concentration of arsenic reported by Sirot *et al.* [316] and the results for pollock, flounder, brill and halibut are all higher than the results obtained by other authors [315, 316, 318]. In all cases, however, the results are in the same range reflecting natural regional variation. This is also reflected in the result for megrim (27.69 µg g^{-1}), which was is in good agreement with the study of Suner *et al.*, which reported values in the range 4.5-33.0 µgg^{-1} [319].

In terms of inorganic arsenic, generally flatfish such as dover sole, John dory, megrim, flounder, dab, sand sole, brill, lemon sole and halibut contain higher inorganic arsenic (0.19 and 1.09 µg g^{-1}) than fine fish, except ling in which the concentration of $InAs^V$ was 0.42 µg g^{-1}. Based on the worldwide literature on uncontaminated areas, although the percentage of inorganic arsenic in marine and estuarine finfish can vary, most samples fall within the range of 0.02-12.20 % of total arsenic [320]. The concentration of inorganic arsenic based upon the dry weight and the percentage of $InAs^V$ are shown in Table 3.8. The values of inorganic arsenic obtained in this study were comparable to those found by other authors. The use of DORM-3 as a certified reference material provided results in good agreement with the certificate value for total arsenic using microwave-assisted acid digestion and enzymolysis (trypsin). A mass balance calculation for each of the samples is summarized in Table 3.9. The total arsenic from

acid digestion of each sample should be equal to both the enzyme extracted total arsenic concentration and the sum of arsenic from species.

Table 3.8 Arsenic concentration in fish (µg g^{-1} ± SD) and percentage of inorganic arsenic.

Fish	Total arsenic using microwave-assisted acid digestion µg g^{-1}	Inorganic arsenic Dry weight µg g^{-1}	$InAs^{V}$ /total arsenic %
Ling	19.44 ± 0.24	0.42 ± 0.01	2.16
Gurnard	12.21 ± 0.62	0.19 ± 0.01	1.56
Grey mullet	3.53 ± 0.19	0.60 ± 0.03	17
Pollock	25.02 ± 1.04	0.22 ± 0.01	0.88
Dover sole	51.32 ± 2.28	0.30 ± 0.01	0.58
John dory	3.61 ± 0.21	<0.027	<0.027
Megrim	27.69 ± 2.63	0.55 ± 0.02	1.99
Flounder	27.10 ± 1.50	0.90 ± 0.11	3.32
Dab	53.10 ± 3.75	0.74 ± 0.02	1.39
Sand sole	33.23 ± 2.4	1.09 ± 0.03	3.28
Brill	15.25 ± 1.32	0.4 ± 0.02	2.62
Lemon sole	75.00 ± 3.62	0.5 ± 0.04	0.67
Halibut	98.80 ± 6.92	0.64 ± 0.04	0.65
DORM-3	6.94 ± 0.36	6.94 ± 0.36	2.45

Table 3.9 Arsenic mass balance for fish samples; mean ± SD (n=3).

Fish	Total arsenic using microwave-assisted acid digestion ($\mu g\ g^{-1}$)	Total arsenic in extract using trypsin $\mu g\ g^{-1}$	Sum of arsenic from species $\mu g\ g^{-1}$
Ling	19.44 ± 0.24	19.99 ±3.4	19.01
Gurnard	12.21 ± 0.62	12.46 ±3.58	12.70
Grey mullet	3.53 ± 0.19	3.73	4.79
Pollock	25.02 ± 1.04	22.51 ±1.97	24.12
Dover sole	51.32 ± 2.28	49.53 ±2.89	52.58
John dory	3.61 ± 0.21	3.27 ±0.21	4.20
Megrim	27.69 ± 2.63	28.10 ±0.33	27.32
Flounder	27.10 ± 1.50	27.61 ±3.04	27.38
Dab	53.10 ± 3.75	50.77 ±0.73	52.24
Sand sole	33.23 ± 2.4	34.83 ±1.71	31.59
Brill	15.25 ± 1.32	15.60 ±0.69	14.18
Lemon sole	75.00 ± 3.62	75.4 ± 3.4	75.24
Halibut	98.80 ± 6.92	98.39 ± 2.37	99.67
DORM-3	6.94 ± 0.36	7.18 ± 0.32	6.92

3.7 Conclusion

The application of ICP-MS coupled to HPLC provided an effective ion-exchange system for the simultaneous separation and detection of inorganic from organic arsenic. A collision reaction cell was used with the HPLC-ICP-MS system to suppress possible polyatomic interferences. The method was successfully applied to separate and detect the arsenical species in the fish samples. There are several factors which affect the separation of the arsenic species such as pH, ionic strength, and the temperature. In addition, the concentration of mobile phase, and the flow rate of the eluent play a role in the separation of the arsenic species. Validation, using certified reference material and mass balance calculation, was used to appraise the accuracy of the methodology used. The limits of detection were determined to be between 0.015 - 0.034 μg g^{-1} for the various arsenic species.

Total arsenic determination in fish was performed using ICP-MS following microwave-assisted acid digestion using concentrated nitric acid working as oxidizing agent and with H_2O_2 to increase the oxidizing power. Arsenic species in fish samples were successfully extracted with high efficiency (90-104 %) using trypsin. Non-toxic AsB was the major species detected in all fish samples; DMA was under the limit of detection (0.022 μg g^{-1}) in the majority of the samples. Except for the John Dory, MMA was detected in all samples. The percentage of $InAs^V$ varied between 0.54-17 % of total extracted arsenic.

Chapter 4

Arsenic speciation in plant based food stuffs using HPLC-ICP-MS

4.1 Total arsenic speciation in rice sample

For populations not exposed to arsenic-contaminated drinking water, food is a potentially important source of dietary arsenic intake [99, 100, 321]. Rice is one of the most popular, main food staples for over half of the world's population. In some Asian countries it provides over 70 % of the daily energy of their daily diet i.e. 0.5 kg (dry weight) per head [322] [98]. Approximately 480 million metric tons of rice is produced annually in the world [323]. The largest rice-producing countries are Republic of China, India, Indonesia, Bangladesh, USA and Vietnam [134]. Rice (*Oryza sativa*) accumulates higher arsenic concentrations compared to other grain crops, most likely because of the high plant availability of arsenic under reduced soil conditions [324]. The predominant arsenic species found in rice are $InAs^{III}$, DMA, MMA and $InAs^{V}$ [325] of the total arsenic present, the inorganic arsenic ranging from 10-90 %[326, 327].

According to Zavala and Duxbury [322] the "global normal" range for arsenic in rice grain is 0.08 to 0.2 µg g^{-1}. The majority of Asian rice has arsenic levels within the normal range (0.080 to 0.200 µg g^{-1}) concentration of arsenic in rice considering that the rice harvested in the environments not contaminated with arsenic. In contrast, rice from the USA and EU has a reputation for high arsenic levels. It was reported that 40 % of rice from U. S. and 20 % of the rice from the EU contained arsenic levels higher than the "normal range" (0.080-0.200 µg g^{-1}) [322]. Toxic limit established for arsenic in rice in Hungary is 0.300 µg g^{-1} while in China it is 0.700 µg g^{-1} [134].. The arsenic levels in rice reported in the literature around the world are presented in Table 4.1.

Table 4.1 Total arsenic concentration in rice reported in some countries around the world.

Country	Arsenic concentration µg g^{-1}	Reference
Bangladesh	<0.010-2.050	326, 327
China	0.310-0.930	134, 327
Hungary	0.116-0.139	134
India	0.030-0.044	322
Italy	0.130-0.377	328
Spain	0.080-0.380	322
Taiwan	<0.100-0.630	327
Vietnam	0.030-0.470	327
USA	0.110-0.660	322, 327
West Bengal	0.04-0.440	327

4.1.1 Experimental

ICP-MS analysis was conducted using an X Series 2 instrument (Thermo Scientific, Hemel Hempstead, UK) as described in Table 4.2. The HPLC instrumentation operating conditions are illustrated in Table 4.2

Table 4.2 ICP-MS and HPLC operating conditions used for the determination of total arsenic and arsenic speciation in rice and vegetable.

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
Dwell time (ms)	ICP-MS	10
	HPLC-ICP-MS	100
Column dimension	250 x 4.1 mm	
Guard column dimension	50 x 4.1 mm	
Packing material	Hamilton resin PRP-X100, 10 µm particle size	
Eluent flow rate	1.1-1.2 mL min^{-1}	
Sample loop	20 µL	
Competitive counter ion	Ammonium dihydrogen phosphate ($NH_4H_2PO_4$)	
Mobile phases (isocratic elution)	20 mM $NH_4H_2PO_4$, pH 6.0, 1 % CH_3OH	

4.1.2 Sample preparation

There are differences in the literature in terms of suggested drying and digestion procedures. Plant samples are usually dried in an oven to release the moisture content [329]. Rice grains were dried in an oven at 80 ºC until constant weight was obtained [265, 330]. Arsenic was determined on the basis of wet weight, then the samples were dried using an oven at 105 ºC to obtain the moisture content [331]. Attention must be paid to choose an appropriate sample size when considering the appropriate weight for homogeneous, accurate representation of the bulk material. In this study different rice types from different origin were collected. Samples (20 g) were then frozen at -40 ºC for 12 h in a freezer and then placed in a freeze drier for 48 h at -40 ºC. All dried samples were ground, using an agate pestle and mortar, to a fine powder and then sieved using a plastic 180 µm mesh sieve to receive a homogenous fraction with a small particle size for analysis.

4.1.2.1 Rice samples and their origin

A total of 17 polished rice samples were analysed. Rice samples were purchased from supermarkets in Kurdistan region-Iraq, Turkey and UK. The rice samples selected are listed in Table 4.3.

Table 4.3 Origin of rice samples used in this study.

Types of rice	Country	Region	Label
Short grain six months matured	Kurdistan-Iraq	Harir	KUH
Short grain five months matured	Kurdistan-Iraq	Akre	KUA1
Short grain six months matured	Kurdistan-Iraq	Akre	KUA2
Hamber short grain rice	Iraq	-	IRQ
Long grain rice	Thailand	-	THA1
Foreign rice			
Sainsbury's long grain rice	Uruguay	-	URU
Sainsbury's Indian basmati rice six months matured	India	-	IND
Tesco organic basmati rice	Pakistan	-	PAK
Thai Glutinous rice	Thailand	-	THA2
Green dragon Thai rice	Thailand	-	THA3
Arrirang	USA	-	USA
Long grain rice	Turkey	Beskiler	TUR1
Effsanel short grain rice	Turkey	Baldido	TUR2
Luts Persin short grain rice	Turkey	Mersin	TUR3
Beskiler long grain rice	Turkey	Gonen	TUR4
Duru long grain rice	Turkey	Balido	TUR5
Pure home Mali short grain rice	Mali	-	MAL

4.1.2.2 Digestion

For total arsenic determination in rice, popular techniques include the use of digestion blocks[330, 332], microwave-assisted digestion[10, 99 333] and accelerated solvent extraction [141]. Techniques often comprise acid digestions, including nitric acid alone [10, 156, 162, 334], a

combination of nitric acid and hydrogen peroxide[145, 160, 322], the use of hydrofluoric acid[335, 336], or perchloric acids [335, 337-339] and a mixture of trifluoroacetic acid and hydrogen peroxide [99]. Enzymatic digestions with α-amylase [162] and protease have also been reported to extract arsenic compounds from the solid sample matrix. Different temperatures have been used to assist the digestion, ramping to final temperatures of 80 ºC [339], 120 ºC [332], 145 ºC [322], 175 ºC [156] and 210 ºC [340]. Total arsenic concentrations in rice samples were extracted using microwave assisted acid digestion as described in section 3.3.2.1.

4.1.2.3 Extraction

The aim of extraction is to extract arsenic species quantitatively from organic matrix without conversion of the arsenic species. The extraction procedures reported in the literature can be divided into either enzymatic extractions or chemical extractions. Degradation of arsenic species can be avoided by using enzymatic extractions because they are mild and offer advantages such as the careful and/or selective hydrolysis the major component of the cell walls (cellulose) and digestion of cell components enzymatically. The mass of material could be decreased significantly, facilitating less dilution of the sample and the ability to determine arsenic species which are not preserved with conventional techniques (water or water/methanol) [124]. However, enzymes extractions also have some disadvantages. The extraction efficiency is sometimes low and variable depending on the sample matrix and the cost of enzymes is high compared to that of chemical reagents.

Chemical extraction for arsenic species determination is usually performed with strong acids or strong bases at high temperatures [100, 341] . Generally high concentrations from 0.2 to 2.0 M of extractants have been used. Even though chemical extractions may cause inter-conversion of $InAs^{III}$ /$InAs^{V}$, it is often not necessary to evaluate these two

species individually in food, as the toxicological and human health issues will be affected by total inorganic arsenic (both $InAs^{III}$ and $InAs^{V}$) [99]. In addition, it has been reported that the concentration of inorganic arsenic determined in rice does not depend on the analytical method applied [342].

In 2007, Foster *et al.* [119] used a procedure using 2% nitric acid to extract arsenic species from marine plants and animal digestive tissues. They showed that dilute nitric acid is able to effectively remove inorganic arsenic from tissue without oxidation of other major species in marine organisms. Sun *et al.* [326] also used 1% of nitric acid for extraction of arsenic species.

The method adopted in this study is based on the work of Sun *et al.* [326]. A 0.50 g sample was accurately weighed out into a 50 mL polypropylene digest tube and 25 mL of 1% of nitric acid added and left overnight. Samples were then extracted in a microwave oven. In the first step of the extraction, the temperature was increased up to 55 ˚C over 5 min and was then held at this temperature for a further 10 min. In the second step of the extraction, the temperature was increased from 55 ˚C to 75 ºC over 5 min and then held for a further 10 min. Finally the temperature for extraction was raised up 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 analysis.

4.1.3 Experimental parameters using HPLC-ICP-MS for rice

Figure 4.1 demonstrates the elution pattern of the four arsenic species under investigation using the conditions derived experimentally from the Hamilton PRP X100 columns. Using a single column with an isocratic elution facilitated the separation of

arsenic species with single run [343]. Baseline separation was achieved successfully and the analysis completed in 850 seconds as shown in Figure 4.1. The chromatographic conditions are illustrated in Table 4.2.

The optimum conditions found were: the Hamilton PRP X100 resin (250 x 4.1 mm) dimension with 10 µm particle size and guard column (50 x 4.1 mm), with a mobile phase 20 mM NH_4HPO_4 containing 1% (v/v) methanol, pH 6, and a mobile flow rate 1.1 mL min^{-1}.

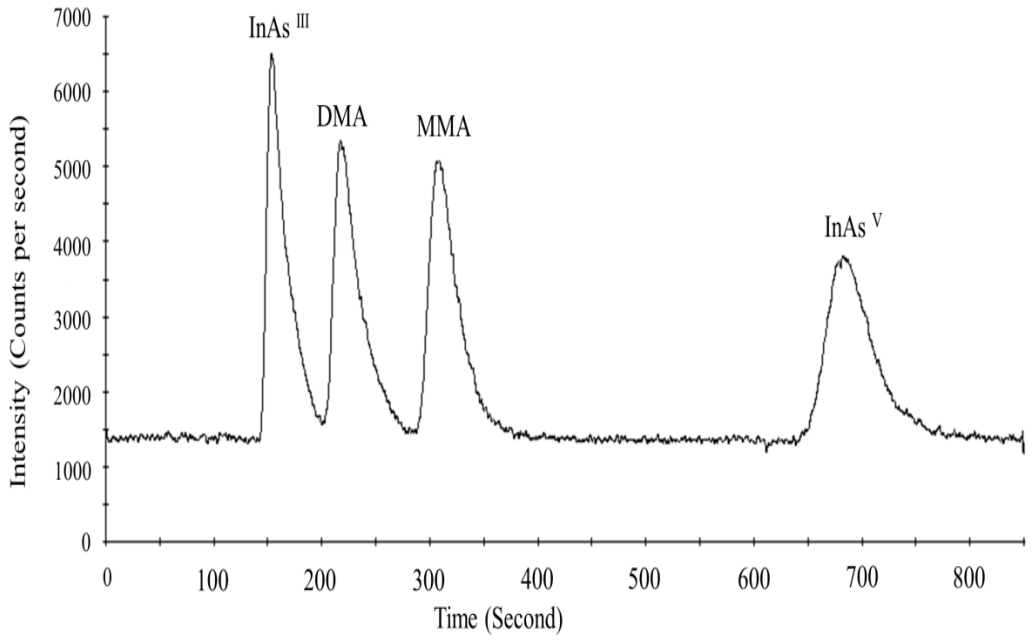


Figure 4.1 Chromatogram of four arsenic species standards in aqueous solution. $InAs^{III}$, DMA, MMA and $InAs^{V}$ 50 µg L^{-1} As of each, employing a Hamilton PRP-X100 anion-exchange HPLC column using conditions described in Table 4.2.

4.1.4 Figures of the merit of the HPLC-ICP-MS method

The chromatogram of a mixture of standards at a concentration of 50 µg L^{-1} (as arsenic) in Fig 4.1 shows complete separation of the arsenic species within 850 seconds. The sensitivity of $InAs^{III}$, DMA, MMA and $InAs^{V}$ was similar at 50 µg L^{-1}. Limits of detection of the procedure are shown in Table 4.4. Detection limits were calculated by three times standard deviation of sample blank (mean of six times the background signal blank of the method) using the conditions in Table 4.2 using 20 mM $NH_4H_2PO_4$ as a mobile phase.

Table 4.4 Detection limits (3 x SD of the background signal of method blank.) of the arsenic species in µg g^{-1} dry weight

Method	$InAs^{III}$	DMA	MMA	$InAs^{V}$
1 % nitric acid	0.006	0.011	0.014	0.012

4.1.5 Study of species inter-conversions using 1 % nitric acid

Figure 4.2 shows the recovery of $InAs^{III}$, DMA, MMA, and $InAs^{V}$ added to the GBW10015 spinach CRM**.** $InAs^{III}$, DMA, MMA, and $InAs^{V}$ were recovered after extraction and chromatography. Redox transformation of all species was minimal with the applied procedure, giving recoveries of 96 and 97 % of $InAs^{III}$ and $InAs^{V}$, respectively. In further tests, 98 % of DMA and 96 % MMA were recovered in the extracts using 1 % HNO_3. Huang *et al.* [344] reported that the preservation of $InAs^{III}$ and $InAs^{V}$ speciation during HNO_3 extraction of rice grains occurred at a narrow range of acid concentrations, *i.e.*,0.28-0.70 M. The released matrix can cause $InAs^{V}$ reduction at HNO_3 concentrations <0.28M and $InAs^{III}$ significant oxidation at HNO_3 concentrations >0.70 M [344].

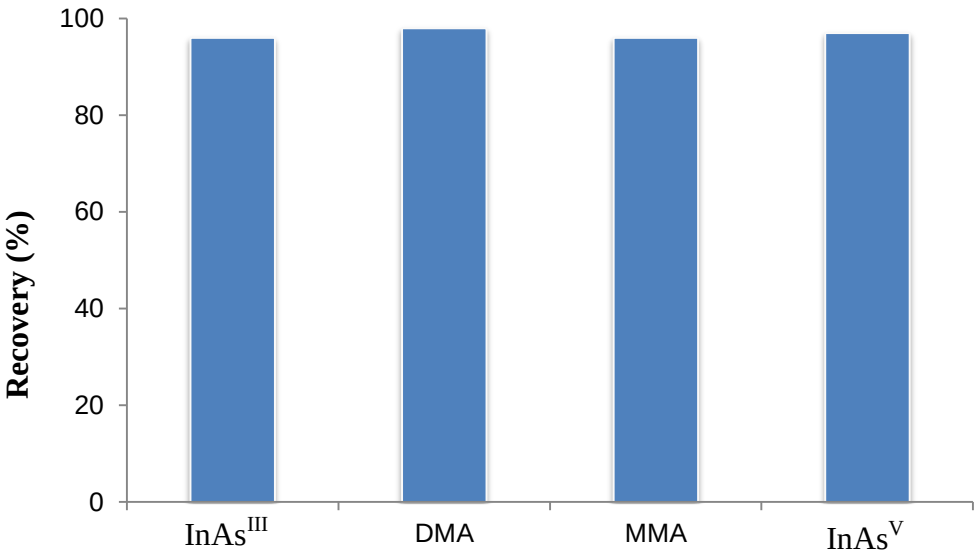


Figure 4.2 Recovery of InAs^{III}, DMA, MMA, and InAs^{V} added to GBW10015-spinach (n=2).

4.1.6 Validation

Analysis of both standards and CRMs was performed in order to validate the optimized extraction methods in rice and vegetable crops. Table 4.5 summarises the total arsenic content in both NIES rice flour and GBW10015 spinach. The concentrations of arsenic in NIES rice flour and GBW10015 spinach were in good agreement with the certified values when applying the proposed method (section 3.3.2.1). Total digestion efficiency of arsenic in NIES and GBW10015 were high compared to the certified values, but still remain within the certified range.

The obtained values for both CRMs were in good agreement with their certified values as U_{Δ} =0.014 µg g^{-1}, 0.034 µg g^{-1} for both NIES-rice flour and GBW10015-spinach > Δ_m 0.006 µg g^{-1} and 0.019 µg g^{-1} for NIES-rice flour and GBW10015-spinach respectively.

Table 4.5 Certified and standard reference material analysis for total arsenic, mean ± standard deviation (n=3).

Materials	Characteristic	Certified value µg g^{-1}	Concentration obtained µg g^{-1}	Digestion efficiency %
NIES	Rice flour unpolished	0.150 *	0.156 ± 0.007	104
GBW10015	Spinach	0.230 ± 0.030	0.250 ± 0.008	108

*indicative value

4.1.7 Results and discussion for total arsenic and arsenic speciation in rice samples

4.1.7.1 Determination of total arsenic in rice samples by ICP-MS.

Total arsenic in NIES rice flour, GBW10015 spinach and rice samples were determined after microwave assisted acid digestion (section 3.3.2.1) by ICP-MS under conditions given in Table 4.2. The results are shown in Table 4.6.

The developed method was applied to 17 rice samples from different origin around the world. The total arsenic concentrations ranged from 0.054 to 0.823 µg g^{-1}. For rice samples grown in the Kurdistan region of Iraq (KU1, KU2, KUH and IRQ) the total arsenic concentrations ranged between 0.054 and 0.149 µg g^{-1}, and for the rice samples grown in Turkey (TUR1, TUR2, TUR3 TUR4 and TUR5) the range was between 0.145 and 0.823 µg g^{-1}. The arsenic content of rice grown in Asian countries such as India, Pakistan, Mali and Thailand was in the range of 0.062 to 0.200 µg g^{-1}. In contrast, rice grown from USA and Uruguay contained arsenic level 0.171 and 0.241 µg g^{-1}, respectively. Rice grown in the Kurdistan of Iraq (KUA1) contained the lowest arsenic

levels with a mean value of 0.054 µg g^{-1}. In contrast rice from Turkey (TUR1) contained the highest arsenic levels in this study (0.823 µg g^{-1}). Rice from India, Pakistan, Mali and Thailand fell into an intermediate category containing arsenic levels higher than rice grown locally in the Kurdistan region of Iraq and lower than of those for rice from the USA, Uruguay and Turkey. The results of this study are in agreement with results obtained from the literature, with Asian rice contained lower arsenic levels than rice from the USA and Europe countries [322]. A "global normal range" for arsenic in rice has been set as 0.080-0.200 µg g^{-1} [322] . Rice samples KUA1, KUA2 and PAK contained arsenic concentration lower than the "global normal range", and rice samples KUH, IRQ, THA1, THA2, THA3, IND, Mal, USA, TUR2 and TUR3 fell within the normal global range for arsenic in rice. The rice samples URU, TUR1 and TUR4 had arsenic levels higher than the global normal range for arsenic in rice. THA1 rice was distributed to every family in Iraq, including the Kurdistan region, during 2012 through a Iraqi national food organisation distributer.

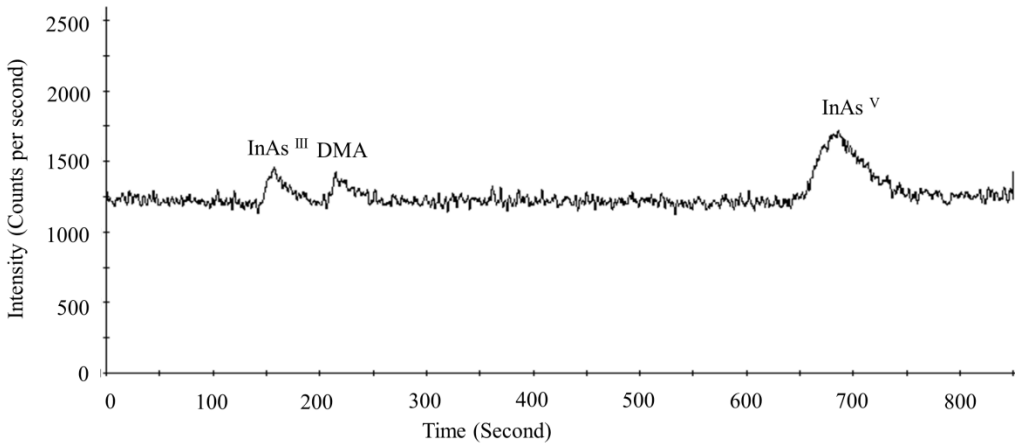
Table 4.6 Results of analysis for arsenic concentration in the rice samples (dry weight); all values are calculated in µg g^{-1} of As, mean ± standard deviation (n=3).

Rice	Microwave assist digestion	$InAs^{III}$	DMA	MMA	$InAs^{V}$	Total arsenic in residue	Total arsenic in extract	Extraction Efficiency %
KUA1	0.054 ± 0.004	0.043 ± 0.001	<0.011	<0.014	0.013 ± 0.0004	<0.009	0.056 ± 0.004	104
KUA2	0.069 ± 0.003	0.040± 0.003	0.023 ± 0.001	<0.014	<0.012	<0.009	0.063 ± 0.006	91
KUH	0.161±0.0006	0.041 ± 0.001	0.039 ± 0.004	<0.014	0.057 ± 0.0022	0.039 ±0.0017	0.156 ± 0.007	97
IRQ	0.149 ± 0.008	0.083± 0.003	<0.011	<0.014	0.079 ± 0.0064	<0.009	0.160 ± 0.008	107
THA1	0.121 ± 0.0037	0.046 ± 0.003	0.038 ± 0.002	<0.014	0.029 ± 0.0024	0.033± 0.004	0.133 ± 0.013	110
THA2	0.191 ± 0.007	0.029 ± 0.001	0.042 ± 0.004	0.030 ±0.001	0.111 ± 0.005	<0.009	0.201 ± 0.002	105
THA3	0.191 ± 0.0068	0.030 ± 0.002	0.035 ± 0.003	<0.014	0.105 ± 0.009	0.010± 0.002	0.182 ± 0.0049	95
PAK	0.062 ± 0.0005	0.028± 0.0001	0.020 ±0.0003	<0.014	0.017 ± 0.0009	<0.009	0.066 ± 0.006	106
IND	0.141 ± 0.011	0.035 ± 0.0007	0.033 ± 0.0006	<0.014	0.063 ± 0.004	<0.009	0.139 ± 0.005	99
MAL	0.200 ± 0.018	0.047 ± 0.0016	0.045 ± 0.002	0.061 ±0.002	0.059 ± 0.005	<0.009	0.214 ± 0.003	107
USA	0.171 ± 0.008	0.063 ± 0.0068	0.020 ± 0.0002	<0.014	0.088 ± 0.0008	<0.009	0.169 ± 0.0054	99
URU	0.241 ± 0.0052	0.036 ± 0.0005	0.071 ± 0.002	<0.014	0.122 ± 0.01	0.011 ± 0.001	0.246 ± 0.0059	102
TUR1	0.823 ± 0.065	0.138 ± 0.0042	0.126 ± 0.011	<0.014	0.561 ± 0.028	<0.009	0.828 ± 0.035	101
TUR2	0.190 ± 0.0086	0.040 ± 0.001	0.055 ± 0.005	0.046 ±0.002	0.078 ± 0.0015	<0.009	0.207 ± 0.017	109
TUR3	0.145 ± 0.0038	0.044 ± 0.004	0.041 ± 0.002	<0.014	0.039 ± 0.0028	<0.009	0.135 ± 0.011	93
TUR4	0.218 ± 0.0036	0.060 ± 0.001	0.050 ± 0.001	0.037 ±0.001	0.075 ± 0.0023	<0.009	0.235 ± 0.018	108
TUR5	0.249 ± 0.018	0.064 ± 0.001	0.059 ± 0.002	0.027 ±0.004	0.060 ± 0.0021	<0.009	0.229 ± 0.014	92
NIES	0.156 ± 0.0073	0.100 ± 0.001	0.027 ± 0.0007	<0.014	0.027 ± 0.0018	0.013 ± 0.013	0.157 ± 0.019	101
GBW10015	0.249 ± 0.0086	0.107 ± 0.008	<0.011	0.034±0.0025	0.113± 0.011	<0.009	0.264 ± 0.018	106

KUA1:Short grain five months matured, KUA2: short grain six months matured, KUH: Short grain six months matured, , IRQ: Hamber short grain rice, THA1: Long grain rice, THA2: Thai Glutinous rice, THA3 Green dragon Thai rice, PAK Tesco organic basmati rice, IND Sainsbury's Indian basmati rice six months matured, MAL: Pure short grain rice home Mali, USA: Arrirang, URU Sainsbury's long grain rice, TUR1: Long grain rice, TUR2: Effsanel short grain rice, TUR3: Luts Persin short grain rice, TUR4: Beskiler long grain rice, TUR5:Duru long grain rice, NIES: unpolished rice flour, GBW10015: Spinach.

4.1.7.2 Determination of arsenic species in rice samples by HPLC-ICP-MS

Because of its low detection limits and selectivity, HPLC-ICP-MS is the most convenient method for the determination of arsenic species. Anion exchange [4] and ion pairing [335] HPLC methods have been used for arsenic speciation analysis. However, separations based on an ion-exchange appear to be more robust and less affected by matrix [345]. An HPLC method adopted in this study is based on the work of Iserte *et al.* [346] which comprises of a Hamilton PRP-X100 column with phosphate buffer at pH 6 (Table 4.2). By applying these conditions good separation of arsenic species such as $InAs^{III}$, DMA, MMA and $InAs^{V}$ in food and other biological samples was achieved. Arsenic species were extracted in different rice samples using 1 % HNO_3 as described in section 4.1.2.3. The main arsenic species determined in rice samples were $InAs^{III}$, $InAs^{V}$, DMA and MMA. The results of the arsenic speciation study are also shown in Table 4.6. The extraction efficiency for each rice sample was evaluated by dividing total arsenic present in the extract after using 1 % HNO_3 to the total arsenic using microwave assisted acid digestion and converting to a percentage. Figure 4.3 shows the chromatogram obtained using the TUR1 rice sample.



Figures 4.3 Chromatogram of TUR1 (Besklier (Turkey)) rice using anionic-exchange HPLC-ICP-MS using conditions described in Table 4.2.

The results showed good extraction efficiency (91-109 %) for total arsenic in the rice samples. The most abundant arsenic species in rice samples KUA1, KUA2, IRQ, THA1, PAK, TUR3 and TUR5 was $InAs^{III}$. However, $InAs^{V}$ was the most abundant arsenic species in rice samples of KUH, THA2, THA3, IND, MAL, USA, URU, TUR1, TUR2 and TUR4. The MMA was under the limit of detection (0.014 µg kg^{-1}) in the majority of rice samples, except for THA2, MAL, URU, TUR2, TUR4 and TUR5. In contrast, DMA was found in the majority of rice samples with the exception of KUA2 and IRQ. The levels of $InAs^{III}$ in the analysed rice samples ranged from 0.028 to 0.138 µg g^{-1} for InAs ^{III}, 0.013 and 0.561 µg g^{-1} for $InAs^{V}$, and the range 0.020 to 0.126 µg g^{-1} for DMA with the exception of KUH and IRQ which were under the LOD (0.011 µg g^{-1}). Although MMA values were below the limit of detection (0.011 µg g^{-1}) in the majority of rice samples were detected it was in the range 0.027 to 0.061 µg g^{-1}. The presence of organic arsenic species (DMA and MMA) in rice samples may confirm the ability of rice plant to metabolize inorganic arsenic to its organic species. Alternatively, the plants may take it up from the environment such as soil or the irrigating water.

The measurement of total inorganic arsenic present in foods is generally sufficient for risk assessment purpose. In all rice samples in this study the majority of arsenic species were inorganic arsenic species representing 50-101% of total arsenic. These results confirm that rice is a bio-accumulative plant for the most toxic arsenic species. However, the developed method is also able to quantify individual inorganic species. The results are summarised in Table 4.7. The highest level of inorganic arsenic was found in rice sample TUR1. This high concentration may be due to the presence of mining activity around the cultivation area. This rice has arsenic level 4 times higher than the global normal range. The mass balance calculations, as described previously, were performed for CRMs and rice samples.

