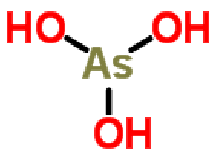
Arsenic, food and DNA – is there a link?

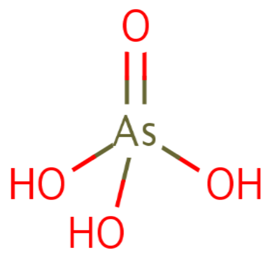
Steve J Hill,

Plymouth University.

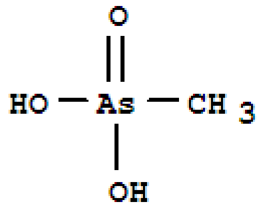
- Overview of arsenic in food.
- Brief look at some UK based examples.
- Methodology
- Case study of Dokan, Kurdistan:
  arsenic speciation in soil, irrigation water and plant tissue.
- DNA studies
- Conclusions.



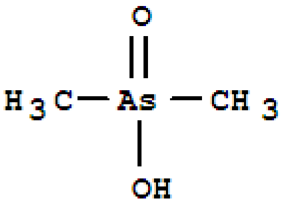
Arsenite (As ^{III})



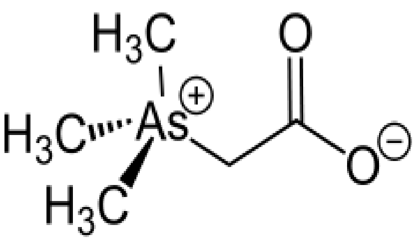
Arsenate (As ^{V})



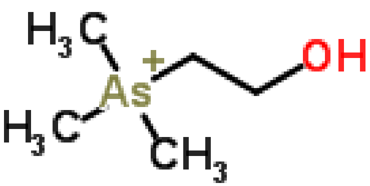
Methyl arsonate (MMA)



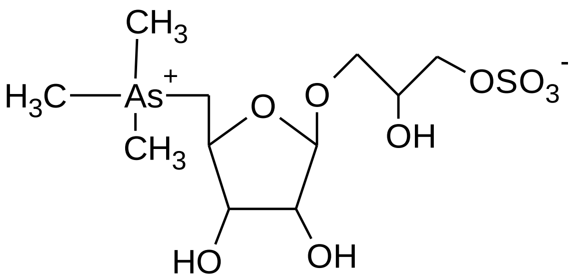
Dimethylarsenate (DMA)



Arsenobetaine (AsB)



Arsenocholine (AsC)

