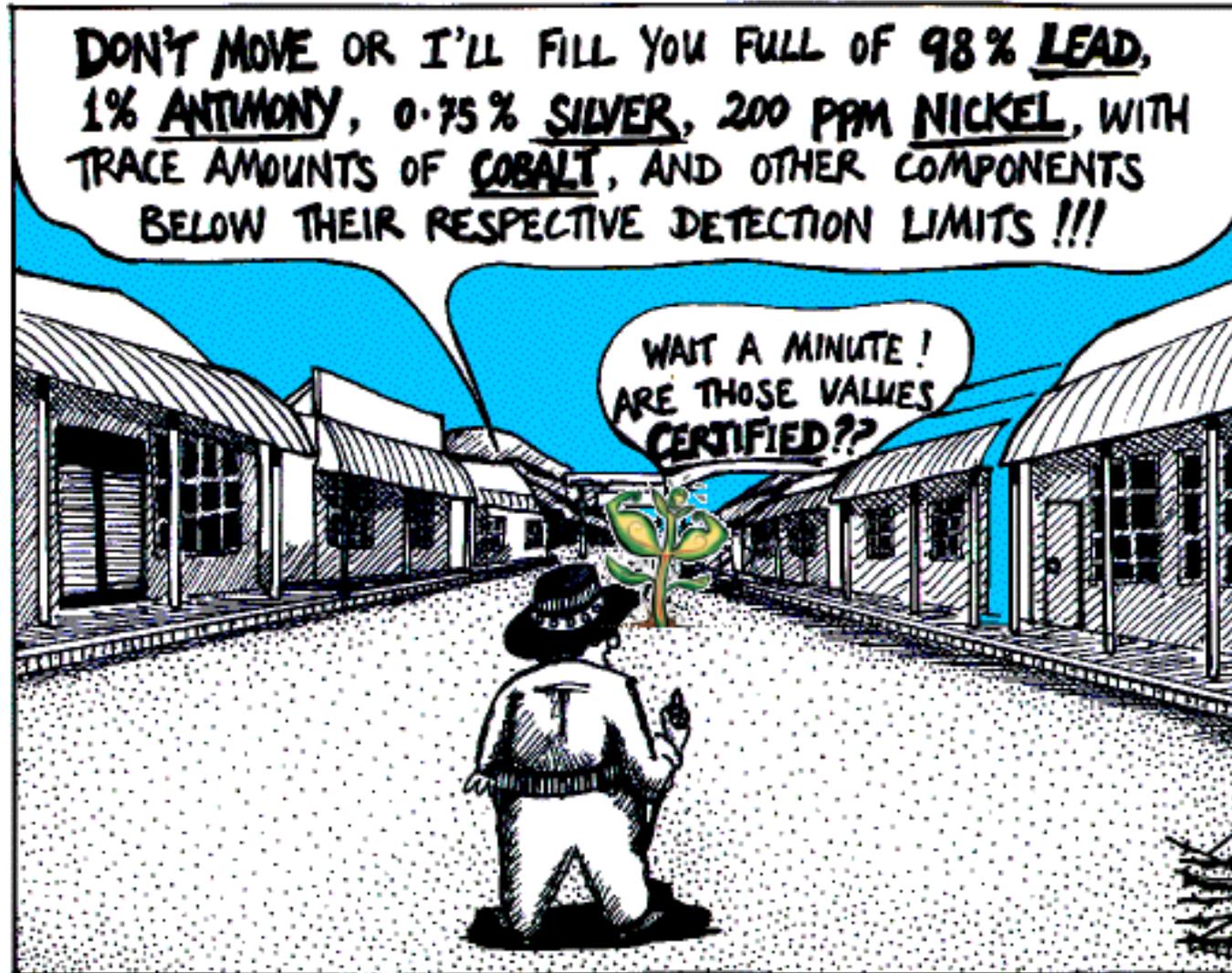


Metal(loid)s and Plants - a complicated relationship

→ Arsenic toxicity

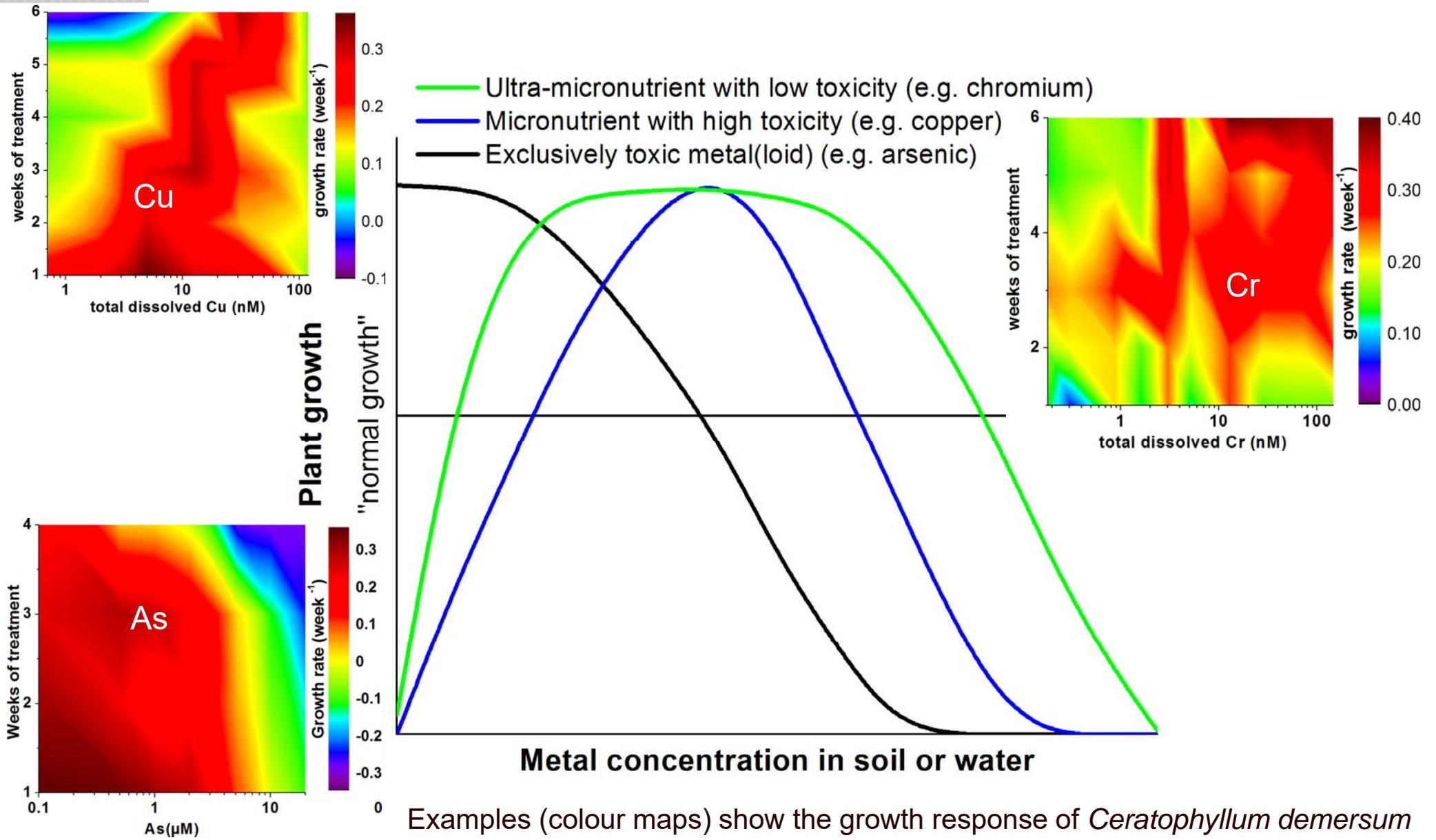


Heavy metal-hyperaccumulation in the Wild West

modified from: <http://strangematter.sci.waikato.ac.nz/>

Hendrik Küpper based on a talk of Seema Mishra, Advanced Course on Bioinorganic Chemistry & Biophysics of Plants, summer semester 2025

Variations of the dose-response principle for different elements



Review (scheme): Küpper H, Kroneck PMH (2005) Metal ions Life Sci 2, 31-62 (modified)

As: Mishra S, Stärk H-J, Küpper H (2014) Metallomics 6, 444-454;

Cr: Küpper H, Stärk H-J, Mattusch J (2021) unpublished;

Cu: Thomas G, Stärk H-J, Wellenreuther G, Dickinson BC (2013) Aquatic toxicology 140-141, 27-36

Arsenic

IA												VIII A					
1											2						
H											He						
1.01											4.00						
II A												III A	IVA	VA	VIA	VII A	
3	4											5	6	7	8	9	10
Li	Be											B	C	N	O	F	Ne
6.94	9.01											10.81	12.01	14.01	16.00	19.00	20.18
11	12											13	14	15	16	17	18
Na	Mg											Al	Si	P	S	Cl	Ar
22.99	24.30											26.98	28.09	30.97	32.06	35.45	39.95
		III B	IV B	VB	VIB	VII B	← VIII B →		IB	II B							
19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
39.10	40.08	44.96	47.87	50.94	52.00	54.94	55.84	58.93	58.69	63.55	65.41	69.72	72.64	74.92	78.69	79.90	83.80
37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
85.47	87.62	88.91	91.22	92.91	95.94	(98)	101.07	102.91	106.42	107.87	112.41	114.82	118.71	121.76	127.60	126.60	131.29
55	56	57	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
132.91	137.33	138.91	178.49	180.95	183.84	186.21	190.23	192.22	195.08	196.97	200.59	204.38	207.20	208.98	(209)	(210)	(222)
87	88	89	104	105	106	107	108	109	110	111	112	113	114	115	116		118
Fr	Ra	Ac	Rf	Db	Sg	Bh	Hs	Mt	Ds	Rg	Uub	Uut	Uuq	Uup	Uuh		Uuo
(223)	(226)	(227)	(261)	(262)	(266)	(264)	(269)	(268)	(271)	(272)	(277)	(284)	(289)	(288)	(292)		(294)



58	59	60	61	62	63	64	65	66	67	68	69	70	71
Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
140.12	140.91	144.24	(145)	150.36	151.96	157.25	158.93	162.50	164.93	167.26	168.93	173.04	174.97
90	91	92	93	94	95	96	97	98	99	100	101	102	103
Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr
232.04	231.04	238.03	(237)	(242)	(243)	(248)	(247)	(251)	(252)	(257)	(260)	(259)	(262)

Arsenic: Applications

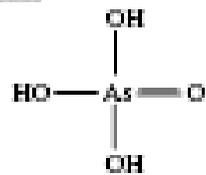
- **Semiconductor industries**
- **Strengthening alloys of copper and lead**
- **Pesticides, in particular herbicides + insecticides**
- **Wood preservatives**
- **Feed additives**
- **Medical uses**
- **Military uses**

- *The **Poison of Kings** and the **King of Poisons***

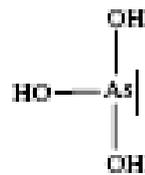


Arsenic trioxide

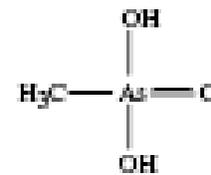
Structures of the most common As compounds



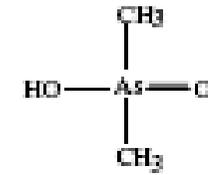
Arsenate
[As(V)]



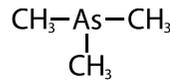
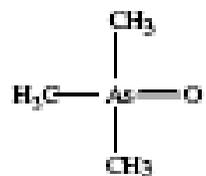
Arsenite
[As(III)]



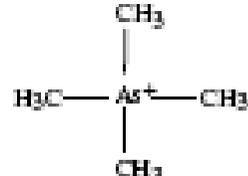
Monomethylarsonic acid
[MMA]



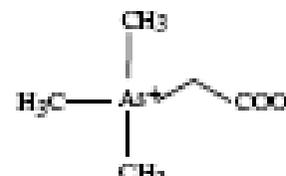
Dimethylarsinic acid
[DMA]



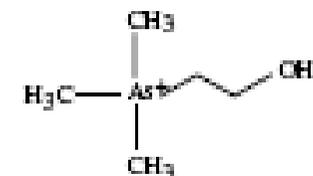
Trimethyl Arsine



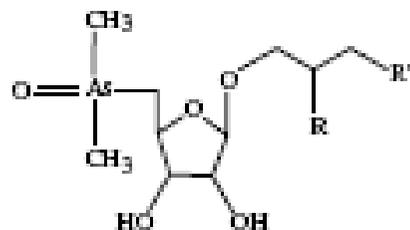
Tetramethylarsonium ion
[TeMA]



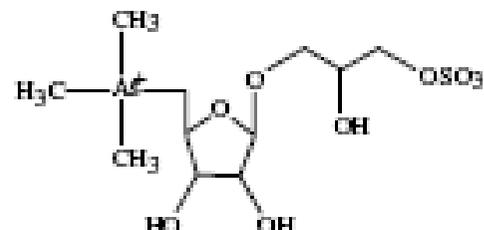
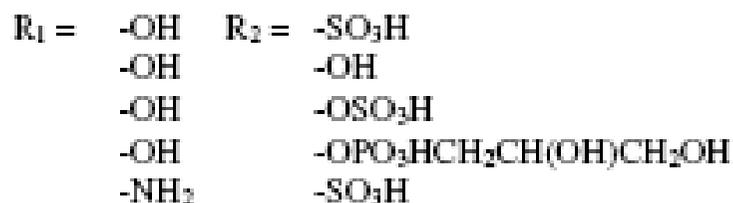
Arsenobetaine
[AB]



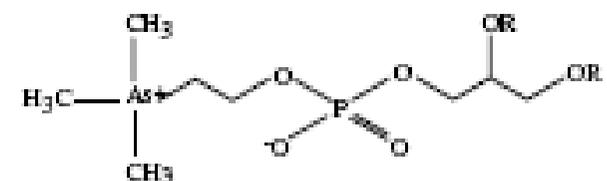
Arsenocholine
[AC]



Dimethylarsinyldribosides



Trimethylarsonioribosides



Glycerolphosphorylarsenocholine R = H
Phosphatidylarsenocholine R = CO(CH₂)_nCH₃

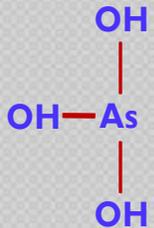
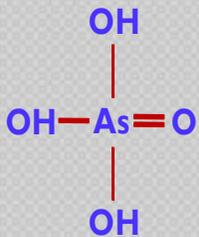
Sources of Arsenic Contamination

- **Through anthropogenic activities such as mining, smelting, phosphate fertilizer, and the use of As-containing pesticides, wood preservatives, and feed additives.**