The results are shown in Table 4.8. Microwave assisted acid digestion was used for the determination of any arsenic remaining in the residue of the rice samples in order to calculate full mass balance calculation.

Table 4.7 Arsenic concentration in rice samples under study (µg g^{-1}), sum of species and percentage of total inorganic arsenic in rice samples.

Rice	Microwave assist digestion	Total arsenic in extract	$InAs^{III}$	$InAs^{V}$	Total inorganic arsenic ($InAs^{III}$ + $InAs^{V}$)	Total inorganic/total As in extracts %
KUA1	0.054	0.056	0.043	0.013	0.056	100
KUA2	0.069	0.063	0.043	<0.012	0.043	68
KUH	0.161	0.156	0.041	0.057	0.098	63
IRQ	0.149	0.160	0.083	0.079	0.162	101
THA1	0.121	0.133	0.046	0.029	0.075	56
THA2	0.191	0.201	0.029	0.111	0.140	70
THA3	0.191	0.182	0.030	0.105	0.135	74
PAK	0.062	0.066	0.028	0.017	0.045	68
IND	0.141	0.139	0.035	0.063	0.098	71
MAL	0.200	0.214	0.047	0.059	0.106	50
USA	0.171	0.169	0.063	0.088	0.151	89
URU	0.241	0.246	0.036	0.122	0.158	64
TUR1	0.823	0.828	0.138	0.561	0.699	84
TUR2	0.190	0.207	0.040	0.078	0.118	57
TUR3	0.145	0.135	0.044	0.039	0.083	61
TUR4	0.218	0.235	0.060	0.075	0.135	57
TUR5	0.249	0.229	0.064	0.060	0.124	54
NIES-rice flour	0.156	0.157	0.1	0.027	0.127	81
GBW10015 -spinach	0.25	0.264	0.107	0.113	0.220	83

Table 4.8 Arsenic mass balance for CRM and rice samples; all values in µg g^{-1} (dry weight) mean ± standard deviation (n=3).

Rice	Microwave assist digestion	Total arsenic in extract	Sum of arsenic from species*
KUA1	0.054 ± 0.004	0.056 ± 0.004	0.056
KUA2	0.069 ± 0.003	0.063 ± 0.006	0.069
KUH	0.161± 0.0006	0.156 ± 0.007	0.176
IRQ	0.149 ± 0.008	0.160 ± 0.008	0.162
THA1	0.121 ± 0.003	0.133 ± 0.013	0.146
THA2	0.191 ± 0.007	0.201 ± 0.002	0.212
THA3	0.191 ± 0.006	0.182 ± 0.004	0.180
PAK	0.062 ± 0.001	0.066 ± 0.006	0.065
IND	0.141 ± 0.011	0.139 ± 0.005	0.131
MAL	0.200 ± 0.018	0.214 ± 0.003	0.212
USA	0.171 ± 0.008	0.169 ± 0.005	0.171
URU	0.241 ± 0.005	0.246 ± 0.005	0.275
TUR1	0.823 ± 0.065	0.828 ±0.035	0.825
TUR2	0.190 ± 0.008	0.207 ± 0.017	0.219
TUR3	0.145 ± 0.003	0.135 ± 0.011	0.124
TUR4	0.218 ± 0.003	0.235 ± 0.018	0.222
TUR5	0.249 ± 0.018	0.229 ± 0.014	0.210
NIES-rice flour	0.156 ± 0.007	0.157 ± 0.019	0.154
GBW10015-spinach	0.250 ± 0.008	0.264 ± 0.018	0.259

*sum of species = sum of the concentrations of $InAs^{III}$, DMA, MMA and $InAs^{V}$ and any arsenic remaining in the residue.

4.1.8 Summery of arsenic speciation in rice

Arsenic speciation in rice samples was achieved using 1 % HNO_3 and anion-exchange HPLC-ICP-MS. A microwave assisted procedure with 1 % HNO_3 was effective in extracting arsenic species from both rice samples and CRMs, with recoveries of between 91-109 %. This approach was used to extracted arsenic species in rice samples which maintains the integrity of species. The limits of detection were adequate for the determination of trace element concentration in investigated rice samples. The results from different rice samples indicated that inorganic arsenic ($InAs^{III}$ and $InAs^{V}$) ranged from 51-101 % of total arsenic while the remainder was mainly DMA. The highest level of inorganic arsenic was found in a sample from Turkey with 0.699 µg g^{-1} ($InAs^{III}$ and $InAs^{V}$), whilst lowest total inorganic arsenic was found in rice from Kurdistan of Iraq which was 0.043 µg g^{-1}.

4.2 Multi-element determination in rice samples

4.2.1 Introduction

It is well established that elements such as Na, K, Ca, Fe and P are present in the daily intake of materials such as milk and dairy products, eggs, meat and meat products, fish and fish products, cereal and cereal product such as wheat flour, corn, breakfast cereals and rice. Vegetables such as tomatoes, potatoes, carrots, lentils and mushrooms and fruit like apples, oranges, apricots and strawberries are also sources of these elements. Table 4.9 shows the content of main elements in the human body [347].

Neural conduction and muscle contraction require elements such as Na, K, Ca and Mg. The solubility and absorbability of many elements from foods in the diet are greatly affected by acids in the stomach. Adults require more than 100 mg per day of elements such as Ca, P, Na, K, Mg, Cl and S which are called dietary macro-minerals [348, 349]. Each of these elements performs a specific function in the body. Physical malfunctions

happen unless these minerals are taken up through the diet on a regular basis [349]. Elements such as Fe, I, Zn, Cu, Cr, Mn, Mo, Co, and metalloids (e.g. B, Se and silica) are also necessary for living organisms [350] since they are basic components of proteins with specific physiological functions. However, these elements may have serious consequences for living organisms if they are in excess or deficiency [351]. Each of these minerals has a biochemical role which is crucial to maintain body functions. For example, the transportation of oxygen to and within the cells is achieved via haemoglobin and myoglobin which contain Fe within their structure. Elements such as, B, Ni, V and Sn which are referred to as ultra-trace minerals have been investigated for a possible biological function [349], but their biochemical roles have not yet been confirmed. Some mineral elements such as Pb, Hg, Cd and Al should, ideally, not be present in the diet because they have been identified as being toxic to the body. However, some essential minerals such as F and Se which have an advantageous biochemical role at proper dietary levels are known to be harmful if consumed in excessive quantities [349].

In the UK there are demands for the mandatory fortification of certain micro-nutrients to bread and flour. It is claimed that fortification of extracted flours with Fe (16.5 $\mu g g^{-1}$), Ca (940 to 1560 $\mu g\ g^{-1}$), thiamine (2.4 $\mu g\ g^{-1}$) and niacin (16 $\mu g\ g^{-1}$) should become compulsory. In higher organisms, the respiratory system and the food chain are the main sources for the intake of trace elements. Accumulation of many hazardous elements or compounds can also occur along the food chain, and consequently this is considered a gateway for persistent toxicants to enter organisms [347].

Table 4.9 Main elements in the human body [347].

Element	Content µg g^{-1}
Ca	10–20
P	6–12
K	2–2.5
Na	1–1.5
Cl	1–1.2
Mg	0.4–0.5

4.2.2 Determination of total elemental composition in rice

The concentration of a range of elements in rice samples under study were determined using ICP-MS (conditions described in Table 4.2). The extraction of these elements from the rice samples was achieved using microwave assisted acid digestion (section 3.3.2.1).

4.2.2.1 Analytical characteristics

Instrumental limits of detection were calculated from the slope obtained from calibration curves and three times standard deviations of 6 replicate blank measurements. The LOD values of the ICP-MS in (µg g^{-1}) were evaluated (3 X SD) as follows: Ag (0.025), Al (0.027), B (0.1), Ba (0.028), Be (0.14), Cd (0.004), Co (0.03), Cr (0.02), Cu (0.023), Fe (0.023), Mn (0.027), Mo (0.029), Ni (0.033), Pb (0.03), Sb (0.068), Se (0.059), Si (0.037), Ti (0.074), V (0.049), Zn (0.024), and As (0.009), which are suitable for the determination trace elements in rice samples.

A certified reference material NIES rice flour was used to validate the accuracy of the developed method for the determination of the traceability of elements in rice samples. The results are shown in Table 4.10 with recoveries ranging from 91-115 %.

There was not significant difference statistically between the found and certified value for elements Mn, Zn, Fe, Cu, Mo, Ni, Cd, Al, Cr, Se and Co as their $\Delta_m < U_\Delta$.

4.2.2.2 Total concentration of element

Sample preparation was performed as described before. All elements were extracted from the rice samples as previously described in section 3.3.2.1. Results obtained for the mean values and standard deviation of elemental concentrations in rice samples are shown in Table 4.11. Three rice sample from the Kurdistan of Iraq (KUH, KUA1, and KUA2), three Thai rice (THA1, THA2 and THA3), five Turkish rice (TUR1, TUR2, TUR3, TUR4 and TUR5) and one rice sample each from Iraq (IRQ) Uruguay (URU), Pakistan (PAK), India (IND) , Mali (MAL) and USA (USA) were analysed and compared (Table 4.11).

The Ag and Be were below limits of detection in all rice samples in this study. The V was below the LOD in all rice samples with the exception of TUR2 (V = 0.082 μg g^{-1}). Sb was below the LOD (0.068 μg g^{-1}) in all rice samples except from TUR 1 and TUR3 (Sb= 0.075, 0.088 μg g^{-1}, for TUR1 and TUR2, respectively). Ti was below the LOD (0.074 μg g^{-1}) in rice samples except for KUH and THA2 rice samples were 0.093 and 0.110 for KUH and THA2 respectively. The B was below the LOD (0.1 μg g^{-1}) in majority of rice samples except samples KUA1, KUA2, IRQ, THA1, TUR1 which ranged from 0.343-2.232 μg g^{-1}. Although Co was under the LOD (0.03 μg g^{-1}) in several rice samples, it ranged between 0.032 to 0.048 μg g^{-1} in rice samples KUA1, KUA2, IRQ, URU, THA2 and THA3. The Kurdistan region of Iraq rice (KUH, KUA1 and KUA2, IRQ) had Al contents higher than other investigated rice samples, and higher concentration of some trace elements such as Cr, Mn and Ni than the other rice samples with the exception of THA1. However, the concentration of Cu was higher than Turkish rice (TUR1, TUR2, TUR3, TUR4 and TUR5) and lower than other international rice samples.

Even though the Cd concentration was found at lower concentration range in some rice samples, in rice samples such as THA1, TUR1, TUR2, TUR3, TUR4, TUR5 and MAL were below the LOD (0.004 µg g^{-1}). The Se and Pb were observed in some rice samples. The concentrations of Si in rice samples TUR1, PAK, THA2, THA3 were higher than these in rice samples TUR2, TUR3, TUR4, TUR5, USA, KUH and MAL (below the LOD). The concentrations of Ba and Zn were found to be similar in all rice samples under investigation. The concentrations of Mo in IND and PAK rice were higher than all other rice samples and TUR2 rice had higher concentration of Fe than other rice samples.

Table 4.10 Results obtained from the analysis of CRM NIES rice flour.

Element	Certified value µg g^{-1}	Experimental value µg g^{-1}	Extraction efficiency %
Mn	40.1 ± 0.2	37 ± 1.4	92
Zn	23.1 ± 0.9	22.9 ± 1.9	99
Fe	11.4 ± 0.8	10.8 ± 1.1	95
Cu	4.1 ± 0.3	3.84 ± 0.3	94
Mo	1.6 ± 0.1	1.64 ± 0.07	103
Ni	0.3 ± 0.03	0.32 ± 0.008	107
Cd	1.82 ± 0.06	1.76 ± 0.05	97
Al	1.5[a]	1.49 ± 0.06	99
Cr	0.08 [a]	0.081 ± 0.009	101
Se	0.07 [a]	0.081 ± 0.009	115
Co	0.007 [a]	0.0072 ± 0.001	103

[a]Indicative value

Table 4.11 Results for mean values and standard deviation of elemental concentrations in 17 rice samples in µg g^{-1}.

Element	Concentration in rice samples µg g^{-1}				
	KUH	KUA1	KUA2	IRQ	THA1
Be	<0.14	<0.14	<0.14	<0.14	<0.14
B	<0.1	0.343 ± 0.03	0.370 ± 0.033	1.231 ± 0.022	2.232 ± 0.177
Al	4.891 ± 0.061	10.487 ± 0.326	13.300 ± 0.566	10.272 ± 0.017	22.732 ±1.792
Si	5.944 ± 0.701	74.520 ± 7.958	100.724 ± 1.578	87.326 ± 3.358	205.976 ± 0.598
V	<0.049	<0.049	<0.049	<0.049	<0.049
Cr	0.160 ± 0.008	0.450 ± 0.034	0.217 ± 0.022	0.399 ± 0.018	0.163 ± 0.017
Mn	9.734 ± 0.410	9.288 ± 0.167	13.762 ± 0.424	6.662 ± 0.223	12.055 ± 0.910
Fe	7.465 ± 0.277	14.296 ± 0.163	15.184 ± 0.185	15.474 ± 1.050	14.710 ± 0.384
Co	<LOD	0.043 ± 0.001	0.048 ± 0.0002	0.036 ± 0.002	<LOD
Ni	0.163 ± 0.016	0.847 ± 0.006	0.773 ± 0.083	0.635 ± 0.049	0.714 ± 0.052
Cu	1.748 ± 0.052	1.484 ± 0.018	2.253 ± 0.057	2.072 ±0.089	1.982 ± 0.165
Zn	11.610 ± 0.727	7.356 ± 0.277	9.620 ± 0.275	8.104 ± 0.389	12.277 ± 0.568
Se	0.065 ± 0.027	<0.059	0.270 ± 0.0064	0.108 ± 0.003	<0.059
Mo	0.670 ± 0.046	0.467 ± 0.009	0.633 ± 0.017	0.436 ± 0.007	0.544 ± 0.040
Ag	<0.025	<0.025	<0.025	<0.025	<0.025
Cd	0.058 ± 0.007	0.013 ± 0.0003	0.015 ± 0.005	0.013 ± 0.001	<0.004
Sb	<0.068	<0.068	<0.068	<0.068	<0.068
Ba	0.086 ± 0.020	0.191 ± 0.022	0.220 ± 0.009	0.248 ± 0.018	0.164 ± 0.003
Ti	0.093 ± 0.001	<0.074	<0.074	<0.074	<0.074
Pb	0.300 ± 0.036	<0.03	<0.03	<0.03	<0.03

Table 4.11 Continued

Element	Concentration in rice samples $\mu g\ g^{-1}$					
	URU	IND	PAK	THA2	THA3	USA
Be	<0.14	<0.14	<0.14	<0.14	<0.14	<0.14
B	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Al	4.888 ± 0.177	0.578 ± 0.001	0.845 ± 0.054	0.956 ± 0.175	3.770 ± 0.32	0.962 ± 0.100
Si	6.861 ± 0.554	11.096 ± 0.101	18.646 ± 0.438	22.472 ± 1.838	23.350 ± 1.508	3.978 ± 0.937
V	<0.049	<0.049	<0.049	<0.049	<0.049	<0.049
Cr	0.028 ± 0.002	0.046 ± 0.001	0.102 ± 0.010	0.045 ± 0.005	0.058 ± 0.001	0.050 ± 0.001
Mn	13.158 ± 0.352	9.620 ± 0.232	8.637 ± 0.016	11.897 ± 0.288	9.380 ± 0.140	10.397 ± 0.456
Fe	6.827 ± 0.161	5.192 ± 0.182	6.051 ± 0.289	2.367 ± 0.072	5.868 ± 0.279	2.970 ± 0.237
Co	0.046 ± 0.002	<0.03	<0.03	0.032 ± 0.001	0.036 ± 0.001	<0.03
Ni	0.214 ± 0.001	0.209 ± 0.009	0.294 ± 0.023	0.157 ± 0.001	0.184 ± 0.020	0.299 ± 0.029
Cu	3.066 ± 0.085	2.564 ± 0.033	2.701 ± 0.024	0.990 ± 0.017	1.190 ± 0.028	1.930 ± 0.104
Zn	13.551 ± 0.309	16.978 ± 1.216	16.940 ± 0.046	18.341 ± 0.366	17.313 ± 0.319	12.125 ± 0.969
Se	<0.059	0.237 ± 0.007	0.157 ± 0.001	0.069 ± 0.002	0.060 ± 0.005	<0.059
Mo	0.692 ± 0.015	1.184 ± 0.031	1.380 ± 0.015	0.384 ± 0.003	0.467 ± 0.011	0.518 ± 0.030
Ag	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025
Cd	0.019 ± 0.001	0.058 ± 0.001	0.048 ± 0.001	0.023 ± 0.002	0.018 ± 0.001	0.018 ± 0.005
Sb	<0.068	<0.068	<0.068	<0.068	<0.068	<0.068
Ba	0.165 ± 0.016	0.151 ± 0.016	0.097 ± 0.003	0.188 ± 0.001	0.297 ± 0.012	0.088 ± 0.001
Ti	<0.074	<0.074	<0.074	0.110 ± 0.004	<0.074	<0.074
Pb	1.542 ± 0.442	<0.03	0.823 ± 0.125	0.341 ± 0.012	0.321 ± 0.079	0.487 ± 0.030

Table 4.11 Continued

Element	Concentration in rice samples µg g^{-1}					
	TUR1	TUR2	TUR3	TUR4	TUR5	MAL
Be	<0.14	<0.14	<0.14	<0.14	<0.14	<0.14
B	0.961 ± 0.131	<0.1	<0.1	<0.1	<0.1	<0.1
Al	3.575 ± 0.264	<0.027	<0.027	<0.027	<0.027	<0.027
Si	142.351 ± 3.751	5.192 ± 0.32	4.809 ± 0.271	9.286 ± 0.718	12.122 ± 0.248	<0.037
V	<0.049	0.082 ± 0.003	<0.049	<0.049	<0.049	<0.049
Cr	<0.02	0.969 ± 0.059	0.121 ± 0.011	0.150 ± 0.011	0.179 ± 0.017	0.217 ± 0.004
Mn	7.334 ± 0.072	6.365 ± 0.531	8.656 ± 0.119	8.084 ± 0.039	8.356 ± 0.452	5.844 ± 0.184
Fe	8.994 ± 0.608	28.151± 0.534	8.742 ± 0.779	8.321 ± 0.389	9.710 ± 0.377	12.863 ± 1.500
Co	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03
NI	0.214 ± 0.019	0.311 ± 0.002	0.078 ± 0.001	0.087 ± 0.004	0.094 ± 0.001	0.202 ± 0.001
Cu	1.171 ± 0.016	1.469 ± 0.034	1.521 ± 0.037	1.802 ± 0.049	1.992 ± 0.088	0.694 ± 0.012
Zn	10.743 ± 0.215	6.900 ± 0.463	5.880 ± 0.480	7.619 ± 0.229	7.940 ± 0.436	8.364 ± 0.413
Se	<0.059	<0.059	<0.059	<0.059	<0.059	<0.059
Mo	0.282 ± 0.013	0.852 ± 0.046	0.495 ± 0.015	0.564 ± 0.009	0.560 ± 0.027	0.387 ± 0.031
Ag	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025
Cd	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
Sb	0.075 ± 0.006	<0.068	0.088 ± 0.003	<0.068	<0.068	<0.068
Ba	0.375 ± 0.020	0.159 ± 0.008	0.052 ± 0.001	0.041 ± 0.005	0.051 ± 0.002	0.217 ± 0.011
Ti	<0.074	<0.074	<0.074	<0.074	<0.074	<0.074
Pb	<0.03	<0.03	0.031 ± 0.001	0.071 ± 0.001	<0.03	0.128 ± 0.020

4.2.4 Summary of multi-element determinations in rice

Total multi-element concentrations in rice samples of different geographical origin were determined using HNO_3/H_2O_2 microwave assisted acid digestion followed by ICP-MS. The applied method was validated using CRM NIES rice flour. Elements such as Be, B, Ag, Co, Cd, V, Sb and Ti were found to be below limits of detection in majority of rice samples. However, elements such as Al, Si, Mn, Fe, Cu and Zn were found at high concentrations, some reaching some μg g^{-1} levels in the analysed rice. The concentrations of Cr, Ni, Se, Mo, Ba and Pb in rice samples were found at low levels.

4.3 Arsenic extraction for speciation in a range of vegetable and crops using HPLC-ICP-MS

4.3.1 Introduction

Inorganic arsenic ($InAs^{III}$ and $InAs^{V}$) is present as dominant arsenic species in the terrestrial plant and vegetables with small amount of organic arsenic species such as DMA and MMA[179]. Other arsenic species have been detected such as tetramethylarsonium (TMA^{+}), AsB, AsC and arsenosugars[130]. Food groups such as vegetable and cereals may lead to arsenic entering the food chain[205].

The vegetable crops were collected from a local market garden area in Devon-UK in November 2012 and washed using Milli-Q water in order to remove the associated soil. Samples were then frozen at -40 ˚C for 12 h in a freezer. These were then placed in a freeze drier for 48 h at -40 ˚C. All dried samples were ground, using an agate pestle and mortar, to a fine powder and then sieved using a plastic 180 μm mesh sieve to retrieve the finer fraction for analysis.

4.3.2 Extraction procedures for the speciation of arsenic in vegetable and crops

The usage of enzyme extraction procedures to maintain the integrity of the arsenic species has been described above in section 4.1.3.3 [124, 352, 353]. An alternative method is to use cellulase to break down the cell wall. Cellulase works effectively in a slightly acidic environment, approximately pH 5 at 37 ºC. Arsenical species in the selected CRMs (pine needle 1575a and NIES rice flour) were extracted using cellulase enzyme in 0.1 M CH_3COONH_4. A 0.5 g sample and 0.5 g cellulase enzyme were directly weighed out into a potter homogenizer, 10 mL of 0.1 M CH_3COONH_4 (pH 5) were added to homogenize with the sample then they were transferred into the centrifuge tube. Another 10 mL of 0.1 M CH_3COONH_4 (pH 5) were added to rinse the homogenizing tube then transferred to the same centrifuge tube. The centrifuge tubes were capped and left in a shaking water bath at 37 ºC for 12 h. The samples were centrifuged at 4000 rpm for 20 min. The extract was quantitatively transferred into a volumetric flask (25 mL) and spiked with In and Ir internal standard solution to give final concentration of 10 μgmL^{-1}. The samples were diluted to volume with 0.1 M CH_3COONH_4. Again the extract was stored in darkness at 4.0 ºC for up to 1 week.

4.3.3 Determination of the mass balance for arsenic in vegetable samples

To obtain data for mass balance calculations, the sum of species in the extracts together with the total arsenic concentration in any residues must be accounted for. In general, the sum of concentrations of arsenic species ($InAs^{II}$, MMA, DMA and $InAs^{V}$) combined with any arsenic, in the residual solids from enzyme extraction, should be equal to the concentration of total arsenic.

4.3.4 Results of the analysis of CRMs and real sample extracts

To check the validity and the quality control of the developed method two different CRMs were used. The digestion technique described in section 3.3.2.1 was applied to determine 'total nitric available' arsenic using pine needle 1575a and NIES rice flour. The results of these CRMs are shown in Table 4.12. Digestion efficiencies were 105 % and 104 % for pine needles 1575a and NIES rice flour, respectively.

The obtained values were in good agreement statistically with the certified values of both pine needle 1575a and NIES rice flour. The found value for both CRMs pine needle 1575a and NIES rice flour were in good agreement with their certified values because their $U_{\Delta} > \Delta_m$.

U_{Δ} =0.0407 µg g^{-1} for pine needle 1575a and 0.014 µg g^{-1} for NIES-rice flour

Δ_m= 0.01 µg g^{-1} for pine needle and 0.006 µg g^{-1} for NIES-rice flour

Table 4.12 Certified Reference Material analysis for total arsenic; mean ± SD (n=3).

Material	Characteristic	Certified value µg g^{-1}	Concentration obtained µg g^{-1}	Digestion efficiency (%)
Pine needle 1575a	Plant	0.210 ± 0.04	0.220 ± 0.004	105
NIES	Unpolished flour rice (high level Cd)	0.150*	0.156 ± 0.007	104

*Indicative value

4.3.5 Detection limit

The detection limits of arsenic species under study are demonstrated in Table 4.13. Detection limits were determined by 3 times standard deviation of the sample blank using HPLC-ICP-MS under the conditions described in Table 4.2.

Table 4.13 Detection limits (3 x standard deviation of sample blank) determined during analysis for $InAs^{III}$, DMA, MMA and $InAs^{V}$ in vegetable crops using HPLC-ICP-MS.

Arsenic species	Detection limit $\mu g\ g^{-1}$
$InAs^{III}$	0.008
DMA	0.019
MMA	0.024
$InAs^{V}$	0.008

4.3.6 Results and Discussion

The aim of this study was to investigate the total and arsenic speciation in vegetable crops grown by local market garden suppliers in areas known to have a history of arsenic mining. A total of 10 different vegetable crops were collected in market garden suppliers around Plymouth in order to investigate their arsenic content. The results are presented in Table 4.14. To maintain quality control two different certified reference materials (pine needles 1575a and NIES rice flour) were analysed simultaneously.

The statutory limit of arsenic in foods in UK is 1 $\mu g\ g^{-1}$ fresh weight [354]. Total arsenic concentrations found in the samples of potato, parsnip, carrot, turnip, swede, beetroot, artichoke and leek, were in the range of 0.023-1.174 $\mu g\ g^{-1}$ all concentrations are on dry

weight basis. The highest level of arsenic was found in leek with a mean value for three replicates of 1.174 ± 0.090 µg g^{-1}, and the lowest level was found in samples of edible part (core) of beetroot (0.023 µg g^{-1}). Arsenic accumulation differs between vegetable species and individuals, the arsenic contents in samples of parsnip and beetroot of two different origins are different (Table 4.14). The results for total arsenic content in this study for leek, 1.174 µg g^{-1}, are higher than that obtained by Al Rmalli *et al.* [355] for the same vegetable, which was between 0.0109-0.0111 µg g^{-1}. The results for total arsenic concentrations in edible part (core) of artichoke in this study, 0.039 µg g^{-1}, was similar to the results of Matos-Reyes *et al.* [356] which was 0.045 µg g^{-1}. The concentrations of total arsenic obtained in edible part of carrots (0.039 µg g^{-1}) and skin (0.073 µg g^{-1}) were higher than the results of Al Ramlli *et al.*[355] which was between 0.0098-0.0104 µg g^{-1} but lower than of Xu's *et al.* [357] and Matos-Reyes's *et al.* [356] study ranged from 0.100 to 0.930 µg g^{-1}.

The arsenic present in the peel was quantified in each of potato, artichoke, parsnip, turnip, beetroot, swede and carrots. In comparison with the total arsenic contents found in edible part (core) shows concentrations of arsenic in the skin between 2 and 12 times higher than the levels found in edible part. The concentration was particularly high in potato (0.541 ± 0.02 µg g^{-1}). Studies conducted by other researchers showed the same behaviour in turnip [358] and carrots[359], with the total arsenic being 2-7 times greater than the inner part of the root.

To investigate the arsenic speciation further, an ion-exchange HPLC method was developed, based on the work of Hunter *et al.* [360]. A Hamilton PRP-100X anion-exchange column was used for HPLC-ICP-MS. The mobile phase for HPLC-ICP-MS was a 20 mM $NH_4H_2PO_4$ solution adjusted to pH 6.0 with aqueous NH_3. The addition of CH_3OH to the mobile phase increases the sensitivity of the produced signal [361, 362].

Addition of organic solvents to the mobile phase is also known to reduce polyatomic interferences in ICP-MS [363]. Arsenic species in the vegetable crops were determined following enzymatic extraction. The use of cellulase breaks down the cell wall releasing the cell contents with no inter-conversion of species. Employing cellulase as an enzyme for digestion of plant tissue as described previously (section 4.3.2) was applied and the results of this method are given in detail in Table 4.14. An example of the chromatographic speciation is shown for leek using the developed anionic-exchange HPLC-ICP-MS method is shown in Figure 4.4.

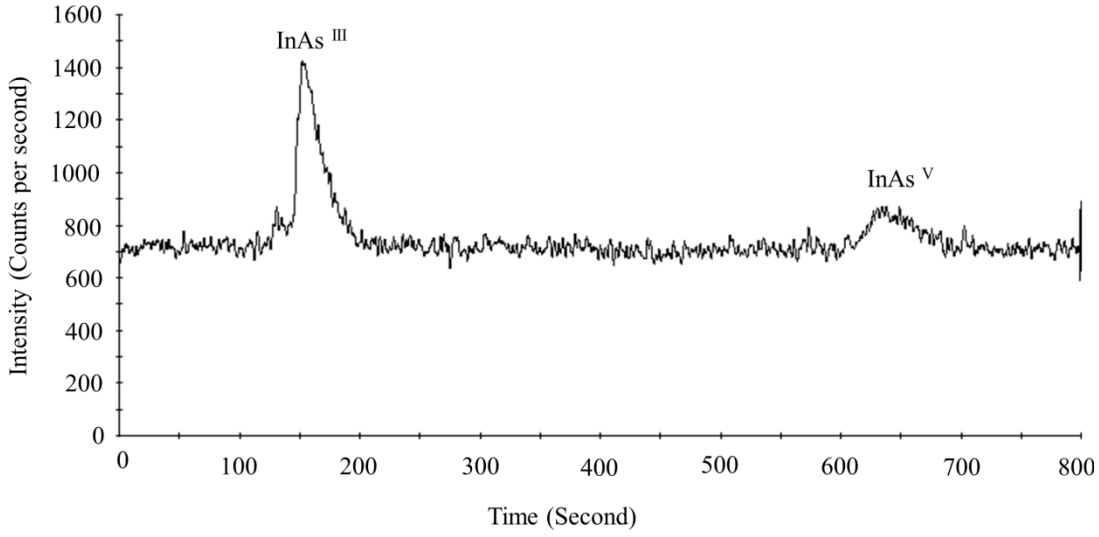


Figure 4.4 Chromatogram of leek sample using anionic-exchange HPLC-ICP-MS, using conditions described in Table 4.2.

Table 4.14 Results of analysis for arsenic concentration in vegetable samples (dry weight); all values are given in µg g^{-1} of arsenic, mean ± SD, (n=3).

Vegetable	Microwave assist digestion	$InAs^{III}$	DMA	MMA	$InAs^{V}$	Total arsenic in residue	Total arsenic in extract	Extraction Efficiency %
Turnip-skin	0.127 ± 0.004	0.043 ±1.23	<0.019	<0.024	0.081 ± 0.003	<0.009	0.128 ± 0.007	101
Turnip-core	0.041 ± 0.003	0.023 ± 0.0003	<0.019	<0.024	0.023 ± 0.001	<0.009	0.046 ± 0.003	112
Beetroot-core (sample A)	0.095 ± 0.004	0.019 ± 0.001	<0.019	<0.024	0.075 ± 0.001	<0.009	0.103 ± 0.003	108
Beetroot-skin (sample A)	0.368 ± 0.006	0.068 ± 0.002	<0.019	0.032 ± 0.003	0.238 ± 0.005	0.039 ± 0.001	0.341 ± 0.004	93
Swede-skin	0.419 ± 0.002	0.103 ± 0.008	0.059 ± 0.007	0.050 ± 0.004	0.165 ± 0.002	0.011± 0.001	0.382 ± 0.004	91
Swede-core	0.054 ± 0.002	0.017 ± 0.001	<0.019	<0.024	0.024 ± 0.001	<0.009	0.052 ± 0.002	96
Carrot –leaf-	0.156 ± 0.004	0.039 ± 0.003	<0.019	<0.024	0.117 ± 0.003	<0.009	0.162 ± 0.01	104
Carrot-core (sample A)	0.039 ± 0.002	0.024 ± 0.002	<0.019	<0.024	0.014 ± 0.001	<0.009	0.042 ± 0.002	108
Carrot-skin- (sample A)	0.073 ± 0.004	0.016 ± 0.001	<0.019	<0.024	0.057 ± 0.001	<0.009	0.077 ± 0.004	105
Parsnip-skin (sample A)	0.204 ± 0.008	0.040 ± 0.003	<0.019	<0.024	0.147 ± 0.001	<0.009	0.204 ± 0.014	100
Parsnip-core (sample A)	0.031 ± 0.002	0.010 ±0.001	<0.019	<0.024	0.019 ± 0.002	<0.009	0.033 ± 0.003	106

Table 4.14 Continued

Vegetable	Microwave assist digestion	$InAs^{III}$	DMA	MMA	$InAs^{V}$	Total arsenic in residue	Total arsenic in extract	Extraction Efficiency %
Leek	1.174 ± 0.09	0.873 ± 0.036	<0.019	<0.024	0.285 ± 0.023	<0.009	1.168 ± 0.1	99
Potato-skin	0.541 ± 0.02	0.160 ± 0.012	<0.019	<0.024	0.370 ± 0.026	0.022 ±0.0007	0.531 ± 0.013	98
Potato-core	0.043 ± 0.001	0.018± 0.002	<0.019	<0.024	0.025 ± 0.001	<0.009	0.42 ± 0.002	98
Parsnip-skin (sample B)	0.132 ± 0.01	0.065 ± 0.003	<0.019	<0.024	0.063 ± 0.004	0.015 ±0.001	0.134 ± 0.006	102
Parsnip-core (sample B)	0.039 ± 0.003	0.012 ± 0.0006	<0.019	<0.024	0.018 ± 0.001	<0.009	0.41 ± 0.002	105
Artichoke-skin	0.268 ± 0.008	0.069 ± 0.001	<0.019	<0.024	0.167 ± 0.015	0.025 ± 0.002	0.255 ± 0.012	95
Artichoke-core	0.038 ± 0.002	0.020 ± 0.001	<0.019	<0.024	0.011 ± 0.0002	<0.009	0.039 ± 0.004	103
Beetroot-skin (sample B)	0.204 ± 0.004	0.069 ± 0.001	<0.019	<0.024	0.135 ± 0.003	<0.009	0.218 ± 0.008	107
Beetroot-core (sample B)	0.023 ± 0.001	0.01 ± 0.001	<0.019	<0.024	0.012 ± 0.001	<0.009	0.029 ± 0.001	126
NIES rice flour	0.156 ± 0.007	0.095 ± 0.0002	0.020 ± 0.001	<LOD	0.053 ± 0.002	<0.009	0.160 ± 0.01	103
Pine needles-1575a	0.220 ± 0.003	0.040 ± 0.003	<0.019	<0.024	0.176 ± 0.007	<0.009	0.219 ± 0.009	100

The extraction efficiency of a particular solvent is defined as the percentage of total arsenic extracted by the solvent from a plant sample. Solvent extraction performed by physical shaking or sonication have been classified as a traditional methods for extracting arsenic from the solid matrices. In order to keep the integrity of arsenic species in the samples, a number of extraction methods comprised of water, methanol, mixtures of both and other ionic extractants have been used [345]. Although water-methanol as an extractant is popular for most terrestrial plants, it's well-known for poor extraction efficiency. Dilute phosphoric acid has been used as a convenient extraction reagent for arsenic species from terrestrial plant. Mixtures of acetonitrile-water and methanol-water-chloroform have also been used. Enzymes such as alpha-amylase for cellulose have also been utilized for the modification of plant matrices[141]. Treatment by this enzyme yielded an extraction efficiency of 104 % for freeze-dried apple [122] and 59 % for rice samples[141]. Overall the results in this study provide indication levels for arsenic in the plant material. Extraction efficiency using cellulase as an extractant for arsenic ranged from 91 % in skin of swede to 113 % for beetroot core (sample B) for all arsenic species.