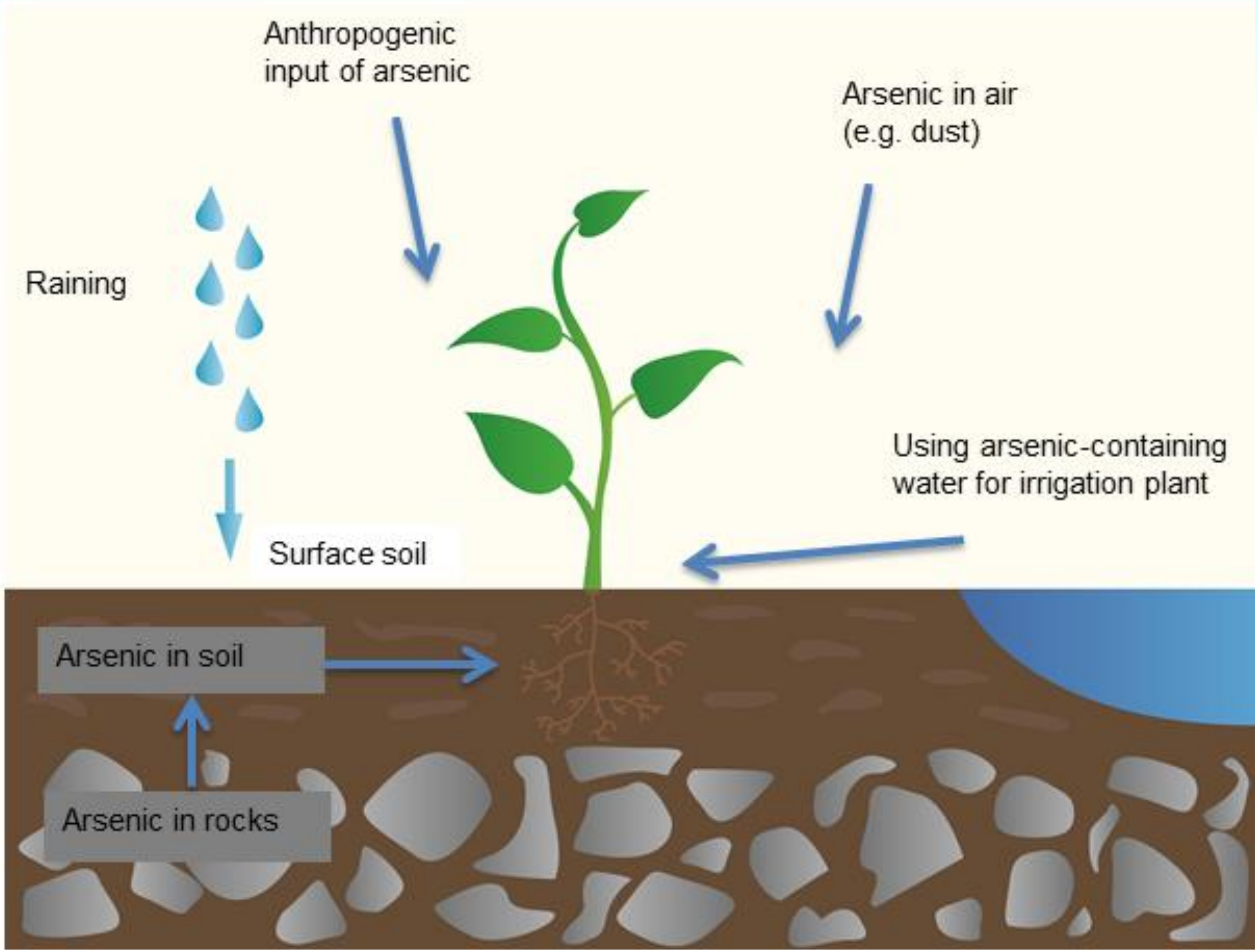


Arsenosugar

Lethal dose LD_{50} values of arsenic species in rat

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

Potential routes for arsenic into food based plants.



The concentration (mg/kg) of inorganic and total arsenic in the 20 food groups of the 2006 UK Total Diet Study

Food group	Inorganic arsenic mg/kg	Total arsenic mg/kg
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

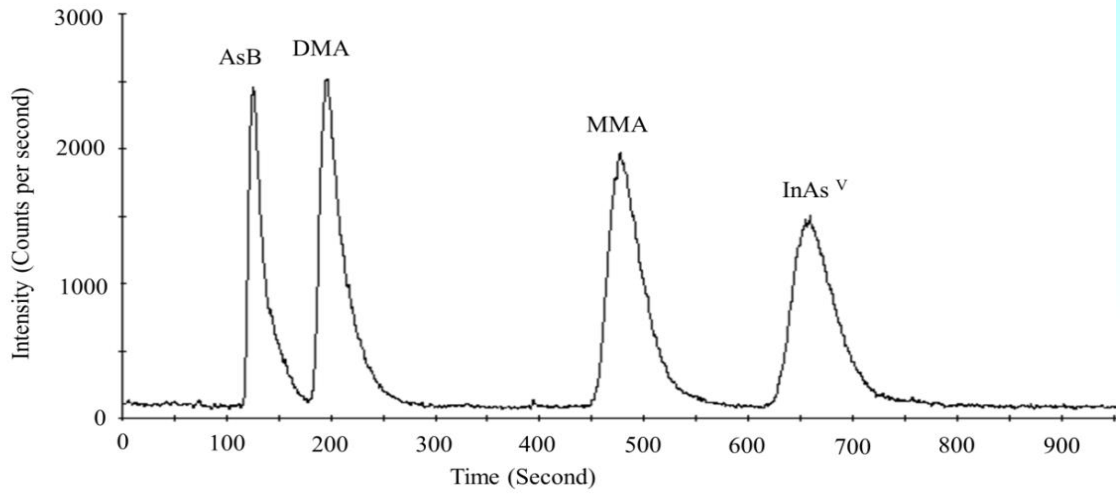
M. Rose, M. Baxter, N. Brereton and C. Baskaran, *Food Additives & Contaminants: Part A*, 2010, **27**, 1380-1404.