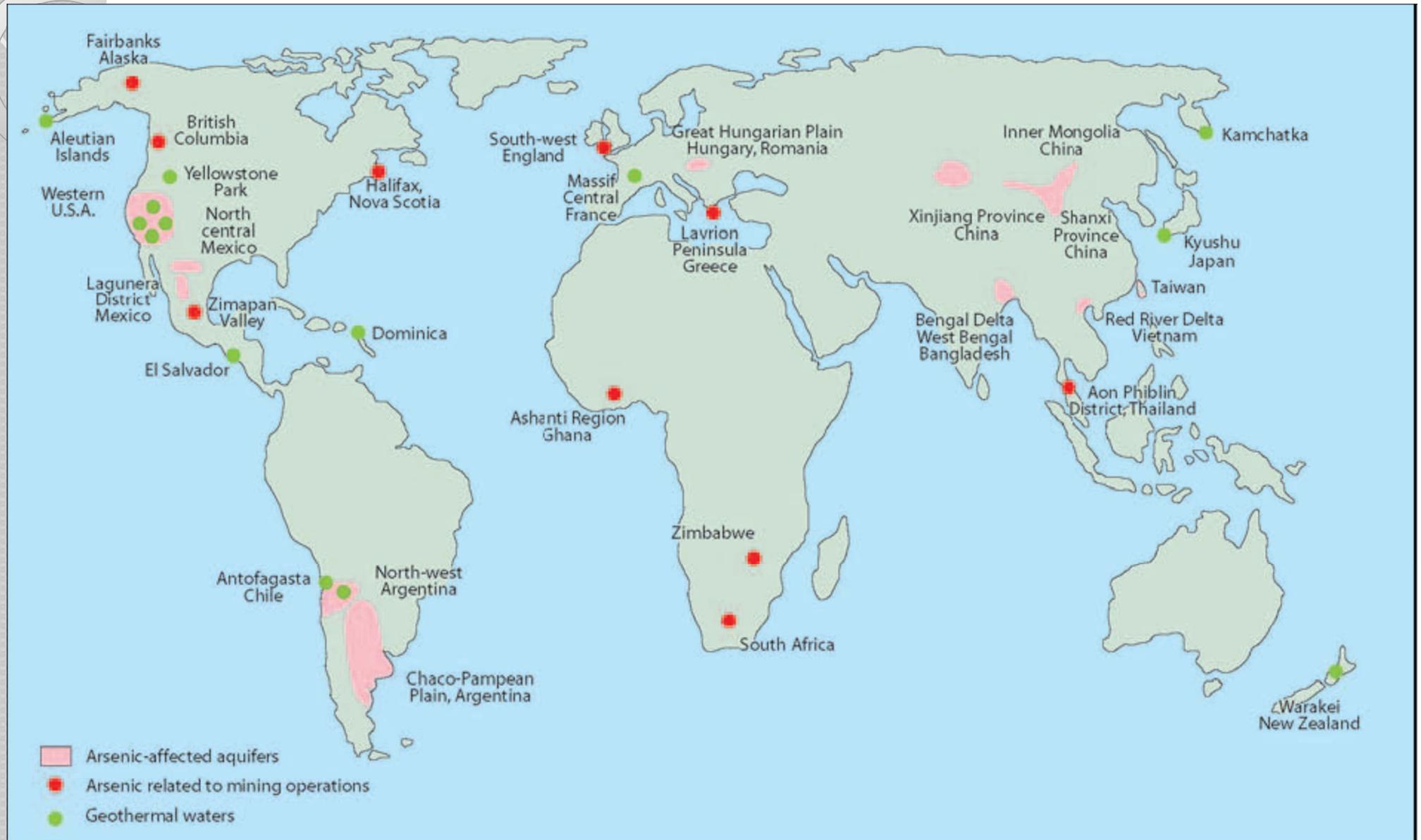
e.g. Contamination in US fields

- **Through natural processes, such as weathering of rocks, volcanic emissions and discharge from hot springs**

e. g. Contamination in South east Asia + USA



Arsenic Contamination



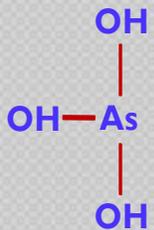
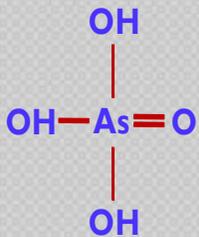
Arsenic Toxicity to Plants

Symptoms

- Reduced germination,
- Inhibited root and shoot growth
- Reduced chlorophylls
- Low grain yield
- Ultimately death

Mechanisms

- Through uptake competition for essential nutrients
- Through substitution of phosphate by iAs(V) in enzyme catalyzed reactions
- By binding of iAs(III) to sulfhydryl group containing enzymes
- ROS generation
- Reduction of iAs(V) to iAs(III) using glutathione as reductant
- Oxidation of iAs(III) to iAs (V) under physiological conditions



Arsenic Toxicity to Plants: Proteomics



Available online at www.sciencedirect.com

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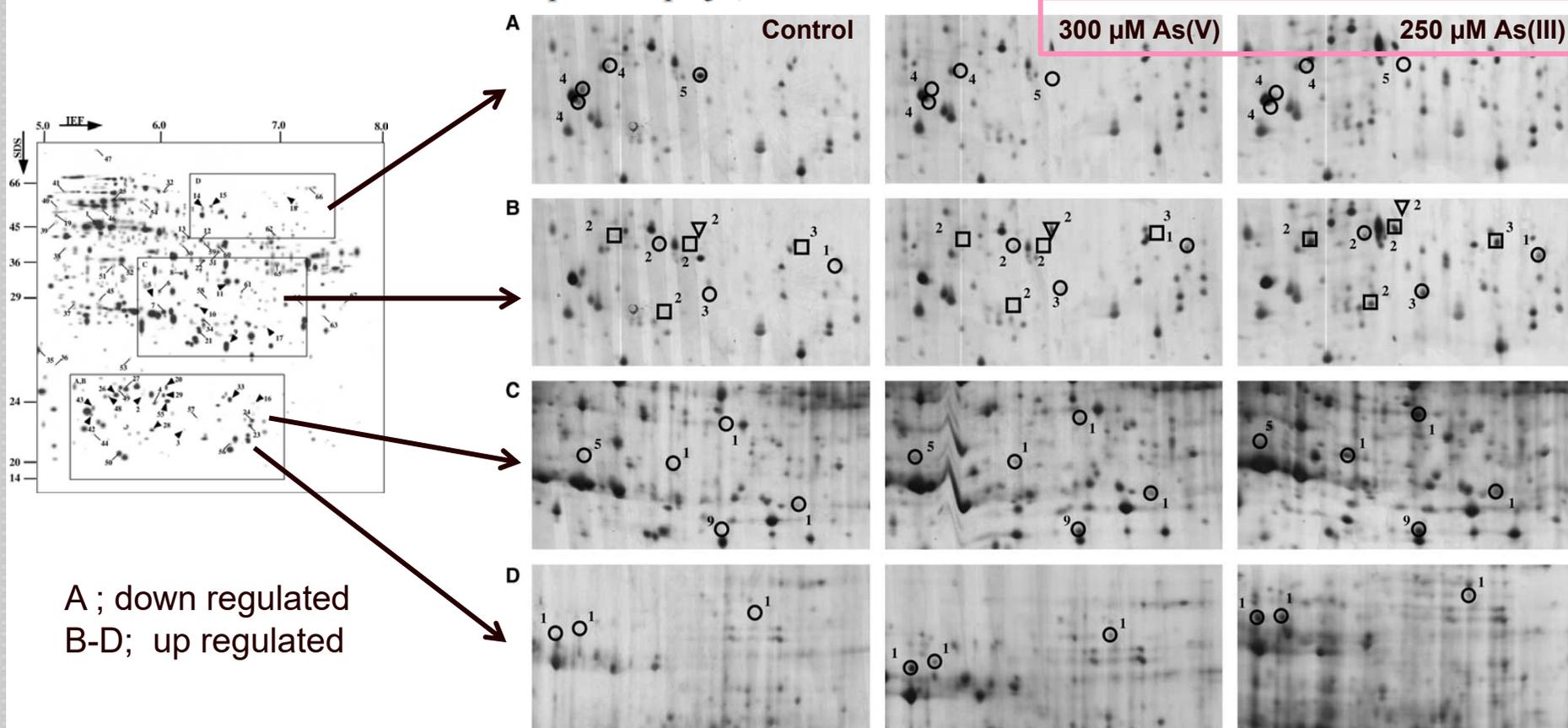
Phytochemistry 66 (2005) 1519–1528

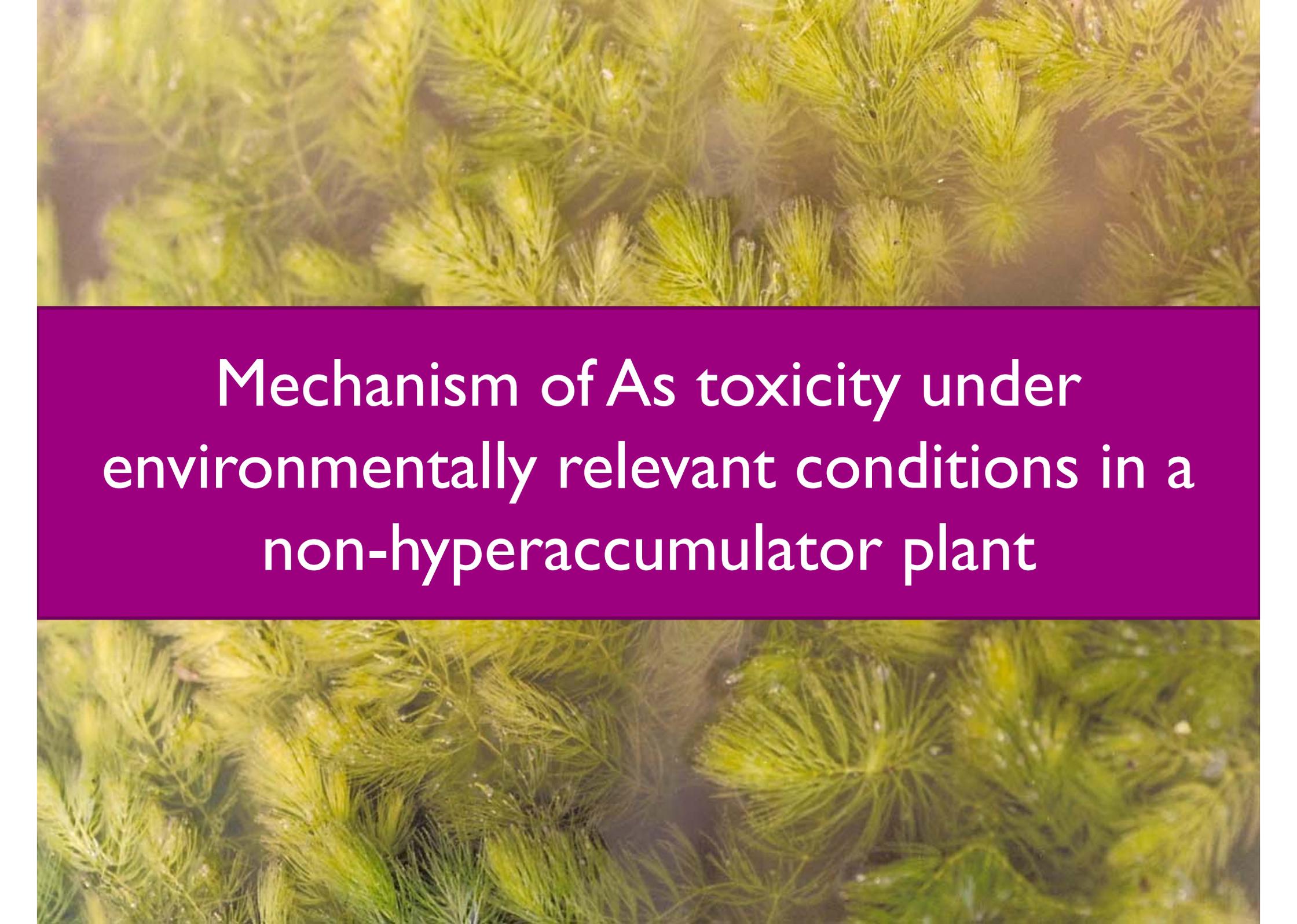
PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Proteome analysis of maize roots reveals that oxidative stress is a main contributing factor to plant arsenic toxicity

Raquel Requejo, Manuel Tena *





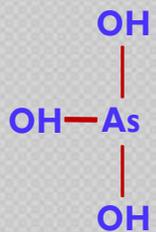
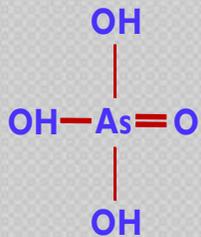
Mechanism of As toxicity under environmentally relevant conditions in a non-hyperaccumulator plant

Ceratophyllum demersum L.

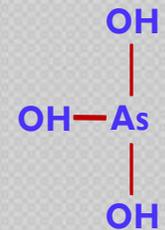
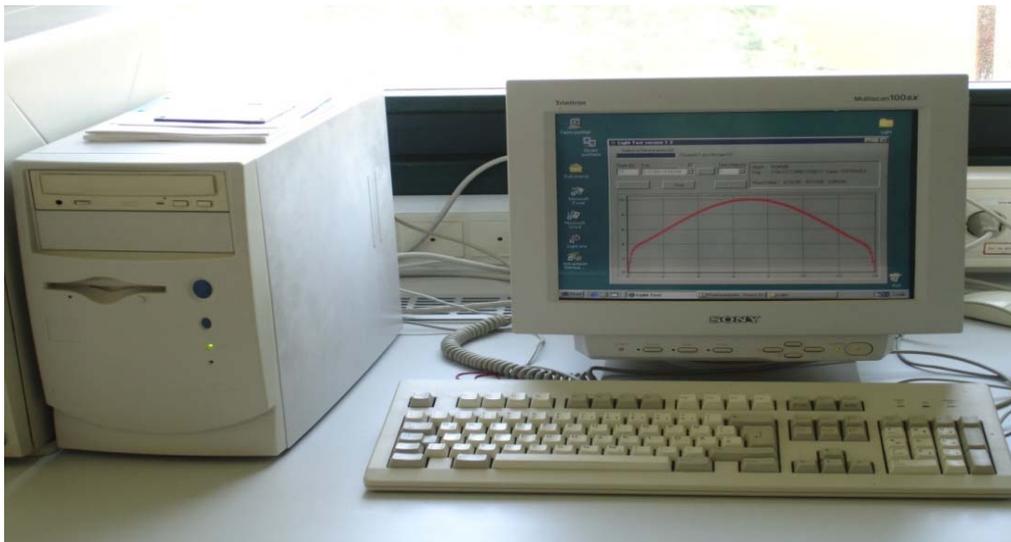
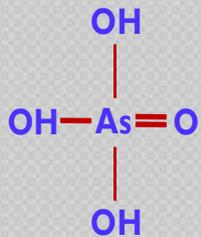


Ceratophyllum demersum is a rootless aquatic weed. Shows rapid growth, has worldwide distribution and can be easily harvested.