There are very few data in the literature for arsenic species content in vegetables. Rose *et al.* [93], using the market basket study method for 20 food groups of the 2006 UK Total Diet Study, detected concentrations of inorganic arsenic in vegetables below 0.01 μg g^{-1} dry weight, values far below the concentrations found in the present study. This is possibly because these plants have been grown in areas that have known of former arsenic mining industry. In most of the samples analysed the inorganic arsenic contents were close to the total arsenic contents, which may indicate limited ability of these plants to methylate absorbed inorganic arsenic from soil. Another possibility of this phenomenon is possibly low uptake of methylated species that may present in soil by these plants or the absence of such compounds in the soils used for growing these plants.

Up to 25 % of the arsenic in food is inorganic, but this is dependent on the type of food crop [364]. The highest results in this study obtained, for leek 0.873 ± 0.036 μg g^{-1} as $InAs^{III}$. Overall, the concentration of $InAs^{III}$ ranged between 0.010 – 0.873 μg g^{-1}. However, the value of $InAs^{III}$ was below the limit of detection (0.009 μg g^{-1}) in the core of beetroot (sample B). The results of $InAs^{V}$ in these vegetable crops were in the range 0.011 to 0.370 μg g^{-1} except in core of beetroot (sample B) where it was below than limit of detection. DMA was only found in the peel from swede with 0.059 μg g^{-1}. MMA in this study was only detected in the skin of beetroot (Sample A) and skin of swede, 0.032 and 0.05 μg g^{-1}, respectively.

The percentages of inorganic arsenic compared to total arsenic ranged from 70 % for a sample of swede skin to values close to 100 % for, leek, potato skin, turnip skin, parsnip core (sample B), artichoke skin, artichoke core, beetroot skin (sample A), parsnip skin (sample A), parsnip core (sample A) and different organs of carrots. Consequently, arsenic in vegetable crops is mainly present in the highly toxic inorganic form, which is opposite to sea food products where the predominant arsenic species are organoarsinical species. In the literature few data are available related to the arsenic speciation in vegetable crops, although majority of them have linked with the total inorganic arsenic contents in vegetable crops and terrestrial plants. The concentration of total arsenic, total inorganic arsenic and percentage of total inorganic of arsenic are demonstrated in Table 4.15. The results of peeled potato were in good agreement with those of Pyles and Woolson [365] who reported that more than a third of extracted arsenic in peeled potato was $InAs^{V}$. Interestingly, both the skin and core of potato $InAs^{V}$ was higher than $InAs^{III}$. Similarly the concentration of $InAs^{V}$ in other vegetable crops was higher than $InAs^{III}$ except from leek and the edible part of artichoke and the inner part of carrot, $InAs^{III}$ was the predominant inorganic form.

The levels of inorganic arsenic in leek are high and in excess might pose a dietary risk although the levels are not a serious cause for concern. The FAO/WHO recommends a maximum daily intake of 150 µg of arsenic for a 70 kg individual per day. If 174 g of this leek were eaten the daily intake would be 159 µg of inorganic arsenic.

A mass balance calculation for each of the samples is presented in the Table 4.16. The total arsenic from acid digestion of each sample should be equal to the sum of enzyme extracted total arsenic concentration and the other arsenic species.

Table 4.15 Arsenic concentration in vegetable crops (μg g^{-1} ± SD) and percentage of inorganic arsenic/total arsenic.

Vegetable	Microwave assist digestion	Total arsenic in extracts	Total inorganic arsenic	% total inorganic arsenic
Turnip-skin	0.127 ± 0.004	0.128 ± 0.007	0.124	97
Turnip-core	0.041 ± 0.004	0.046 ± 0.002	0.046	100
Beetroot-core (sample A)	0.095 ± 0.003	0.103 ± 0.003	0.094	91
Beetroot-skin (sample A)	0.368 ± 0.005	0.341 ± 0.004	0.306	90
Swede-skin	0.419 ± 0.002	0.382 ± 0.004	0.268	70
Swede-core	0.054 ± 0.002	0.052 ± 0.002	0.041	79
Carrot -leaf	0.156 ± 0.004	0.162 ± 0.01	0.156	96
Carrot-core (sample A)	0.039 ± 0.002	0.042 ± 0.002	0.038	90
Carrot-skin (sample A)	0.073 ± 0.003	0.077 ± 0.004	0.073	95
Parsnip-skin (sample A)	0.204 ± 0.008	0.204 ± 0.014	0.187	92
Parsnip-core (sample A)	0.031 ± 0.002	0.033 ± 0.003	0.029	88
Leeks	1.174 ± 0.09	1.168 ± 0.1	1.158	99
Potato-skin	0.541 ± 0.02	0.531 ± 0.013	0.530	100
Potato-core	0.043 ± 0.001	0.042 ± 0.002	0.043	102
Parsnip-skin (sample B)	0.132 ± 0.009	0.134 ± 0.006	0.128	96
Parsnip-core (sample B)	0.039 ± 0.003	0.041 ± 0.002	0.03	73
Artichoke-skin	0.268 ± 0.008	0.255 ± 0.012	0.236	93
Artichoke-core	0.038 ± 0.0008	0.039 ± 0.004	0.031	79
Beetroot-skin (sample B)	0.204 ± 0.004	0.218 ± 0.008	0.204	94
Beetroot-core (sample B)	0.023 ± 0.002	0.029 ± 0.003	0.022	76
NIES rice flour	0.156 ± 0.007	0.16 ± 0.001	0.148	93
GBW10015-spinach	0.22 ± 0.003	0.219 ± 0.009	0.216	99

Table 4.16 Arsenic mass balance for vegetable crops; µg g^{-1} ± SD (n=3).

Vegetable	Microwave assist digestion	Total arsenic in extracts	Sum of arsenic from species
Turnip-skin	0.127 ± 0.004	0.128 ± 0.007	0.124
Turnip-core	0.041 ± 0.004	0.046 ± 0.002	0.046
Beetroot-core (sample A)	0.095 ± 0.003	0.103 ± 0.003	0.094
Beetroot-skin (sample A)	0.368 ± 0.005	0.341 ± 0.004	0.377
Swede-skin	0.419 ± 0.002	0.382 ± 0.004	0.388
Swede-core	0.054 ± 0.002	0.052 ± 0.002	0.041
Carrot -leaf	0.156 ± 0.004	0.162 ± 0.01	0.156
Carrot-core (sample A)	0.039 ± 0.002	0.042 ± 0.002	0.038
Carrot-skin (sample A)	0.073 ± 0.003	0.077 ± 0.004	0.073
Parsnip-skin (sample A)	0.204 ± 0.008	0.204 ± 0.014	0.187
Parsnip-core (sample A)	0.031 ± 0.002	0.033 ± 0.003	0.029
Leeks	1.174 ± 0.09	1.168 ± 0.1	1.158
Potato-skin	0.541 ± 0.02	0.531 ± 0.013	0.552
Potato-core	0.043 ± 0.001	0.042 ± 0.002	0.043
Parsnip-skin (sample B)	0.132 ± 0.009	0.134 ± 0.006	0.143
Parsnip-core (sample B)	0.039 ± 0.003	0.041 ± 0.002	0.030
Artichoke-skin	0.268 ± 0.008	0.255 ± 0.012	0.261
Artichoke-core	0.038 ± 0.0008	0.039 ± 0.004	0.031
Beetroot-skin (sample B)	0.204 ± 0.004	0.218 ± 0.008	0.204
Beetroot-core (sample B)	0.023 ± 0.002	0.029 ± 0.003	0.022
NIES rice flour	0.156 ± 0.007	0.16 ± 0.001	0.168
GBW10015-spinach	0.22 ± 0.003	0.219 ± 0.009	0.216

4.3.7 Summary of arsenic speciation in vegetables

In this study arsenic speciation in different vegetable crops was investigated. HPLC-ICP-MS with collision reaction cell has been used to measure arsenical species in collected vegetable crops. Cellulase has been used to extract arsenic species. The extraction efficiency ranged between 91 and 126%, indicating that most of the arsenic present in the sample was extracted and speciated. The method performance was satisfactory when it was applied to vegetable crops with a wide range of arsenic concentration. Only $InAs^{V}$ and $InAs^{III}$ were detected in vegetable crops with low total arsenic concentrations. In samples with high total arsenic concentration DMA was only found in the skin of swede while MMA has also been found in the skin of both swede and beetroot (sample A). The values found for inorganic arsenic were close to the total arsenic, which may indicate that the toxic arsenic species in vegetables is predominant species. However, traces of organic species have also been detected in some vegetable crops. The high levels of total inorganic arsenic in some vegetable, was reflect the growing conditions of these vegetable crops in agriculture soils that are associated with past mining history.

4.4 Conclusion

The vast majority of population around the world uses rice as a main food for their diet. Rice is one of the main foods which provides arsenic to human diet. Total arsenic, arsenic species, and total multi-elements (including Ag, Al, B, Ba, Be, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Si, Ti, V and Zn) were determined in rice samples from Kurdistan in Iraq and different geographical origin (including Iraq, Thailand, Uruguay, India, Pakistan, USA, Turkey and Mali) . This is the first study that has been conducted in the rice samples from the Kurdistan region of Iraq and in which HPLC-ICP-MS has been used to separate different arsenic species ($InAs^{III}$, DMA, MMA and $InAs^{V}$) in the rice samples. The arsenic contents in the rice samples from the Kurdistan region of Iraq were below the 'normal global range' for arsenic which was set to be 0.08-0.200 μg g^{-1}. However, the rice samples URU, TUR1, TUR4 and TUR5 contained higher arsenic than the 'normal global range' for arsenic. Inorganic arsenic species ($InAs^{III}$ and $InAs^{V}$) were found to be dominant arsenic species representing 51-100 % of the total arsenic contents, while organic arsenic species (DMA and MMA) were also detected in rice samples under study. In terms of total multi-elements in the analysed rice samples, Al, Si, Mn, Fe, Cu and Zn elements were found to be with high levles, while elements such as Cr, Ni, Se, Mo, Ba and Pb in the rice samples were found at low concentrations.

Arsenic speciation in a range of vegetables (including turnip, beetroot, swede, carrot, artichoke, leek, parsnip and potato) was performed using HPLC-ICP-MS after extracting arsenic species using cellulase. These samples were collected in two different local markets in areas known to have a mining history in Devon-UK. The results showed that total arsenic contents in the skin of the vegetables (turnip, beetroot, swede, carrot, artichoke, parsnip and potato) were higher than those the core of the same vegetables, this was consistent with previous studies. Vegetables from different locations contain variable arsenic contents, for example beetroot and parsnip contained

different arsenic concentrations as they were purchased from different locations. Inorganic arsenic species ($InAs^{III}$ and $InAs^{V}$) were the dominant species in these vegetable crops comprising 70 – 102 % of the total arsenic contents. Both methods were validated using CRMs NIES rice flour and pine needles 1575a for both rice and vegetable crop samples, respectively.

Chapter 5

A study of arsenic speciation on soil, irrigation water and plant tissue: A case study of vegetables and crops.

5.1 Introduction

It has been shown previously that a range of foodstuffs contain arsenic. Although it is well understood that information on the total element concentration is insufficient when assessing the toxicity, total levels may be used as an indicator, particularly for food of non-marine origin. The combination of natural processes such as weathering reactions, biological activities and volcanic emissions as well as anthropogenic sources govern the mobilization of arsenic in any ecosystem [366] .

The determination of plant available arsenic species in soil is very important for understanding the uptake, transferred and accumulation processes of arsenic in plants, for evaluating the potential health risk and evaluation and management of environmental risk and safety.

In this study, both total arsenic and arsenic speciation were determined in selected vegetables and plants collected at different location in the Kurdistan region of Iraq (Figure 5.1). This is thought to be the first study on arsenic speciation in vegetables and crops grown in this region. The aim of the study was to better understand how the local habitat may impact on the uptake, transformation, and accumulation of arsenic in plants and indigenous food stuffs. Figure 5.2 shows a diagram of potential routes for arsenic into food based plants.

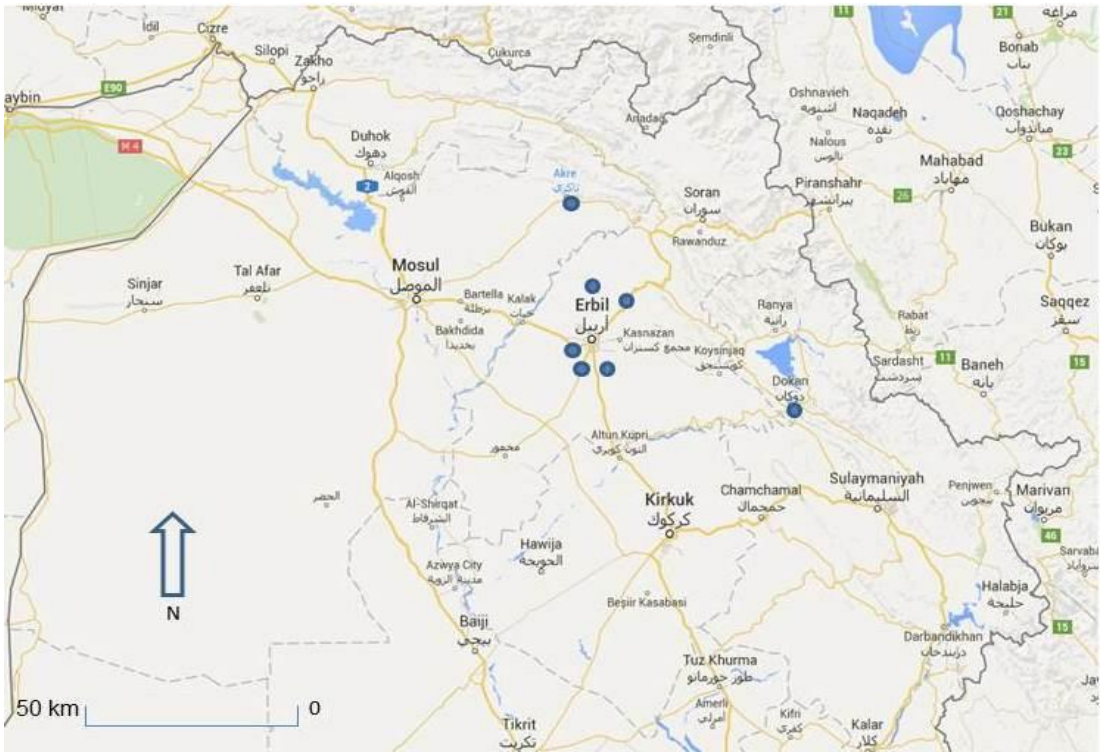


Figure 5.1 Sampling station (●) Kurdistan of Iraq, 2014.

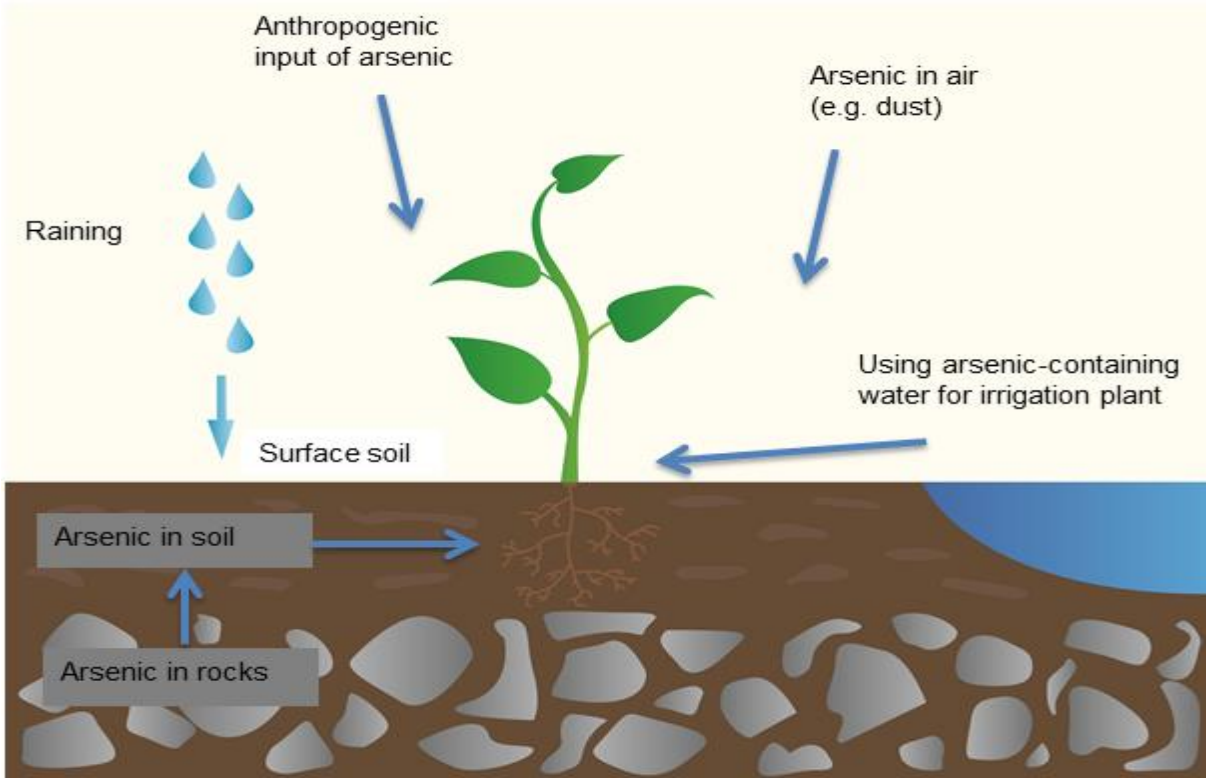


Figure 5.2 Potential routes for arsenic into food based plant.

5.2 Water

Arsenic is often present in groundwater and in several countries the concentrations exceed international health standards. In some contaminated areas the concentrations of arsenic in ground water can reach as high as hundreds of μg L^{-1}. Millions of people use ground waters contaminated with arsenic concentration ≥ 50 μg L^{-1} [17, 78], i.e. significantly higher than the World Health Organization (WHO) maximum permissible limit in drinking water which is 50 μg L^{-1} and the value has been reduced to 10 μg L^{-1} [79]. Ground water contaminated with arsenic has been reported in 20 countries around the world [78]. The levels of arsenic in uncontaminated groundwater usually range from 1-2 μg L^{-1} [21]. Surface freshwater such as river and lake can have variable arsenic concentration by more than four orders of magnitude depending on the source, availability and geochemistry of the catchments [17]. Arsenic is present in the earth's crust at an average concentration of 2-5 μg g^{-1} [367], although its relative abundance in fresh water is not known. This is important as human exposure to elevated arsenic is often associated with drinking water. Bangladesh, India, Argentina, Chile, Mexico, China and the USA have high levels of arsenic in groundwater[368]. Drinking water contaminated with Arsenic is a major global concern, with over 100 million people affected, including up to 57 million in Bangladesh alone [369]. Arsenic is present predominately as $InAs^{III}$ and $InAs^{V}$ in ground and surface water utilized as drinking water supplies [18, 370]. The distribution of arsenic species in water depends on several factors for instance pH, salinity, pK_a, redox potential [371]. However, organic species such as DMA and MMA have also been detected in ground water [372, 373]. A relationship has also found between elevated arsenic exposure through drinking water and the prevalence of skin, lung, and bladder cancers based on the epidemiological studies of populations of Taiwan, Chile and Argentina exposed to high concentrations of arsenic [367].

Surface freshwater such as river and lake water can have variable arsenic concentration by more than four orders of magnitude depending on the source, availability and geochemistry of the catchments [17]. Normal concentrations of arsenic in river water are in the range of 0.1-2.1 $\mu g L^{-1}$ with an average concentration of 0.8 $\mu g\ L^{-1}$ [374]. Geothermal input, evaporation, and ground water contamination may lead to the high concentrations of arsenic in rivers. For example, extremely high concentration of arsenic (up to 21000 $\mu g\ L^{-1}$) have been found in the River Lao of northern Chile possibly attributed to the above mentioned reasons [375]. Studies have revealed that high levels of dissolved Fe(II) and low SO_4^{2-} in ground water may have caused high concentrations of arsenic[368]. It has been found that water samples in some different districts of West Bengal contained arsenic in the range of 100-1000 $\mu g\ L^{-1}$ [376]. Contamination of ground water may occur naturally as a result of dissolving of minerals associated with weathered rocks, ash and soils of volcanic activity. It has been evaluated that the ratio of natural to anthropogenic atmosphere addition of arsenic is 60:40 [374]. In addition, anthropogenic activities including herbicides, mining and wood treatment operations may be involved in the presence of this element in ground water [273, 377, 378].

5.2.1 Water sample collection

The irrigation water samples were collected in Kurdistan region of Iraq from the side farm well close to where the plants and soil samples were collected. The samples were stored in HDPE bottles. The pH of the water samples were adjusted with nitric acid to 2.0 in order to preserve any arsenic species and prevent interchanges between species.

5.2.2 Results and discussion of water samples

Total arsenic concentrations in the irrigating water samples were measured using ICP-MS (conditions described in Table 3.1). The total concentration of arsenic in the irrigation water in all areas under study ranged from 0.54 to 2.4 $\mu g\ L^{-1}$ which is

significantly below the recommended limiting value for arsenic in drinking water by the WHO (10 µg L^{-1}) [379, 380]. The lowest concentration of arsenic (0.54 µg L^{-1} As) was found in water used for irrigating vegetables such as chard- *Beta vulgaris subsp,* radish-*Raphanus sativus*, spinach- *Spinacia oleracea*, Garden cress-*Lepidium sativum L* and Celery-*Apium graveolens*, while water (W5) used for irrigating beetroot- *Beta vulgaris* contained the highest value of arsenic (2.39 µg L^{-1} As). Water samples (W2, W3 and W4) used for irrigating Egyptian leek-*Allium kurrat. schweinf* contained concentrations in the range of 0.664-0.697 µg L^{-1} arsenic. Water samples W6, W7 and W8 which were used for growing vegetables such as potato- *Solanum tuberosum*, rice- *Oryza sativa* and broad bean-*Vicia Faba* have different geographical origin and contained arsenic concentrations between 0.576-1.152 µg L^{-1} arsenic. Other plants such as Arum-*Arum spp.*, Mallow-*Malva parviflora* and Sunflower-*Gundelia tournefortii*. are cultivated naturally depending on rain water.

Because the concentration of arsenic in water is very low and near the limit of detection using HPLC-ICP-MS, speciation on the water was not performed. At this level, irrigation water was not felt to be a significant contributor, but would be a facilitator at the root surface. Results are shown in Table 5.1.

Table 5.1 Concentrations of arsenic in irrigating water samples and sampling locations. The vegetable crops grown in each region are also shown.

Water sample	Location	area	Label	Concentration ($\mu g\ L^{-1}$ ± SD (n=3)	Vegetable or crop
Water 1	South west Arbeel	Turaq	W1	0.54 ± 0.01	Chard-*Beta vulgaris subsp.*, spinach-*Spinacia oleracea*, radish-*Raphanus sativus*, Garden cress-*Lepidium sativum L* and Celery- *Apium graveolens*
Water 2	South west Arbeel	Turaq	W2	0.664 ± 0.025	Egyptian leek-*Allium Kurrat Schweinf*
Water 3	South Arbeel	South industrial	W3	0.697 ± 0.02	Spring onion-*Allium fistulosum*
Water 4	South Arbeel	Bahar	W4	0.683 ± 0.06	Wild mint-*Mentha longifolia*
Water 5	South east Arbeel	Pirdawd	W5	2.4 ± 0.12	Beetroot-*Beta vulgaris*
Water 6	South east Arbeel	Awena	W6	1.152 ± 0.07	Potato-*Solanum tuberosum*
Water 7	North west Arbeel	Akre	W7	0.576 ± 0.02	Rice-*Oryza sativa*
Water 8	South east Arbeel	Dokan	W8	1.06 ± 0.07	Broad bean-*Vicia Faba*
-	-	-	-	-	Arum-*Arum spp**
-	-	-	-	-	Mallow-*Malva parviflora**
-	-	-	-	-	Sunflower-*Gundelia tournefortii.**

*Rain-dependent

5.3 Soil sample

5.3.1 Introduction

The occurrence of arsenic in vegetables in non-marine food crops and the accumulation of arsenic through the food chain can be due to either using pesticides laden with arsenic or using polluted soil in agriculture [381]. The occurring of arsenic species in soils ultimately depends on several factors such as the form and amounts of sorbing components of the soil, the redox potential, and the overall pH [382]. Moreover, pyrite and sphalerite minerals in mine tailings, spoils and sludge process can be affected by weathering processes which produce poorly crystalline Fe oxides as this process has strong influence on soil quality and phytotoxicity in soil [383]. Other sources of arsenic in soil can be industrial , use of pesticides, herbicides, wood preservations, mine wastes [384], dust from the burning of fossil fuels and disposal of industrial wastes[361]. However, soils from areas in the world have been contaminated with arsenic as a result of using groundwater containing high levels of arsenic for irrigating [385]. The arsenic contents of soil occur at different concentration according to the region. Normally, arsenic content in non-contaminated soils occurs at levels ranging from 1-40 µg g^{-1}; however, high arsenic concentrations measured more than 20 µg g^{-1} may be sampled in some old industrial and mining sites [386], comprising an overall mean of 5 - 6 µg g^{-1} [387, 388]. Some anthropogenic activities may assimilate high arsenic levels (10000-20000 µg g^{-1}) in some agriculture areas and human habitats, posing high levels of risk to human health, plants and microorganisms [389]. Soil guideline Value (SGV's) in the UK have been set at 20 µg g^{-1} per dry weight of soil for residential area and allotments whereas the value for commercial or industrial land is 500 µg g^{-1} per dry weight of soil [388]. Dissolving and transformation of this high concentration of arsenic is achieved under different environmental conditions. Nevertheless, an immediate environmental risk can be posed

by a small quantity of arsenic in polluted soils or mining areas due to the low water solubility of arsenic species [390].

Even though the main arsenic species that have been found in soil are $InAs^{III}$ and $InAs^{V}$, DMA and MMA may be detected due to microorganisms-mediated oxidation-reduction reactions [385]. Aerobic and anaerobic microorganisms can methylate inorganic arsenic to produce methylated arsenic species such as MMA, DMA and TMAO [391]. There are several factors controlling the production of volatilized arsenic (organic and inorganic) by microorganisms such as the individual arsenic species, concentration, soil moisture, temperature, organic materials, other elements, microbial growth and capacity of arsenic volatilization [392]. The existance of organic arsenic species in soil may be attributed to different sources such as anthropogenic sources, methylation, atmospheric deposition, terrestrial plants and terrestrial animals [391]. In general, research has focused on the pentavalent forms of arsenic in soil because of their high reactivity in the environment [393]. Essentially, $InAs^{V}$ occurs naturally in soil at pH 6-8 in the form of oxyanions $H_2AsO_4^-$ and $HAsO_4^{2-}$ (pKa = 6.8) under oxidising conditions. At pH levels below 9.2 under reducing conditions, $InAs^{III}$ is recognized as the major arsenic species and exists as H_3AsO_3 [384]. The toxicity and mobility of arsenic in soil thus depends on its chemical form as $InAs^{III}$ is the most mobile species [384, 394].

It has been documented[389, 395] that biological availability or potential toxicity measurements, which are crucial for possible environmental impacts, is not represented by total arsenic in soil. 'Plant available' for absorption or "bioavailable" of arsenic can pose risk to plants.

5.3.2 Soil sample collection

Soil samples were collected from the same agriculture sites used to grow the vegetables and crops in April 2014. Soil samples were collected in the vicinity of each type of plant, and stored in a specific HDPE bottle. All samples were then freeze-dried. The soil samples were ground using an agitate pestle mortar and passed through 180 µm nylon mesh. The identification of the soil samples collected and the vegetables grown are shown in Table 5.2.

Table 5.2 Location and identification of soils used to cultivate plant and vegetable crops.

Soil sample	Location	Area	Liable	Vegetable or crop
Soil 1	South west Arbeel	Turaq	So1	Chard-*Beta vulgaris subsp.*
Soil 2	South west Arbeel	Turaq	So2	Celery-*Apium graveolens*
Soil 3	South west Arbeel	Turaq	So3	Garden cress-*Lepidium sativum L.*
Soil 4	South west Arbeel	Turaq	So4	Radish-*Raphanus sativus,*
Soil 5	South west Arbeel	Turaq	So5	Spinach-*Spinacia oleracea*
Soil 6	South west Arbeel	Turaq	So6	Egyptian leek-*Allium Kurrat. Schweinf.*
Soil 7	South Arbeel	South industrial	So7	Spring onion-*Allium fistulosum*
Soil 8	South Arbeel	Bahar	So8	Wild mint-*Mentha longifolia*
Soil 9	South east Arbeel	Pirdawd	So9	Beetroot-*Beta vulgaris*
Soil 10	South east Arbeel	Awena	So10	Potato-*Solanum tuberosum*
Soil 11	North west Arbeel	Akre	So11	Rice-*Oryza sativa*
Soil 12	South east Arbeel	Dokan	So12	Broad bean-*Vicia Faba*
Soil 13	South east Arbeel	Qopaqran	So13	Arum-*Arum spp**
Soil 14	South east Arbeel	Qopaqran	So14	Mallow-*Malva parviflora**
Soil 15	North Arbeel	Pirmam	So15	Sunflower-Gundelia *tournefortii.**

5.3.3 Sample preparation

5.3.3.1 Aqua regia extraction procedure

Total 'aqua regia-extractable' arsenic in the soil samples was extracted using a modified version of the LGC protocol [396] according to the following steps:

1. Sample (approx. 1.0 g) should be weighed accurately into a pre-cleaned beaker and transferred to a large pre-cleaned digestion tube.

2. The beaker should then be re-weighed so that an accurate weight of sample transferred to the tube could be obtained.

3. Hydrochloric acid (8 mL) and nitric acid (2 mL) should then be added and the sample allowed to stand at room temperature for at least one hour so that easily oxidised material could be destroyed.

4. After that, the digestion tubes should be placed in a Tecator digestion block (Foss Teactor ^{TM} Digestor, Sweden) and heated at 110 ºC for two hours.

5. After cooling, the extracts were transferred quantitatively without filtering to pre-cleaned 100 mL capacity volumetric flasks and diluted to volume with de-ionised waster.