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

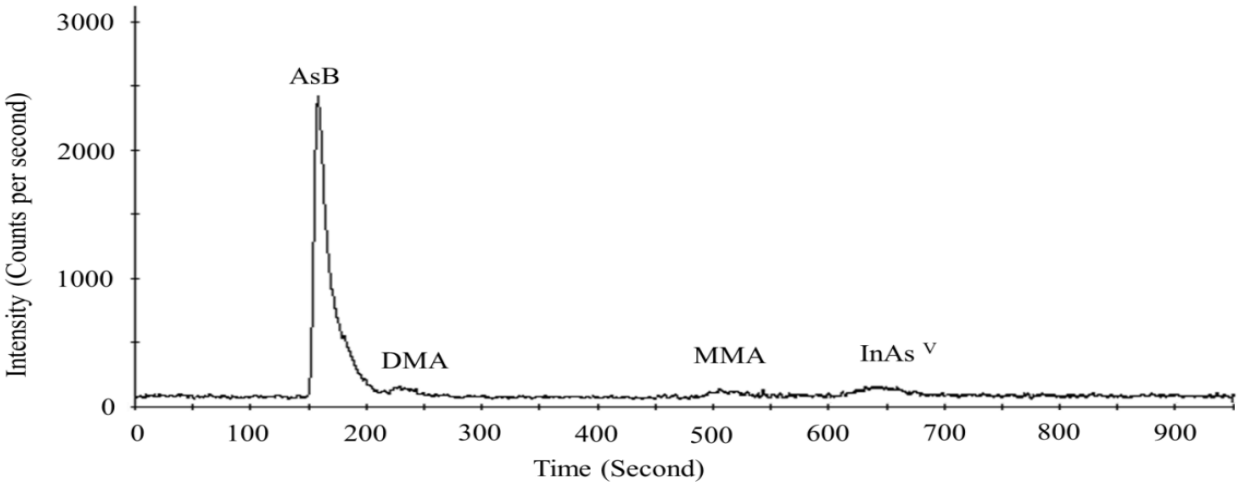
ICP-MS	X Series 2 (Thermo Scientific) will collision cell	
	Peristaltic pump speed ml min^{-1}	1.1
	Nebulizer type	V-groove
	Spray chamber	Sturman-Masters
	Radio frequency power (W)	1350
Gas flows/L min^{-1}	Coolant	13
	Auxiliary	0.75
	Nebulizer	1.0
H_2 addition	Gas flow (mL. min^{-1}) 7 % H_2 in He	3.6
(m/z)	As	75
	In	115
	Ir	193
Dwell time (ms)	ICP-MS	10
	HPLC-ICP-MS	100

HPLC conditions for 1 % HNO_3 extracts

Column dimension	250 x 4.6 mm
Guard column dimension	50 x 4.6 mm
Packing material	Hamilton resin PRP-X100, 10 µm particle size
Eluent flow rate	1.1 mL min^{-1}
Sample loop	20 µL
Mobile phases:	20mM Ammonium dihydrogen phosphate ($NH_4H_2PO_4$) – pH 6.0
	Sodium sulphate – pH 10.2



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 sodium sulfate.



Chromatogram of sand sole using anionic-exchange HPLC-ICP-MS, using sodium sulfate

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

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

* Aqua regia extractable content

A case study of arsenic speciation in soil, irrigation water and plant tissue.

Location: Dokan, SE of Arbeel in Kurdistan, Iraq.

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	5.01 ± 0.19	0.718 ± 0.055	<0.119	<0.084	4.228 ± 0.160	0.113 ± 0.003	4.98 ± 0.1	99
So5	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
So6	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
So7	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
So8	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
So9	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
So10	5.32 ± 0.042	0.564 ± 0.0007	<0.119	<0.084	4.584 ± 0.22	<0.027	5.3 ± 0.3	100
So11	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
So12	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
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	104

Concentrations of arsenic in irrigation water samples *(vegetable crops grown in each region also shown).*

Water sample	Location	area	Label	Concentration (μg 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

Determination of arsenic concentration in different organs of vegetable crops.