It has been successfully used in biological life support systems on space flights
(Blüm et al., 1994)

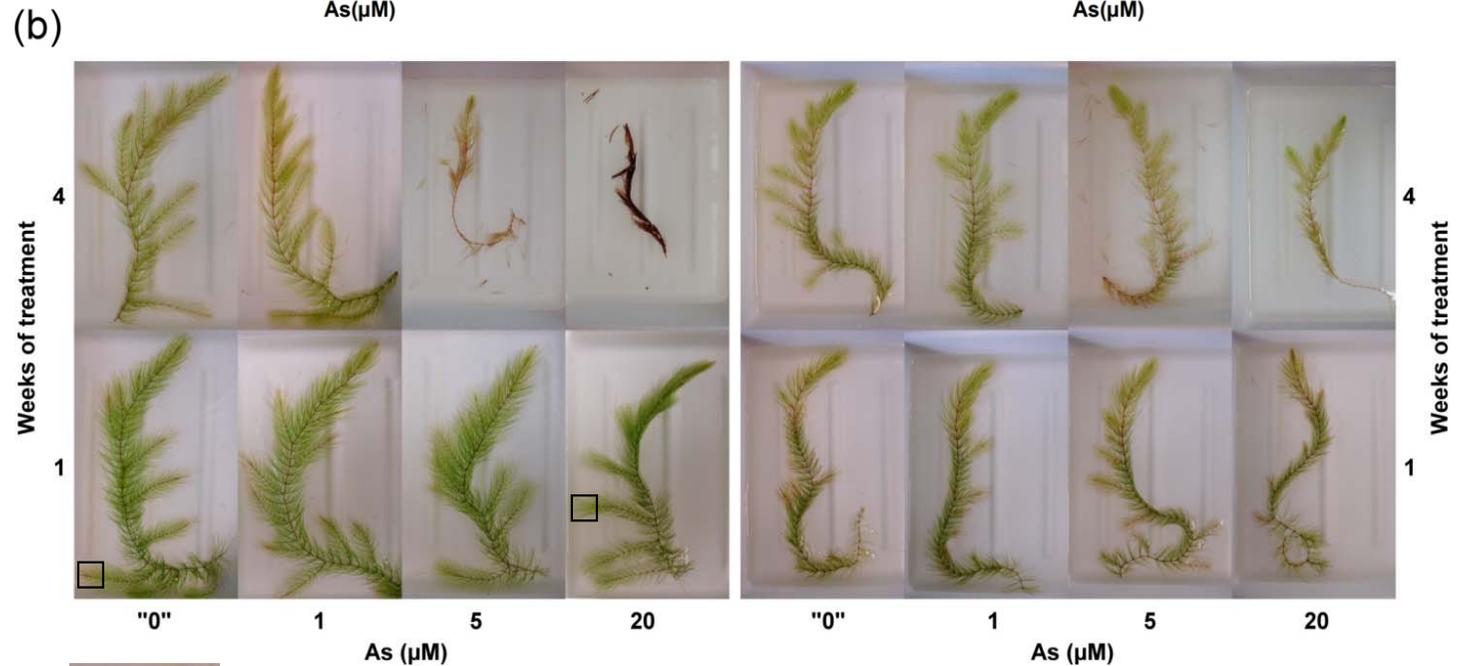
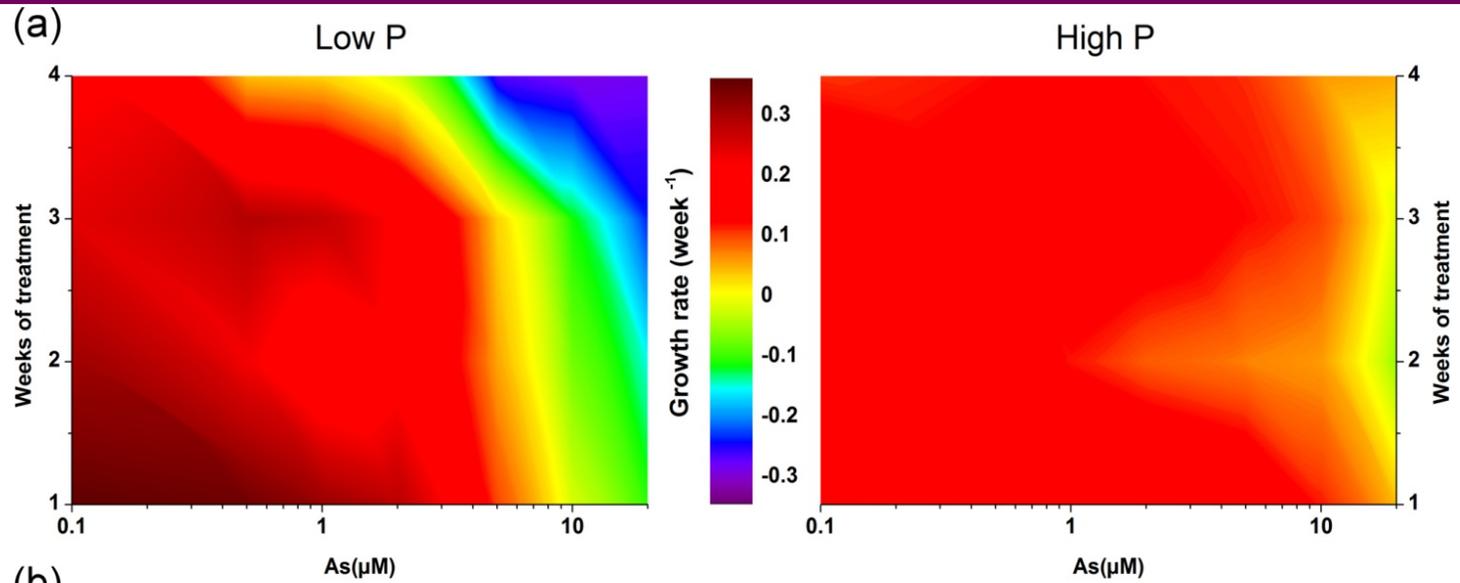
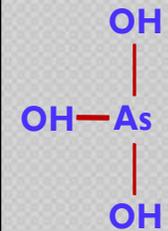
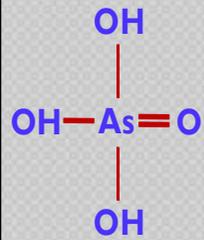


Simulation of environmental conditions



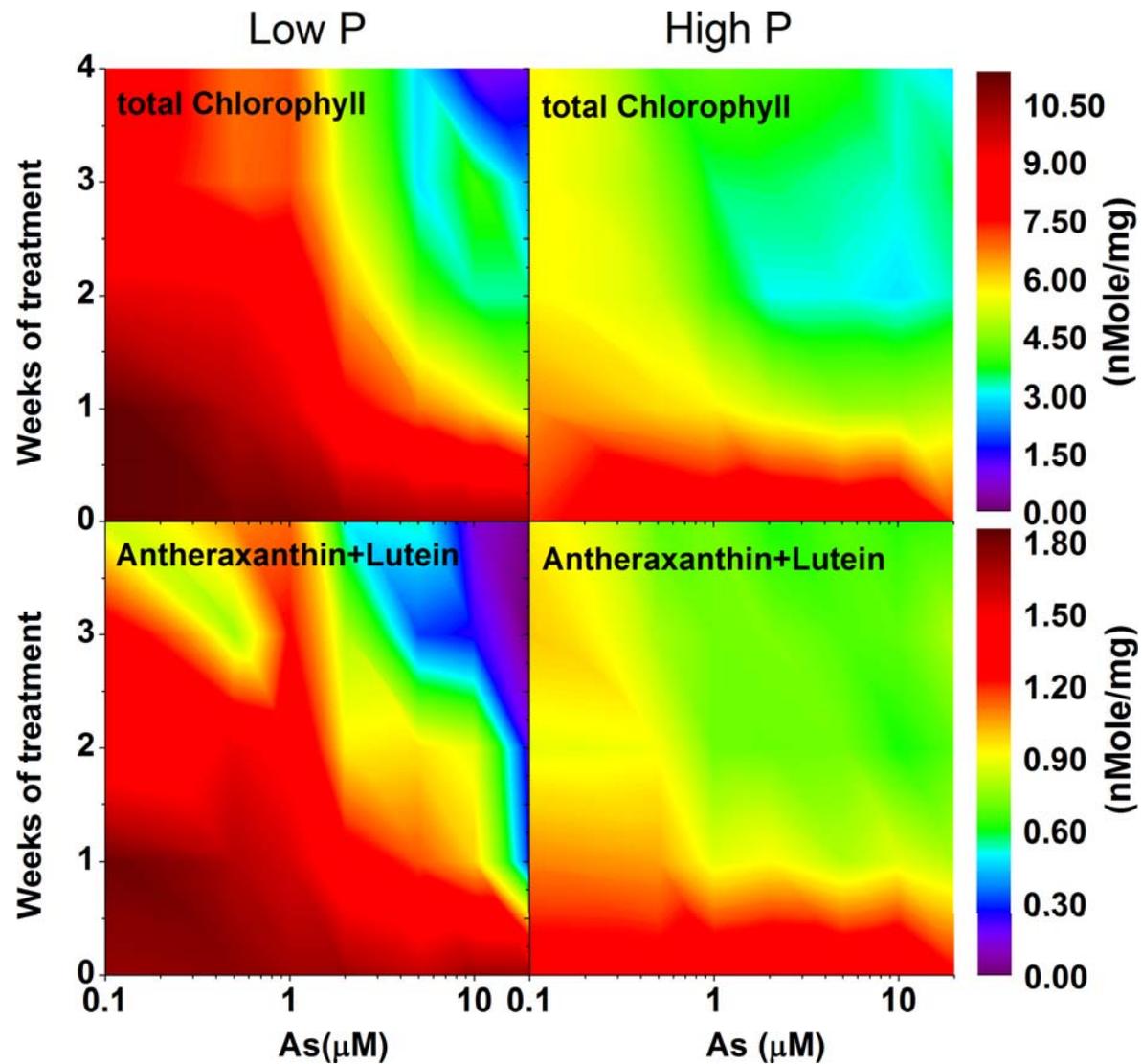
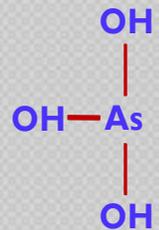
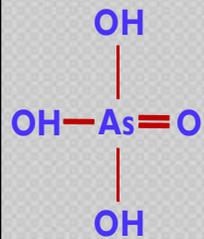
- Exposure to environmentally relevant arsenic concentrations (0.5 to 20 μM ; within the range of contaminated areas e.g. in India)
- Long exposure time of 4 weeks
- Sinusoidal light and temperature (18-24 $^{\circ}\text{C}$) cycles
- Low plant biomass to liquid ratio
- Continuous flow to achieve constant concentrations of each element
- Two environmentally relevant levels of phosphate