6. Three replicates of each sample should be prepared together with certified reference materials. The certified reference material should be prepared in the same way. A procedural blank should also be prepared in the same way, but omitting the sample.

5.3.3.2 Extraction of arsenic species using 1 M phosphoric acid

The phosphoric extraction method used was modified from Lou *et al.*'s [397] procedure for arsenic speciation in soil using phosphoric acid and ascorbic acid to prevent any species interchanges during extraction. Extraction of arsenic was performed as follows:

1. Sample (0.5 g) was weighed into a digestion tube and phosphoric acid (1 M, 25 mL) and ascorbic acid 0.1 M added.

2. The samples were digested using microwave-assisted extraction 80 ºC for a period of 20 minutes.

3. The liquid phase was then transferred to a 50 mL capacity centrifuge tube.

4. A blank was also prepared in a similar fashion, but omitting the sample.

5. The extracted samples were centrifuged at 2000 rpm for a period of 20 minutes and the supernatant decanted into pre-cleaned volumetric flasks.

6. The soil pellet was then re-suspended with a further 20 mL of water, centrifuged again at 2000 rpm for 20 minutes and the supernatant combined with the extracts obtained previously.

7. The samples were then diluted to volume using Milli-Q water.

8. Once prepared, the extracts were analysed for "total arsenic". On this occasion, calibration standards were matrix matched with the appropriate amount of 1 M phosphoric acid and 0.1 M ascorbic acid.

5.3.3.3 BCR sequential extraction (three-step) procedure for the determination of extractable trace metal (arsenic) contents in soil and BCR 701

Before applying the standardised BCR procedure for sequential extraction, the following solutions were prepared:

Solution A (acetic acid, 0.11 M): In a fume cupboard, 25 ± 0.1 mL of glacial acetic acid was added to about 0.5 L of Milli-Q water in a 1 L graduated polypropylene or polyethylene bottle and made up to 1 L with Milli-Q water. 250 mL of this solution

(acetic acid, 0.43 M) was taken and diluted to 1 L with milli-Q water to obtain an acetic acid solution of 0.11 M.

Solution B (hydroxylammonium chloride (hydroxylamine hydrochloride), 0.5 M). 34.75g of hydroxylammonium chloride was dissolved in 400 mL Milli-Q water. This was transferred in to a 1L volumetric flask containing 25 mL of 2 M HNO_3 and made up to 1 L with Milli-Q water. Prepare this solution in the same day the extraction is carried out.

Solution C (hydrogen peroxide, 300 mg g^{-1}, i.e., 8.8 M). The hydrogen peroxide was used as supplied by the manufacturer, i.e., acid-stabilised to pH 2-3.

Solution D (ammonium acetate, 1.0 M). This solution was prepared using ammonium acetate and adjusted pH to 2 ± 0.1 with concentrated HNO_3.

Blanks

Analytical blank solutions for the As, Cd, Cr, Cu, Ni, and Zn solutions were prepared as follows:

i) **Vessel blank**. One vessel from each batch was taken through the cleaning procedure, and 40 mL of solution A added. This was then analysed along with sample solutions from step 1 (detailed below).

ii) **Reagent blank**. A sample of each batch of solutions A, B, C and D was analysed.

iii) **Procedural blank.** With each batch of extractions, a blank sample (i.e., a vessel with no soil) was carried through the complete procedure and analysed at the end of each extraction step.

Step 1. 40 mL of solution A was added to 1 g soil in an 50 mL centrifuge tube. This was stoppered and agitated on a shaker for 16 hours at 22 ± 5 ºC (overnight). No delay was allowed between the addition of the extractant solution and the beginning of the shaking. The extracts were then separated from the solid residue by centrifugation at 3000 g for 20 min and the supernatant liquid decanted into a polyethylene container. This was either analysed immediately, or stored in a refrigerator at about 4 ºC prior to analysis. The residue was washed by adding 20 mL milli-Q water, shaking for 15 min at 3000 rpm. The supernatant was decanted and then discarded, taking care not discard any of solid residue.

Step2. 40 mL of a freshly prepared solution B was added to the residue from step 1 in the centrifuge tube. This was resuspended by manual shaking, stoppered and then extracted by mechanical shaking for 16 hours at 22 ± 5 ºC (overnight). No delay was allowed between the addition of the extractant solution and the beginning of the shaking. The extract from the solid residue was separated by centrifugation and decantation as in step 1. The extract was retained in a stoppered polyethylene container, as in step 1, for analysis. The residue was washed by adding 20 mL milli-Q water, shaking for 15 min on the shaker and centrifuging for 20 min at 3000 rpm. The supernatant was then decanted and discarded, taking care not to discard any of the solid residue.

Step 3. Small aliquots (10 mL) of solution C were added carefully to the residue in the centrifuge tube to avoid losses due to a possible violent reaction. The vessel was loosely capped and digested at room temperature for 1 hour with occasional manual shaking. The digestion is continued for another hour at 85 ± 2 ºC in a water bath, with occasional shaking for the first 30 min. The volume is then reduced to less than 3 mL by further heating of the uncovered tube. A further aliquot of 10 mL of solution C in added and again heated in a covered vessel at 85 ± 2 ºC for 1 hour, with occasional shaking for the

first 30 min. The cover was removed and the volume of liquid reduced to about 1 mL avoiding complete dryness. 50 mL of solution D was added to the cool moist residue and shaken for 16 hours at 22 ± 5 ºC (overnight). No delay occurred between the addition of the extractant solution and the beginning of the shaking. The extract was then separated from the solid residue by centrifugation and decantation as in step 1.

5.3.3.4 Results and discussion

5.3.3.4.1 Total arsenic and arsenic speciation in soil

Fifteen different soils used to cultivate the different vegetable crops were collected at the same sites. Total arsenic in the soil was extracted using an aqua regia extraction method (section 5.3.3.1). The concentrations of arsenic in the soil samples were determined using ICP-MS (conditions described in chapter three Table 3.1). The results are presented in Table 5.3. The accuracy of the aqua regia digestion procedure for total arsenic determinations was verified using a certified reference material loam soil-ERM-CC141. Analysis of this certified reference material in 3 replicates revealed satisfactory accuracy, with the recovery for arsenic in loam soil 98 %. The obtained result (7.33 µg g^{-1}) for this CRM using aqua regia procedure was valid compare to its certified value (7.55 µg g^{-1}). There was not significant difference statistically between the obtained value and certified value for arsenic for loam soil ERM-CC141 as its UΔ (1.632 µg g^{-1}) > Δm (0.17 µg g^{-1}).

The concentrations of arsenic in soils ranged from 2.88 to 6.21 µg g^{-1}. The concentrations of arsenic in the soils of this study were within the global average concentration of arsenic in uncontaminated soil which is between 5 and 6 µg g^{-1} [388]. The lowest concentration of arsenic in the analysed soil in this study was found in soil So11 (2.88 µg g^{-1}), which was used to cultivate rice. While the highest concentration of

arsenic in analysed soils under study was found in soil So6 (6.21 µg g^{-1}), which was used to cultivate Egyptian leek-*Allium kurrat schweinf.*

Phosphoric acid was used as the extraction reagent owing to its ability to extract arsenic species effectively [15, 387, 395, 398, 399] .Therefore, 1 M phosphoric acid and 0.1 M ascorbic acid were used to extract the arsenic species from the soil samples. HPLC-ICP-MS (Chapter 4 Table 4.2) was then used to speciate arsenic species in the soils using $NH_4H_2PO_4$ as mobile phase. The stability of the arsenic species ($InAs^{III}$ and $InAs^{V}$) under the applied extraction conditions was also checked by spiking the CRMs with these arsenic species standards into the loam soil (CRM). The results demonstrated good stability for both species after the extraction with a variability of only 2 % for both species which considering the analytical uncertainty. It has been reported in the literature that adding ascorbic acid to the extraction media help to prevent inter-conversion of arsenic species during extraction process[400-402].

The chromatogram obtained for soil So1 is shown in Figure 5.3. Results for the species found in soil ($InAs^{III}$, DMA, MMA and $InAs^{V}$) are shown in Table 5.3. The speciation results show that $InAs^{III}$ and $InAs^{V}$ were present in all soils, whilst MMA and DMA were below limits of detection of the method. In all soil samples $InAs^{V}$ was present at higher concentration than $InAs^{III}$. The concentrations of $InAs^{V}$ ranged from 2.307 to 5.873 µg g^{-1}. The highest level of $InAs^{V}$ in soil was found in soil10 (5.522 µg g^{-1}) used to cultivate potato, while the lowest concentration was found in soil So11 (2.307 µg g^{-1}) which was used to cultivate rice plant. The highest levels of $InAs^{III}$ was found to be 0.756 µg g^{-1} in So1 soil which used to cultivate chard, whereas So 15 had lowest level of $InAs^{III}$ (0.260 µg g^{-1}) which was used for Sunflower-*Gundelia tournefortii*.

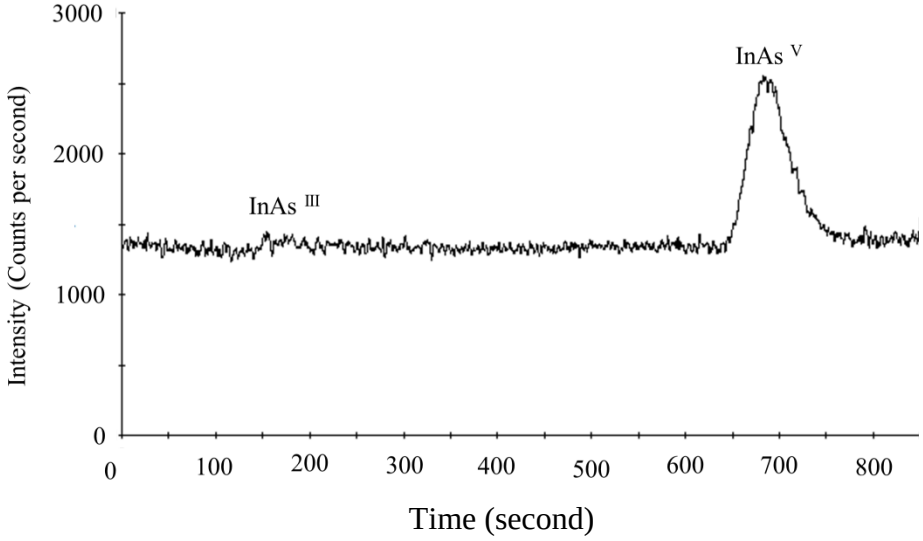


Figure 5.3 Chromatogram obtained from soil So1 using anion-exchange HPLC-ICP-MS, using the conditions described in Table 4.2.

Table 5.3 Concentrations of total arsenic and arsenic species in soil samples used to cultivated different vegetable and crops, all values are µg g^{-1} ± SD (n=3).

Soil type	Aqua regia extractable As	$InAs^{III}$	DMA	MMA	$InAs^{V}$	Residue	Total As in extracts	Extraction efficiency
So1	5.450 ± 0.04	0.756 ± 0.037	<0.119	<0.084	4.487 ± 0.110	0.224 ± 0.007	5.23 ± 0.212	96
So2	5.32 ± 0.37	0.662 ± 0.048	<0.119	<0.084	4.474 ± 0.142	0.135 ± 0.003	5.15 ± 0.146	97
So3	5.25 ± 0.26	0.549 ± 0.048	<0.119	<0.084	4.132 ± 0.277	0.696 ± 0.063	5.46 ± 0.15	104
So4	5.83 ± 0.03	0.556 ± 0.022	<0.119	<0.084	4.568 ± 0.131	0.497 ± 0.006	5.21 ± 0.125	89
So5	5.01 ± 0.19	0.718 ± 0.055	<0.119	<0.084	4.228 ± 0.160	0.113 ± 0.003	4.98 ± 0.1	99
So6	6.21 ± 0.02	0.371 ± 0.039	<0.119	<0.084	5.414 ± 0.161	0.320 ± 0.023	5.83 ± 0.15	94
So7	6.11 ± 0.04	0.545 ± 0.041	<0.119	<0.084	5.482 ± 0.417	0.556 ± 0.030	6.1 ± 0.21	100
So8	3.92 ± 0.02	0.281 ± 0.014	<0.119	<0.084	3.573 ± 0.072	0.152 ± 0.019	3.9 ± 0.09	99
So9	5.41 ± 0.18	0.278 ± 0.019	<0.119	<0.084	4.763 ± 0.124	0.372 ± 0.020	5.19 ± 0.14	96
So10	6.04 ± 0.086	0.344 ± 0.010	<0.119	<0.084	5.522 ± 0.036	0.138 ± 0.027	5.9 ± 0.19	98
So11	2.88 ± 0.056	0.373 ± 0.023	<0.119	<0.084	2.307 ± 0.093	0.105 ± 0.004	2.68 ± 0.06	93
So12	5.32 ± 0.042	0.564 ± 0.0007	<0.119	<0.084	4.584 ± 0.22	<0.027	5.3 ± 0.3	100
So13	6.09 ± 0.08	0.532 ± 0.019	<0.119	<0.084	5.275 ± 0.120	0.405 ± 0.045	6.17 ± 0.19	101
So14	4.2 ± 0.23	0.509 ± 0.038	<0.119	<0.084	3.750 ± 0.077	0.126 ± 0.003	4.36 ± 0.12	104
So15	5.59 ± 0.06	0.260 ± 0.020	<0.119	<0.084	5.043 ± 0.081	0.247 ± 0.015	5.32 ± 0.13	95
Loam soil	7.33 ± 0.42	1.186 ± 0.003	<0.119	<0.084	5.873 ± 0.003	0.112 ± 0.01	7.17 ± 0.32	98
BCR 701	23.77 ± 1.84	8.45 ± 0.34	<0.119	<0.084	17.17 ± 0.11	<0.027	26 ± 0.69	109

5.3.3.4.2 'Plant available' arsenic in soil

Sequential extraction schemes are the methods of choice to assess the trace element in soil and sediments and have been used widely to determine the properties of these environmental samples[403-405]. A three-step extraction scheme has been established by the Community of Reference (BCR, Measurements and Testing Programme SM&T) in order to coordinate different extraction schemes [113].

This standard BCR protocol was applied here to assess plant available arsenic in the soils. Because the value for arsenic 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 values of other metals (Cd, Cr, Cu, Ni and Zn) were measured and compared in order to validate the applied procedure. However, Alvarez *et al.* [406] have measured the arsenic concentration in BCR 701 using the sequential extraction procedure and they performed just steps 1 and 2. The concentrations of arsenic in steps 1 and 2 were 2.100 ± 0.100 µg As g^{-1} (mean ± SD) and 18 ± 1 µg As g^{-1} (mean ± SD), respectively. The results presented here are comparable to those in Alvarez *et al.*'s study. Detection limits (three times the standard deviation of the blank, divided by the slope of the calibration graph) are presented in Table 5.4. The standard sediment reference material was analyzed using 3 replicates. The extractable contents and recoveries in each step of the standard reference material used are presented in Table 5.5.

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 %.

From step 1 the obtained values for Cr, Cu and Ni in BCR 701 were statistically in good agreement with their certified values, while Cd and Zn showed significant differences with their certified values. In step 2 statistical agreement obtained for Cr and Ni as their UΔ>Δm. There was not good agreement statistically between obtained and certified values for Cd, Cu and Zn, however, and the extraction efficiency of both Cu and Zn were within acceptable range (90 % for both elements); this is because of the precision of measured elements was higher than the certified elements. In step 3 statistical agreement was obtained (UΔ>Δm) for Cd, Cr, Ni, and Zn between measured and certified values with the exception of Cu because its Δm (15.5) value was higher than UΔ (4.4). In total aqua-regia extractable elements in BCR 701 the concentrations of elements are given as indicative values. There was no significant difference between indicative values and measured values for Cu and Cd as their Δm values were smaller than UΔ. Since the Δm is bigger than UΔ for Cr, Ni and Zn the results indicated that there was significant differences between their indicative values and measured values for these elements, although the extraction efficiency of Zn was in good agreement (90 %) due to the high precision of the measured value.

It is noted that the total element contents are 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 [407]. Although a phosphoric acid procedure was applied to extract the arsenic species in the studied soils, this is not an indication that the total arsenic is mobile and/or that vegetable and crop plants can take up all of the arsenic species. The extractable contents and recoveries in each step of the analysed soils under study are given in Table 5.6. The maximum amount of arsenic which could potentially be mobilised in the soils under study (pseudo-total content) was between 2.88 to 6.21 $\mu g g^{-1}$. Overall, between 17.52 to 34.4 % of arsenic in the analysed soils were 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 7.87 to 21.14 % arsenic were 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 [408]. The 'reducible fraction' extracted from the soils under study gave between 2.96 and 13.26 % arsenic, which is considered mostly bound to the structure of primary and secondary minerals. In comparison, the BCR-701 material retained 74 % of its arsenic with this fraction. The arsenic associated with the 'organic materials and sulphides' fraction in the studied soils were between 0.51 to 2.55 %. It is assumed that the metallic bound species in this phase could be retained longer, but may be immobilized by a decomposition process [409]. A soluble fraction 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 as plant available ('bioavailable') because it is associated with stable humic substances. These only slowly release small quantities of metals [405]. Arsenic in the studied soil was found to be bound mainly within the residual fraction (from 54.16 to 91.67 %).

A comparison of the sum of the arsenic concentrations in the sequential extraction steps with this aqua regia metal concentrations showed a good agreement for all soils (Table 5.7).

Table 5.4 Detection limits for analysis in the sequential extraction reagents (μg g^{-1}).

Analyte	Step 1	Step 2	Step 3	Aqua regia
Cd	0.002	0.003	0.001	0.006
Cr	0.006	0.008	0.008	0.001
Cu	0.004	0.002	0.002	0.015
Ni	0.003	0.006	0.008	0.001
Zn	0.008	0.004	0.003	0.002
As	0.008	0.005	0.003	0.003

Table 5.5 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 %
Step 1				
	Cd	5.95 ± 0.11	7.34 ± 0.35	81
	Cr	2.57 ± 0.22	2.26 ± 0.16	113
	Cu	53.9 ± 3.85	49.3 ±1.7	109
	Ni	13.89 ± 3.6	15.4 ± 0.9	91
	Zn	165 ± 5	205 ± 6	80
	Fe	140 ± 3	Not certified	-
	Mn	166 ± 0.7	Not certified	-
	As	2.1 ± 0.1	Not certified	-
	As % of total	8.4	-	-
Step 2				
	Cd	2.72 ± 0.2	3.77 ± 0.28	72
	Cr	49.4 ± 0.67	45.7 ± 2	108
	Cu	112 ± 1.4	124 ± 3	90
	Ni	26.3 ± 0.31	26.6 ± 1.3	99
	Zn	103 ± 0.77	114 ± 5	90
	Fe	6990 ± 46	Not certified	-
	Mn	121.6 ± 2.1	Not certified	-
	As	17.6 ± 0.4	Not certified	-
	As % of total	74	-	-
Step 3				
	Cd	0.26 ± 0.03	0.27 ± 0.06	96
	Cr	136 ± 14	143 ± 7	95
	Cu	39.5 ± 1	55 ± 4	72
	Ni	14.6 ± 1.9	15.3 ± 9	95
	Zn	42 ± 2	46 ± 4	91
	Fe	944.4 ± 75.8	Not certified	-
	Mn	19.3 ± 0.35	Not certified	-
	As	2.2 ± 0.17	Not certified	-
	As % of total	9	-	
Aqua-regia extractable				
	Cd	13 ± 0.2	11.7 ± 1 [a]	111
	Cr	227.3 ± 2.5	272 ± 20 [a]	84
	Cu	281 ± 1.8	275 ± 0.013 [a]	102
	Ni	86 ± 1	103 ± 4[a]	83
	Zn	410 ± 3	454 ± 19[a]	90
	Fe	18597 ± 221	Not certified	-
	Mn	486 ± 5	Not certified	-
	As	23.8 ± 1.8	Not certified	-

a Indicative value

Table 5.6 Summery of the results obtained by sequential extraction and aqua regia digestion for arsenic in soil samples.

Soil type	Aqua regia extractable As µg g^{-1}	Step 1 µg g^{-1}	As % of total	Step 2 µg g^{-1}	As % of total	Step 3 µg g^{-1}	As % of total	Step 4 total arsenic in residues µg g^{-1}	As % of total
So1	5.45 ± 0.044	0.95 ± 0.09	17.43	0.263 ± 0.01	4.83	0.094 ± 0.01	1.72	4.33 ± 0.61	79.45
So2	5.32 ± 0.37	1.03 ± 0.04	19.36	0.198 ± 0.02	3.72	0.1 ± 0.007	1.88	4.16 ± 0.04	78.20
So3	5.25 ± 0.26	1.11 ± 0.11	21.14	0.274 ± 0.02	5.22	0.128 ± 0.01	2.44	4.04 ± 0.01	76.95
So4	5.83 ± 0.03	1.14 ± 0.17	19.55	0.18 ± 0.02	3.09	0.03 ± 0.01	0.51	3.89 ± 0.13	66.72
So5	5.01 ± 0.19	1.01 ± 0.04	20.16	0.23 ± 0.01	4.59	0.104 ± 0.01	2.08	4.03 ± 0.06	80.44
So6	6.21 ± 0.02	1.0 ± 0.13	16.10	0.3 ± 0.02	4.83	0.073 ± 0.01	1.18	4.2 ± 0.55	67.63
So7	6.11 ± 0.042	1.1 ± 0.1	18.00	0.2 ± 0.02	3.27	0.046 ± 0.006	0.75	4.29 ± 0.09	70.21
So8	3.92 ± 0.02	0.73 ± 0.1	18.62	0.52 ± 0.03	13.27	0.1 ± 0.006	2.55	3.05 ± 0.2	77.81
So9	5.41 ± 0.18	0.77 ± 0.01	14.23	0.16 ± 0.01	2.96	0.072 ± 0.007	1.33	2.93 ± 0.2	54.16
So10	6.04 ± 0.086	0.95 ± 0.05	15.73	0.437 ± 0.01	7.24	0.025 ± 0.007	0.41	3.95 ± 0.09	65.40
So11	2.88 ± 0.56	0.41 ± 0.02	14.24	0.35 ± 0.03	12.15	0.044 ± 0.005	1.53	1.97 ± 0.05	68.40
So12	5.32 ± 0.42	0.60 ± 0.08	11.28	0.222 ± 0.01	4.17	0.11 ± 0.007	2.07	4.43 ± 0.04	83.29
So13	6.09 ± 0.08	0.77 ± 0.06	12.64	0.674 ± 0.01	11.07	0.048 ± 0.004	0.79	4.27 ± 0.1	70.11
So14	4.2 ± 0.23	0.7 ± 0.03	16.67	0.15 ± 0.01	3.57	0.045 ± 0.002	1.07	3.85 ± 0. 5	91.67
So15	5.59 ± 0.06	0.44 ± 0.04	7.87	0.63 ± 0.01	11.27	0.05 ± 0.003	0.89	3.91 ± 0.05	69.95
BCR 701	23.77 ± 1.84	2.00 ± 0.1	8.41	17.55 ± 0.39	74	2.2 ± 0.17	9.0	5.33 ± 0.61	22.42

Table 5.7 Comparative results of the aqua regia digestion and the BCR sequential extraction procedure of the soil samples (n=3), based on the arsenic content (μg g^{-1})

Soil	Aqua regia extractable As	Sum of species (step 1+2+3+4)	Recovery (%) (sum of species from extract/aqua regia extractable)
So1	5.45 ± 0.04	5.637	103
So2	5.32 ± 0.37	5.488	103
So3	5.25 ± 0.26	5.552	106
So4	5.83 ± 0.03	5.24	90
So5	5.01 ± 0.19	5.374	107
So6	6.21 ± 0.02	5.573	90
So7	6.11 ± 0.04	5.636	92
So8	3.92 ± 0.02	4.4	112
So9	5.41 ± 0.18	3.932	73
So10	6 ± 0.08	5.362	89
So11	2.88 ± 0.56	2.774	96
So12	5.32 ± 0.42	5.362	89
So13	6.09 ± 0.08	5.762	95
So14	4.2 ± 0.23	4.745	113
So15	5.59 ± 0.06	5.03	90
BCR 701	23.77 ± 1.84	27.08	114

5.4 Arsenic and arsenic speciation in different organs of vegetable crops

The 'availability' of arsenic to plants depends mainly on the texture and chemical composition of the soil. The uptake of arsenic from soil to plant depends on plant species. There are several factors which govern the arsenic species found in plants such as arsenic species in the soil, the ability of the compounds to enter the plant, (actively or passively), the plant ability to synthesize arsenic species and the existence of arsenic species outside surface of the plant roots [58].

Anionic arsenic species are adsorbed efficiently by iron and aluminium oxide in acidic soil. While in alkaline soil anionic arsenic species to a lesser extent are adsorbed by calcium oxides [410, 411]. Hence, anionic arsenic species available to crops grown in acidic soil are generally less than those in alkaline soil [412]. A competition may occur between arsenate and phosphate in their uptake by plants because they have similar chemical properties. Therefore, a high uptake of arsenate may be observed in the presence of a low content of phosphate in soils [410]. The arsenic concentration in the edible part of mushroom species *Laccaria amethystina* was 1000 μg g^{-1} which was sampled from arsenic contaminated soil. Interestingly, the major arsenic species was DMA which is less toxic than inorganic arsenic species [413]. The absorption of arsenic can occur from soils or deposited matter on leaves.

The average concentration of arsenic in plants is 3.6 μg g^{-1} because not all arsenic is taken up by most plants [414]. This is due to majority of arsenic in soils being insoluble like phosphate and therefore with low availability to plants despite its concentration and toxicity to organisms [415]. Many studies on arsenic in plants have focused on the transformation of arsenical pesticides in crops such as rice, tomato, apple or carrot. More than one arsenic species have been identified in plant tissues growing on both arsenic-contaminated and uncontaminated sites. A range of arsenic species have been

found in plant tissues, for example, inorganic $InAs^{III}$ and $InAs^{V}$, methylated arsenic species, AsB and arseno-sugars. Stress in the plants, including growth inhibition is usually caused by arsenate [416]. Some plant species have developed tolerance mechanisms which allow them to grow in contaminated soils with a very high arsenic content [61]. Plant tolerance is normally 2 $\mu g\ g^{-1}$ [417].

Although inorganic arsenic species are generally toxic to plants, organic arsenic species are considered to be even more toxic [58]. The mechanisms of arsenic uptake by plant roots have not been clearly illustrated, although it can happen either via uptake by phosphate transporters in mycorrihzal fungus [75] or direct uptake by plant roots[145]. Arsenate is a phosphate analogue and transported across the plasma membrane through a phosphate cotransport systems. It competes with phosphate inside cytoplasm, for example replacing phosphate in ATP to produce unstable ADP-As, and causes the disruption of energy flows in cells [58]. Reduction of arsenate to arsenite has also been reported in plants [418]. This may occur either non-enzymatically by glutaredoxin [419] or enzymatically by specific arsenate reductase [420].

Different hypotheses have been reported to explain the metabolism and presence of arsenic species in plants. Organic arsenic species can be taken up from soil solution following microbial activity [421]. The plants themselves may also methylate arsenic. Alternatively, arsenic species could potentially be transformed by microbial endophytes[66], or the metabolism of inorganic arsenic can coincidentally happen *via* induction constitutive enzymes. In order to defend against potentially toxic levels of essential metals, a range of enzymatic process is stimulated in plants. For example, in response to Cd and Hg the plant may be stimulated to produce phytochelatins, glutathione and superoxide dismutase. Arsenate may coincidentally induce such

responses, permitting the plants to constitutively detoxify low levels of arsenic contamination [422, 423].

5.4.1 Results and discussion

Microwave assisted acid digestion (Chapter 3 section 3.3.2.1) was used to extract total arsenic from freeze-dried samples of different organs of different plant species and ICP-MS (Table 4.2) was used to measure total arsenic. Results are presented in Table 5.8, HPLC-ICP-MS (conditions shown in Table 4.2) was utilized for speciation. The chromatographic conditions which are described in Chapter 4, Table 4.2 were successfully used to separate arsenic species. The chromatogram obtained for the root of rice plant is shown in Figure 5.4. The results of this study indicate that the plant species show different tendencies to accumulate arsenic. Generally high concentrations of arsenic are to be found in the roots of plants. However, in certain plant species this situation is different. From the obtained results for the plant species under study, they can be categorized into four different groups. First group includes rice-*Oryza sativa*, sunflower-*Gundelia tournefortii.*, Egyptian leek-*Allium kurrat. schweinf.*, chard- *Beta vulgaris subsp.*, radish- *Raphanus sativus.*, potato- *Solanum tuberosum*, spring onion-*Allium fistulosum*, celery-*Apium graveolens* and garden cress-*Lepidium sativum L*. This group is characterized that the highest concentrations of arsenic being found in the root of the plant and the lowest arsenic concentration was found in the grain or the leaf of the same plant. However, group 2 which contains only mallow-*Malva parviflora* has the opposite pattern, with the highest concentrations of arsenic compartmentalized in the leaf of the plant rather than the root of the same plant. This is consistent with previous findings that arsenic concentrates in the leaves more than flowers and stalks of the same plant such as in nasturtium (*Tropaeolum majus*)[424, 425] . Group 3 consists of broad bean-*Vicia Faba* and wild mint-*Mentha longifolia* which have lower arsenic concentrations in the stem compared to the root and leaf of the same plant species. Finally, group 4

includes beetroot- *Beta vulgaris*, spinach- *Spinacia oleracea* and Arum-*Arum spp*. Here, the stem of the plant species accumulated more arsenic than in other organs of the same plant species. This pattern has been reported in turnips[425].

In general the concentration of arsenic in plants varies between <0.01- 5 µg g^{-1} (dry weight basis) [25]. The arsenic concentration in different vegetables from the UK (Cornwall, England) market in a previous study was reported to be in the range 0.01-3.88 µg g^{-1} [357]. The concentration of total arsenic and arsenic species measured in different parts such as root, stem, leaf, skin and/or grain within different vegetable crops in this study are shown in Table 5.8. Figure 5.4 shows the chromatogram of arsenic species found in the root of the rice plant.

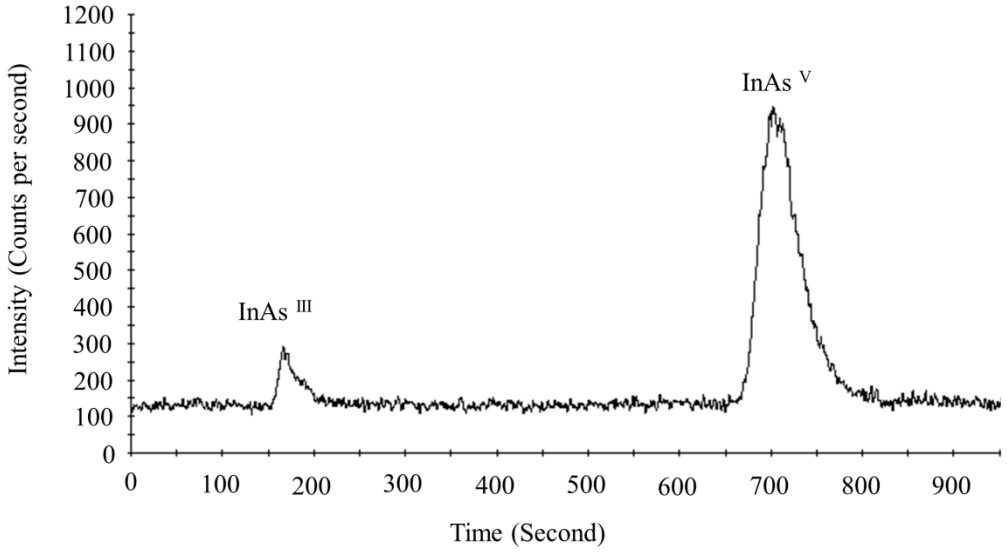


Figure 5.4 Chromatogram for the root of the rice plant using anionic-exchange HPLC-ICP-MS and using conditions illustrated in section 4.1.2.3.

Table 5.8 Results of analysis for arsenic concentration in different organs of vegetable crops (dry weight); all values are given in µg g^{-1} of arsenic mean ± SD (n=3).