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									
Rice									
Spring onion									
Radish									
Chard									
Egyptian Leek									
Sunflower									
Mallow									
Garden cress									
Wild Mint									
Board bean									
Beetroot									
Arum									

Arsenic concentration in different organs of vegetable crops – POTATO (dry weight); all values are given in µg g^{-1} of arsenic mean ± SD (n=3).

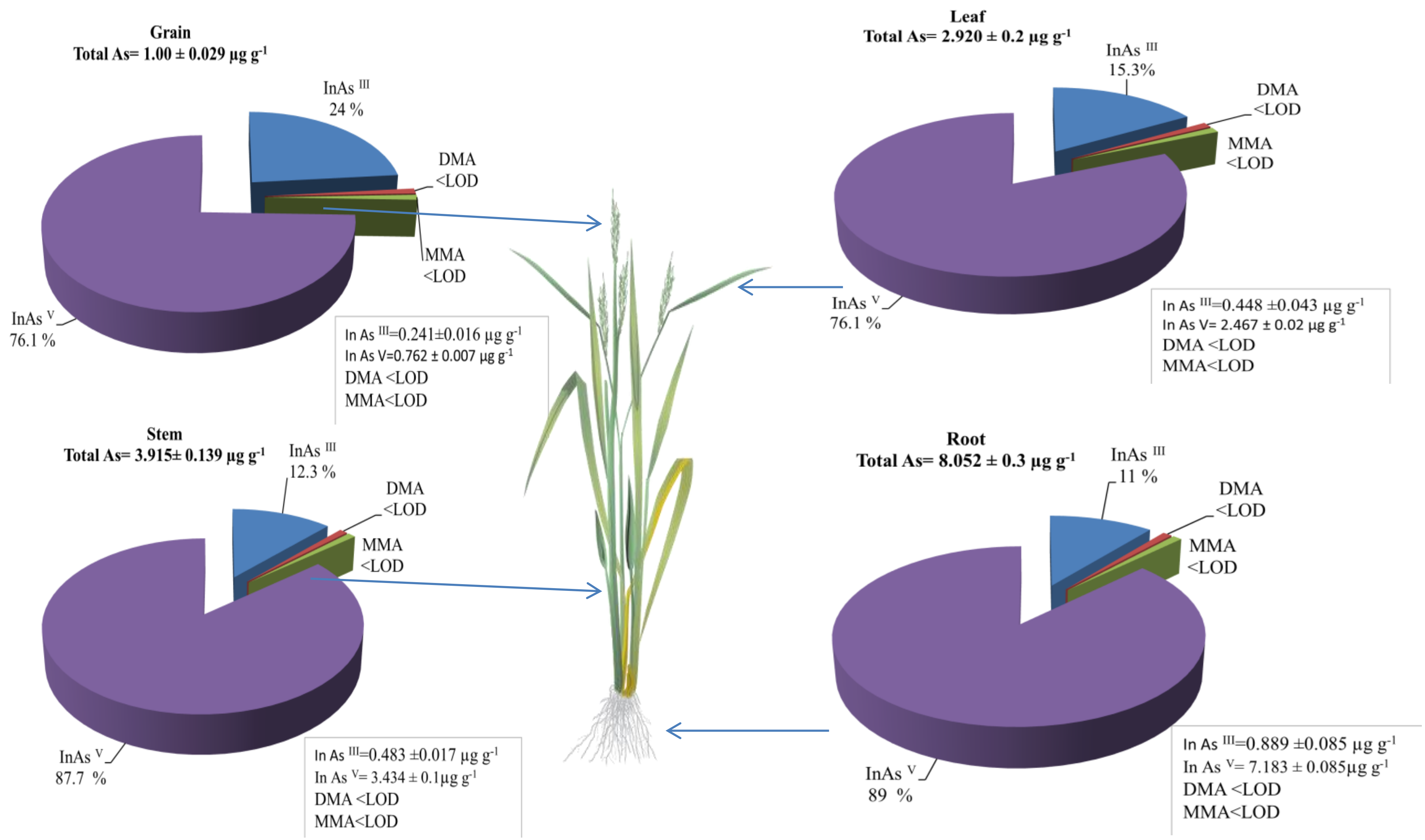
Vegetable	Microwave assist digestion	$InAs^{III}$	DMA	MMA	$InAs^{V}$	Total arsenic in residue	Total arsenic in extracts	Efficiency of extraction %	Sum of arsenic from species
Potato									
Root	0.337 ± 0.003	0.150 ± 0.008	<0.011	<0.014	0.200 ± 0.012	<0.009	0.355 ± 0.015	105	0.350
Skin	0.392 ± 0.015	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