Growth rate of As exposed plants

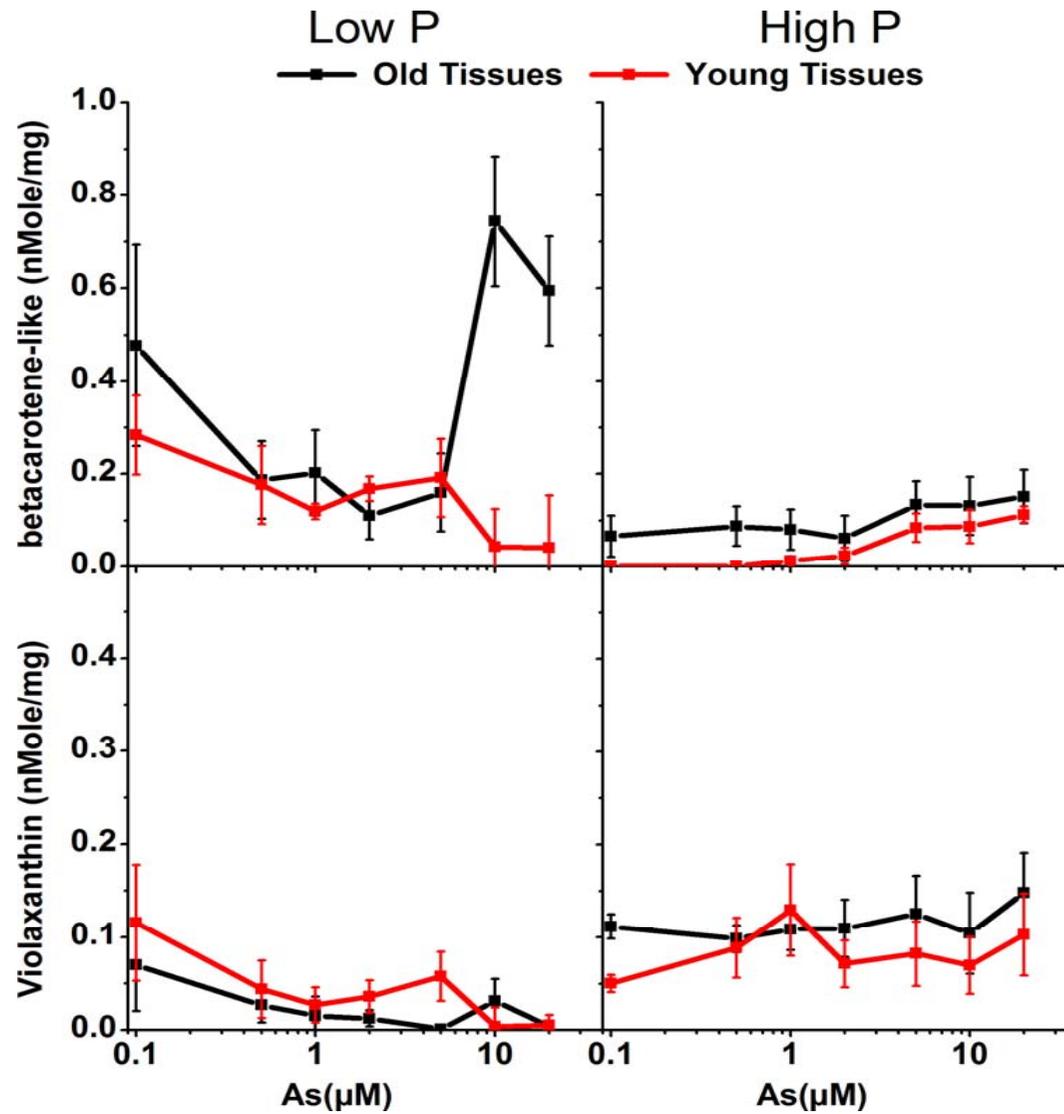


Mishra S, Stärk H-J, Küpper H (2014)
 Metallomics 6, 444-454

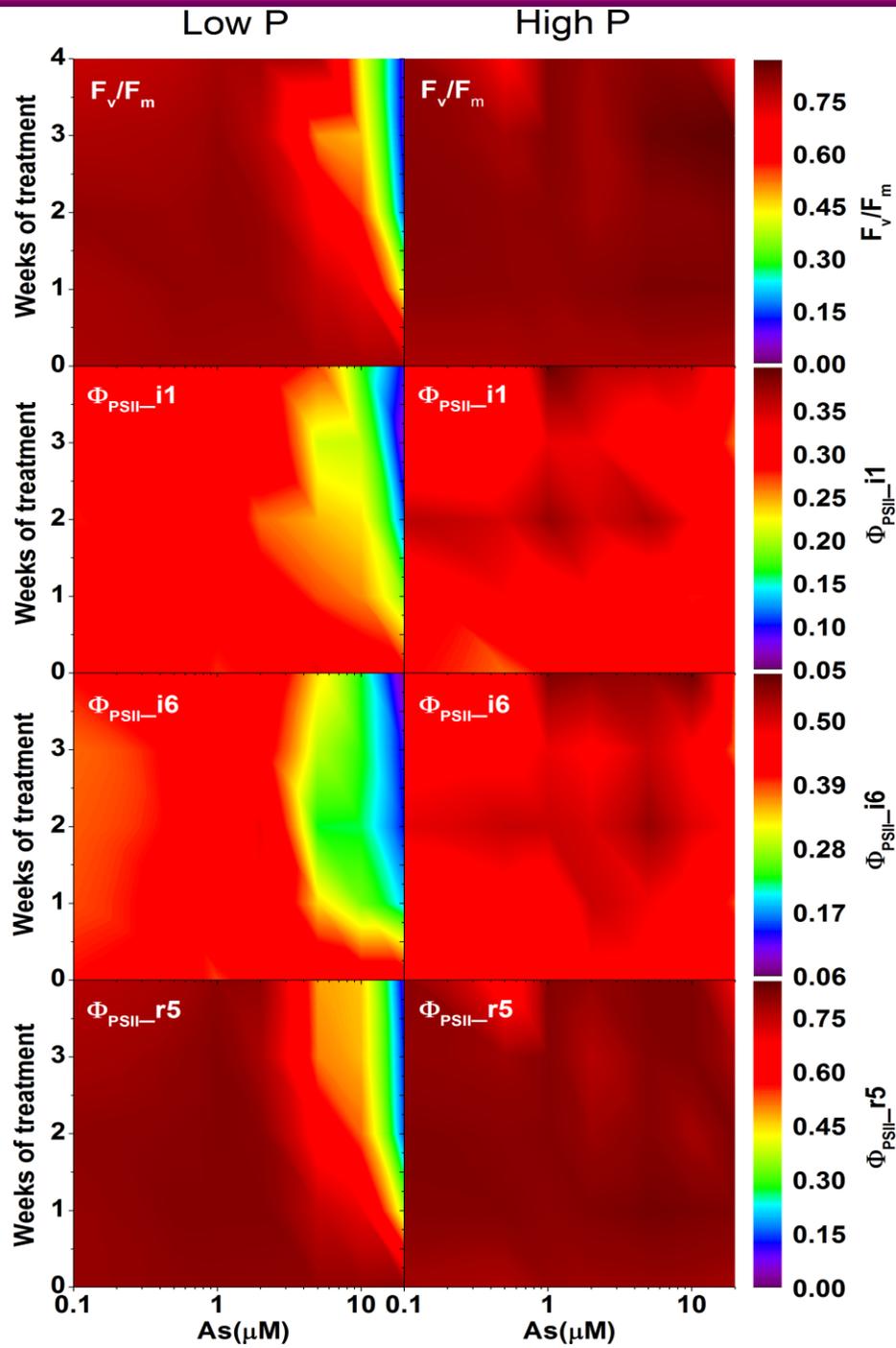
Effect of As on pigments



Effect of As on pigments

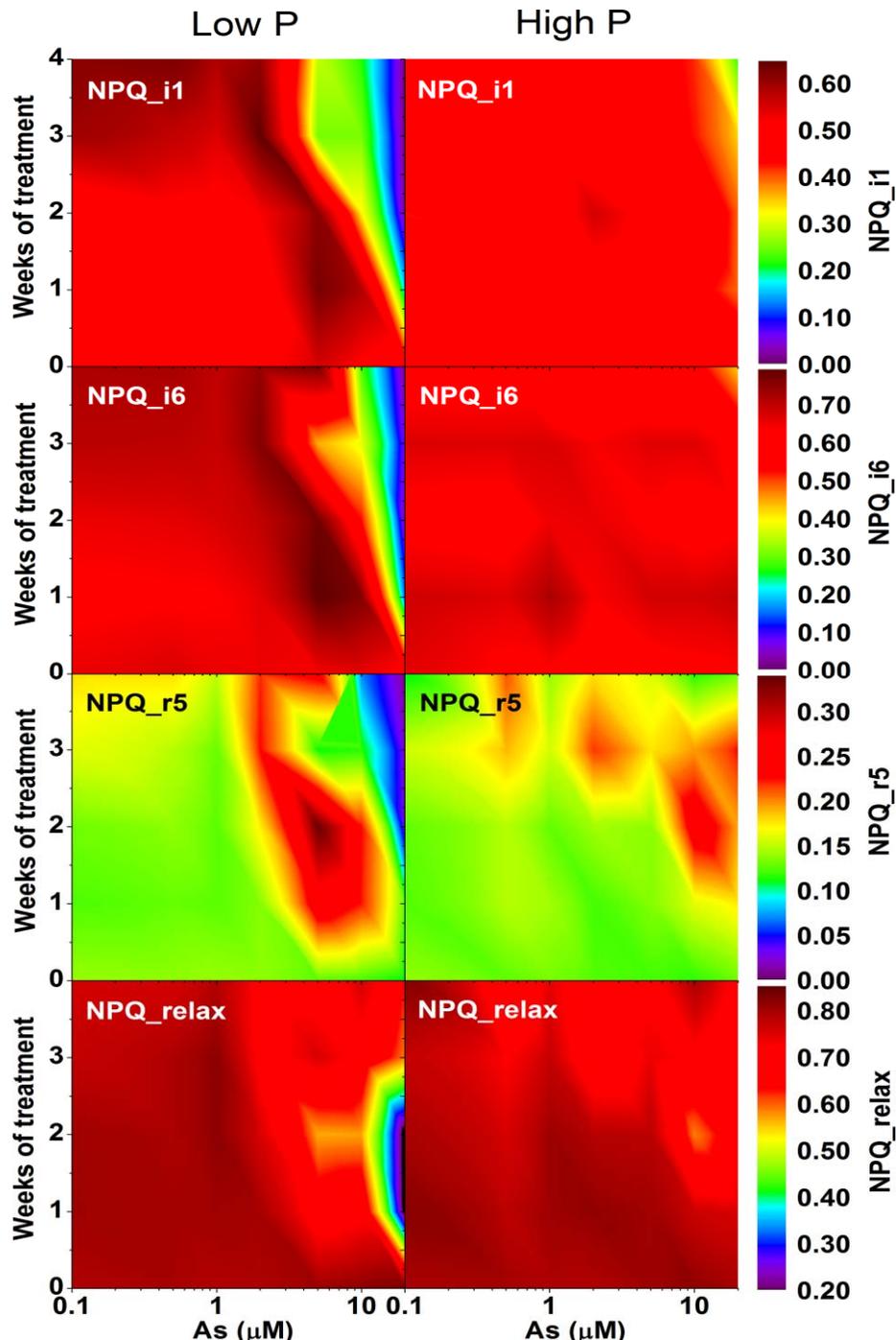


Effect of As on Photosynthetic Parameters



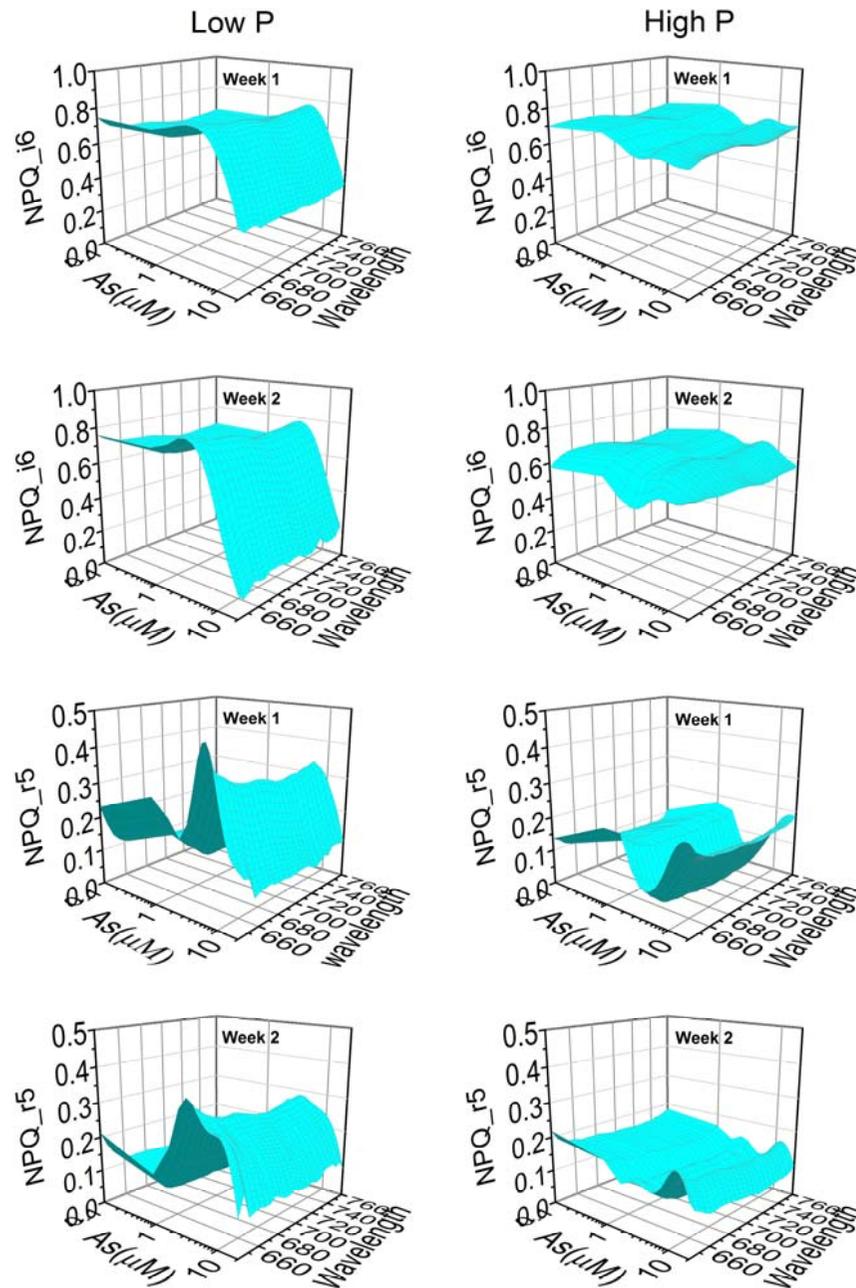
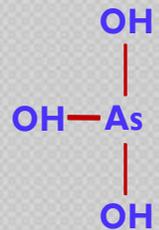
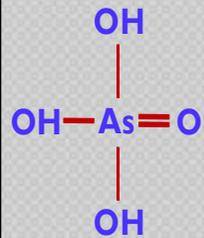
Effect of Arsenic on
photochemical parameters

Effect of As on Photosynthetic Parameters



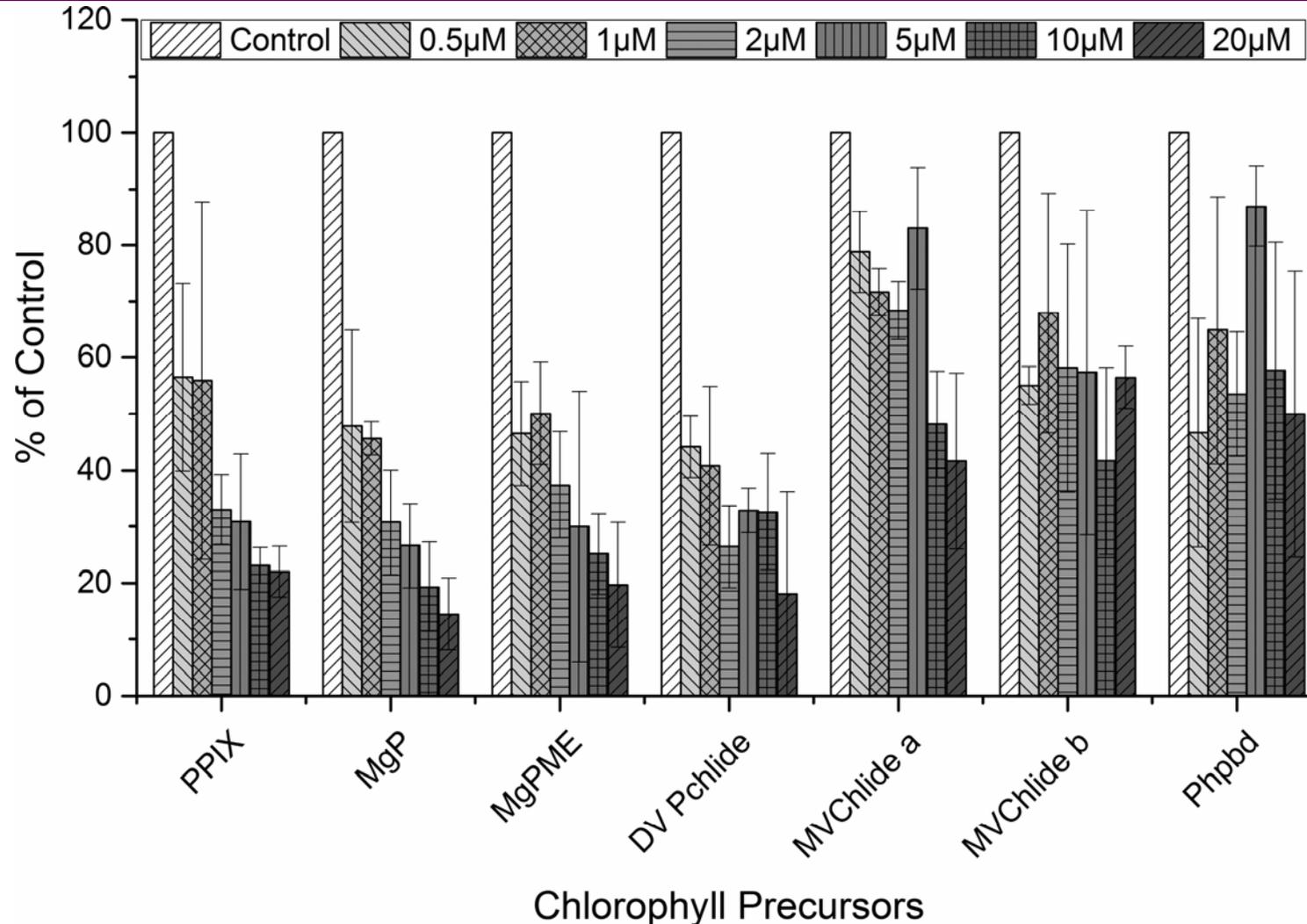
Effect of Arsenic on
non- photochemical parameters

Effect of As on Photosynthetic Parameters



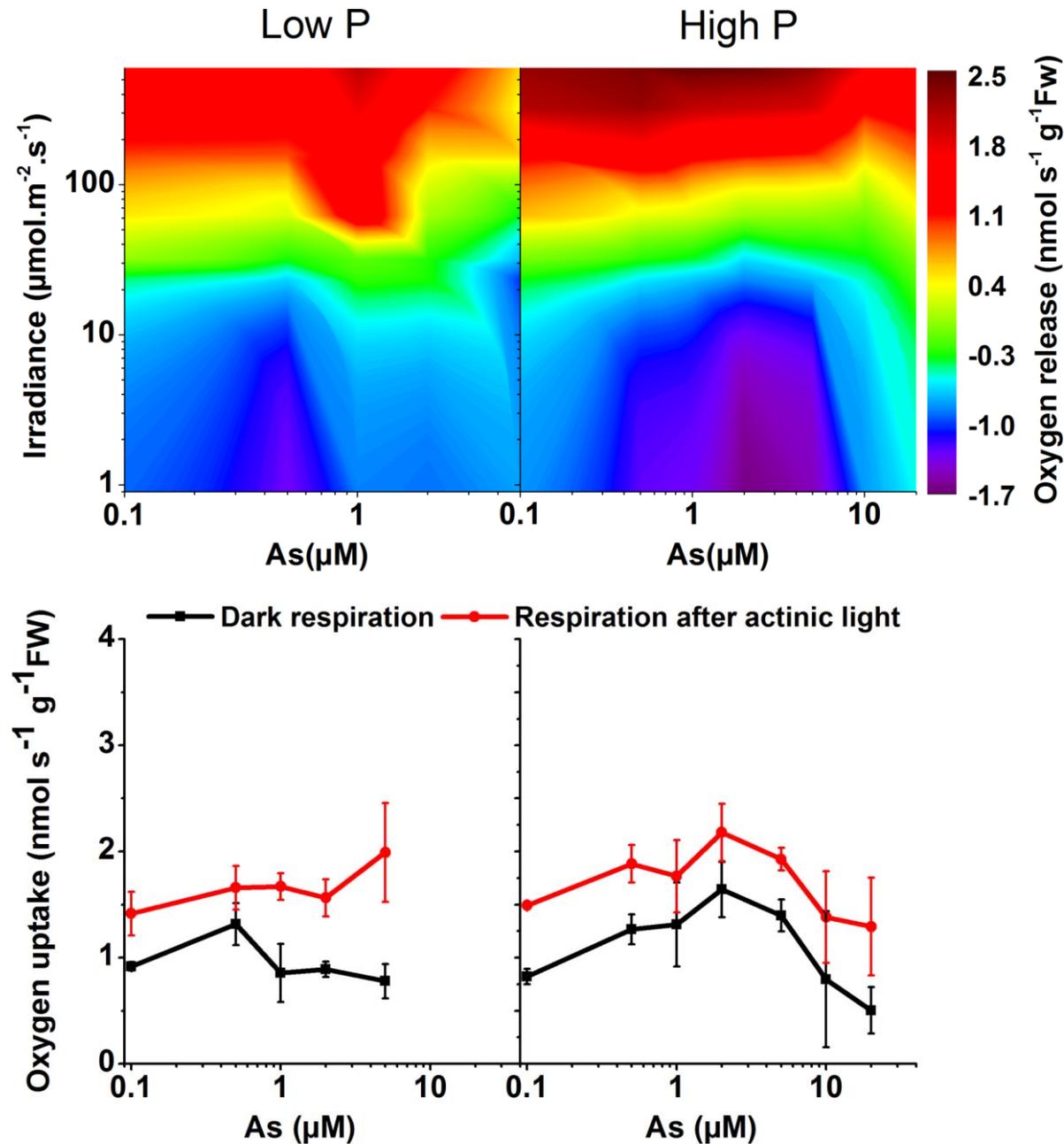
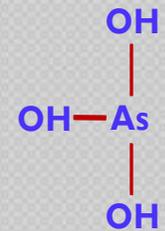
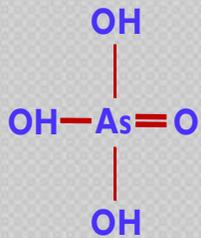
Effect of As on non-photochemical quenching measured in a spectrally resolved way