Vegetable	Microwave assist digestion	$InAs^{III}$	DMA	MMA	$InAs^{V}$	Total arsenic in residue	Total arsenic in extracts	Efficiency of extraction %	Sum of arsenic from species
Rice									
Root	8.284 ± 0.539	0.889 ± 0.014	<0.011	<0.014	7.183 ± 0.085	0.226 ± 0.009	8.052 ± 0.300	97	8.298
Stem	4.005 ± 0.264	0.483 ± 0.017	<0.011	<0.014	3.434 ± 0.100	0.120 ± 0.006	3.915 ± 0.139	98	4.037
Leaf	2.932 ± 0.052	0.448 ± 0.043	<0.011	<0.014	2.467 ± 0.020	0.066 ± 0.003	2.920 ± 0.200	100	2.981
Grain	1.017 ± 0.089	0.241 ± 0.016	<0.011	<0.014	0.762 ± 0.07	<0.009	1.000 ± 0.020	98	1.003
Spring onion									
Root	2.072 ± 0.024	0.739 ± 0.041	<0.011	<0.014	1.279 ± 0.077	0.047 ± 0.003	2.024 ± 0.161	98	2.065
Bulb	0.160 ± 0.011	0.083 ± 0.004	<0.011	<0.014	0.066 ± 0.005	<0.009	0.153 ± 0.012	96	0.149
Stem	0.702 ± 0.022	0.209 ± 0.013	<0.011	<0.014	0.509 ± 0.014	<0.009	0.716 ± 0.038	102	0.718
Leaf	0.594 ± 0.048	0.092 ± 0.007	0.085 ± 0.004	0.094 ± 0.003	0.293 ± 0.012	<0.009	0.588 ± 0.050	99	0.564
Radish									
Root	0.672± 0.041	0.360 ± 0.014	<0.011	<0.014	0.238 ± 0.007	0.104 ± 0.003	0.597 ± 0.018	89	0.702
Skin	0.428 ± 0.026	0.251 ± 0.017	<0.011	<0.014	0.127 ± 0.012	0.053 ± 0.002	0.388 ± 0.008	91	0.431
Core	0.396 ± 0.024	0.194 ± 0.005	<0.011	<0.014	0.157 ± 0.014	0.056 ± 0.004	0.374 ± 0.006	94	0.407
Stem	0.331 ± 0.017	0.163 ± 0.005	<0.011	0.051 ± 0.002	0.124 ± 0.003	<0.009	0.351 ± 0.014	106	0.338
Leaf	0.184 ± 0.011	0.058 ± 0.001	<0.011	0.048 ± 0.003	0.100 ± 0.007	<0.009	0.200 ± 0.005	109	0.206

Table 5.8 Continued

Vegetable	Microwave assist digestion	$InAs^{III}$	DMA	MMA	$InAs^{V}$	Total arsenic in residue	Total arsenic in extracts	Efficiency of extraction %	Sum of arsenic from species
Potato									
Root	0.337 ± 0.003	0.150 ± 0.008	<0.011	<0.014	0.200 ± 0.012	<0.009	0.355 ± 0.015	105	0.350
Skin	0.392 ± 0.015	0.048 ± 0.001	<0.011	<0.014	0.287 ± 0.004	0.027 ± 0.001	0.373 ± 0.029	95	0.362
Core	0.052 ± 0.004	0.014 ± 0.001	<0.011	<0.014	0.027 ± 0.001	<0.009	0.045 ± 0.005	87	0.041
Stem	0.247 ± 0.020	0.082 ± 0.005	<0.011	<0.014	0.132 ± 0.010	<0.009	0.235 ± 0.008	95	0.214
Leaf	0.208 ± 0.011	0.088 ± 0.004	<0.011	<0.014	0.125 ± 0.006	<0.009	0.225 ± 0.007	108	0.213
Chard									
Root	0.578 ± 0.030	0.161 ± 0.006	<0.011	<0.014	0.328 ± 0.018	0.074 ± 0.004	0.496 ± 0.045	86	0.563
Stem	0.387 ± 0.012	0.117 ± 0.006	<0.011	<0.014	0.266 ± 0.022	<0.009	0.378 ± 0.019	98	0.383
Leaf	0.183 ± 0.014	0.037 ± 0.002	<0.011	<0.014	0.150 ± 0.005	<0.009	0.193 ± 0.003	105	0.187
Garden cress									
Root	0.498 ± 0.012	0.204 ± 0.014	<0.011	<0.014	0.296 ± 0.021	<0.009	0.512 ± 0.013	103	0.500
Stem	0.278 ± 0.010	0.130 ± 0.007	<0.011	<0.014	0.159 ± 0.004	<0.009	0.302 ± 0.017	108	0.289
Leaf	0.211 ± 0.010	0.042 ± 0.001	<0.011	<0.014	0.159 ± 0.015	0.013 ± 0.001	0.198 ± 0.008	94	0.214
Egyptian leek									
Root	1.860 ± 0.103	0.868 ± 0.026	<0.011	<0.014	1.018 ± 0.051	0.047 ± 0.001	1.939 ± 0.003	104	1.933
Leaf	0.288 ± 0.009	<0.006	<0.011	<0.014	0.279 ± 0.012	<0.009	0.290 ± 0.019	101	0.279

Table 5.8 Continued

Vegetable	Microwave assist digestion	$InAs^{III}$	DMA	MMA	$InAs^{V}$	Total arsenic in residue	Total arsenic in extracts	Efficiency of extraction %	Sum of arsenic from species
Celery									
Root	0.328 ± 0.015	0.101 ± 0.006	<0.011	<0.014	0.210 ± 0.007	0.033 ± 0.001	0.295 ± 0.003	90	0.344
Stem	0.219 ± 0.01	0.053 ± 0.003	<0.011	<0.014	0.166 ± 0.009	<0.009	0.219 ± 0.008	100	0.219
Leaf	0.102 ± 0.008	<0.006	<0.011	<0.014	0.098 ± 0.004	<0.009	0.098 ± 0.008	96	0.098
Sunflower									
Root	0.504 ± 0.018	0.043 ± 0.001	<0.011	<0.014	0.224 ± 0.017	0.258 ± 0.018	0.266 ± 0.019	53	0.525
Stem	0.262 ± 0.010	0.072 ± 0.007	<0.011	<0.014	0.194 ± 0.007	<0.009	0.286 ± 0.019	109	0.266
Leaf	0.086 ± 0.003	<0.006	<0.011	<0.014	0.086 ± 0.006	<0.009	0.084 ± 0.007	98	0.086
Mallow									
Root	0.144 ± 0.005	0.045 ± 0.003	<0.011	<0.014	0.095 ± 0.004	<0.009	0.134 ± 0.002	93	0.140
Stem	0.276 ± 0.011	<0.006	<0.011	<0.014	0.274 ± 0.012	<0.009	0.278 ± 0.012	101	0.274
Leaf	0.542 ± 0.011	0.337 ± 0.016	<0.011	<0.014	0.197 ± 0.011	<0.009	0.533 ± 0.021	98	0.534
Wild mint									
Root	0.868 ± 0.022	0.250 ± 0.003	<0.011	<0.014	0.591 ± 0.018	0.040 ± 0.002	0.839 ± 0.039	97	0.881
Stem	0.196 ± 0.003	0.033 ± 0.001	<0.011	<0.014	0.149 ± 0.004	<0.009	0.185 ± 0.014	94	0.182
Leaf	0.382 ± 0.012	0.128 ± 0.006	<0.011	<0.014	0.248 ± 0.009	<0.009	0.373 ± 0.017	98	0.376

Table 5.8 Continued

Vegetable	Microwave assist digestion	$InAs^{III}$	DMA	MMA	$InAs^{V}$	Total arsenic in residue	Total arsenic in extracts	Extraction efficiency %	Sum of As species
Broad bean									
Root	2.065 ± 0.034	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	2.059
Stem	0.212 ± 0.006	0.035 ± 0.003	0.050 ± 0.004	0.044 ± 0.003	0.062 ± 0.005	0.029 ± 0.001	0.191 ± 0.008	90	0.220
Leaf	0.489 ± 0.040	0.091 ± 0.006	<0.011	0.101 ± 0.001	0.232 ± 0.011	0.072 ± 0.005	0.415 ± 0.001	85	0.496
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	0.26
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	0.126
Beetroot									
Root	0.190 ± 0.015	0.058 ± 0.002	<0.011	<0.014	0.129 ± 0.003	<0.009	0.196 ± 0.012	103	0.187
Skin	0.330 ± 0.023	0.107 ± 0.005	<0.011	<0.014	0.250 ± 0.006	<0.009	0.354 ± 0.028	107	0.357
Core	0.181 ± 0.005	0.025 ± 0.001	<0.011	<0.014	0.170 ± 0.003	<0.009	0.196 ± 0.007	108	0.195
Stem	0.317 ± 0.019	0.079 ± 0.003	<0.011	<0.014	0.243 ± 0.015	<0.009	0.328 ± 0.026	103	0.322
Leaf	0.218 ± 0.021	<0.006	<0.011	<0.014	0.217 ± 0.012	<0.009	0.217 ± 0.013	100	0.217
Spinach									
Root	0.265 ± 0.014	0.098 ± 0.004	<0.011	<0.014	0.157 ± 0.007	<0.009	0.274 ± 0.004	103	0.255
Stem	0.575 ± 0.017	0.126 ± 0.006	<0.011	<0.014	0.434 ± 0.022	<0.009	0.565 ± 0.022	98	0.560
Leaf	0.179 ± 0.009	0.057 ± 0.005	<0.011	<0.014	0.116 ± 0.005	<0.009	0.180 ± 0.013	101	0.173
Arum									
Root	0.261 ± 0.012	0.042 ± 0.001	<0.011	<0.014	0.195 ± 0.005	0.014 ± 0.001	0.242 ± 0.011	93	0.251
Stem	0.341 ± 0.031	0.077 ± 0.003	<0.011	<0.014	0.225 ± 0.010	0.029 ± 0.002	0.311 ± 0.020	91	0.331
Leaf	0.185 ± 0.017	0.046 ± 0.003	<0.011	<0.014	0.121 ± 0.005	0.012 ± 0.001	0.171 ± 0.017	92	0.179

- **Rice-** ***Oryza sativa***

Arsenic concentrations in rice plant increased in the order grain<leaf<stem<root. The speciation of arsenic in rice identified $InAs^{III}$ and $InAs^{V}$ as major arsenic species in root, stem, leaves and grain, whereas DMA and MMA were below the LOD (DMA 0.011 µg g^{-1} and MMA 0.014 µg g^{-1}) in different parts of the rice plant. Organic arsenic species were not detected in the cultivated soil. $InAs^{V}$ was the major species found in the analysed soil So 11 which was used to cultivate the rice plant (2.307 µg g^{-1}) with smaller quantities of $InAs^{III}$ (0.373 µg g^{-1}). The arsenic 'plant available' in the analysed soil So 11 was 0.41 µg g^{-1} (14.23 % of the total arsenic in the analysed soil (2.88 µg g^{-1})). In the rice plant $InAs^{III}$ and $InAs^{V}$ species decreased from the root to the grain with $InAs^{V}$ as a dominant species in each part of the rice plant. The concentration of arsenic in rice grain was five times higher than the global range concentration of arsenic in rice which could possibly cause risk to human consumption of rice. This high concentration could be due to the presence of an oil well near the paddy rice. The high concentration of arsenic in the stem and leaf of rice could pose a potential health risk to cattle and sheep, because rice stem and leaf used as fodder in the local area. This could then lead to increased levels of arsenic in humans as a result of the consumption of cattle and sheep products Full distribution of arsenic species in different parts of rice plant is presented in Figure 5.5.

- **Spring onion-*Allium fistulosum.***

In the spring onion plant under study (Table 5.8), the total concentration of arsenic decreased from the root to the leaf except for the 'bulb' of spring onion which had the lowest concentration of arsenic contain compare to the other parts. $InAs^{V}$ was the major species in the soil So 7 (5.482 µg g^{-1}), with lower concentration of $InAs^{III}$ (0.545 µg g^{-1}). The plant available arsenic in the analysed soil So7 was 1.1 µg g^{-1} (18 % of the total arsenic in the soil (6.11 µg g^{-1})). $InAs^{V}$ and $InAs^{III}$ were the major arsenic species in all

parts of spring onion and decreased gradually from the root to the leaf with the exception in the 'bulb' which accumulated lower quantities. DMA (0.085 µg g^{-1}) and MMA (0.094 µg g^{-1}) species were only found in the leaf of spring onion. These results suggested that the 'bulb' of the spring onion could be 'the safest' part to human health. Full distribution of arsenic species in different parts of spring onion plant is shown in Figure 5.6.

- **Radish-*Raphanus sativus L.***

The soil So4 was used to cultivate radish and the speciation results showed that $InAs^{V}$ was the most abundant arsenic species (4.568 µg g^{-1}). $InAs^{III}$ was found at 0.556 µg g^{-1}. The BCR extraction procedure revealed that 19.55 % of 5.825 µgg^{-1} (aqua regia available arsenic) was available in the 'plant available' arsenic form. Although the predominant arsenic species in the cultivated soil was $InAs^{V}$, in the root of radish plant arsenic was mainly found in the form of $InAs^{III}$ (60.3 %). $InAs^{V}$ represented 39.8 % of total arsenic in the root with organic arsenic species (MMA and DMA) being below the LOD (more details are shown Figure 5.7). $InAs^{III}$ decreased in the radish plant in the following sequence root>skin>core>stem>leaf. The $InAs^{V}$ concentration in radish plant had a similar pattern except in the skin had the lowest arsenic content. The DMA was below the LOD (0.011 µg g^{-1}) in all parts of the radish plant. MMA was found in both stem and leaf of radish plant, 0.051 and 0.048 µg g^{-1}, respectively.

- **Potato-*Solanum tuberosum***

In the potato plant, $InAs^{V}$ was the predominant arsenic species in the cultivated soil So 10 (5.522 µg g^{-1}), with less $InAs^{III}$ (0.334 µg g^{-1}). Arsenic 'plant available' was only 15.83 % of the total aqua regia arsenic available. The results presented in Figure 5.8 show that $InAs^{III}$ comprised 42.2 % of the total arsenic concentration while $InAs^{V}$ comprised 56.3 %. The organic arsenic species (DMA and MMA) were below LOD

(DMA 0.011 µg g^{-1} and MMA 0.014 µg g^{-1}). In the potato skin, potato core, potato stems and potato leaves, the distribution of arsenic was similar (Figure 5.8) with the majority of the arsenic existing as inorganic arsenic forms and the organic arsenic species (DMA and MMA) which were below the LOD (DMA 0.011 µg g^{-1} and MMA 0.014 µg g^{-1}). $InAs^{V}$ was the predominant species in potato root, skin, core, stem and leaf (comprising 55.5 to 76.9 %) followed by $InAs^{III}$ (12.86 to 42.2 %).

- **Chard-*Beta vulgaris subsp.***

The soil So1 was used to cultivate chard. The speciation results using phosphoric acid revealed that $InAs^{V}$ was the major arsenic species (4.487 µg g^{-1}) in the soil with a lesser amount of $InAs^{III}$ (0.756 µg g^{-1}). DMA and MMA were below the LOD (DMA 0.119 µg g^{-1} and MMA 0.084 µg g^{-1}) in the analysed soil. 17.43 % of total arsenic (5.450 µg g^{-1}) in So 1 was present as 'plant available arsenic'. In the chard root, $InAs^{V}$ was the dominant arsenic species (66.1 %) followed by $InAs^{III}$ (32.4 %) whereas DMA and MMA were below than LOD (DMA 0.011 µg g^{-1} and MMA 0.014 µg g^{-1}) (Figure 5.9). In the chard stem, the distribution of arsenic was similar to the chard root with $InAs^{V}$ being the predominant species (70.3 %), followed by $InAs^{III}$ (30.9 %) while organic arsenic species such as DMA and MMA were below the LOD (DMA 0.011 µg g^{-1} and MMA 0.014 µg g^{-1}). In the chard leaf, the majority of arsenic present was $InAs^{V}$ (77.7 %) followed by $InAs^{III}$ which comprised 19.1 % of total arsenic present in the leaf.

- **Garden cress-*Lepidium sativum L.***

The soil So3 was used for the cultivation of Garden cress. $InAs^{V}$ (4.132 µg g^{-1}) was the predominant arsenic species in the soil while $InAs^{III}$ was present in small quantity (0.549 µg g^{-1}). The 'plant available' arsenic in the soil was relatively high (21.14 % of total arsenic. The distribution of arsenic species in Garden cress root, stem and leaf are shown in Figure 5.10. $InAs^{III}$ was the minor arsenic species in all parts of the plant.

Whereas $InAs^{V}$ comprised 57.8, 52.6 and 80.3 % of total arsenic of root, stem and leaf respectively. Organic arsenic species DMA and MMA were below the LOD (DMA 0.011 µg g^{-1} and MMA 0.014 µg g^{-1}) in each of root, stem and leaf of Garden cress.

- **Egyptian leek-*Allium Kurrat. Schweinf.***

$InAs^{V}$ was the major species found in the cultivated soil So6 (5.414 µg g^{-1}) used to grow Egyptian leek. Smaller quantities of $InAs^{III}$ (0.371 µg g^{-1}) were found. It was observed that 16.1 % arsenic (6.21 µg g^{-1}) was present in the easily extractable form considered 'plant available'. In the Egyptian leek plant root, the majority of arsenic present was as $InAs^{V}$. The proportion of $InAs^{V}$ found in the root of this plant was 52.5 % (1.018 µg g^{-1}) of the total arsenic. The percentage of As^{III} measured in the root was 44.7 % of the total arsenic present. MMA and DMA were below the LOD (DMA 0.011 µg g^{-1} and MMA 0.014 µg g^{-1}). Only $InAs^{V}$ was found in the leaf and other species such as $InAs^{III}$, DMA and MMA were below the LOD ($InAs^{III}$ µg g^{-1}, 0.006 DMA 0.011 µg g^{-1} and MMA 0.014 µg g^{-1}). (Figure 5.11).

- **Celery-*Apium graveolens***

The soil So2 was used to cultivate celery. The major arsenic species in the soil was $InAs^{V}$ (4.474 µg g^{-1}) and $InAs^{III}$ was found as minor arsenic species (0.662 µg g^{-1}). DMA and MMA were below than LOD (DMA 0.119 µg g^{-1} and MMA 0.084 µg g^{-1}). In spite of high arsenic concentration present in the analysed So2 (5.32 µg g^{-1}) only 19.3 % (1.03 µg g^{-1}) was 'plant available'. In the root of celery, the most abundant arsenic species was $InAs^{V}$. Both $InAs^{V}$ and $InAs^{III}$ concentration in celery decreased in order root>stem>leaf (Figure 5.12). MMA and DMA were below the LOD (DMA 0.011 µgg^{-1} and MMA 0.014 µg g^{-1}).

- **Sunflower-*Gundelia tournefortii*.**

Sunflower was grown in soil So15. Arsenic speciation using phosphate revealed that the major arsenic species in the soil was $InAs^{V}$ (5.043 µg g^{-1}) while the minor species was $InAs^{III}$ (0.26 µg g^{-1}). However, DMA and MMA were below the LOD (DMA 0.119 µgg^{-1} and MMA 0.084 µg g^{-1}). Only 7.87 % of total arsenic in the soil (5.59 µg g^{-1}) was in the form 'plant available'. In this study, the extraction efficiency for arsenic using 1 % HNO_3 was adequate for the stem and leaf (109 % from the stem and 98 from the leaf), however for the root the recovery was poor at 53 %. This may indicate that this extractant is not suitable for the root of the Sunflower. Overall, $InAs^{V}$ was the major arsenic species and comprised 84.2 % of the total extracted arsenic. $InAs^{III}$ represented most of the remaining arsenic (16.1 %) (Figure 5.13). Both DMA and MMA were below than LOD (DMA 0.011 µg g^{-1} and MMA 0.014 µg g^{-1}). $InAs^{V}$ was the most abundant arsenic species in the sunflower stem (67.8 %) followed by $InAs^{III}$ (25.7 %). MMA and DMA were again below the LOD (DMA 0.011 µg g^{-1} and MMA 0.014 µg g^{-1}). In the Sunflower leaf, $InAs^{V}$ (100%) was the major species while other species such as $InAs^{III}$, DMA and MMA were below the LOD (DMA 0.011 µg g^{-1} and MMA 0.014 µgg^{-1}).

- **Mallow-*Malva parviflora***

Mallow showed a different ability to compartmentalize arsenic within itself compared with previous plants. Here, high arsenic concentration was found in the leaf instead of the root. Mallow was grown in Soil So14. $InAs^{V}$ was the predominant arsenic species (3.75 µg g^{-1}) with $InAs^{III}$ at 0.509 µg g^{-1}. DMA and MMA were below than LOD (DMA 0.119 µg g^{-1} and MMA 0.084 µg g^{-1}). 16.66 % of the total arsenic (4.2 µg g^{-1}) was present as 'plant available' arsenic. The majority of arsenic found in the mallow root was $InAs^{V}$ (70.8 %) followed by $InAs^{III}$ (33.5 %), DMA and MMA were below the LOD (DMA 0.011 µg g^{-1} and MMA 0.014 µg g^{-1}). In the stem $InAs^{V}$ represented the

only arsenic species found (98.1 %). $InAs^{III}$, DMA and MMA were below the LOD ($InAs^{III}$ 0.006 µg g^{-1}, DMA 0.011 µg g^{-1} and MMA 0.014 µg g^{-1}). The percentage of observed $InAs^{III}$ in the leaf was 63.2 % of the total arsenic present (0.542 µg g^{-1}) whereas $InAs^{V}$ was only 36.9 %. DMA and MMA were below the LOD (DMA 0.011 µg g^{-1} and MMA 0.014 µg g^{-1}) (Figure 5.14).

- **Wild mint-*Mentha longifolia***

Wild mint-*Mentha longifolia* and broad bean *Vicia Faba* belong to the third group of plants where the arsenic concentrations in the stem of the plants are less than both root and leaf.

The soil So8 was used to cultivate *Mentha longifolia*. The predominant arsenic species was found in the analysed soil was $InAs^{V}$ (3.573 µg g^{-1}) whilst $InAs^{III}$ was found as a minor species (0.281 µg g^{-1}). DMA and MMA were found to be below the LOD in So8 soil. Despite the low concentration of arsenic in the soil, 18.62 % of total arsenic (3.92 µg g^{-1}) in the soil was found in the form of 'available' arsenic. $InAs^{V}$ was the dominant arsenic species in the wild mint and $InAs^{V}$ comprised 70.4 % of total arsenic (0.839 µg g^{-1}) present in the root (Figure 5.15). The proportion of $InAs^{III}$ in the root was found to be 29.7 % of the total arsenic present. DMA and MMA were below than LOD (DMA 0.011 µg g^{-1} and MMA 0.014 µg g^{-1}). In Wild mint stems and Wild mint leaves, the distribution of arsenic species was similar, with the majority of arsenic existing as $InAs^{V}$ (80.5 and 68.4 % for stems and leaves, respectively). The percentages of $InAs^{III}$ in the wild mint stems and wild mint leaves were 17.8 and 34.3 % for stems and leaves , respectively. DMA and MMA were found to be below the LOD (DMA 0.011 µg g^{-1} and MMA 0.014 µg g^{-1}) in both stems and leaves in wild mint.

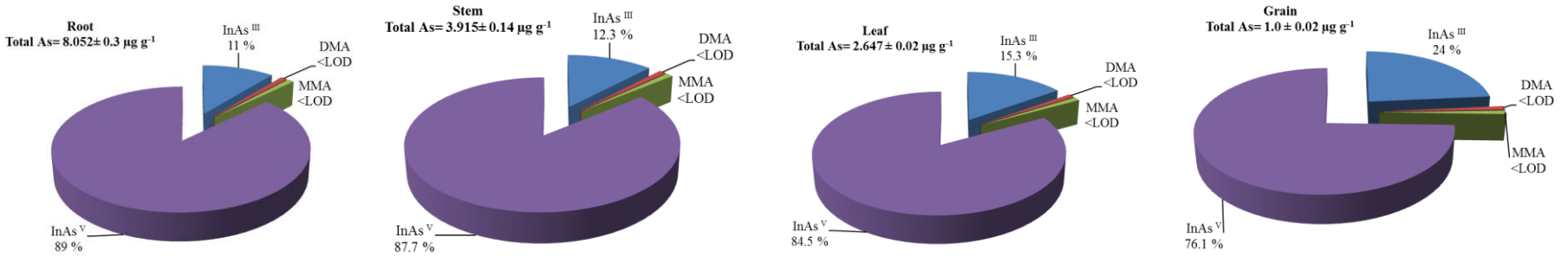


Figure 5.5 Distribution and speciation of arsenic species in rice plant -*Oryza sativa.*

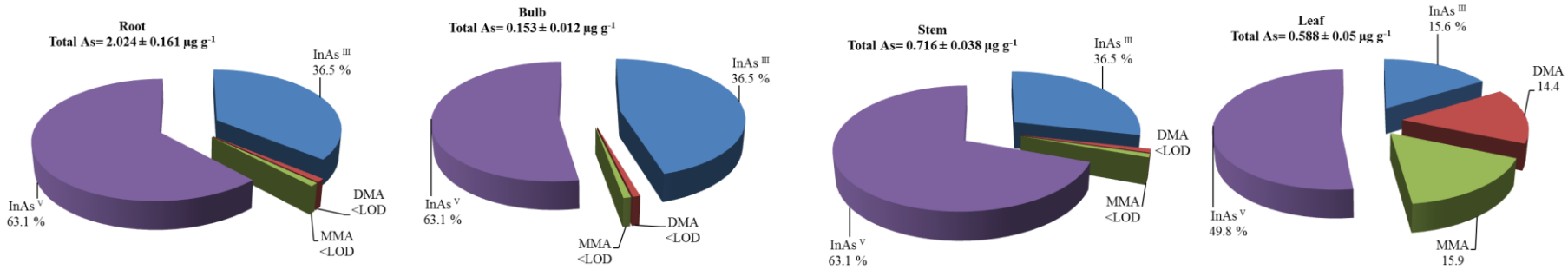


Figure 5.6 Distribution and speciation of arsenic species in spring onion- *Allium fistulosum*.

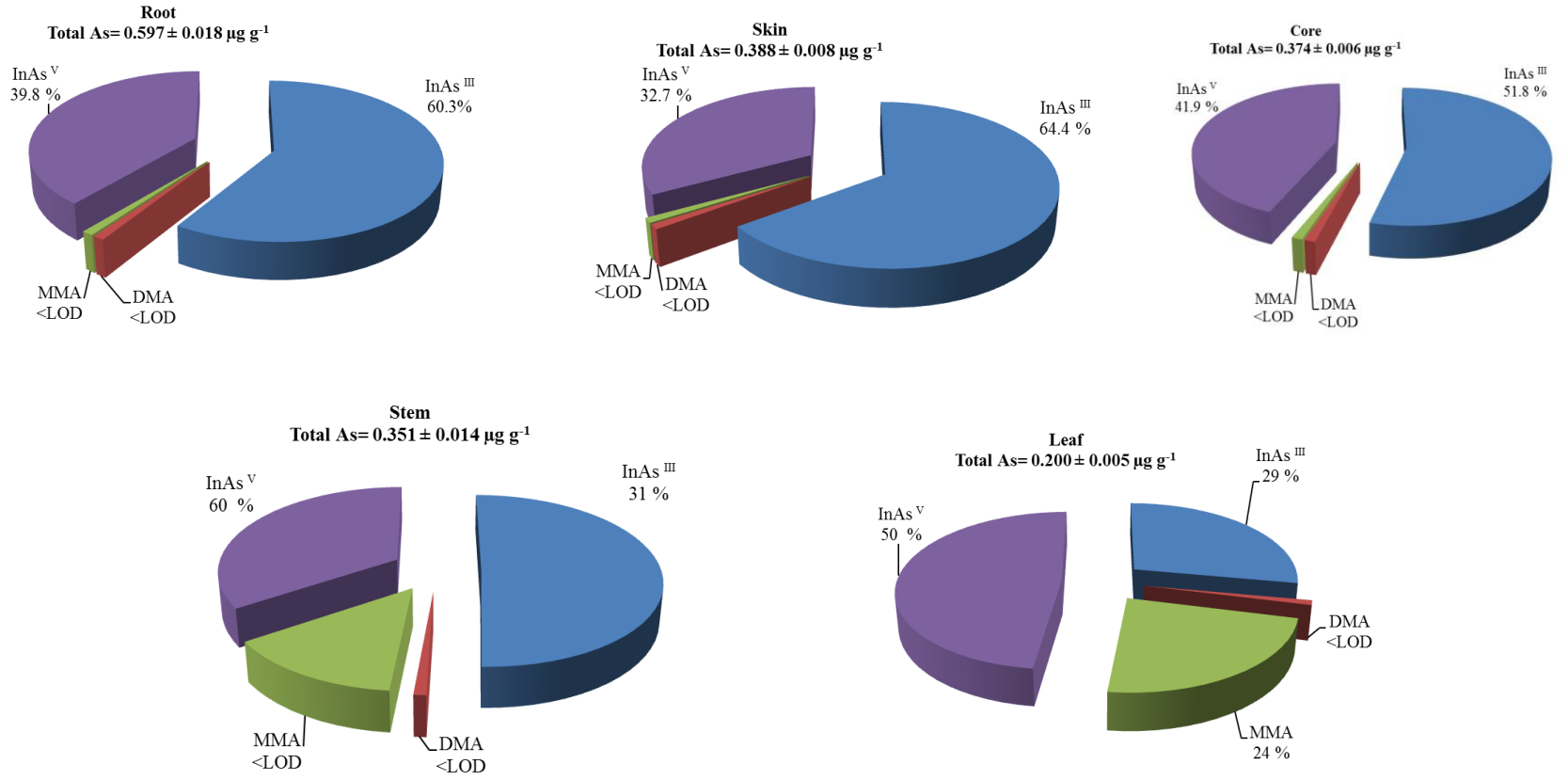


Figure 5.7 Distribution and speciation of arsenic in Radish- *Raphanus sativus L*.

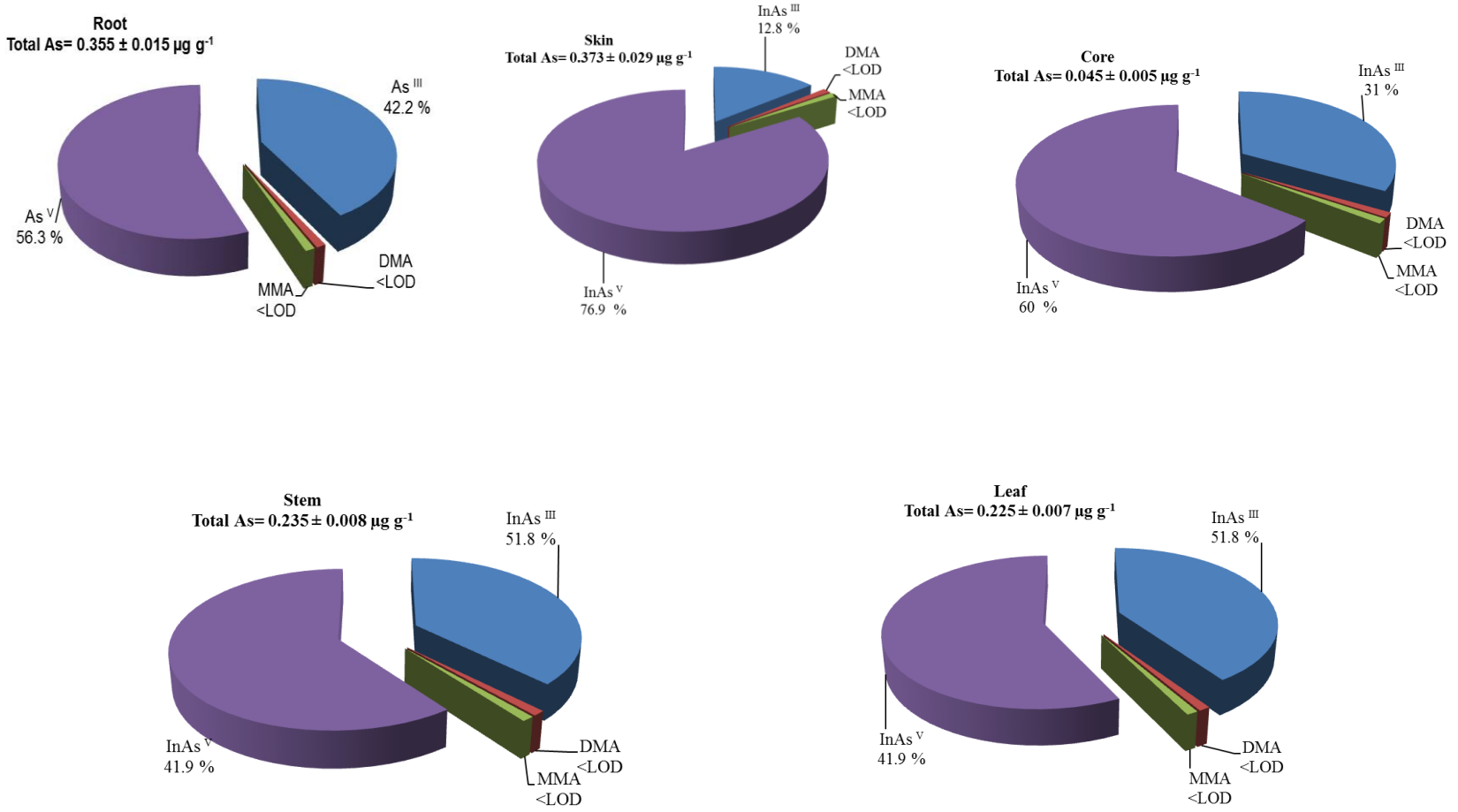


Figure 5.8 Distribution and speciation of arsenic in potato- *Solanum tuberosum*.