Concentration of As in cultivation soil, plant sample (μg As g^{-1} dry mass basis), and in irrigation water (μg As L^{-1}) mean ± SD (n=3)

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

Results of analysis for arsenic speciation in the Broad bean plant (n=3)

Vegetable: Broad bean	Microwave assist digestion μg g^{-1} ± SD	As ^{III} μg g^{-1} ± SD	DMA μg g^{-1} ± SD	MMA μg g^{-1} ± SD	As ^{V} μg g^{-1} ± SD	Total arsenic in residue μg g^{-1} ± SD	Total arsenic in extracts μg g^{-1} ± SD	Efficiency of total species extraction %
Root	2.065 ± 0.033	0.324 ± 0.014	0.041 ± 0.007	0.068 ± 0.004	1.585 ± 0.087	0.041 ± 0.001	2.024 ± 0.175	98
Stem	0.212 ± 0.006	0.035 ± 0.003	0.050 ± 0.004	0.044 ± 0.003	0.062 ± 0.005	0.030 ± 0.001	0.191 ± 0.008	90
Leaf	0.489 ± 0.04	0.091 ± 0.006	<LOD	0.101 ± 0.001	0.232 ± 0.011	0.072 ± 0.005	0.415 ± 0.001	85
Pod	0.258 ± 0.017	0.049 ± 0.004	0.070 ± 0.006	0.027 ± 0.002	0.082 ± 0.002	0.032 ± 0.001	0.232 ± 0.006	90
Bean	0.133 ± 0.009	0.009 ± 0.001	0.022 ± 0.003	0.055 ± 0.003	0.024 ± 0.002	0.016 ± 0.001	0.114 ± 0.011	86
Spinach GBW10015	0.249 ± 0.008	0.107 ± 0.008	<LOD	0.034 ± 0.002	0.113 ± 0.011	<LOD	0.264 ± 0.018	104



Distribution and concentration of arsenic species in rice plant-*Oryza sativa*

Arsenic concentration in different compartments of selected plants

Group	Plant species	Order of distribution
Group 1	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*	Root>stem>leaf>grain
Group 2	mallow-*Malva parviflora*	Leaf>stem>root
Group 3	broad bean-*Vicia Faba* and wild mint-*Mentha longifolia*	Root>stem<leaf
Group 4	beetroot- *Beta vulgaris*, spinach- *Spinacia oleracea* and Arum-*Arum spp*	Root<stem>leaf

Cellular level compartmentalisation of arsenic.

- Known that As^{V} is a phosphate analog – uptake through phosphate transport proteins.
- Also known that As^{III} is a silicic acid analogue – uptake through xylem system.

However, few studies on cellular level compartmentalisation of As in vegetative systems.

Plant genomic DNA extraction using CTAB

- A cetytrimethylammonium bromide (CTAB) buffer was prepared.
- Final solution pH adjusted to 5.0
- 0.5g of freeze-dried plant material placed into centrifuge tube with 5ml of CTAB
- Incubated for 15 mins at 55^{o}C then centrifuge to spin down cell debris.
- Add choroform : iso amyl alcohol mixture, mix and centrifuge again.
- Upper aqueous layer contains the DNA.

- Aqueous DNA phase transferred and mixed with ammonium acetate and ethanol.
- Tube inverted to precipitate the DNA.
- Arsenic in this ‘crude’ DNA extract was measured after dissolution with nitric acid.
- The extract was then washed several times with ethanol prior to final dissolution of the refined extract.