Effect of As on Chlorophyll Biosynthesis

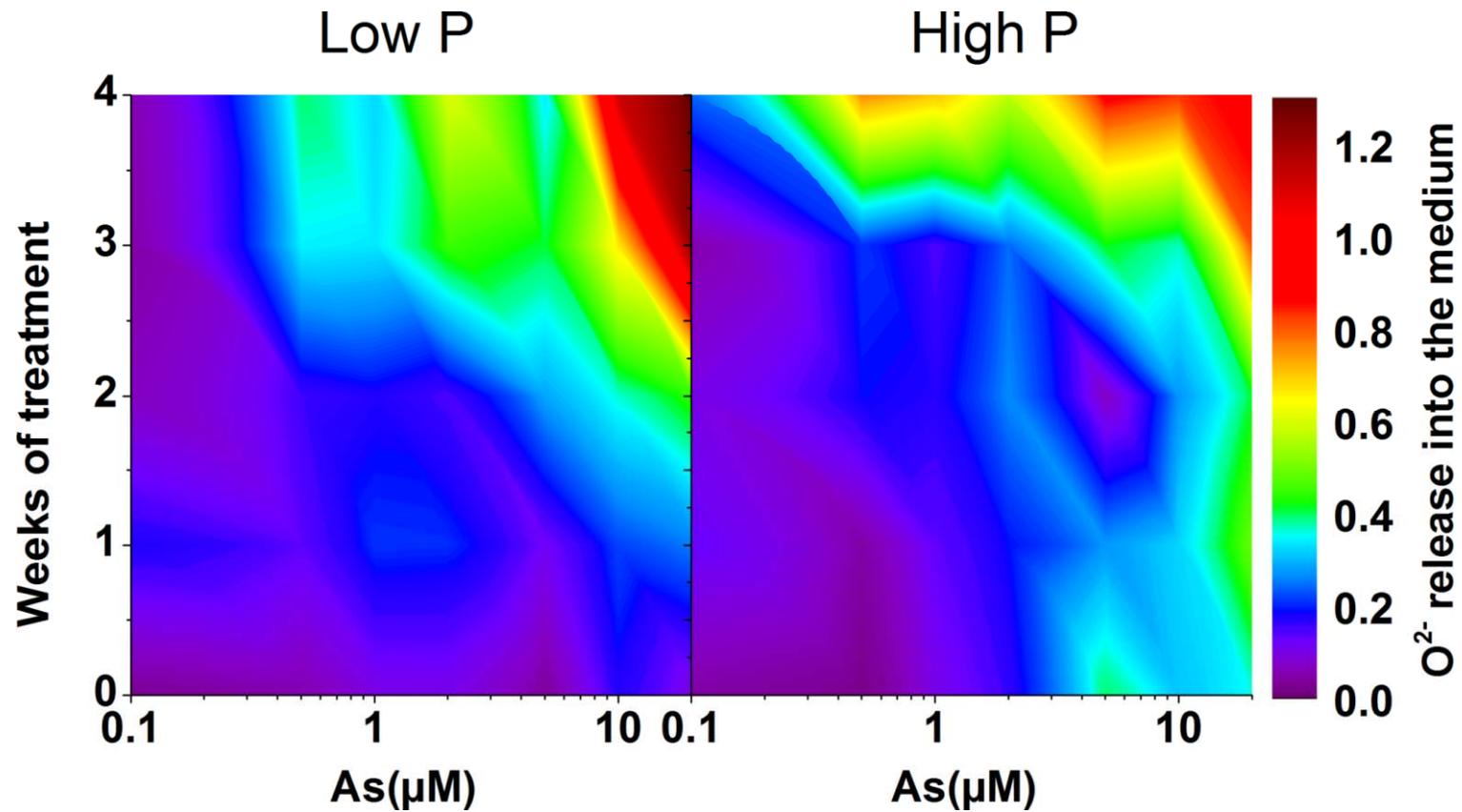


- A) Analysis of precursors of chlorophyll and degradation metabolites revealed that the observed decrease in chlorophyll concentration was associated with hindered biosynthesis, and was not due to degradation
- B) The results indicate that the pathway was blocked upstream of tetrapyrrole synthesis.

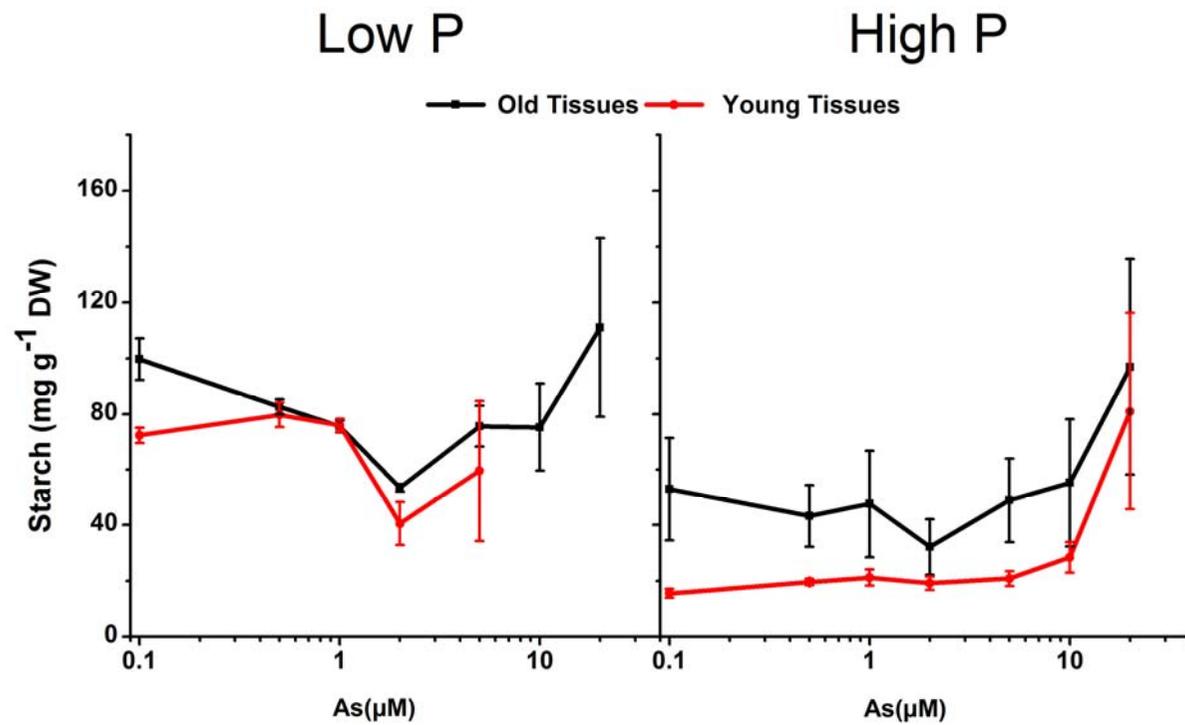
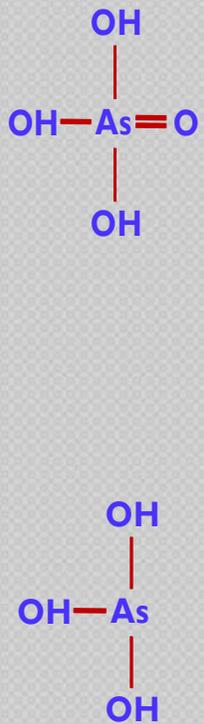
Effect of As on photosynthesis and respiration



Production of Superoxide upon As exposure



Effect on level of starch

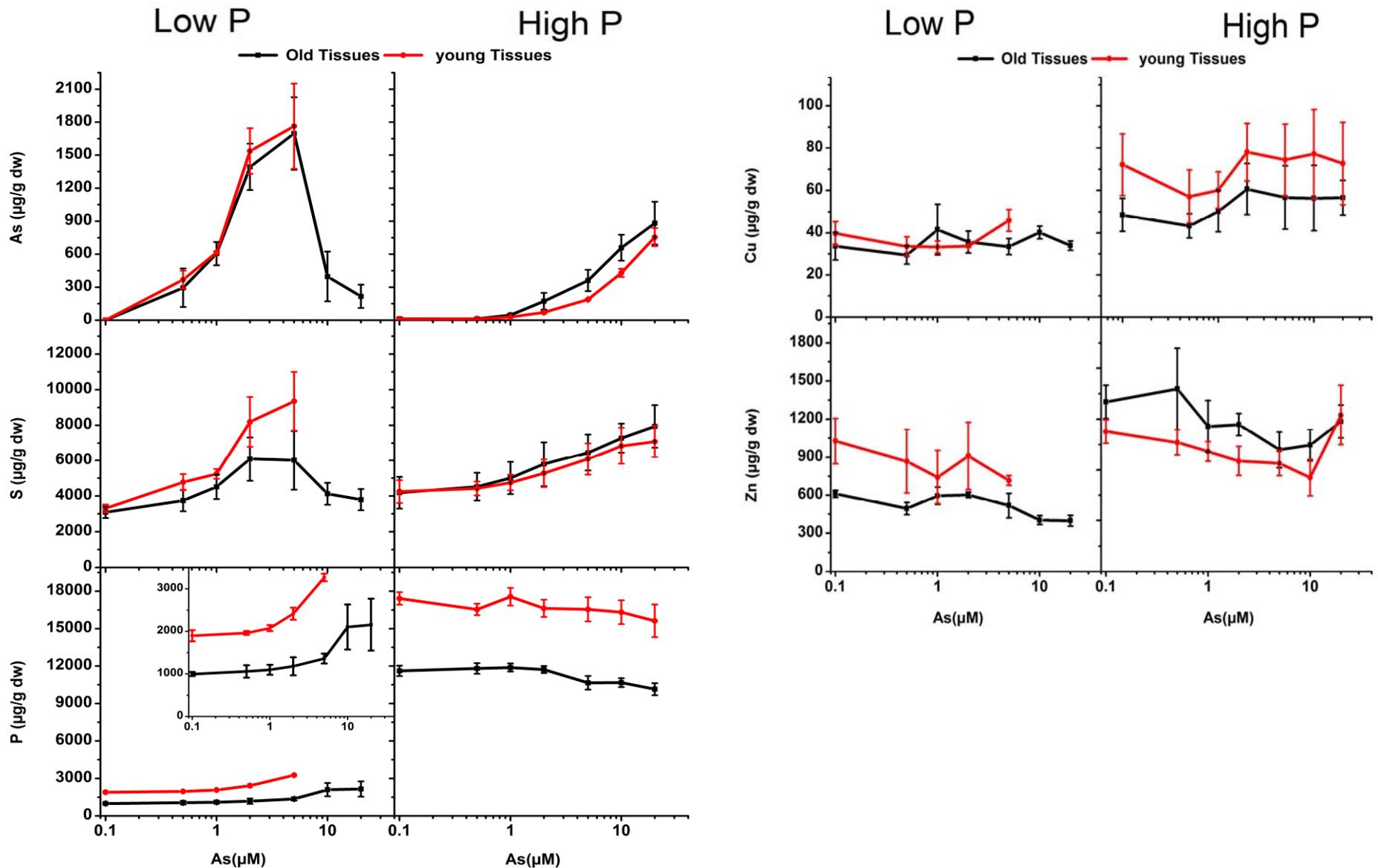
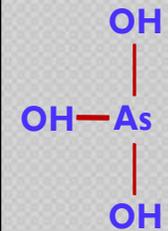
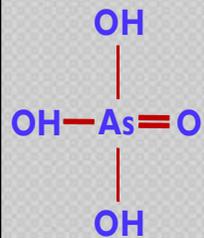




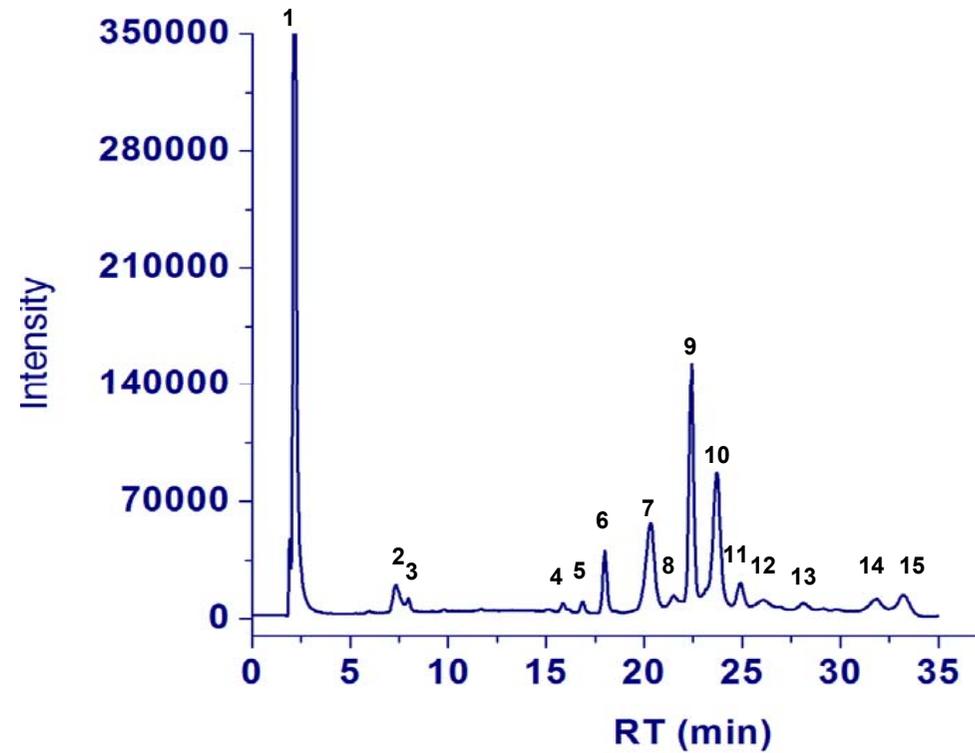
Accumulation, Distribution & Speciation of Arsenic in *C. demersum*

(HPLC-ICP-MS-ESI-MS, μ -XRF, μ -XANES)