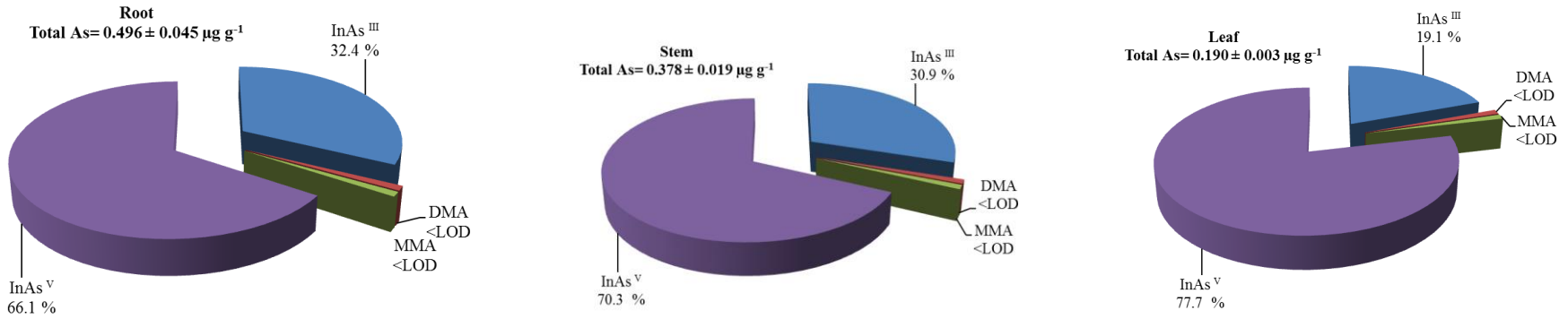


Figure 5.9 Distribution and speciation of arsenic in chard- *Beta vulgaris subsp*.

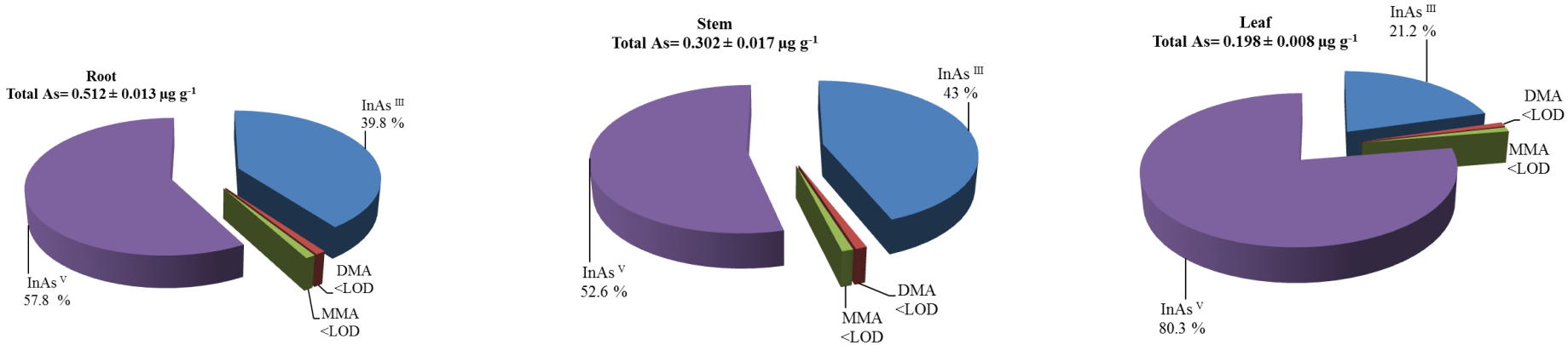


Figure 5.10 Distribution and speciation of arsenic in Garden cress -*Lepidium sativum L.*

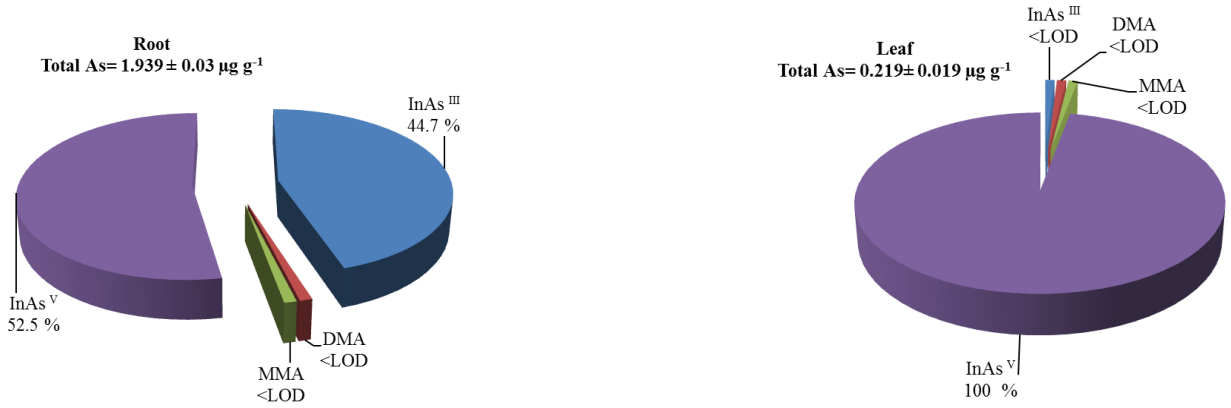


Figure 5.11 Distribution and speciation of arsenic in Egyptian leek- *Allium Kurrat. Schweinf.*

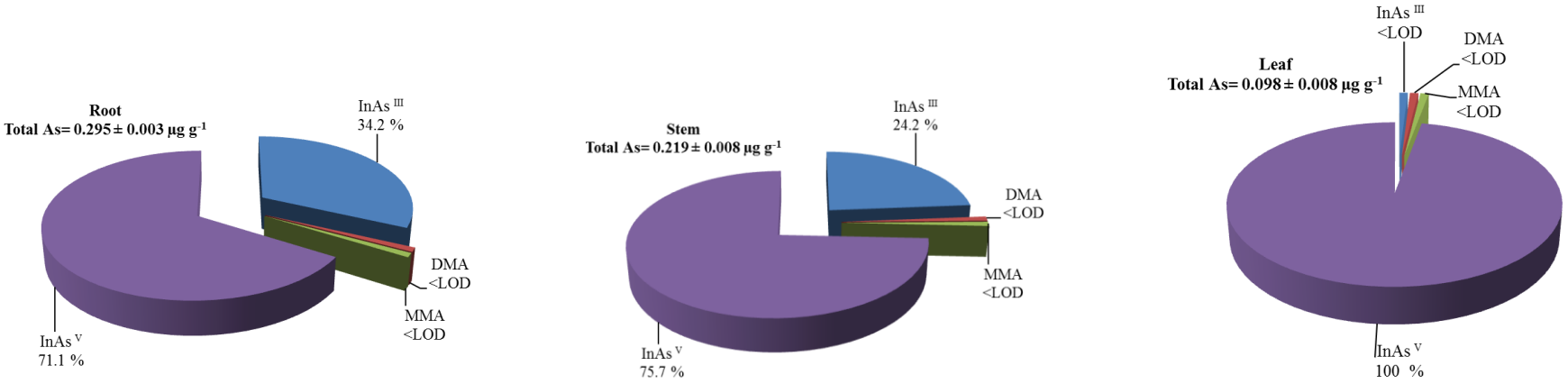


Figure 5.12 Distribution and speciation of arsenic species in Celery-*Apium graveolens.*

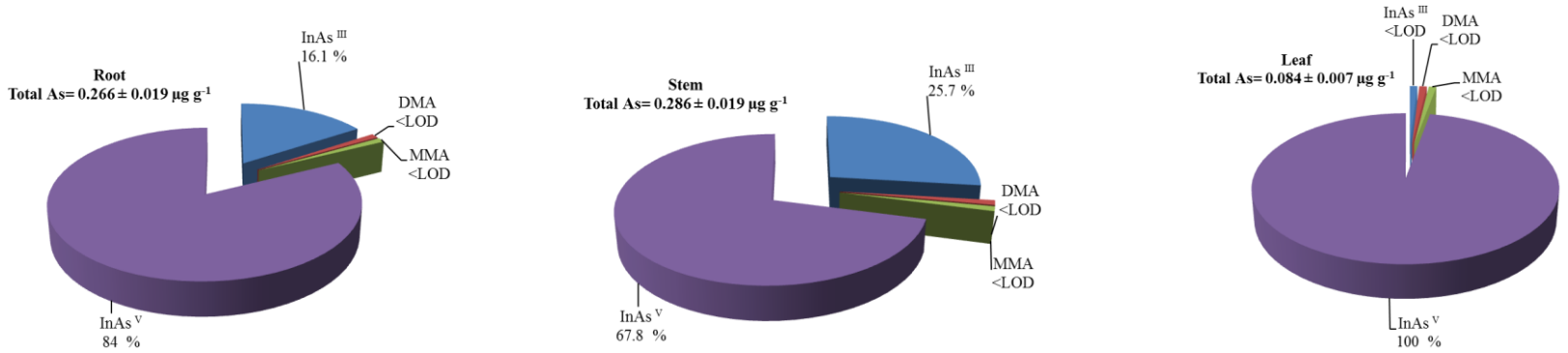


Figure 5.13 Distribution and speciation of arsenic in Sunflower- *Gundelia tournefortii*.

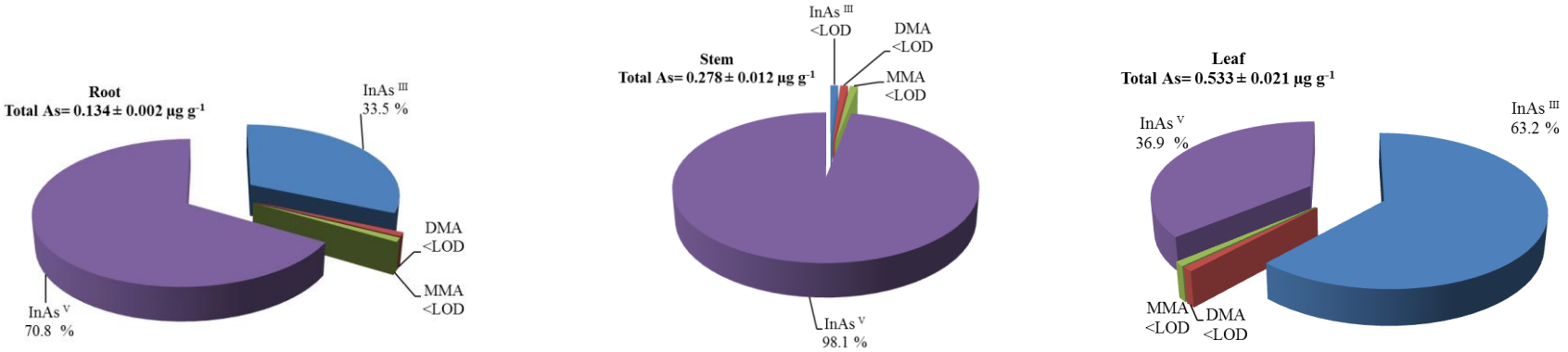


Figure 5.14 Distribution and speciation of arsenic in Mallow- *Malva parviflora*.

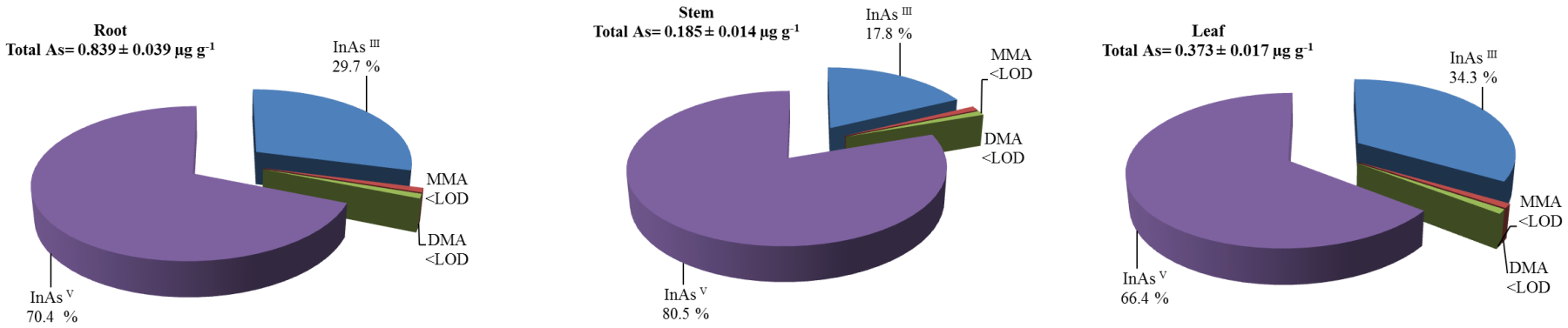


Figure 5.15 Distribution and speciation of arsenic in Wild mint-*Mentha longifolia.*

- **Broad bean-*Vicia Faba***

Although the phosphoric acid procedure was applied to extract arsenic species from the soil with $InAs^{V}$ (4.584 µg g^{-1}) being a major species, this is not an indication that the arsenic was mobile and the broad bean plant can take up all arsenic species. The maximum amount of arsenic which could potentially be mobilised in the soil under study (pseudo-total content) was 5.32 µg g^{-1} (So12). Overall only some 17 % of arsenic in the soil So2 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 study, it was observed that 11 % arsenic (0.6 µg g^{-1}) was present in an easily acid-soluble extractable form. The 'reducible fraction' extracted from the soil under study gave 4 % arsenic (0.22 µg g^{-1}), which is considered mostly bound to the structure of primary and secondary minerals. In comparison, the BCR-701 material released 74 % of its arsenic with this fraction. The arsenic associated with the 'organic materials and sulfides' fraction in the Soil So2 studied was only 2 % (0.1 µg g^{-1})

Inorganic arsenic ($InAs^{III}$ and $InAs^{V}$) were the major species found in the root, stem, leaf and pod of the broad bean plant, whilst the organic arsenic species (MMA and DMA) were the major species in the bean. $InAs^{III}$, $InAs^{V}$, 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 (0.011 µg g^{-1}). In the root of the plant, inorganic arsenic ($InAs^{III}$ and $InAs^{V}$) represented the predominant species (94 %) compared with the organic arsenic species which was the minor arsenic species. $InAs^{V}$ was found to be the major arsenic species (78%) with smaller amounts of $InAs^{III}$ (16%). DMA and MMA consisted of only 2% and 3% of total arsenic concentrations in the root, respectively.

In the stem of the plant 51 % of total arsenic was found as inorganic forms ($InAs^{III}$ and $InAs^{V}$) and $InAs^{V}$ was predominant representing 32.4 % of the total arsenic. In the leaf, $InAs^{V}$ was found to be the major species of arsenic accounting for 55.9 % of the total of arsenic, but $InAs^{III}$ was also present as a minor species DMA was below the limit of detection (0.011 µg g^{-1}).

In the pod of the broad bean, 35.3 % of total arsenic in the pod was $InAs^{V}$ while MMA was a minor species with 11.6 % of the total arsenic concentration. The results indicate that DMA was higher than $InAs^{III}$ and MMA in the same part of the broad bean plant. In contrast, in the bean the organic arsenic species were the main arsenic species and MMA was found to be 48.2 % of the total arsenic concentration. $InAs^{III}$ (7.8 %) represented the lowest concentration of the arsenic species. The highest levels of both $InAs^{V}$ and $InAs^{III}$ 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.

It is interesting to note that, while the readily extractable arsenic from the Soil So12 was only nearly 0.6 µg g^{-1}; (11.27 % of the 5.32 µg g^{-1} aqua regia total), the levels of arsenic 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 5.16 and the distribution of arsenic species in different

‘compartments’ of broad bean plant is shown in Figure 5.17. 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 $InAs^{V}$ content decreases from the root to the bean both in absolute concentration and as a fraction.

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

Arsenical route system	Compartment	Total As in compartment shown µg g^{-1}	$InAs^{III}$ µg g^{-1}	$InAs^{V}$ µg g^{-1}	Total inorganic As µg g^{-1}	DMA µg g^{-1}	MMA µg g^{-1}	Total organic As 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

Figure 5.16 Schematic diagram of the distribution of total arsenic, the various arsenic species and possible metabolite routes for different parts of the broad bean plant and its soil.

- **Beetroot-*Beta vulgaris***

The last group contains beetroot, spinach and Arum which are characterized by high arsenic concentrations in the stem compared to other parts of the same plant. So9 soil was used to cultivate the beetroot plant. $InAs^{V}$ (4.763 µg g^{-1}) was the most abundant arsenic species in the soil, while $InAs^{III}$ was 0.278 µg g^{-1} and DMA and MMA were below the LOD (DMA 0.119 µg g^{-1} and MMA 0.084 µg g^{-1}). Based on the results of this study, when using BCR protocol only 14.23 % of total arsenic (5.41 µg g^{-1}) was available as easily acid-soluble extractable form in the soil that used to cultivate beetroot plant. In the beetroot root the majority of observed arsenic was $InAs^{V}$. $InAs^{V}$ in the root represented 65.8 % of the total, with $InAs^{III}$ making up the remaining arsenic present. DMA and MMA were below the LOD (DMA 0.011 µg g^{-1} and MMA 0.014 µg g^{-1}) (Figure 5.18). In the beetroot skin the predominant arsenic species was $InAs^{V}$

(70.6 %) followed by $InAs^{III}$ (30.2 %). Again MMA and DMA were below the LOD (DMA 0.011 µg g^{-1} and MMA 0.014 µg g^{-1}). In the beetroot core the majority of arsenic present was as $InAs^{V}$ (86.7 %). The percentage of detected $InAs^{III}$ was 12.7 % of total arsenic recovered. DMA and MMA were below the LOD (DMA 0.011 µg g^{-1} and MMA 0.014 µg g^{-1}). $InAs^{V}$ and $InAs^{III}$ were the dominant arsenic species in the beetroot stem. $InAs^{V}$ comprised 74 % of total arsenic concentration in the stem while $InAs^{III}$ comprised 24 %. MMA and DMA were below the LOD (DMA 0.011 µg g^{-1} and MMA 0.014 µg g^{-1}). In beetroot leaf, $InAs^{V}$ represented 100 % of the total arsenic in the leaf.

- **Spinach-*Spinacia oleracea***

The soil So5 was used to cultivate spinach. The majority of the arsenic present in the soil was $InAs^{V}$ (4.228 µg g^{-1}) while the remaining was $InAs^{III}$ (0.718 µg g^{-1}). The BCR fractionation procedure was applied on So5 and the results showed that 20.5 % of the total arsenic in the soil (5.01 µg g^{-1}) was present as 'plant available'. In the spinach root and stem the distribution of arsenic was similar with the majority of arsenic observed as inorganic arsenic ($InAs^{III}$ and $InAs^{V}$). Organic arsenic (DMA and MMA) were below the LOD (DMA 0.011 µg g^{-1} and MMA 0.014 µg g^{-1}) (Figure 5.19). Arsenate was the most abundant arsenic species in both roots and stems (57.2 % and 76.8% for roots and stems respectively) while the remainder was $InAs^{III}$ in both spinach root and stems (35.7 % and 29.3 % for roots and stems respectively). In the spinach leaves, the majority of arsenic present was $InAs^{V}$. The proportion of $InAs^{V}$ in the spinach leaves was 64.4 % of the total arsenic recovered while $InAs^{III}$ comprised 31.6 % of the total arsenic. DMA and MMA were below the LOD (DMA 0.011 µg g^{-1} and MMA 0.014 µg g^{-1}).

- **Arum-*Arum spp.***

Arum was grown in Soil So13. Arsenic speciation in soil was performed using phosphate and ascorbic acid. $InAs^{V}$ (5.275 µg g^{-1}) was observed as a major arsenic species in So13, while the remainder was $InAs^{III}$ (0.532 µg g^{-1}). After fractionation of the soil using the BCR protocol only 12.64 % of total arsenic in soil (6.10 µg g^{-1}) was 'plant available'. In Arum the distribution of arsenic species in all parts of the plant (root, stem and leaf) was similar. The majority of recovered arsenic species was $InAs^{V}$ followed by $InAs^{III}$. While DMA and MMA were below than the LOD (DMA 0.011 µg g^{-1} and MMA 0.014 µg g^{-1}) in each of root, stem and leaf of Arum plant (Figure 5.20). In the Arum root the proportion of observed $InAs^{V}$ was 80.5 % of total arsenic (0.242 µg g^{-1}) while $InAs^{III}$ was 17.3 %. In the Arum stem $InAs^{V}$ comprised 72.3 % of total arsenic present in the stems while $InAs^{III}$ comprised 31.1 %. DMA and MMA were below the LOD. In Arum spp. leaves, $InAs^{V}$ (70.7 %) was the predominant arsenic species followed by $InAs^{III}$ 26.9 %, DMA and MMA were below the LOD (DMA 0.011 µg g^{-1} and MMA 0.014 µg g^{-1}).

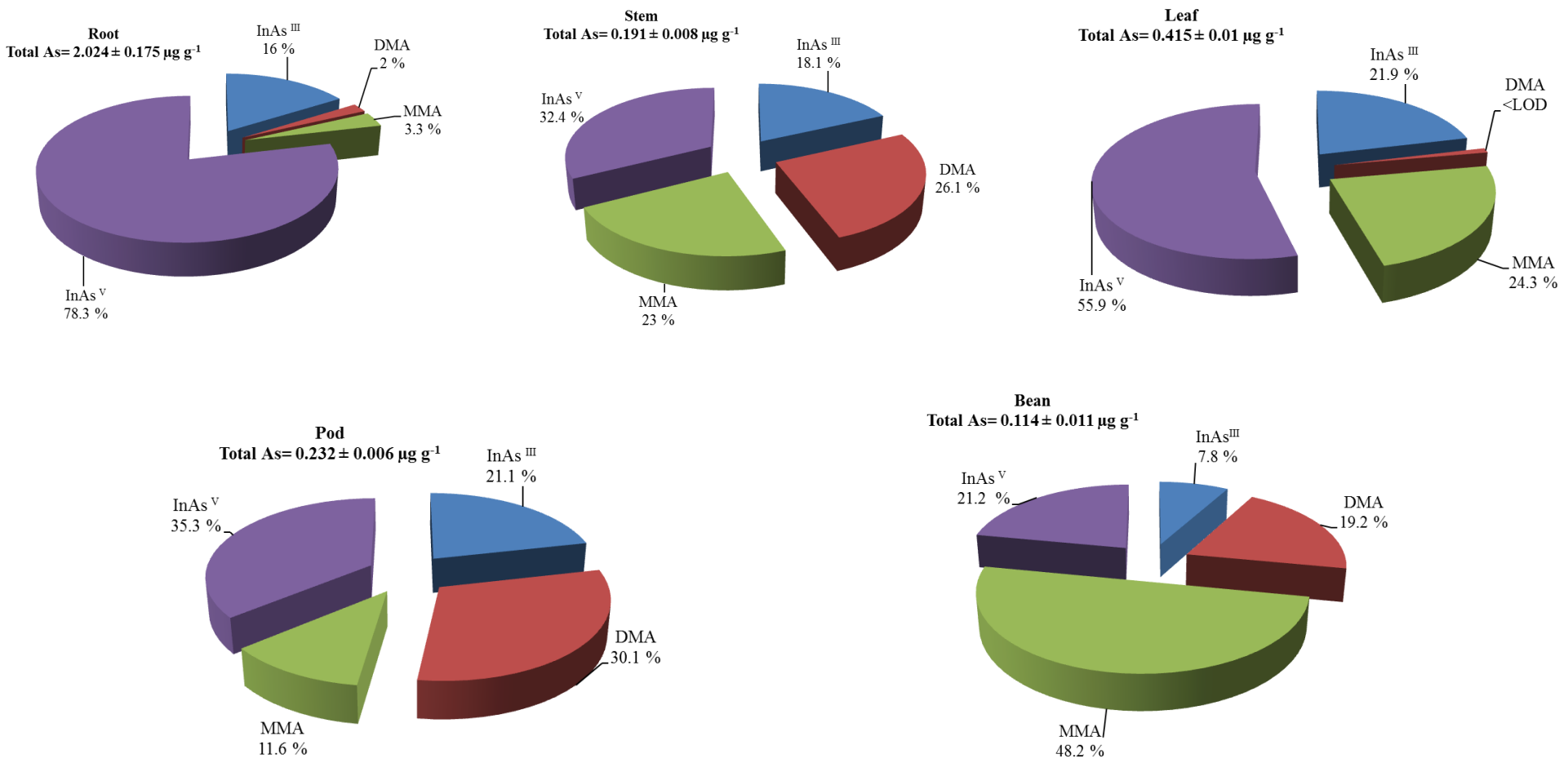


Figure 5.17 Distribution and speciation of arsenic in broad beans-*Vicia Faba*.

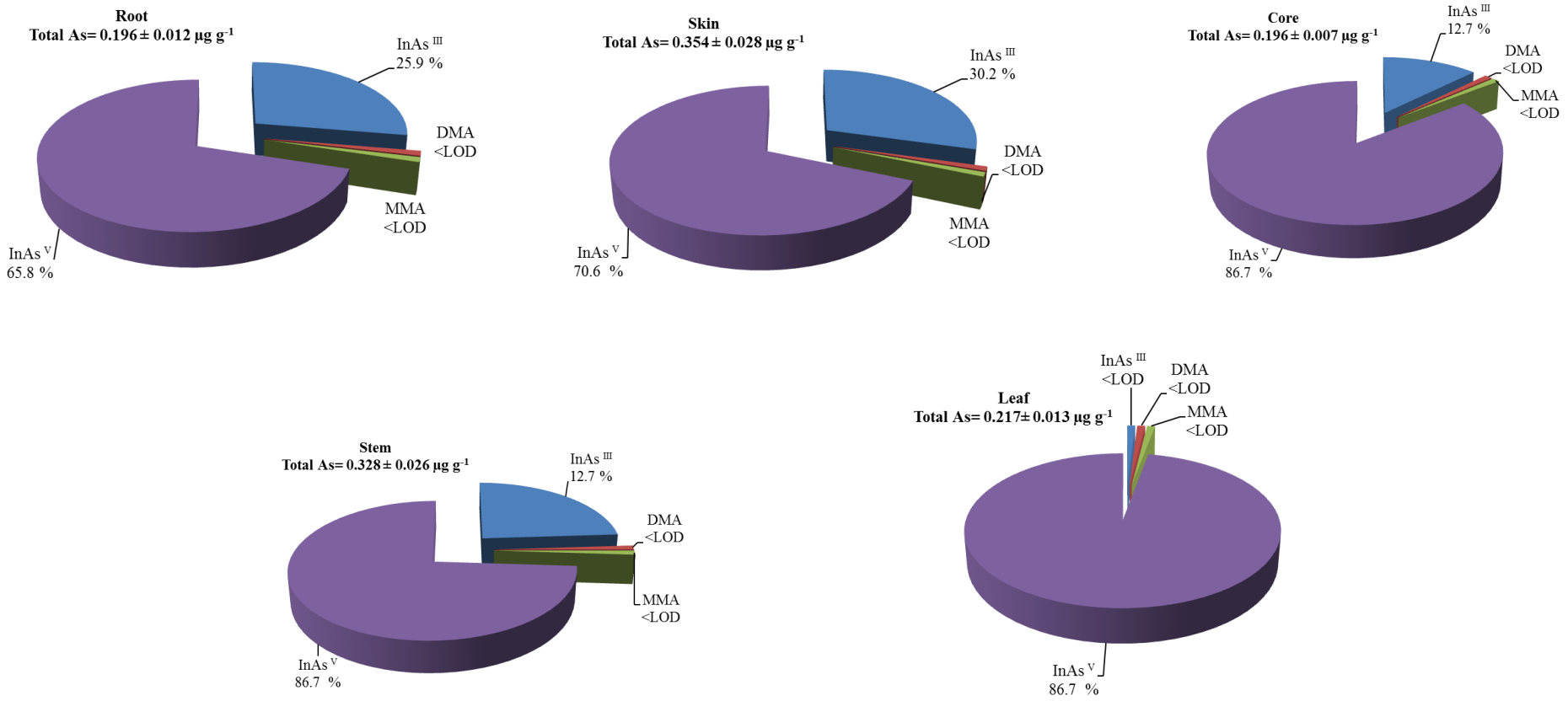


Figure 5.18 Distribution and speciation of arsenic in beetroot- *Beta vulgaris*.

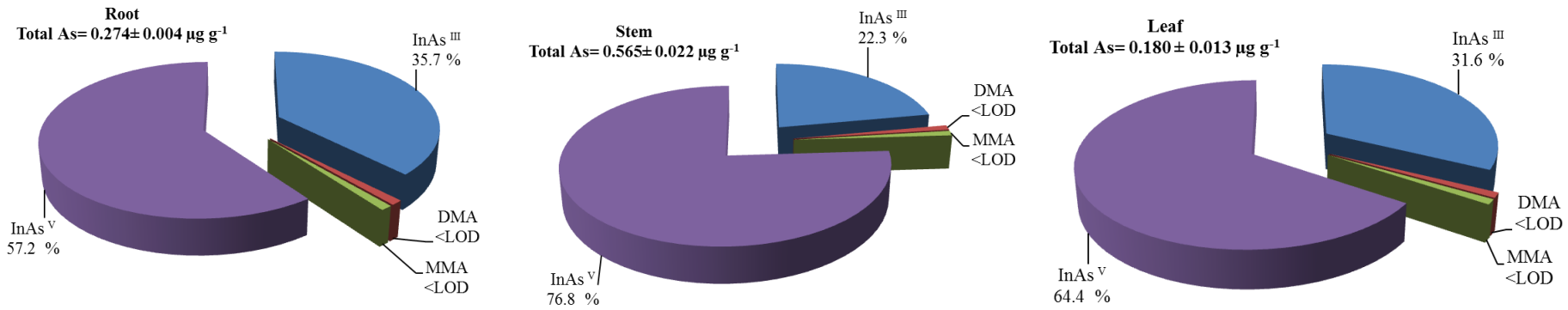


Figure 5.19 Distribution and speciation of arsenic in spinach- *Spinacia oleracea.*

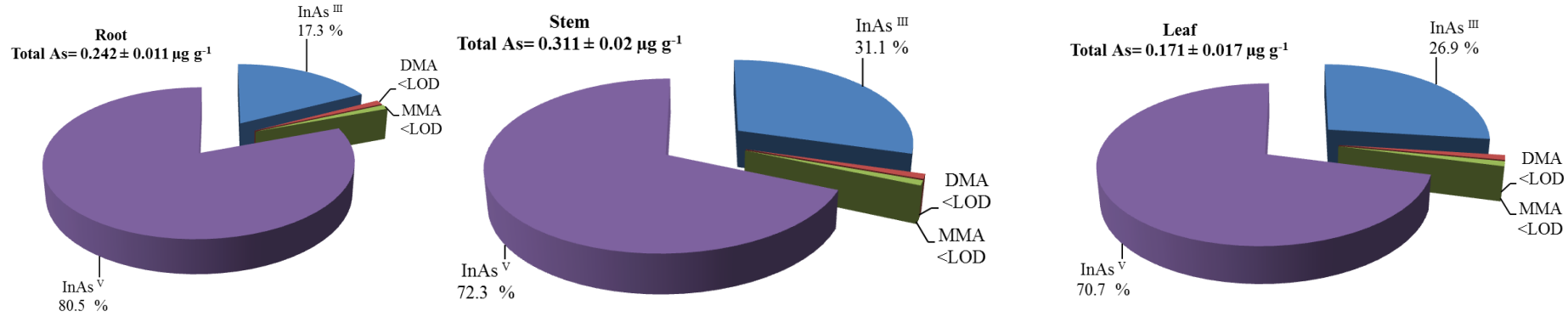


Figure 5.20 Distribution and speciation of arsenic in Arum- *Arum spp.*

5.5 Conclusion

The aim of this study was to investigate both the total arsenic and arsenic species in different parts of common vegetable food crops. These crops were grown in Kurdistan region of Iraq. Since the arsenic levels in the plant reflect the local growing environment, soil in which the plants 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 transformation and accumulation of arsenic in the plants. This study provided useful information for better understanding the distribution of arsenic species in different parts of the same plant (root, skin, core, stem, leaf, etc.).

The concentration of arsenic in the water samples was very low and near the limit of detection using ICP-MS. Therefore speciation of the arsenic in the water was not performed. At this level, the irrigation water was not felt to be a significant contributor of arsenic to the growing plants but it would be a facilitator at the root surface assisting arsenic mobility. The concentration of total arsenic observed in the soil samples from different areas of agriculture land used to cultivate the plant crops ranged from 2.88 to 6.21 μg g^{-1}. Thus the soil samples were not contaminated with arsenic. Phosphoric acid was used to extract arsenic species from the soil samples, and to prevent any possible arsenic species transformation ascorbic acid was used. The results of soil analysis revealed that the predominant arsenic species in all soils used to cultivate plant crops was $InAs^{V}$ followed by $InAs^{III}$. DMA and MMA were not observed in any agriculture soil. The extraction procedure was validated using a loam soil (ERM-CC141) certified reference material.