Total arsenic in different compartments of plant (ROOT) 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	Total arsenic in plant using microwave assisted acid digestion	Without washing Total arsenic in DNA extract	Washing with 70% ethanol Total arsenic in DNA extract
Rice	8.284 ± 0.539	0.199 ± 0.005	0.188 ± 0.014
Spring onion	2.072 ± 0.024	0.030 ± 0.002	<0.019
Radish	0.672± 0.041	0.022 ± 0.003	<0.019
Potato	0.337 ± 0.003	0.036 ± 0.003	<0.019
Chard	0.578 ± 0.030	0.014 ± 0.003	<0.019
Egyptian leek	1.860 ± 0.103	0.032 ± 0.003	<0.019
Sunflower	0.504 ± 0.018	0.027 ± 0.001	<0.019
Mallow	0.144 ± 0.005	<0.019	<0.019
Wild mint	0.868 ± 0.022	0.021 ± 0.002	<0.019
Broad bean	2.065 ± 0.034	<0.019	<0.019
Beetroot	0.190 ± 0.015	<0.019	<0.019
Arum	0.261 ± 0.012	0.027 ± 0.001	<0.019

Total arsenic in different compartments of plant (STEM) 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 Stem	Total arsenic in plant using microwave assisted acid digestion	Without washing Total arsenic in DNA extract	Washing with 70% ethanol Total arsenic in DNA extract
Rice	4.005 ± 0.264	0.09 ± 0.006	0.067 ± 0.005
Spring onion	0.702 ± 0.022	0.021 ± 0.003	<0.019
Radish	0.331 ± 0.017	<0.019	<0.019
Potato	0.247 ± 0.020	<0.019	<0.019
Chard	0.387 ± 0.012	<0.019	<0.019
Sunflower	0.262 ± 0.010	<0.019	<0.019
Mallow	0.276 ± 0.011	<0.019	<0.019
Wild mint	0.196 ± 0.003	<0.019	<0.019
Broad bean	0.212 ± 0.006	<0.019	<0.019
Beetroot	0.317 ± 0.019	0.02 ± 0.002	<0.019
Arum	0.341 ± 0.031	<0.019	<0.019

Total arsenic in different compartments of plant (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 Leaf	Total arsenic in plant using microwave assisted acid digestion	Without washing Total arsenic in DNA extract	Washing with 70% ethanol
Rice	2.932 ± 0.052	0.048 ± 0.005	0.036 ± 0.001
Spring onion	0.594 ± 0.048	0.026 ±0.001	<0.019
Radish	0.184 ± 0.011	<0.019	<0.019
Potato	0.208 ± 0.011	0.021 ± 0.001	<0.019
Chard	0.183 ± 0.014	0.025 ± 0.003	<0.019
Egyptian leek	0.288 ± 0.009	0.026 ± 0.002	<0.019
Sunflower	0.086 ± 0.003	<0.019	<0.019
Mallow	0.542 ± 0.011	<0.019	<0.019
Wild mint	0.382 ± 0.012	<0.019	<0.019
Broad bean	0.489 ± 0.040	0.042 ± 0.002	<0.019
Beetroot	0.218 ± 0.021	0.026 ± 0.001	<0.019
Arum	0.185 ± 0.017	0.034 ± 0.003	<0.019

Conclusions:

- The distribution of total and arsenic species in plant material depends on the individual plant species.
- Arsenic concentration in different compartments of plants in this limited study fell into four groups.
- Preliminary studies show 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.

- We believe that arsenate can replace phosphate, more specifically the phosphate linkages of the DNA fraction, especially when the 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, in very recent work, taking the washed DNA and dissolving in TRIS EDTA buffer prior to speciation by ion chromatography ICP-MS, we found that although some As^{V} was released, the roots stem and leaf compartments all retained a very similar proportion of the As^{V} (41±1 %) implying that this fraction may be ‘incorporated’ into the DNA.

Acknowledgements

I would like to acknowledge the contribution of Bashdar Sadee and Michael Foulkes to the work presented today.

Thank you for listening.