Elemental analysis of As exposed plants

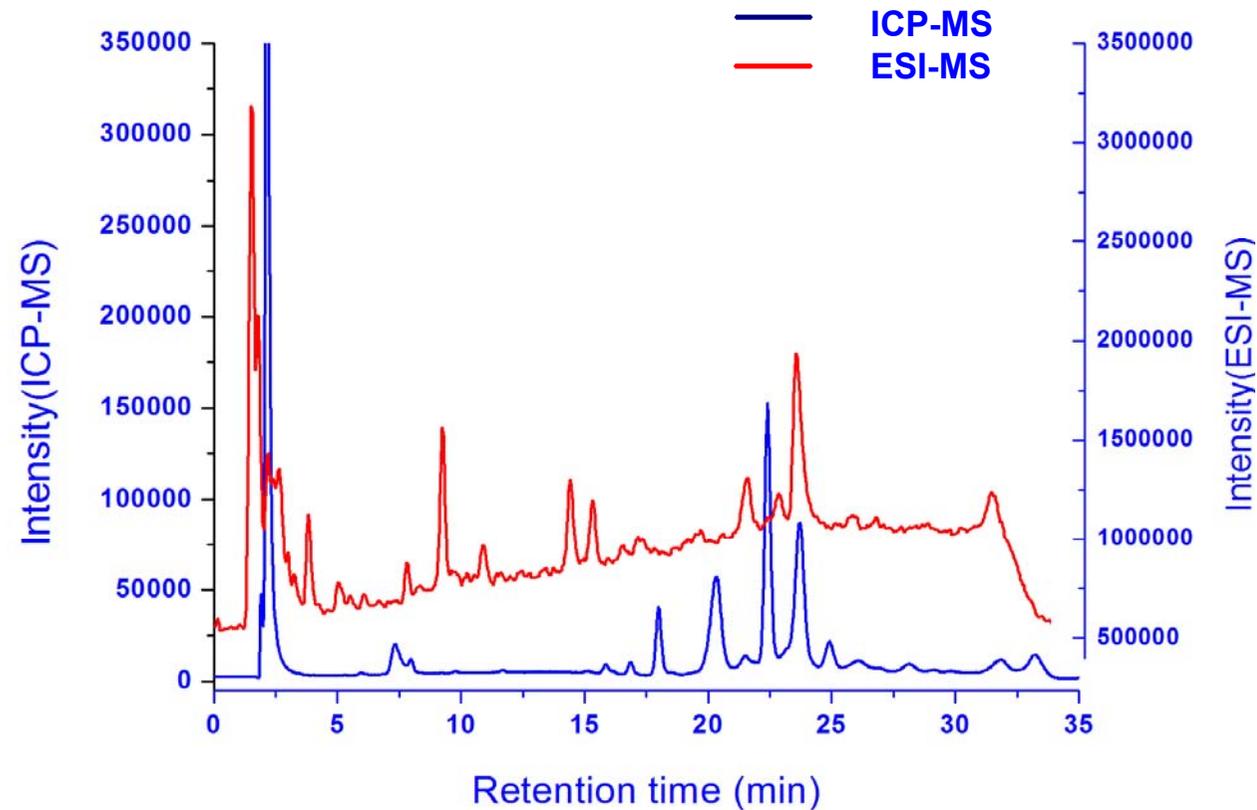


As Speciation Analysis



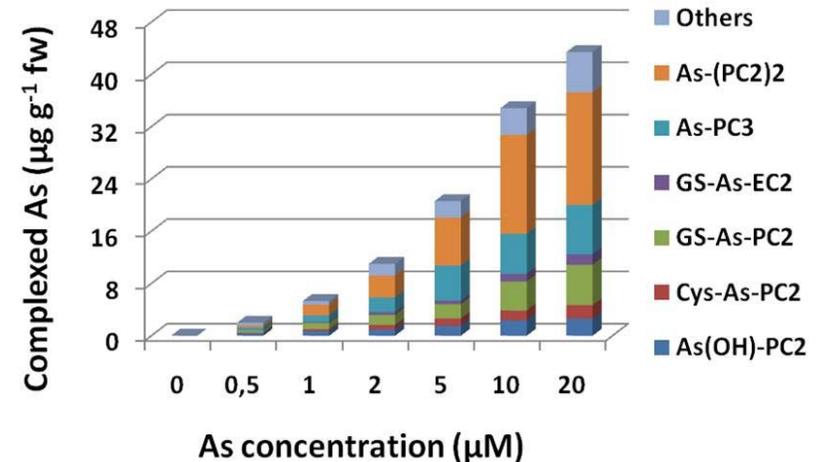
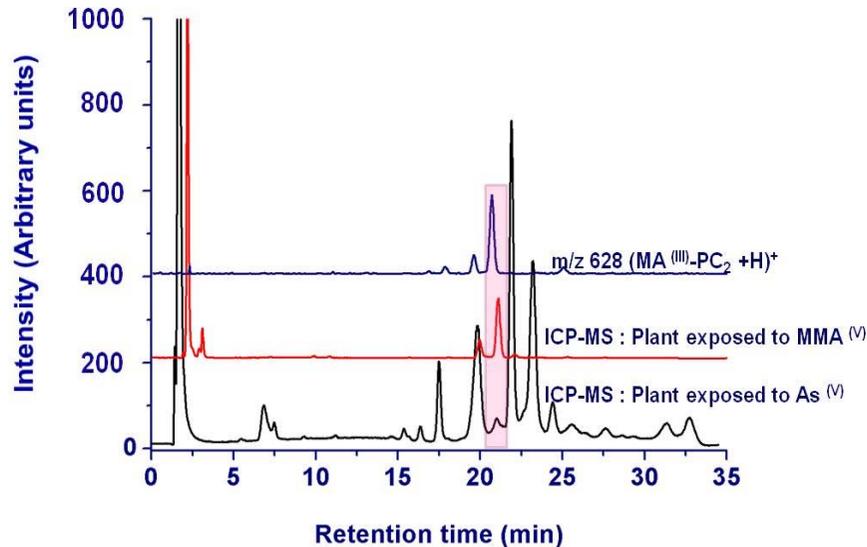
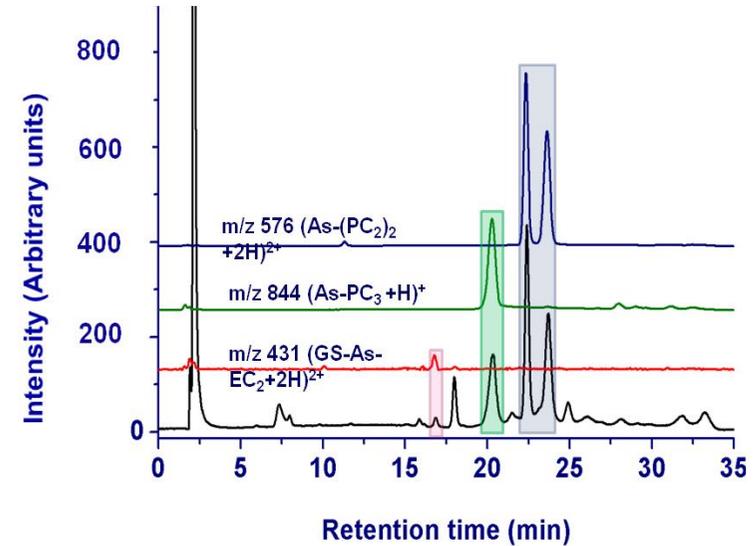
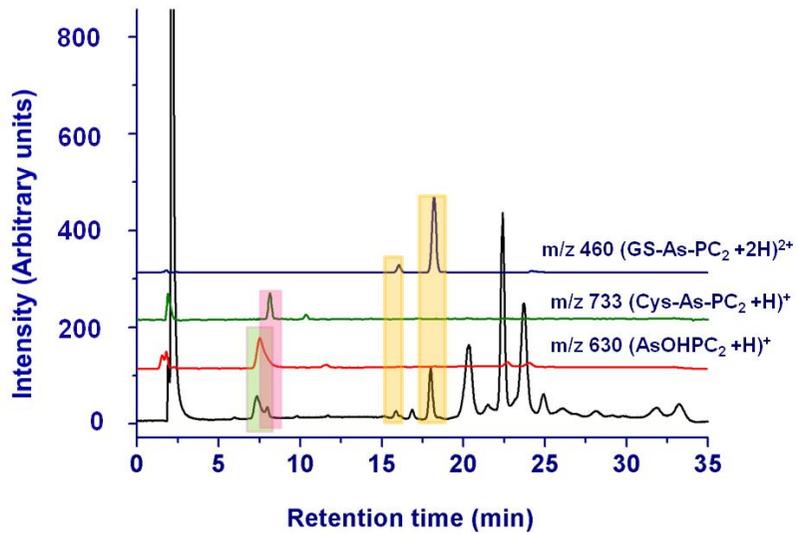
Speciation of As in Plant extract through RP-HPLC (C18) coupled to ICP-MS

As Speciation Analysis



HPLC-ICP-MS and ESI- MS (scan mode) chromatograms of As exposed plant extract

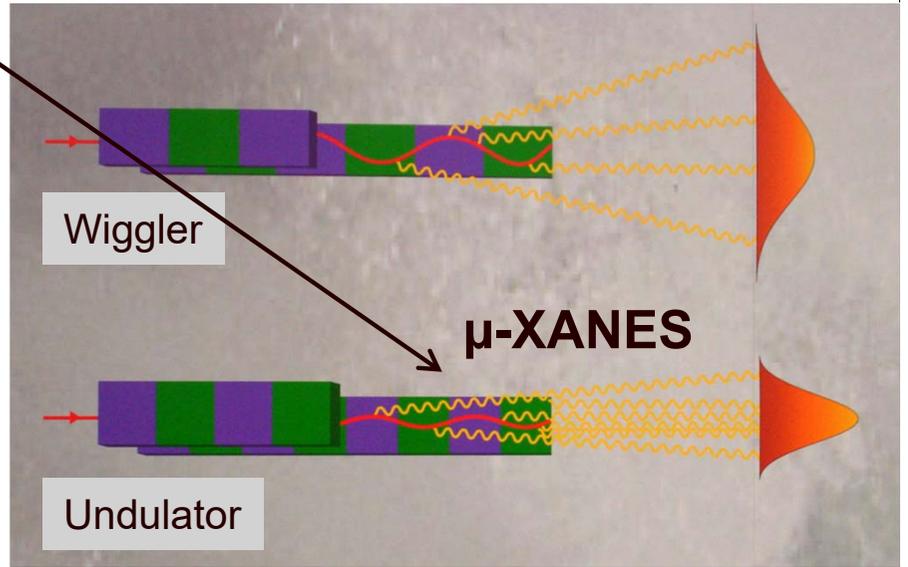
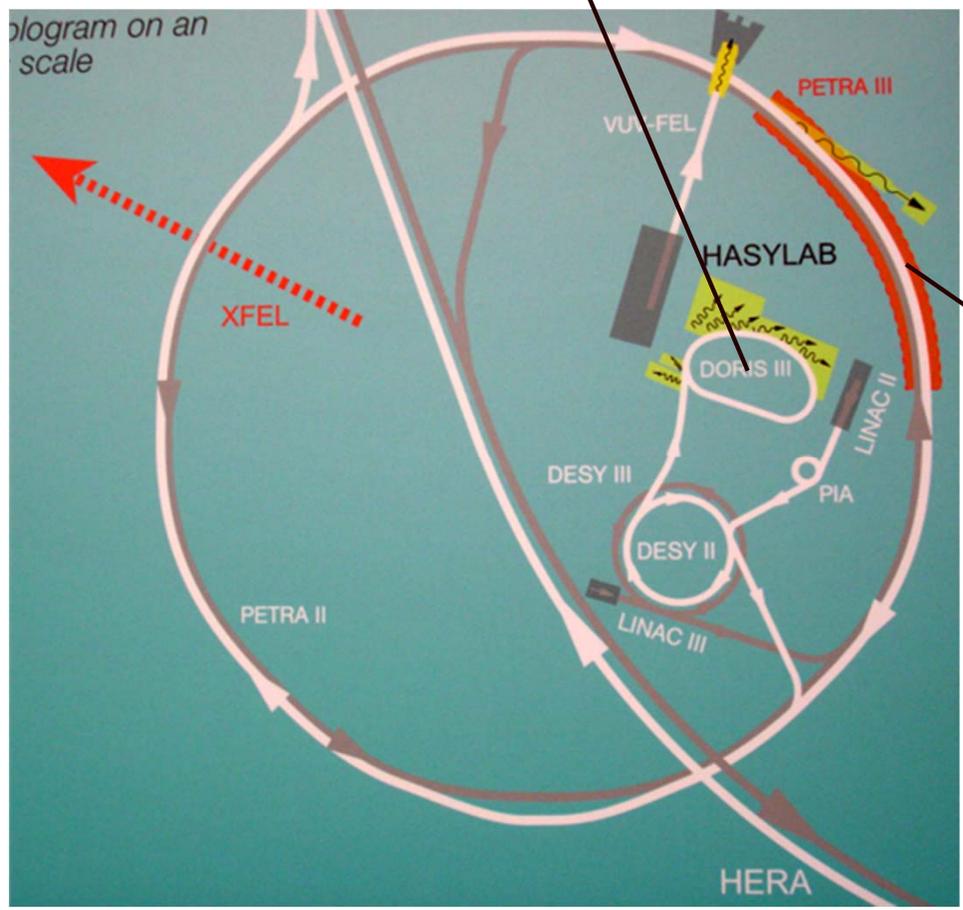
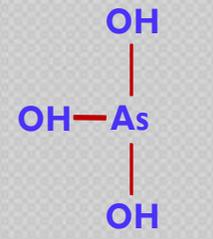
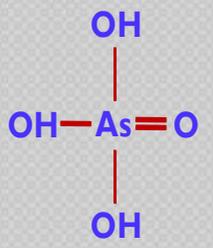
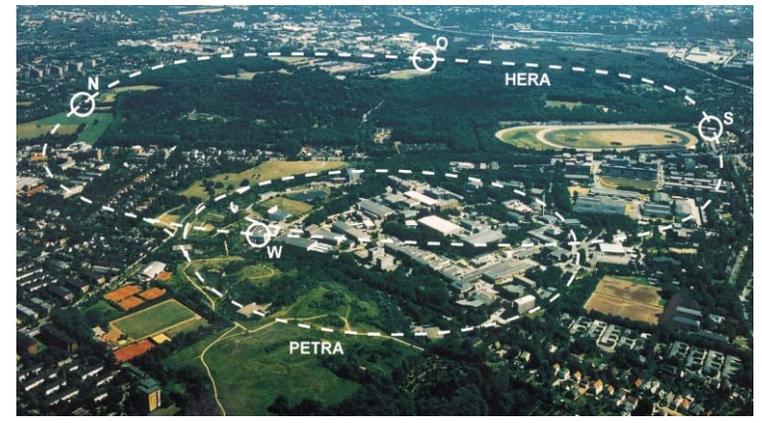
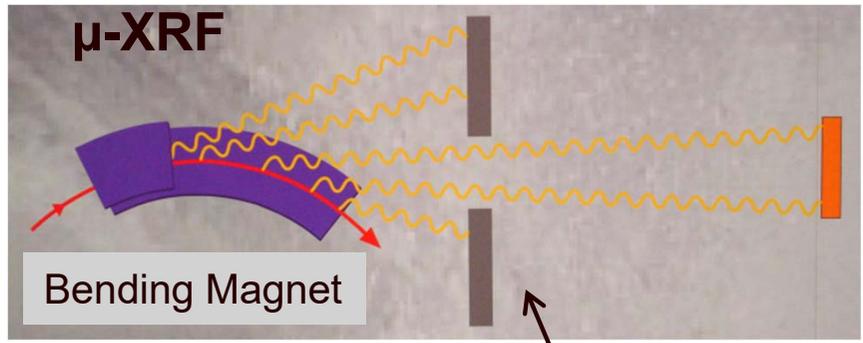
As Speciation Analysis