The BCR method was applied to evaluate the various chemical forms present in the soils. It was observed that ‘plant available’ arsenic in the soils ranged from 7.78 % (4.2

µg g^{-1}) to 21.14 % of the total arsenic present in soil (5.25 µg g^{-1}). The extraction procedure was validated by using BCR -701.

Microwave assisted acid digestion using HNO_3/H_2O_2 was effective for the determination of total arsenic concentration in crop plants. The distribution of arsenic in different parts of crop plant such as root, stem, leaf and grain depended on the species of plant. The results of this study demonstrated that the distribution of arsenic in crops such as rice, spring onion, radish, potato, chard, garden cress, Egyptian leek, celery and sunflower was in the order root>stem>leaf>grain. In mallow it was in the order root<stem<leaf. The distribution of arsenic in plant crops such as wild mint and broad bean was in the order root>stem<leaf while in plant crops such as beetroot, spinach and Arum was in the order root<stem>leaf. The variation in the distribution of arsenic in different crops explains the accumulation patterns within different parts of each crop plant. Dilute HNO_3 (1 %) was used effectively to extract arsenic species from different parts of the crop plants with an extraction efficiency between 86 to 109 % (except in the root of sunflower) in which only 53 % of arsenic species were recovered. Inorganic arsenic ($InAs^{III}$ and $InAs^{V}$) was the predominant arsenic species in all crop roots, stems and leaves while DMA and MMA were below the LOD. In rice grain, bulb of spring onion and broad bean pod the majority of arsenic was inorganic arsenic ($InAs^{III}$ and $InAs^{V}$) while in the bean of broad bean the majority of arsenic was organic species (DMA and MMA). Inorganic arsenic species ($InAs^{III}$ and $InAs^{V}$) were the dominant arsenic species in radish skin, potato skin and beetroot skin. The majority of observed arsenic in the radish core, beetroot core and potato core was inorganic form. This study demonstrates the importance of robust methodology when assessing the potential risks from arsenic in food crops.

Chapter 6

The determination of total arsenic and its speciation in cellular DNA fractions from highly contaminated vegetative plants

6.1 Introduction

Arsenic speciation is an important parameter to assess. It can help in our estimation of the arsenic uptake mechanisms by plants and animals [61, 426] and help in our understanding of the term 'toxicity'. In general, inorganic arsenic species, $InAs^{III}$ and $InAs^{V}$, are more toxic than organic arsenic (for example MMA and DMA) species. It is known that $InAs^{V}$, which is a phosphate analogue, can occur as $H_2AsO_4^-$ or $HAsO_4^{2-}$ dependent upon pH, and may enter the root plant symplast through phosphate transport proteins [340, 427]. $InAs^{III}$, which is considered a silicic acid analogue, exists as H_3AsO_3 at pH <8 , is known to enter into the root symplast and loaded into the xylem system through the aquaporin channels LSi1 and LSi2 placed along the endodermal and exodermal root cell [428]. In general, the concentration of arsenic in plant tissues varies between <0.01 and 5 μg g^{-1} (dry weight basis) [25]. Different plant species can take up arsenic and distribute it within various parts of the same plant; sometimes referred to as compartmentalisation. The arsenic concentrations in different vegetables and fruits collected from the southwest and northeast UK in a basket survey were reported to be in the range 0.001- 0.142 and 0.001- 0.056 μg g^{-1} fresh weight for the southeast and northeast respectively [160]. Inorganic arsenic species are often present as the dominant arsenic species in 'terrestrial' plants and vegetables together with small amounts of organic arsenic species such as DMA and MMA[179]. Other arsenic species have been detected such as TMA^+, AsB, AsC and arsenosugars in some terrestrial plants [130]. However, one survey found the inorganic arsenic in the edible tissues of vegetables

including carrot, garlic, potato, and beetroot ranged from 28 up to 100 % of the total arsenic content [205].

It is noted that while a study in Science from 2010 [429] suggested a Gammaproteo bacterium (GFAJ-1) was able to use arsenic instead of phosphorus to sustain growth (in opposition to the universally-held role of phosphorus in biological systems), this theory has since been disproved in the more recent Science paper of 2012 [430]. This paper demonstrated that the GFAJ-1 bacterium is an arsenic-resistant and still phosphate-dependent organism. However, despite obvious effects from high levels of arsenic upon growth and certain biotransformation processes being noted from root to stem to leaf and flower / grain, few studies have covered the cellular level compartmentalisation of arsenic and arsenic species in vegetative / floral systems.

An investigation into the processing of inorganic and organo-arsenic species and their quantification within selected foods contaminated from high arsenic-containing environments, at a cellular level, was undertaken. This included extraction experiments to help identify where vegetative DNA processes interact with these arsenic species and compartmentalise them (whether associated-weakly bound to DNA, or incorporated-within DNA forming part of its structure, potentially replacing phosphate linkages) in order to reduce their toxic effects within the plant. The instrumental techniques of ICP-OES, ICP-MS and HPLC-ICP-MS were used to measure the total and species concentrations of arsenic in cellular fractions and where necessary, levels of phosphorus and phosphate were measured as part of the comparative process after using DNA extraction techniques from contaminated vegetable-based foods.

A preliminary study on measurement of total arsenic in extracted vegetative DNA was first performed on the different ‘compartments’ (root, stem and leaf) of major plant species collected from Kurdistan of Iraq. These experiments included the plants, rice-

Oryza sativa, spring onion-*Allium fistulosum,* radish-*Raphanus sativus*, potato-*Solanum tuberosum,* Egyptian leek-*Allium kurrat. schweinf.*, chard-*Beta vulgaris subsp.*, -sunflower-Gundelia *tournefortii,* mallow-*Malva parviflora,* wild mint-*Mentha longifolia*, broad bean-*Vicia Faba*, beetroot-*Beta vulgaris,* and Arum-*Arum spp*..

From this study, a rice sample including its different 'compartments' such as root, stem and leaf was chosen to become the focus. This was because of three factors: i) it is an important staple food for more than half of the world's population[431] ii) different compartments of the rice samples were contaminated with high levels of arsenic iii) arsenic in the DNA extracts were above the LOD of the developed methodology. Measurement of the arsenic and its species in the different compartments of the rice plant could differentiate whether they are associated with or integrated within the DNA structure. A method was first developed based upon the extraction of vegetative DNA in arsenic-contaminated rice plants. This method was then used to measure the arsenic and arsenic species within the DNA of the other compartments of the rice plant sample to identify whether they are 'associated' or 'incorporated'.

6.2 Experimental

6.2.1 Samples used in this study

A list of the samples included in the DNA study are shown in Table 6.1 (sampling locations are detailed in Figure 5.1 and Table 5.1 of Chapter 5).

Table 6.1 The vegetable crops used in the DNA study.

Common name	Scientific name
Rice	*Oryza sativa*
Spring onion	*Allium fistulosum*
Radish	*Raphanus sativus L*
Potato	*Solanum tuberosum*
Egyptian leek	*Allium kurrat schweinf*
Chard	*Beta vulgaris subsp*
Sunflower	*Gundelia tournefortii*
Mallow	*Malva parviflora*
Wild mint	*Mentha longifolia*
Broad bean	*Vicia Faba*
Beetroot	*Beta vulgaris*
Arum	*Arum spp*

6.2.2 Instrumentation

6.2.2.1 Instrumentation

An X Series 2 ICP-MS instrument (Thermo Scientific, Hemel Hempstead, UK) was used for both arsenic and phosphorus detection. Collision cell technology was used to eliminate the possible argon chloride interference. An iCAP 7400 series (Thermo Scientific) was also used for phosphorus determination. The operating conditions employed are described in Table 6.2. For the ICP-MS analysis, caesium, indium and iridium were used as internal standards for all samples at a final concentration of 10 μgL^{-1}. The mass spectrometer was set to sample arsenic ion intensity (peak jumping option) at mass 75 and phosphorus ion intensity ($^{31}P^{+}$) at m/z 31. For internal standardisation the signal intensity was sampled at m/z 133 ($^{133}Cs^{+}$), m/z 115 ($^{115}In^{+}$) and m/z 193 ($^{193}Ir^{+}$).

6.2.2.2 Chromatographic conditions used for the HPLC-ICP-MS Method in the determination of arsenic species and phosphate in extracted DNA

Chromatographic separations were carried out using a Jasco chromatographic pump (Japan) with a 250 x 4.1 mm column packed with 10 µm particle size Hamilton PRP-X100 anion exchange resin. A 50 X 4.1 mm guard column packed with the same material was used to protect the 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 connected the column outlet directly with the inlet to the nebulizer. An Oakton pH meter (Eutech Instruments, Singapore) was used to take pH readings. Two mobile phases were employed for HPLC-ICP-MS speciation measurements: i) 6.5 mM sodium sulfate (pH 10.2-10.5) in 5 % CH_3OH for the phosphorus/phosphate system and the comparative arsenic species and ii) a phosphate buffer (20 mM ammonium dihydrogen

phosphate in 1 % CH_3OH adjusted to pH 6.0 with ammonia) for the arsenic system. The injection volume used for both chromatographic measurements was 20 µL. The ion intensities at m/z 75 and 31 were monitored continually during the analysis and quantification was performed using peak area against known standards.

6.2.3 Chemicals and Reagents

Analytical reagent grade chemicals and Milli-Q water (18 MΩ cm) were used throughout. Total arsenic standards were prepared from the high purity stock solution 100 µg mL^{-1} in 5 % HNO_3 (CPI international, USA). Arsenic oxide ($InAs^{III}$) was obtained from Aldrich (Milwaukee, USA). The DMA, sodium sulphate anhydrous and Tris were purchased from Sigma (Gillingham, Dorset, UK), and MMA was purchased from Greyhound Chromatography and Allied Chemicals (Birkenhead, Merseyside, UK). The salt $Na_2HAsO_4.7H_2O$ ($InAs^{V}$), In, Ir, sodium chloride, polyvinyl pyrrolidone (PVP), iso amyl alcohol and ammonium dihydrogen orthophosphate (AnalaR grade) were obtained from VWR International (MERCK, Lutterworth, Leicestershire, UK). Hydrogen peroxide 37% and nitric acid 70% were purchased from Merck (Poole, Dorset, UK). Cetyltrimethylammonium bromide (CTAB), chloroform, EDTA and HCl were purchased from Fisher (Loughborough, Leicestershire, UK). Ethanol was obtained from Rathburn (Walkerburn, Scotland).

Table 6.2 ICP-MS and ICP-OES operating conditions used for the determination of arsenic and phosphorus in DNA extracts of plant sample.

ICP-MS	X Series 2	
	Peristaltic pump speed/ mL min^{-1}	1.2
	Nebulizer type	V-groove
	Spray chamber	Sturman-Masters
	Radio frequency power	
	Forward power/W	1400
Gas flows ($L\ min^{-1}$)	Coolant	13
	Auxiliary	0.75
	Nebulizer	1.0
	Collision cell gas flow (mL min^{-1}) 7 % H_2 in He	3.6
Dwell time (ms)	ICP-MS	10
	HPLC-ICP-MS	100
ICP-OES	iCAP 7400 (Thermo Scientific)	
	Peristaltic pump speed mL /min	1.1
	Nebulizer type	Burgener (MiraMist)
	Spray chamber	Cyclone
	Exposure time (s)	2
	Radio frequency power (W)	1150
	Viewing height (mm)	12
Phosphorus	Wavelength (nm)	177.4
Gas flows/L min^{-1}	Coolant	12
	Auxiliary	0.5
	Nebulizer	0.5

6.3 Plant genomic DNA extraction using CTAB

A cetyltrimethylammonium bromide (CTAB) protocol was adopted for isolation of high-quality genomic DNA from all plant samples [432]. The CTAB buffer was prepared using 2.0 g of CTAB, 10 mL 1 M Tris (pH 8.0), 4 mL of 0.5 M EDTA (pH 8), 28 mL 5 M NaCl, 40.0 mL H_2O and 1 g polyvinyl pyrrolidone (vinylpyrrolidine homopolymer) MW 40,000 (PVP). The final solution was adjusted to pH 5.0 with HCl and made up to 100 mL with Milli-Q water. Freeze-dried 'plant tissue' 0.5 g was weighed into a 15 mL plastic centrifuge tube and 5 mL of CTAB was added. The CTAB/plant tissue was incubated for about 15 min at 55 ºC in a water bath. After incubation, the CTAB/plant extract mixture was spun at 3500 rpm for 15 min to spin down the cell debris. To each tube 2 mL of a chloroform: iso amyl alcohol mixture (24:1) was added and the contents mixed by inversion. After mixing, the tubes were spun at 3500 rpm for 5 min.

The upper aqueous phase (which contains the required DNA) was transferred to a clean plastic centrifuge tube. To each tube 250 µL of ammonium acetate followed by 2.5 mL of ice cold absolute ethanol was added. The tubes were inverted slowly several times to precipitate the DNA. Generally DNA can be seen to precipitate out of solution. Alternatively the tubes can be placed for 1 h at -20 ºC after the addition of ethanol to help precipitate the DNA.

The precipitated DNA was transferred into clean plastic centrifuge tubes. The precipitated DNA is considered to be a 'crude DNA' extract at this stage and the total arsenic in this crude DNA can be measured, without washing, using ICP-MS after digestion with nitric acid. The total arsenic in a fully processed DNA pellet was determined using ICP-MS after washing with 70 % of ice cold ethanol. To wash the primary extractable DNA, the precipitated DNA was transferred into plastic centrifuge tubes containing 0.5 mL of ice cold 70 % ethanol and slowly inverted. The precipitate

was isolated by spinning the tube at 3500 rpm for 1 min to form a pellet. The supernatant was removed; the DNA pellet was washed by adding two further changes of ice cold 70 % ethanol. The DNA washed pellets were then either i) digested in nitric acid for total arsenic or ii) dissolved in 10 mM Tris-HCl (pH 8) and 1 mM EDTA (pH 8) [433] for speciation analysis using HPLC-ICP-MS. The applied procedure is shown in a schematic diagram in Figure 6.1.

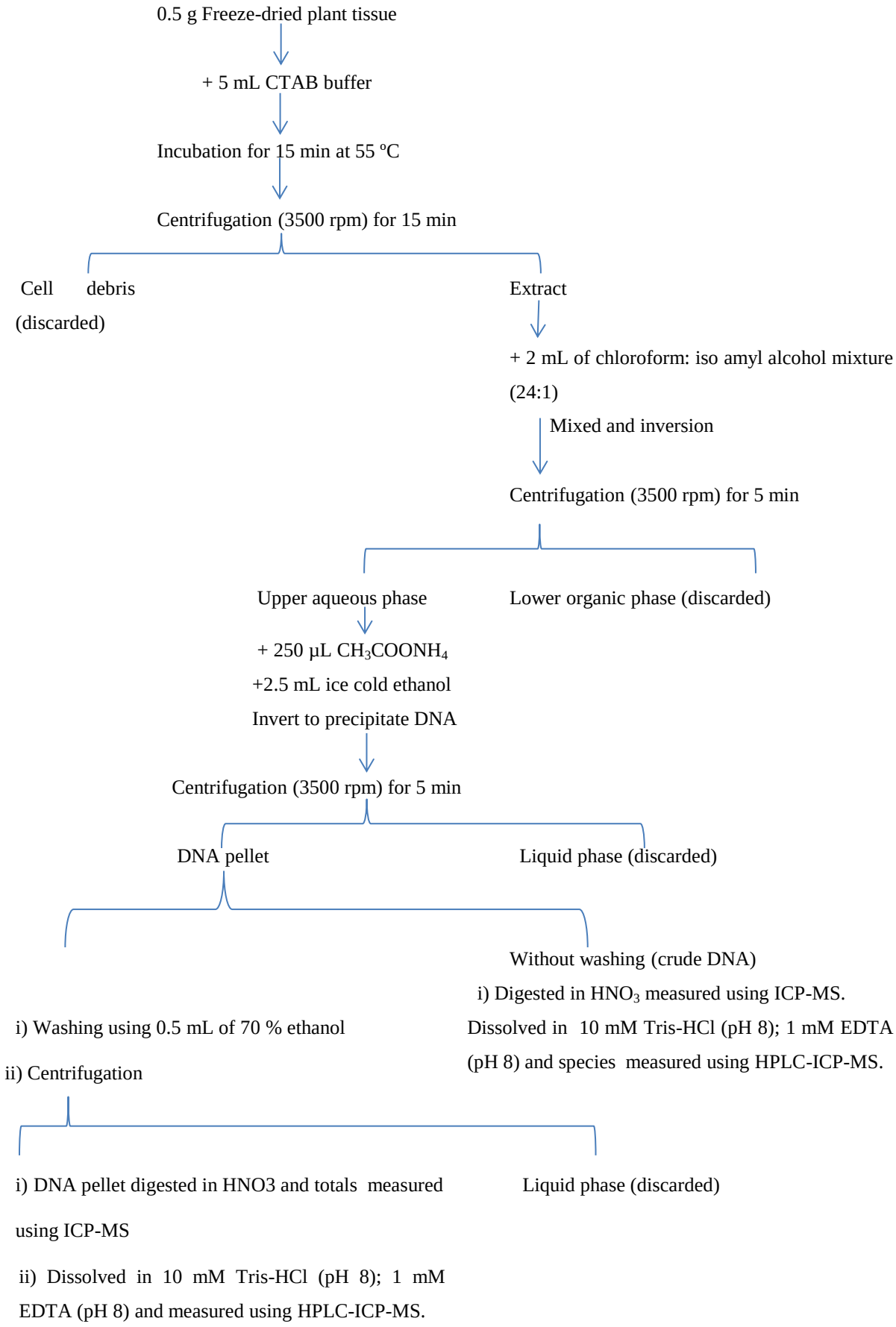


Figure 6.1 Schematic diagram for extraction of DNA from vegetative plants using CTAB buffer for analytical measurement of arsenic and arsenic species.

6.4 Limits of detection

Detection limits for arsenic species were calculated from the analysis of six replicates of the 'procedural' blank samples which were measured together with the plant following standard calibration of the instrument. The results from these sample blanks were used to calculate the limit of detection (LOD) using the following equation:

LOD=3 X SD ; where SD is the standard deviation of the procedural blank sample values in concentration units.

Table 6.3 shows the limits of detection of six measurements of the sample blank (10 mM Tris buffer pH 8 and 1 mM EDTA pH8) as was used to extract arsenic species in the DNA extract of plants).

Table 6.3 Detection limits (3 X standard deviation of the sample blank) determined during analysis of total arsenic and arsenic species in vegetative DNA extracts using ICP-MS and HPLC-ICP-MS.

Arsenic species	**Detection limit (n=6)** $\mu g\ g^{-1}$
$InAs^{III}$	0.004
$InAs^{V}$	0.006
Total arsenic	0.019

6.5 Results and discussion

6.5.1 Preliminary study for total arsenic levels in DNA extracts from plant samples

Extraction of the DNA from the different 'compartments' (root, stem and leaf) of the 12 different plant species was performed in order to measure the total arsenic in their extracted DNA. The DNA pellet (extracted using conditions described in section 6.3 and shown in Figure 6.1) from each compartment of these vegetative plants was then either i) dissolved in concentrated HNO_3 for subsequent ICP-MS analysis (conditions shown in Table 6.2) to measure the total arsenic content in the digested extracts or ii) the extracted DNA from the same compartment of the same plant (three replicates) underwent further purification by washing, using 70 % ethanol and then the total arsenic in these washed DNA extracts was measured using ICP-MS after digestion in concentrated HNO_3.

The results obtained are shown in Table 6.4. Only the rice plant provided an arsenic concentration in the DNA extracts that was sufficiently above the LOD (0.019 μg g^{-1}) for root, stem and leaf samples in both cases; i.e. without washing and with washing using 70 % ethanol. Therefore, the rice plant was selected for further investigation. It is noted that the concentration of arsenic in the extracted DNA in the different compartments from these other plants, being at or below the LOD, may indicate that the DNA of these plants was less affected by arsenic.

Table 6.4 Total arsenic in different compartments of plant (root, stem and leaf) compared with total arsenic in the DNA extracts from the different compartments (root, stem and leaf) of plant samples; all values are in µg g^{-1} ± SD.

Sample	Root			Stem			Leaf		
	Total arsenic in plant using microwave assisted acid digestion	Without washing total As in DNA extract	Washing with 70% ethanol total arsenic in DNA extract	Total As in plant using microwave assisted acid digestion	Without washing total As in DNA extract	Washing with 70% ethanol total As in DNA extract	Total As in plant using microwave assisted acid digestion	Without washing total As in DNA extract	Washing with 70% ethanol total As in DNA extract
Rice	8.284 ± 0.539	0.199 ± 0.005	0.188 ± 0.014	4.005 ± 0.264	0.09 ± 0.006	0.067 ± 0.005	2.932 ± 0.052	0.048 ± 0.005	0.036 ± 0.001
Spring onion	2.072 ± 0.024	0.030 ± 0.002	<0.019	0.702 ± 0.022	0.021 ± 0.003	<0.019	0.594 ± 0.048	0.026 ±0.001	<0.019
Radish	0.672± 0.041	0.022 ± 0.003	<0.019	0.331 ± 0.017	<0.019	<0.019	0.184 ± 0.011	<0.019	<0.019
Potato	0.337 ± 0.003	0.036 ± 0.003	<0.019	0.247 ± 0.020	<0.019	<0.019	0.208 ± 0.011	0.021 ± 0.001	<0.019
Chard	0.578 ± 0.030	0.014 ± 0.003	<0.019	0.387 ± 0.012	<0.019	<0.019	0.183 ± 0.014	0.025 ± 0.003	<0.019
Egyptian leek	1.860 ± 0.103	0.032 ± 0.003	<0.019	-	-	-	0.288 ± 0.009	0.026 ± 0.002	<0.019
Sunflower	0.504 ± 0.018	0.027 ± 0.001	<0.019	0.262 ± 0.010	<0.019	<0.019	0.086 ± 0.003	<0.019	<0.019
Mallow	0.144 ± 0.005	<0.019	<0.019	0.276 ± 0.011	<0.019	<0.019	0.542 ± 0.011	<0.019	<0.019
Wild mint	0.868 ± 0.022	0.021 ± 0.002	<0.019	0.196 ± 0.003	<0.019	<0.019	0.382 ± 0.012	<0.019	<0.019
Broad bean	2.065 ± 0.034	<0.019	<0.019	0.212 ± 0.006	<0.019	<0.019	0.489 ± 0.040	0.042 ± 0.002	<0.019
Beetroot	0.190 ± 0.015	<0.019	<0.019	0.317 ± 0.019	0.02 ± 0.002	<0.019	0.218 ± 0.021	0.026 ± 0.001	<0.019
Arum	0.261 ± 0.012	0.027 ± 0.001	<0.019	0.341 ± 0.031	<0.019	<0.019	0.185 ± 0.017	0.034 ± 0.003	<0.019

6.5.2 Total arsenic and arsenic speciation in rice plant

Microwave assisted acid digestion was used to extract total arsenic in all selected plants (details given in section 3.3.2.1) and arsenic species were extracted from plants under study using 1 % HNO_3 (conditions given in section 4.1.2.3). The different 'compartments' were analysed for their total and arsenic species using both ICP-MS and HPLC-ICP-MS respectively. The values for total arsenic and arsenic speciation in the rice sample are presented in Figure 6.2.

The concentration of total arsenic in the different parts of the rice plant (root, stem, leaf and grain) ranged between 1.017 and 8.284 µg g^{-1}. The majority of arsenic in the rice plant was compartmentalized in the root which was 8.284 µg g^{-1}, while the lowest concentration of arsenic was found in the grain (1.017 µg g^{-1}). This is consistent with previous findings that arsenic concentrates in roots of rice more than stem, leaf and grain of the same plant [145, 434]. In the rice plant the $InAs^{III}$ and $InAs^{V}$ species decreased from the root to the grain with $InAs^{V}$ being the dominant species in each part of the rice plant. The arsenic in the rice grain (1.017 ± 0.09 µg g^{-1}) was five times higher than the 'global range' concentration of arsenic in rice. This could possibly cause a risk to humans by consumption of this rice. According to Zavala *et al.* [322] the global "normal" range for arsenic in rice grain is 0.08 to 0. 20 µg g^{-1}.

6.5.2.1 Method Development for speciation study of arsenic in DNA

The chemical form of 'arsenic' within the DNA from the selected rice plant was of particular interest because of the level encountered in the plant and because of the possibility of inorganic arsenic becoming incorporated into the DNA structure replacing phosphate linkages. However, the levels of the total arsenic in the DNA from the rice plant are low compared with the levels of $InAs^{III}$ and $InAs^{V}$ from the plant. Table 6.5

shows the total arsenic in DNA from each compartment (root, stem and leaf) of the rice plant together with the concentration of arsenic species for the rice plant as a whole.

Table 6.5 Concentration of arsenic species in the rice plant and the total arsenic in DNA extracts of the different compartments of the rice plant; all values are in µg g^{-1}.

Sample	$InAs^{III}$ in plant	$InAs^{V}$ in plant	DMA	MMA	Total As in DNA without washing	Total As in DNA washing with 70 % ethanol
Rice-root	0.889 ± 0.014	7.183 ± 0.085	<0.011	<0.014	0.199 ± 0.005	0.188 ± 0.014
Rice-stem	0.483 ± 0.017	3.434 ± 0.1	<0.011	<0.014	0.090 ± 0.006	0.067 ± 0.005
Rice-leaf	0.448 ± 0.043	2.467 ± 0.02	<0.011	<0.014	0.048 ± 0.005	0.036 ± 0.001

It is important to note that the levels of total arsenic in the DNA from the rice root are considerably greater than the individual organic arsenic levels from DMA and MMA found in that compartment of the plant. The suggestion that the arsenic associated with DNA from the rice root includes an inorganic form would however require further studies to confirm.

A speciation methodology for arsenic in the extracted DNA was therefore developed, and included using the different compartments of the rice plant. This was in order to help identify whether arsenic is just associated (weakly bound) or actually incorporated within the DNA framework itself.

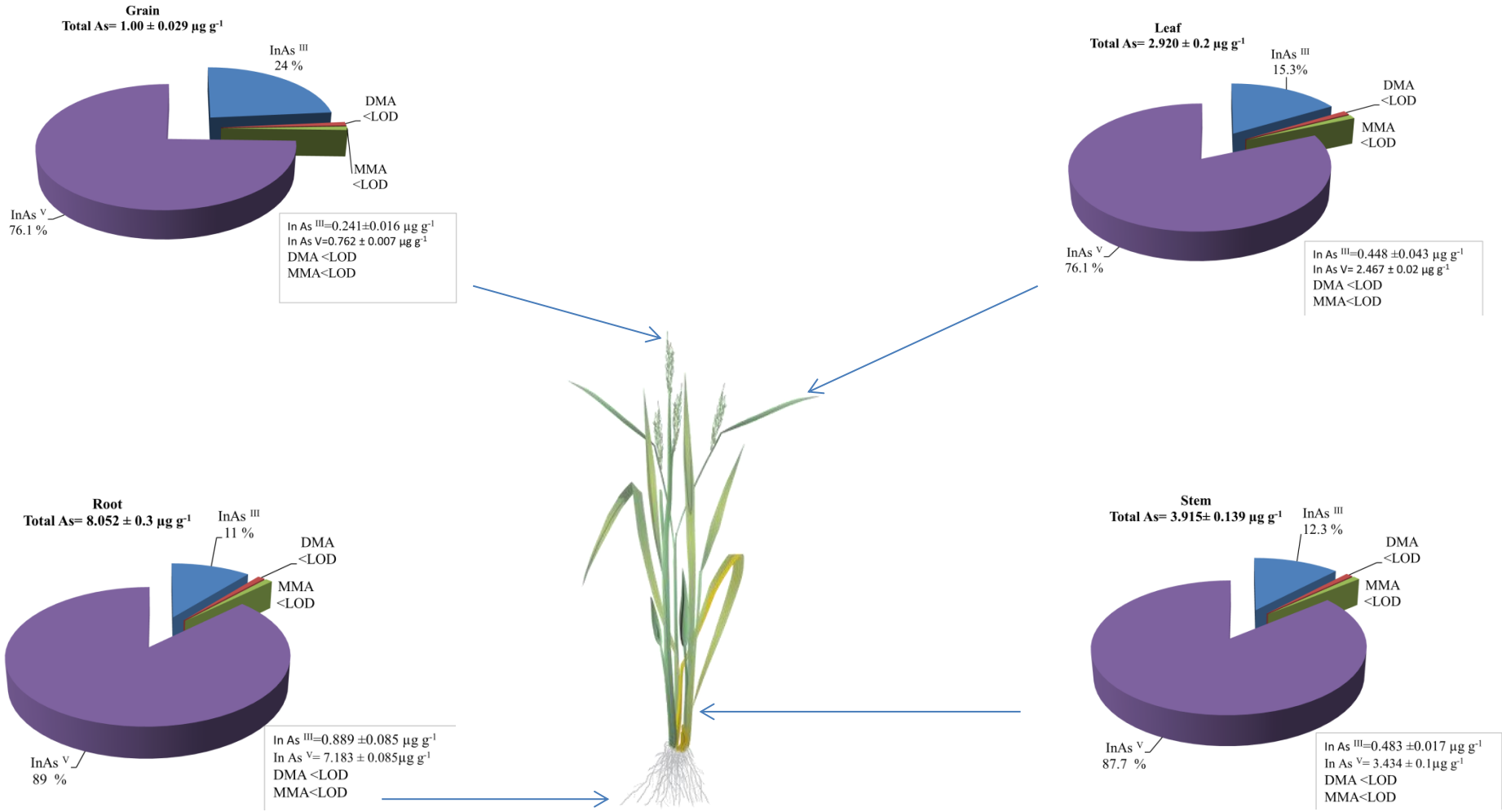


Figure 6.2 Distribution and concentration of arsenic species in rice plant-Oryza sativa.

6.5.3 Analysis of cellular DNA extracts from the rice plant for arsenic and phosphorus content

A CTAB buffer was used to extract DNA from the selected rice plant. The extracted DNA was digested in HNO_3 in order to measure the total arsenic and phosphorus content using ICP-MS. To partially validate the method, procedural blanks were performed with each extraction step as there is no appropriate certified reference material for arsenic in extracted nucleic acid. The purity of the standard phosphate used in the speciation studies was checked against the phosphorus standard calibration using ICP-OES (conditions shown in Table 6.2). The ICP-OES instrument was also used to measure the total phosphorus content of the DNA extracts in each sample to double verify the concentration of phosphate data obtained using HPLC-ICP-MS. The development of the extraction methodology to identify whether arsenic is associated with, or integrated within the vegetative DNA (part of structure potentially replacing phosphate linkage) in different parts of rice plant was undertaken.

6.5.3.1 Total arsenic in rice DNA

The extraction conditions shown in the Figure 6.1 were used to extract DNA from the different compartments of the rice plant sample. Total arsenic in the extracted DNA (without and with washing using 70 % ethanol) was then measured. The results are shown in Tables 6.5 and 6.6. They show that the concentrations of the total arsenic in the extracts of DNA increased with increasing total arsenic content in different compartments of the plant itself. The highest concentration of arsenic in the extracted DNA from the rice plant was found in the root. As the total concentration of arsenic in different parts of the rice plant decreased from the root to the leaf the concentration of total arsenic in the extracted DNA decreased in the same order (root>stem>leaf).

6.5.3.2 Effect of washing DNA extracts on arsenic content

From the root of the rice plant, the concentration of total arsenic in extracted DNA was 0.199 μg g^{-1} (Table 6.5). This was without washing the DNA pellet. When the DNA pellet was washed twice with 70 % ethanol the concentration of the total arsenic in the extracted DNA decreased to 0.188 μg g^{-1}. This result shows how 70 % ethanol washings can remove arsenic that is weakly associated with the DNA. This is only some 5.5 % therefore washing with 70 % ethanol was used throughout the rest of the experiments.

6.5.3.3 Speciation of arsenic and phosphorus in rice DNA

In order to investigate the relationship between arsenic species and cellular fractions, including DNA, from the rice plant grown in an elevated arsenic-bearing environment, HPLC-ICP-MS was used. However, the mobile phase used in previous work had been $NH_4H_2PO_4$ buffer. This required changing if phosphorus was to also be measured. An alternative system previously used has been sodium sulfate as the competitive species in anion exchange chromatography. Sodium sulfate was therefore used as the mobile phase to separate both $InAs^{III}$, $InAs^{V}$ (Figure 6.3 and 6.4) and phosphate (Figure 6.5 and 6.6). It is noted that DMA and MMA were below the LOD (0.011 and 0.014 μg g^{-1}, for DMA and MMA respectively) in the different compartments of rice plant under study. Therefore, $InAs^{III}$, $InAs^{V}$ and phosphate were measured in the extracted rice DNA using HPLC-ICP-MS.