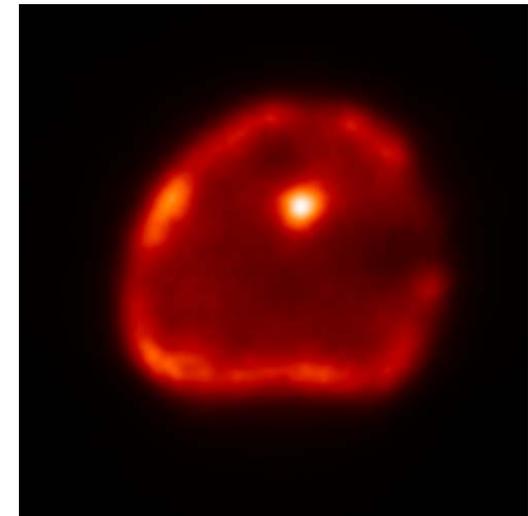
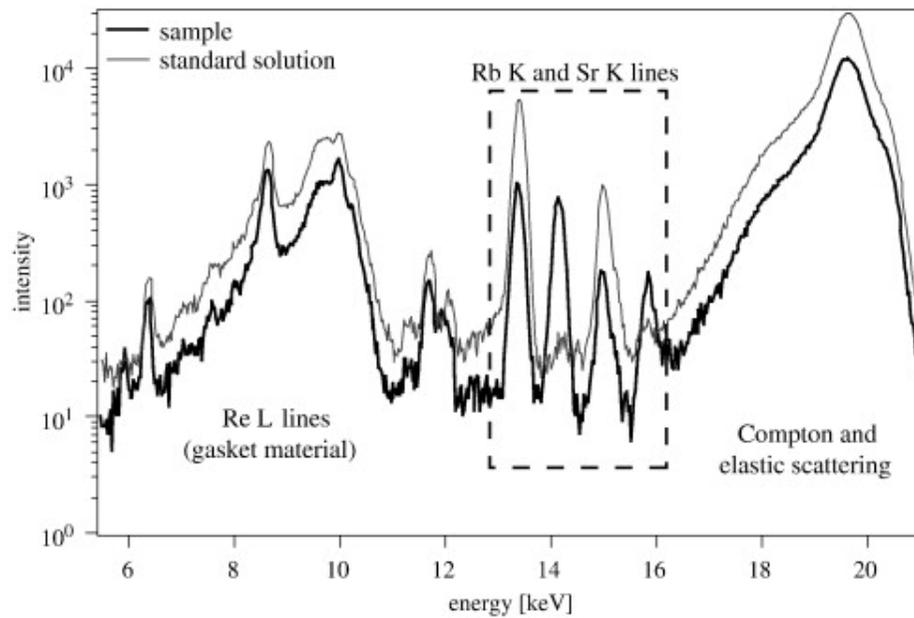
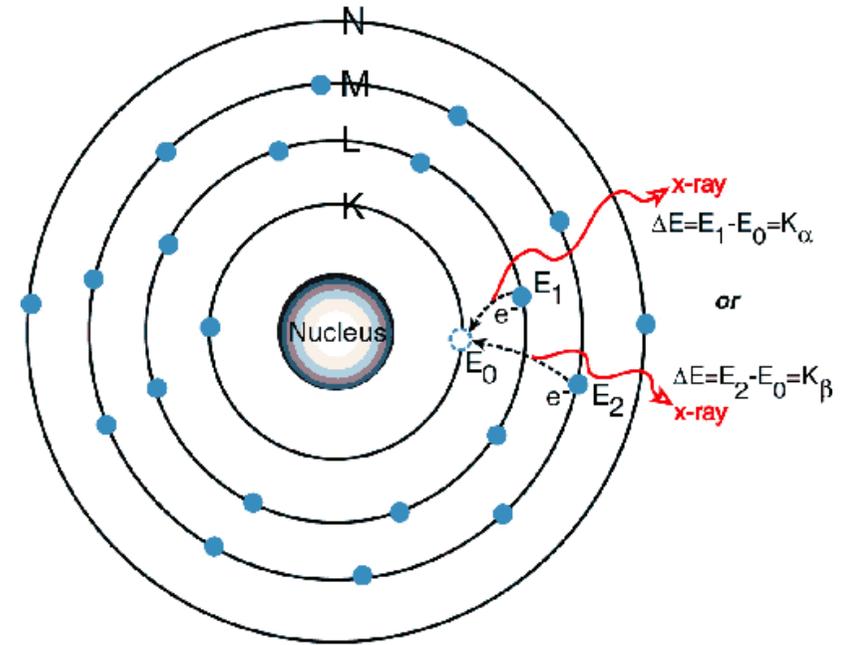
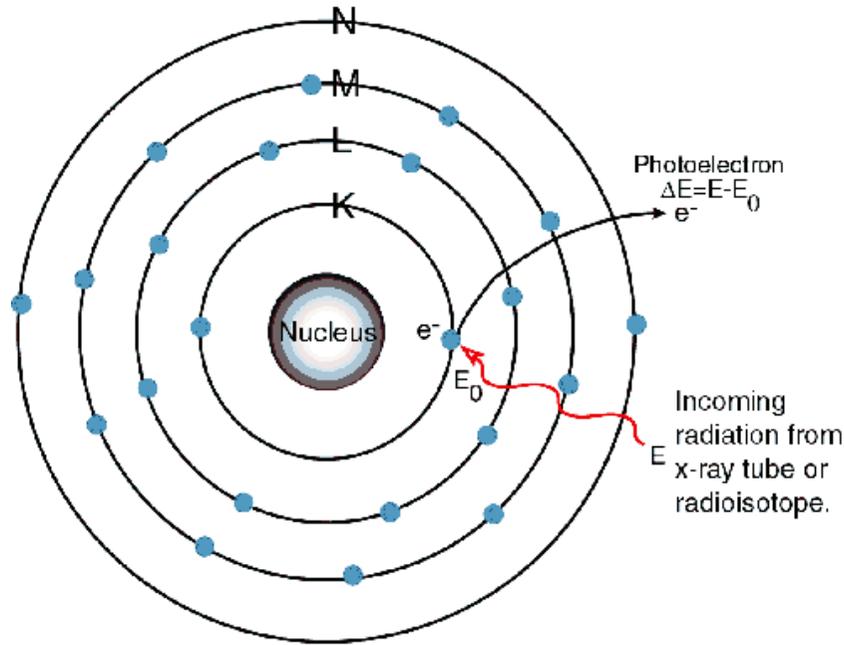
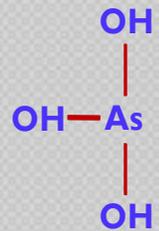
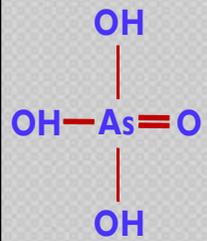
Separation of As-PC complexes; m/z traces of As-PC species measured by ESI-MS and m/z 75 (As) measured by ICP-MS

Quantification of various As species (based on ICP-MS data)

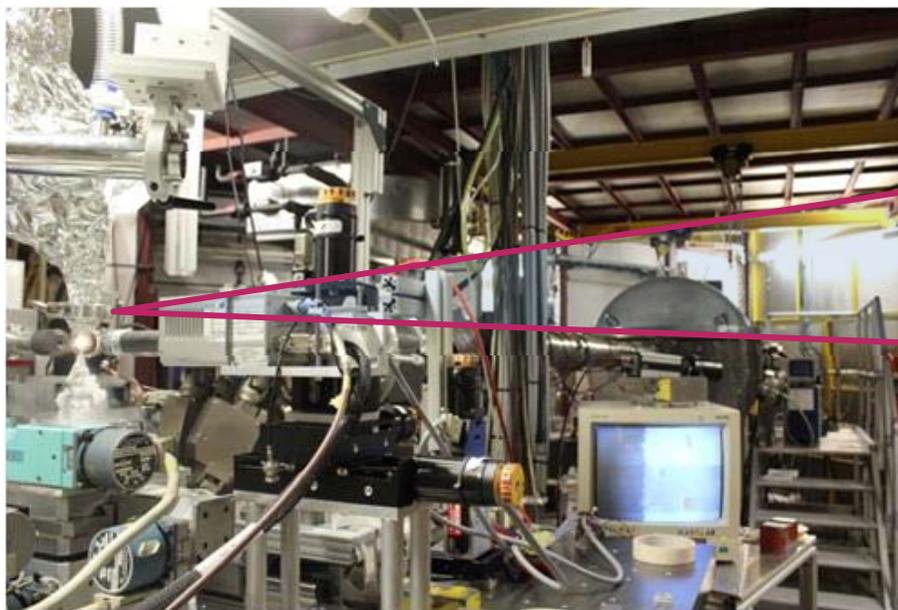
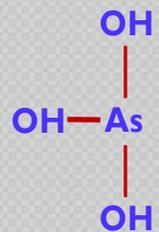
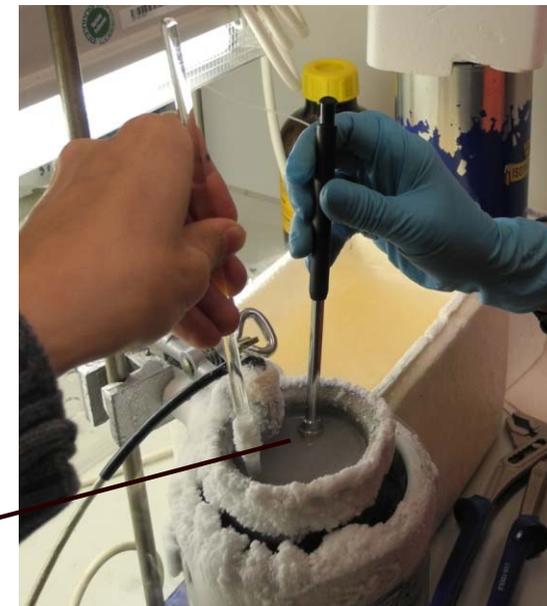
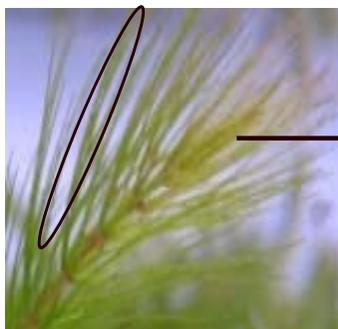
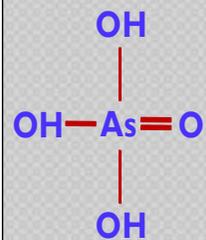
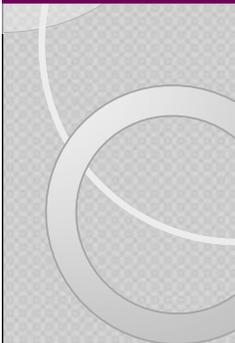
μ -XRF and μ -XANES



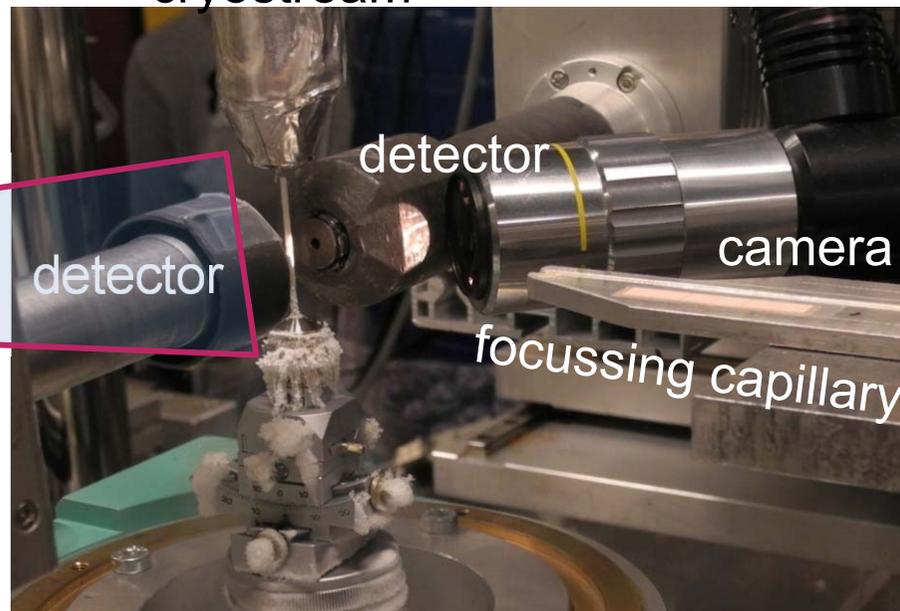
μ -XRF



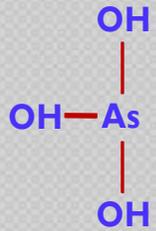
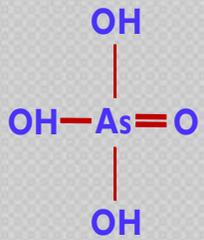
Sample preparation and instrumental setup for μ -XRF and μ -XANES



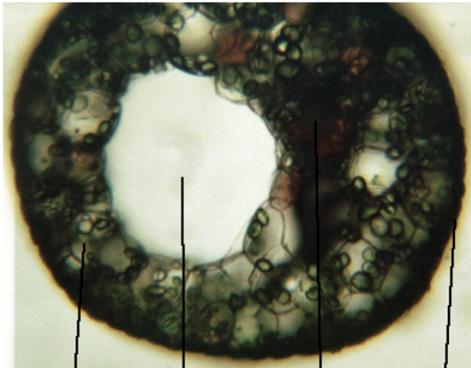
cryostream



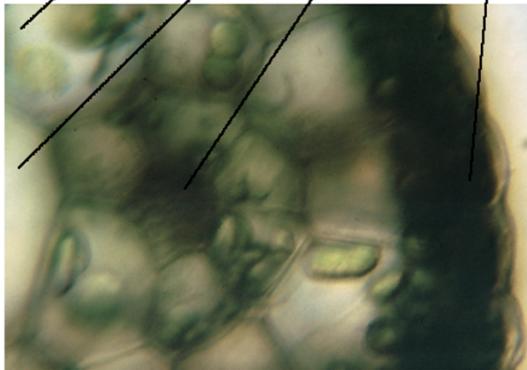
Distribution of As and its effect on Copper and Zinc