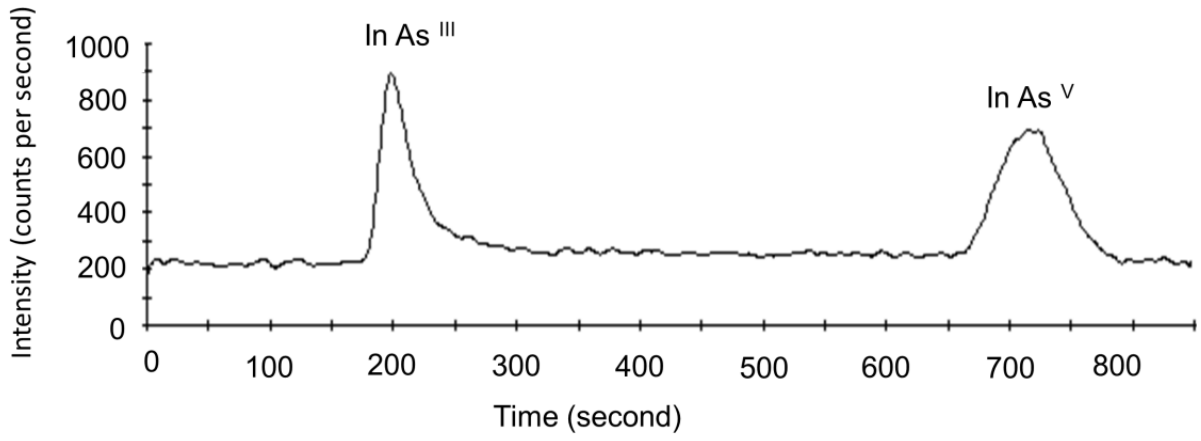


Figure 6.3 Chromatogram of $InAs^{III}$ and $InAs^{V}$ in aqueous solution. $InAs^{III}$ and $InAs^{V}$ 50 µg L^{-1} arsenic, employing a Hamilton PRP-X100 anion-exchange HPLC column using 6.5 mM sodium sulfate as a mobile phase at pH 10.2.

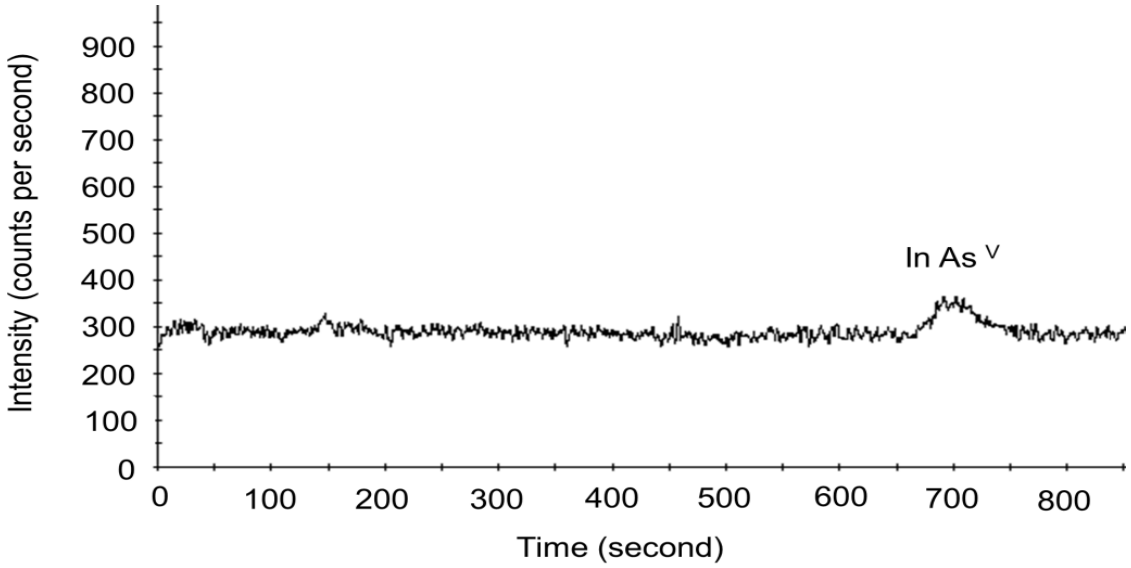


Figure 6.4 Chromatogram of arsenic species in DNA extracts from the rice root sample employing a Hamilton PRP-X100 anion-exchange HPLC column using 6.5 mM sodium sulfate as a mobile phase at pH 10.2.

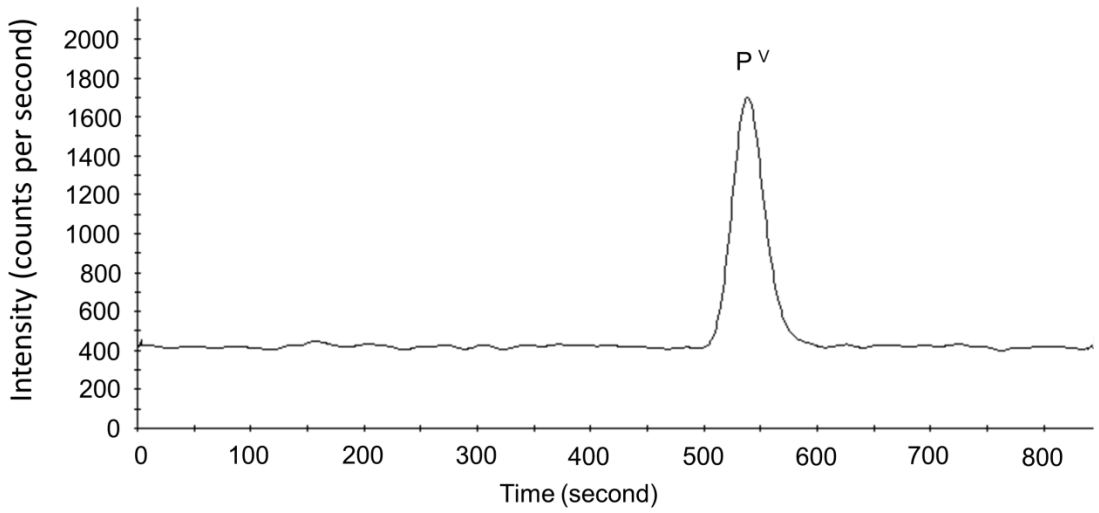


Figure 6.5 Chromatogram of phosphate in aqueous solution. Phosphate 5000 µgL^{-1}, employing a Hamilton PRP-X100 anion-exchange HPLC column using 6.5 mM sodium sulfate as a mobile phase pH 10.2.

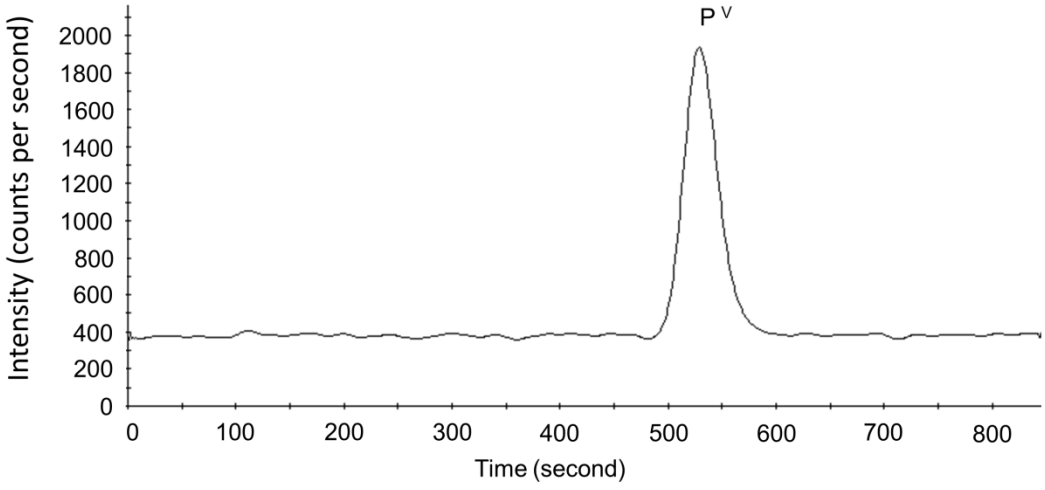


Figure 6.6 Chromatogram of phosphate in DNA extracts from the root of the rice sample employing a Hamilton PRP-X100 anion-exchange HPLC column using 6.5 mM sodium sulfate as a mobile phase pH 10.2.

6.5.3.4 Dissolution of DNA extracts for speciation studies

Pellets of DNA from the rice plant compartments, root, stem and leaf, were dissolved using 10 mM Tris and 1 mM EDTA at pH 8 in order to extract both arsenic species and phosphate. Spiking experiments were conducted to check the stability of the arsenic species using this buffer solution. The recovery of both $InAs^{III}$ and $InAs^{V}$ were 102 % and 101 %, respectively.

It is thought that this is the first trial on the determination of arsenic and arsenic species in whole DNA extracts from plants. When the extracts of DNA from the root of the rice were injected through the anion-exchange column (a Hamilton PRP-X100) not all of the extracted arsenic (species) was detected. Some of the arsenic (species) was retained on the column when using sulfate as the mobile phase and there was therefore a shortfall in the arsenic mass balance. One possible explanation is that this is because of DNA being such a large molecular species with many negative charges because of the presence of phosphate groups in its backbone [435]. These may become bound very strongly with the stationary phase of the column and not easily be eluted. Therefore the sum of arsenic species in the chromatographic separation from the injected DNA extracts using HPLC-ICP-MS was lower than the total arsenic measured from the extracts of DNA measured using ICP-MS. As a result one can hypothesis that these arsenic species which can be detected on the chromatogram are only relatively weakly associated with DNA molecules (because they can easily be removed from the DNA itself using only competitive sulfate ions).

Those arsenic species which are retained on the column can be considered as integrated or incorporated within the DNA, possibly part of the structure and which have potentially replaced phosphate linkages in the vegetative DNA of the plants. In this case $InAs^{V}$ as arsenate which is a phosphate analogue [436] could replace phosphate in the

DNA molecule's backbone when in a highly arsenic contaminated environment. This is consistent with Wolfe-Simon *et al.*'s [429] study which suggested that arsenate could be a viable substitute for phosphate in the DNA of the *Halomonadaceae* GFAJ-1 strain which can grow in the presence of $InAs^{V}$ and possibly in the absence of suitable levels of phosphate.

A similar phenomenon occurred when the phosphate concentration from the same compartmentalised DNA extracts was measured. The concentrations of total phosphorus measured using ICP-MS in DNA extracts were higher than the phosphorus present as phosphate, determined using the same conditions as that used for arsenic species determined using HPLC-ICP-MS. The results are shown in Table 6.6.

6.5.3.5 Estimation of arsenic incorporated within DNA extracts

In the root of the rice plant the total concentration of arsenic in the washed DNA extract dissolved using Tris and EDTA buffer was 0.196 µg g^{-1} as shown in Table 6.6, This is in good agreement with the total arsenic concentration in the extracted and washed DNA digested in HNO_3 (0.188 µg g^{-1})**.** Arsenic, 0.115 µg g^{-1}, as arsenate was found to be weakly 'associated' with the DNA (Figure 6.4). However, the concentration of arsenic in the extracted DNA which may be incorporated within the DNA itself (that which could replace phosphate linkages) was found by difference to be ~ 0.081 µg g^{-1}. The concentration of phosphorus as phosphate detected in the DNA extracts of the root of the rice after chromatographic separation was 98 µg g^{-1}. This is considered to be phosphate just 'associated' with the DNA extract (Figure 6.6). If the arsenic species incorporated within DNA is compared with the phosphorus as phosphate incorporated (integrated) within DNA (217 µg g^{-1}) then the arsenic incorporated within DNA is 0.03 % of the phosphorus level (as phosphate in the DNA backbone) incorporated within the DNA extracted from the root of the rice plant. Results indicate that $InAs^{V}$

associated with DNA from the root was 58.7 % of the total arsenic from the extracted DNA. The remaining arsenic, which could be $InAs^V$ incorporated within DNA was some 41.3 % of the total arsenic in the extracted DNA.

In the stem, the total arsenic concenrtation in the DNA extract was 0.072 µg g^{-1}. Arsenic, 0.042 µg g^{-1}, as arsenate was the only arsenic species found to be just weakly associated with the DNA fraction. The remaining 0.030 µg g^{-1} of arsenic, was incorporated within the DNA from the stem; possibly replacing phosphate linkages. The $InAs^V$ which is weakly associated with DNA represented 58.3 % of the total arsenic from the extracted DNA while some 41.6 % of arsenic was 'incorporated' within the DNA fraction. Finally in the leaf of the rice plant, a small quantity of $InAs^V$, some 0.020 µg g^{-1} was found to be just associated with the DNA structure while 0.014 µg g^{-1} of $InAs^V$ was integrated within the DNA fraction, possibly replacing phosphate linkages in the DNA from the leaf. The percentage of $InAs^V$ associated with DNA fraction was 58.8 % of the total arsenic found in the extracted DNA in the leaf while the other fraction, some 41 % was found to be incorporated within the DNA fraction.

It is of particular note that the ratio of the hypothesised to be 'incorporated' arsenic as arsenate in the DNA from the three independent compartments of root, stem, and leaf are all in the very close range 41 ± 1 %. This value may tell us something about the coping strategy that a rice plant adopts to deal with arsenic in highly contaminated environments.

6.5.3.6 Removal of arsenic and phosphorus-retained species from column

After several injections of DNA onto the column, an attempt to release the retained species was undertaken. A cleaning solution (1 % 6 M HNO_3 in CH_3OH) which is recommended to clean but also to stabilise the Hamilton PRP-X100 column, was injected instead of the sample. Both $InAs^V$ as arsenate and phosphorus as phosphate

were detected as coming from the ‘retained DNA fraction’ held on the column. Both these species ($InAs^{V}$ and phosphate) are considered to be strongly ‘incorporated’ within the DNA fraction. This harsh solution of acid (1 % HNO_3 6 M) in alcohol was therefore beginning to strip the strongly bound $InAs^{V}$ from the DNA together with phosphate, as the DNA was being denatured. This could be further evidence of how in high arsenic bearing environments, plant phosphate linkage molecules in its DNA can be replaced by $InAs^{V}$ as arsenate.

Table 6.6 Results of analysis for total arsenic and phosphorus and their species, concentrations in DNA extracts dissolved in 10 mM Tris and 1 mM EDTA from the different parts of rice samples (root, stem and leaf); all values are calculated in µg g^{-1} of arsenic and phosphorus, mean ± standard deviation (n=3).

Sample	Microwave assist digestion total arsenic	Total arsenic in DNA extracts using HNO_3 digestion	Total arsenic in DNA extracts using 10 mM Tris and 1.0 mM EDTA*	$InAs^{III}$ weakly associated with DNA	$InAs^{V}$ weakly associated with DNA	$InAs^{V}$ possibly incorporated within DNA	$InAs^{V}$ % 'associated' with DNA	$InAs^{V}$ % 'incorporated' within DNA	Total P in DNA extracts using 10 mM Tris and 1 mM EDTA	Total Phosphate after chromatographic separation*	Phosphorus as phosphate possibly incorporated within the DNA
Rice-root	8.284 ± 0.539	0.188 ± 0.014	0.196 ± 0.008	<0.004	0.115 ± 0.01	0.081	58.7	41.3	315 ± 24	98 ± 3.5	217
Rice-stem	4.005 ± 0.264	0.067 ± 0.005	0.072 ± 0.007	<0.004	0.042 ± 0.002	0.030	58.3	41.6	110 ± 6	88 ± 3	22
Rice-leaf	2.932 ± 0.052	0.036 ± 0.001	0.034 ± 0.002	<0.004	0.020 ± 0.0002	0.014	58.8	41.1	328 ± 5	195 ± 8	133

P: Phosphorus: *Sulfate was used for speciation of arsenic and phosphate in DNA chromatography

6.6 Conclusion

The determination of the total arsenic content and arsenic species in rice was accomplished using ICP-MS and HPLC-ICP-MS. The main arsenic species in plants analysed during this study were $InAs^{V}$ and $InAs^{III}$, while DMA and MMA were below the LOD (DMA 0.011 µg g^{-1} and MMA 0.014 µg g^{-1}) in plant samples. The distribution of total and arsenic species in rice was in the order of root>stem>leaf>grain with $InAs^{V}$ the predominant arsenic species in all compartments of rice plant.

A novel study investigating the relationship between arsenic species and selected cellular fraction including DNA was conducted using extracts acquired from vegetative foods containing elevated levels of arsenic. A preliminary study was conducted using a CTAB buffer to extract DNA from the different 'compartments' (root, stem and leaf) of selected vegetative foods including rice-*Oryza sativa*, spring onion-*Allium fistulosum*, radish-*Raphanus sativus*, potato-*Solanum tuberosum*, Egyptian leek-*Allium Kurrat. Schweinf.*, chard-*Beta vulgaris subsp.*, sunflower-Gundelia *tournefortii.*, mallow-*Malva parviflora*, wild mint-*Mentha longifolia*, broad bean-*Vicia Faba*, beetroot-*Beta vulgaris*, and Arum-*Arum spp*. in order to determine the total arsenic in the extracted DNA.

The results showed that the DNA extracts of rice (including root, stem and leaf) contained arsenic above the LOD (0.019 µg g^{-1}) of the initial methodology. Then, HPLC-ICP-MS was used to identify the arsenic species and the phosphate in the DNA extracts of the different compartments of the rice plant. In rice plant, the concentration of total arsenic in DNA extracts decreased with decreasing total arsenic concentration in the three compartments, from the root to the leaf of the same plant. It was found in this study that $InAs^{V}$ as arsenate could replace phosphate, more specifically the phosphate linkages of the DNA fraction, especially when the vegetative plant contains a high inorganic arsenic concentration. It was also found that the concentration of arsenic as

arsenate ‘associated’ with DNA in rice plant decreased with decreasing arsenic concentration from the root to the leaf of the same plant. Finally, it was noted that the % fraction of ‘incorporated’ arsenic in DNA from the root, stem and leaf are all very similar range, being 41±1 %.

Chapter 7

Conclusions and future work

7.1 Conclusions

Chemical speciation is well established for studying arsenic in food, since total element measurements are insufficient to assess the toxicological and biological impact of the element in the environment. Inorganic arsenic species such as $InAs^{III}$ and $InAs^{V}$ are considered toxic, while DMA and MMM are less toxic and AsB is considered to be a non-toxic arsenic specie. This study focussed on developing a robust and routine method for the determination of arsenic trace and its speciation in a range of indigenous food stuffs.

The experimental design was based on the separation of toxic from relatively non-toxic species of arsenic in food staples such as such as fish, rice and vegetable crops. A robust method was developed to separate arsenic species using chromatographic separation utilizing a high capacity anion-exchange based column. The pKa value of the species, buffering capacity and ionic strength of the mobile phase were the main factors that affected the separation of arsenic species using HPLC. Two chromatographic systems were developed. The experimental parameters of the first chromatographic system using sulphate as a mobile phase were; anion-exchange Hamilton resin PRP-X100 (250 X 4.6 mm) packed with 10 µm particle size; flow rate 1.2 mL min^{-1}; sample loop 20 µl and 6.5 mM Na_2SO_4 5 % (v/v) CH_3OH, pH 10.2 as a mobile phase. The second chromatographic system comprised of a Hamilton PRP-X100 anion exchange (250 X 4.6 mm) packed with 10 µm particle size;20 mM $NH_4H_2PO_4$, 1 % (v/v) CH_3OH, pH 6.0 as mobile phase; flow rate 1.1 mL min^{-1}; 20 µl injected sample volume. Both

chromatographic systems were used in isocratic mode. Methanol was added to the mobile phase in order to increase the sensitivity of the determination of As species.

To facilitate the extraction of arsenic species, both enzymatic and chemical extraction reagent were used to extract arsenic species in fish, vegetable, rice and plant samples. Enzymatic extractants such as trypsin and cellulase were utilized to extract arsenic species in a range of fish and vegetables, respectively, with the aid of mechanical shaking in a water bath at 37 ºC. Arsenic speciaton in rice and vegetative plants collected from the Kurdistan region of Iraq was performed using 1 % HNO_3 (v/v) assisted with microwave energy to extract arsenic species from these food samples. Phosphoric extractant (1M) with ascorbic acid was used to extract arsenic species from soils that used to cultivate the vegetable plants under study, while a BCR three step extraction method was used to fractionate arsenic species from the soil samples. In majority of cases the methodology was optimised and the resultant extraction efficiencies were generally higher than 90 % when compared with the total arsenic concentrations determined using HNO_3/H_2O_2 (microwave assisted acid digestion). Finally, CTAB buffer was used to extract vegetative DNA in vegetative plants contained high arsenic contents, to facilitate the determined of arsenic in the extracted DNA.

Mass balances were calculated to include a full elemental mass balance analysis to aid an estimate of the associated toxicology. The mass balance calculations were also conducted for the CRMs used (DORM-3 fish protein, NIES rice pine needle 1575a, flour, GBW10015 spinach, BCR 701, ERM-CC141 loam soil), fish, rice, vegetable crops and also vegetative plants. The mass balance results were in good agreement with the certified and reference values based upon both organic and inorganic species. The

validation of the applied methods was checked using the selected CRMs according to the sample matrix.

The determination of arsenic species in foodstuff is clearly of interest because of consensus regarding arsenic in the diet. Seafood is an important source of protein, and thus an important food staples for many people. Arsenic speciation in the fish samples selected in this study was successfully performed with high extraction efficiencies between 90-104 % using trypsin. AsB, was found to be the dominant arsenic species in ling, gurnard, grey mullet, pollock, Dover sole, John dory, megrim, flounder, dab, sand sole, brill, lemon sole and halibut. DMA and MMA were also found in the samples. The percentage of $InAs^{V}$ in these fish samples ranged between 0.54 -8.05 % of total extracted arsenic (3.53-98.80 µg g^{-1}). DORM-3 fish protein was used to validate the developed method.

In vegetables, a range of edible vegetable crops were analysed to investigate total and arsenic speciation. Initial studies considered vegetable crops grown by local market garden suppliers in Devon-UK known to be in areas with a history of arsenic mining. Cellulase was utilized to extract arsenic species from the cell content of the investigated vegetable crops (leek, potato, carrot, parsnip, artichoke, beetroot, swede and turnip). Inorganic arsenic species were demonstrated to be the dominant arsenic species in analysed vegetables with levels ranging from 0.022 µg g^{-1} to 1.158 µg g^{-1}. The extraction efficiencies using cellulase in vegetables were between 91 and 126 % of the total arsenic using microwave assisted acid digestion. The concentration of arsenic quantified in the peel of turnip, beetroot (sample A), swede, carrot, parsnip (sample A), potato, parsnip (sample B), beetroot (sample B), artichoke and beetroot (sample B) were higher than those in the core of the same vegetables. Similar results have been reported other studies [358, 359]. DMA was only found in the skin of swede, while MMA was found

to be in the peel of both beetroot (sample A) and swede. The method used for the study on vegetable was validated using CRMs pine needle 1575a.

Arsenic determination in rice is of importance due to being as a staple food for over half of the world's population. The total arsenic and its speciation and total multi-element concentration in rice samples of different geographical origin were assessed. For the first time, total arsenic in rice samples grown in the Kurdistan region of Iraq has been determined. This study demonstrated the concentrations of total arsenic in rice samples are different based on their geographical origin. For example, rice grown in the Kurdistan region of Iraq contained low arsenic content in comparison with rice from other origins. The highest concentration of arsenic in rice in this study was found in rice from Turkey which was found to be 0.823 μg g^{-1}. This particular high arsenic concentration may reflect the impact of the presence of mining activity near the paddy rice. The arsenic species in the rice were extracted using 1 % HNO_3 in a microwave oven to give extraction efficiencies of between 91-109 %. The majority of arsenic in this study was found to be inorganic arsenic species, consisting 51-100 % of total arsenic found in each sample. Organic arsenic species such as DMA and MMA species were also found in analysed rice samples with variable concentrations. Finally, a multi-element analysis to include Ag, Al, B, Ba, Be, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Si, Ti, V and Zn in rice samples of different origin was conducted. The validation of the applied method was undertaken using CRM NIES rice flour for both arsenic and also other elements.

A case study was undertaken to investigate both the total arsenic and arsenic species in different parts of a range of vegetable collected in the Kurdistan of Iraq. This is the first time crops from this region have been investigated. The crops included rice-*Oryza sativa,* spring onion-*Allium fistulosum,* radish-*Raphanus sativusL.,* potato-*Solanum*

tuberosum, Egyptian leek-*Allium Kurrat. Schweinf.,* chard-*Beta vulgaris subsp.,* -sunflower-Gundelia *tournefortii.,* spinach-*Spinacia oleracea,* Garden cress-*Lepidium sativum L.,* Celery- *Apium graveolens* mallow-*Malva parviflora,* wild mint-*Mentha longifolia,* broad bean-*Vicia Faba,* beetroot-*Beta vulgaris,* and Arum-*Arum spp*.

Since the arsenic levels in the plant may reflect the local growing environment, soil in which these crops were cultivated together with the irrigation waters used were both included in the study. The aim was to understand how the local habitat may impact on the uptake, and help elucidate the accumulation and transformation of arsenic in the plants.

The total concentrations of arsenic in the irrigation waters were found to be between 0.536 2.039 µg L^{-1}. This is significantly below the recommended limiting value for arsenic in drinking water by the WHO (10 µg L^{-1}). However, at these levels, irrigation waters were not felt to be a significant source of arsenic to the growing plant, but may be a facilitator at the root surface and soil interface, assisting arsenic mobility. The total 'aqua-regia arsenic' available in analysed soils were between 2.88 - 6.21 µg g^{-1}. Again at these levels of arsenic, the analysed soils can be classified as uncontaminated soils with arsenic. Phosphoric acid and ascorbic acid were used to perform arsenic speciation in the analysed soils with $InAs^{V}$ being as predominant arsenic species with low concentrations of $InAs^{III}$ while both DMA and MMA were below the LOD (0.119 and 0.084 for DMA and MMA respectively) in the analysed soils. A microwave assisted-acid digestion using HNO_3/H_2O_2 was then used to extract total arsenic from different organs of the crop plants.

The compartmentalization of total arsenic in different organs of the same plant was found to be plant dependent. It was found that in some of crop plants the distribution of arsenic had the following order: root>stem>leaf>grain, while in other plants the

distribution of arsenic was in the opposite order with root accumulating the lowest arsenic content in comparison with other organs. In addition, a further distribution order was found in two plants. Here it followed the sequence: root>stem<leaf and root<stem>leaf. Speciation of the arsenic in the crop plants under study was performed using 1 % HNO_3 with the aid of a microwave technique. Inorganic arsenic species were found to be the dominant arsenic species in the majority of the analysed crop plants under study. Validation of the applied method was conducted using GWB10015 spinach. A three step sequential fractionation extraction procedure was performed to evaluate the 'arsenic plant available' in the analysed soils. The results of this study showed that the percentage of 'plant available arsenic' was of between 7.87-21.14 % of the total arsenic aqua-regia available (2.88-6.21 μg g^{-1}). In this case the method was validated using CRM BCR 701.

Finally, a novel experiment was designed in order to investigate where arsenic species interact with plant DNA. The aim was to determine whether it is found in the intra or extra cellular fraction. This experiment was performed using a CTAB buffer to extract the DNA from plants with a high arsenic content. A preliminary study was performed to extract DNA from the different 'compartments' of selected foods including rice-*Oryza sativa,* spring onion-*Allium fistulosum,* radish-*Raphanus sativus*, potato-*Solanum tuberosum,* Egyptian leek-*Allium Kurrat. Schweinf.*, chard-*Beta vulgaris subsp.*, -sunflower-Gundelia *tournefortii.,* mallow-*Malva parviflora,* wild mint-*Mentha longifolia*, broad bean-*Vicia Faba*, beetroot-*Beta vulgaris*, and Arum-*Arum spp*. in order to determine the total arsenic in the extracted DNA. The results showed that only the DNA extracts of rice (including root, stem and leaf) contained arsenic above the LOD (0.019 μg g^{-1}) of the methodology. It was found that total arsenic in the extracted DNA decrease from the root to the leaf as the total arsenic in the rice plant decreased in the same direction.

In order to determine whether inorganic arsenic species are incorporated within or just associated with DNA extracts of plant material, the extracts were injected through an anion-exchange chromatography system using a Hamilton PRP-X100 column and Na_2SO_4 as mobile phase. This strongly suggested that $InAs^V$ could replace phosphate and become 'incorporated' within the DNA, more specifically the phosphate linkages of the DNA fraction. This was especially the case when the plant contains high levels of arsenic. It was also found that the concentration of inorganic arsenic ($InAs^V$) associated with DNA selectively obtained from the root, stem and leaf of the rice plant decreased with decreasing total arsenic concentration obtained from the root, stem and leaf of the same plant. A narrow, near-constant ratio for these two arsenic values was further evidence for the structural incorporation of the inorganic arsenic into the DNA.

7.2 Future work

This work has demonstrated that accurate speciation methods are required for the determination of arsenic in environmentally important samples. However, the methodology is designed to focus on the key arsenic species. Further work is required to extend these studies to include other arsenic species such as arseno sugars often found in marine materials. This could be further extended to toxicity studies, perhaps using model gut systems, to provide a more comprehensive account of the implications of some of the less well studied arsenic species in food, particularly those originating in marine based products by the changing of chromatographic system with optimization of chromatographic conditions.

The study of rice and vegetables could also be extended to include different types of cooking practice. For example, cooking in an excess of water or parboiling the samples. Comparisons could then be made with uncooked samples. A similar exercise could be conducted on sample preparation practices. The current study also showed the different

distribution pathways for the arsenic in individual crops. This study could also be extended to see if crops could be systematically classified with respect to the partitioning characteristic.

A total diet study would also be useful to examine the dietary exposure of people to different types of food. The study could include different fish samples and the rice sample and vegetables from a single region. This would be of particular interest in areas that have a reputation of being highly contaminated with chemicals during the war in the Kurdistan region of Iraq. A total diet study has not been performed in the Kurdistan region of Iraq.

Finally, the method developed to enable the arsenic associated with DNA to be detected, could be further developed to look at other molecules from extracted vegetative DNA. This method could comprise a suitable chromatographic separation technique for elution of the complete DNA molecule, hyphenated with very sensitive and versatile detectors such as ICP-MS. A suitable mobile phase and column-type will be needed to separate the 'whole DNA molecule' in order to determine the actual concentration of intra-cellular arsenic species associated within the DNA fraction. This methodology could be used as further evidence for the compartmentalisation of arsenic in the cellular fraction of vegetative crop plants. A modified HPLC-ICP-MS system should be developed to identify the major eluted 'DNA species' in order to monitor both arsenic and phosphorus in a range of plants with high levels of arsenic.

The separation and identification of DNA fraction with profiling, using electrophoresis and based upon a soft-laddering process would be particularly interesting with regard to identifying arsenic species specifically from plants with high arsenic contents. A sensitive and accurate speciation method for the identification of arsenic in these plants

needs to be developed in order to identify arsenic within the soft DNA breakdown fractions.

Electron microscopy technique could be used to identify arsenic and arsenic species within cells of plants deliberately grown in very high arsenic concentrations.

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B. A. Sadee, S. Hill, M. Foulkes, (2012). Total arsenic and arsenic speciation in indigenous food stuffs, Biogeochemistry Research Centre (BGC), 4th Annual Conference, Plymouth, UK.

B. A. Sadee, S. Hill, M. Foulkes, (2013). Arsenic in our food: A study of arsenic speciation in a range of common food stuffs, Biogeochemistry Research Centre (BGC), 5th Annual Conference, Plymouth, UK.

B. A. Sadee, S. Hill, M. Foulkes, (2014). Determination of total arsenic and arsenic speciation in a range of vegetables and crops measured by HPLC-ICP-MS, Biogeochemistry Research Centre (BGC), 5th Annual Conference, Plymouth, UK.

Poster presentation

B. A. Sadee, S. Hill, M. Foulkes, (2013). Total arsenic and arsenic speciation in fish, The Postgraduate Society Annual Conference, Plymouth University, Plymouth, UK.

B. A. Sadee, S. Hill, M. Foulkes, (31 August - 4 September 2014). Determination of total arsenic and arsenic speciation in local market garden vegetable crops. 17th BNASS/14th Trace Spe – The TANDEM conference, University of Aberdeen, Aberdeen, UK.

B. A. Sadee, S. Hill, M. Foulkes, (31 August - 4 September 2014). The determination of total arsenic and arsenic speciation in some Kurdish vegetables and plants. 17th BNASS/14th Trace Spe – The TANDEM conference, University of Aberdeen, Aberdeen, UK.

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The determination of arsenic speciation in different organs of broad bean (*Vicia Faba*) using HPLC-ICP-MS (submitted for publication in Food Chemistry (Jan 2016).

The determination of total arsenic and its speciation in cellular DNA fractions from highly contaminated vegetative plants. (in draft)