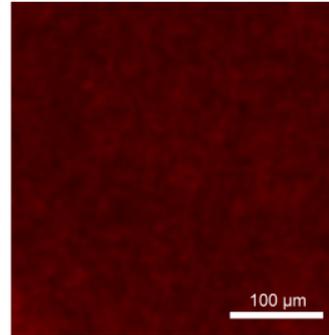
microscopic image of control leaf



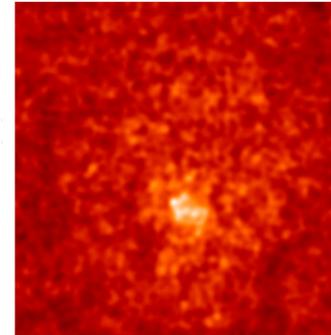
mesophyll
central cavity
vein
epidermis



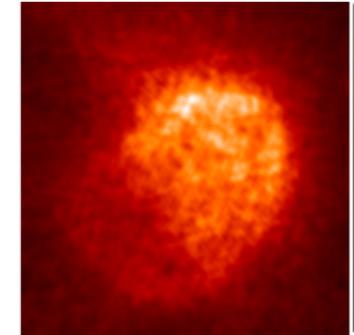
0 μM
yml



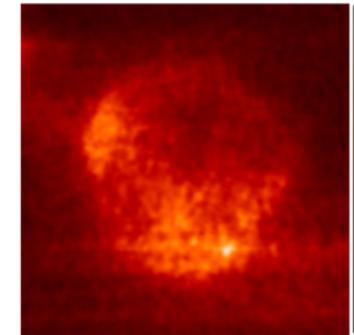
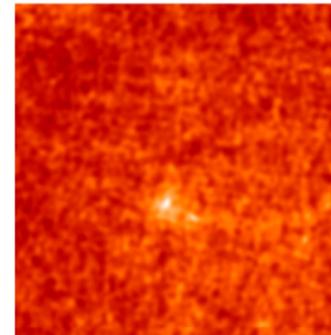
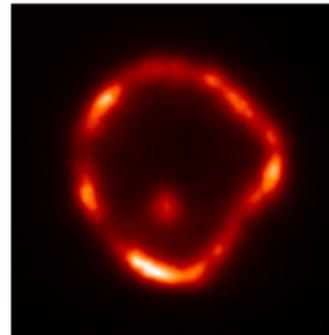
Cu



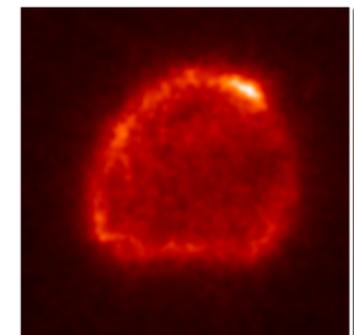
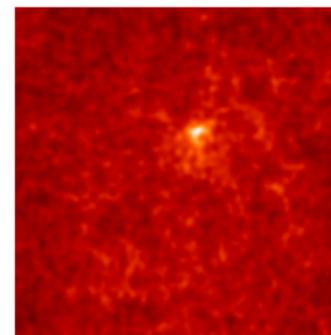
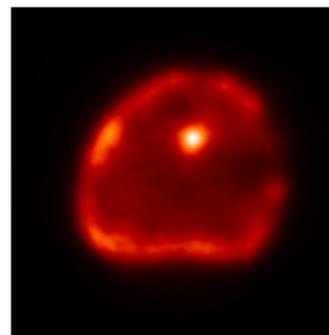
Zn



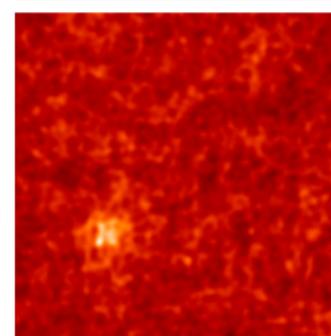
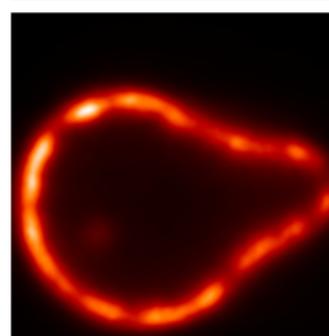
1 μM
yml



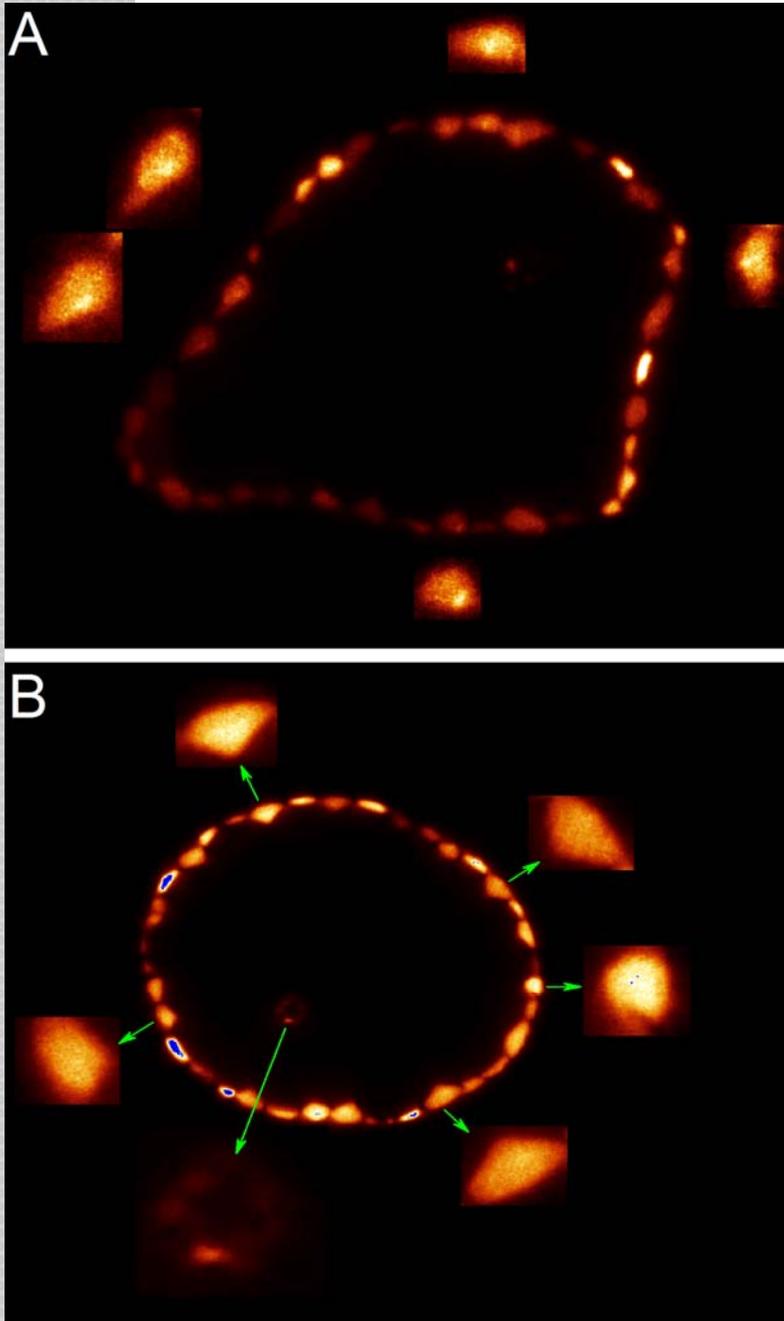
5 μM
yml



5 μM
ml



Sub-cellular distribution of As in *C. demersum* leaves



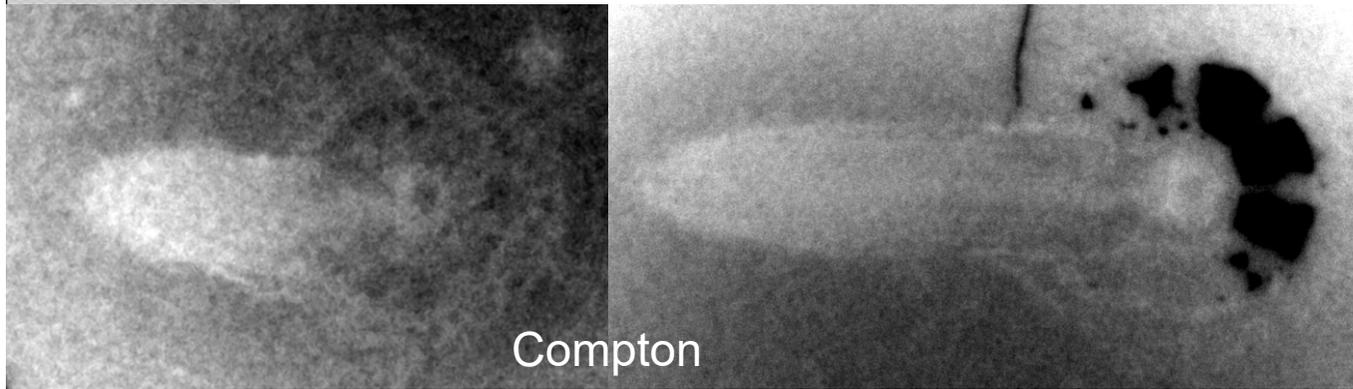
2 phase response to As toxicity

- A) Initially, at sublethal concentrations (1 μM As), As is accumulated mainly in the nucleus
→ genotoxicity (besides inhibition of Chl biosynthesis)

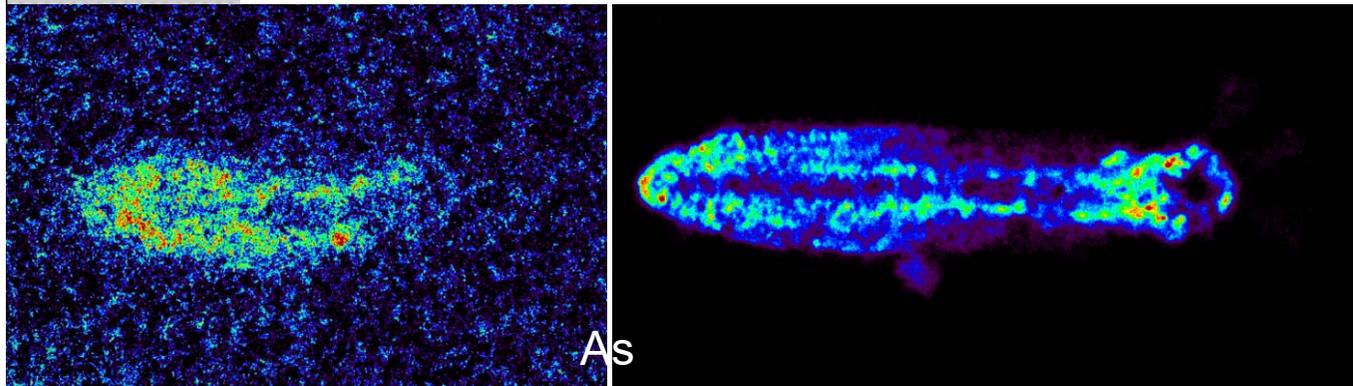
- B) At lethal concentration (5 μM As), As fills the whole cell
→ various types of damage

As distribution in rice roots

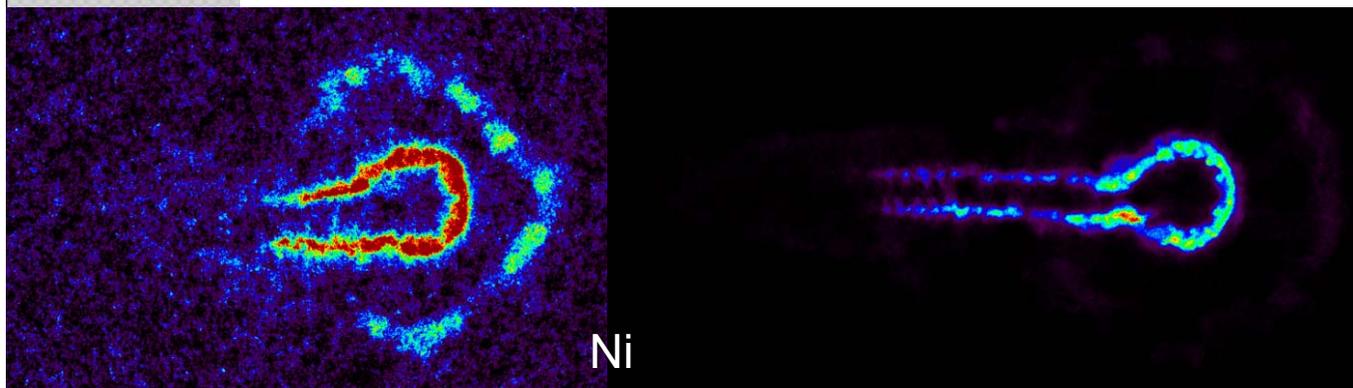
1



Compton



As



Ni

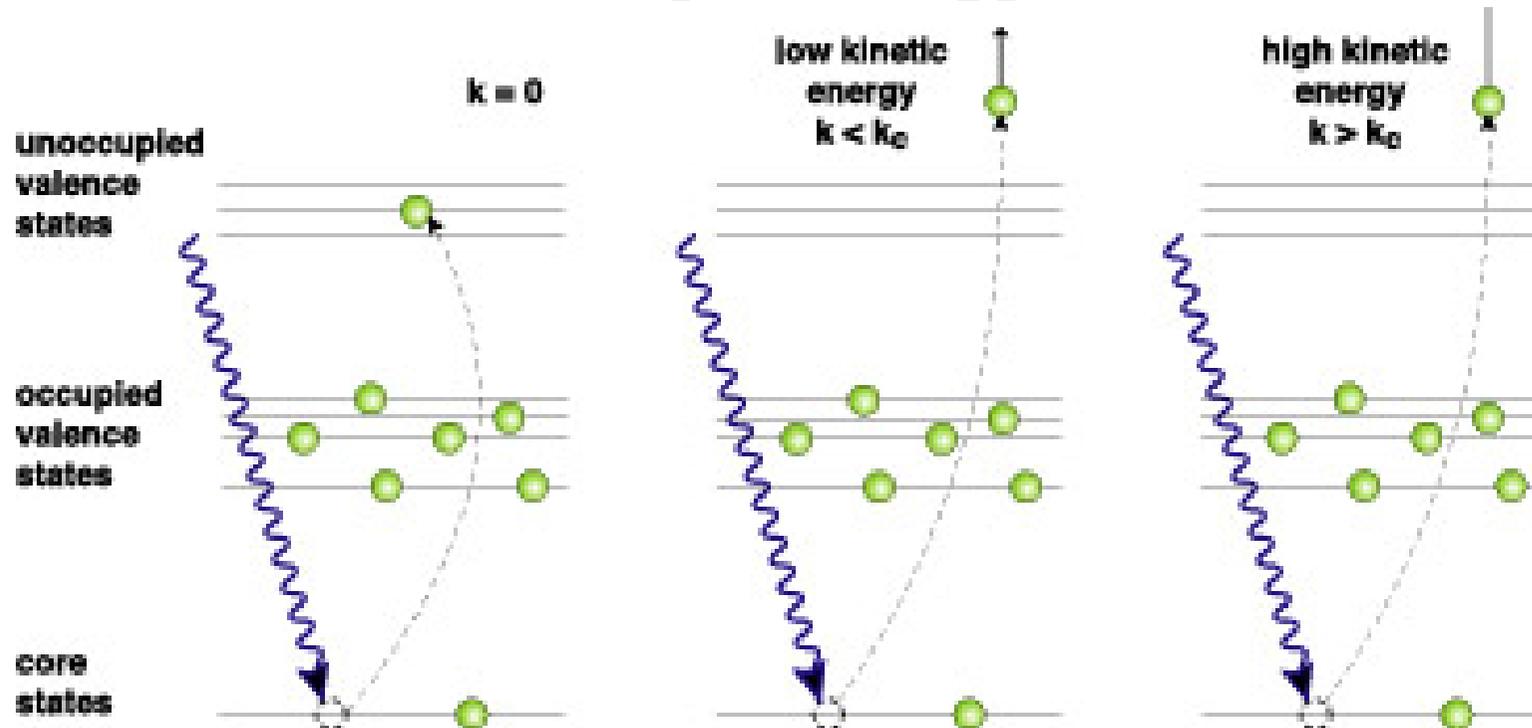
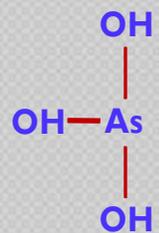
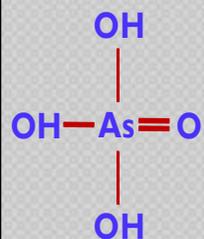
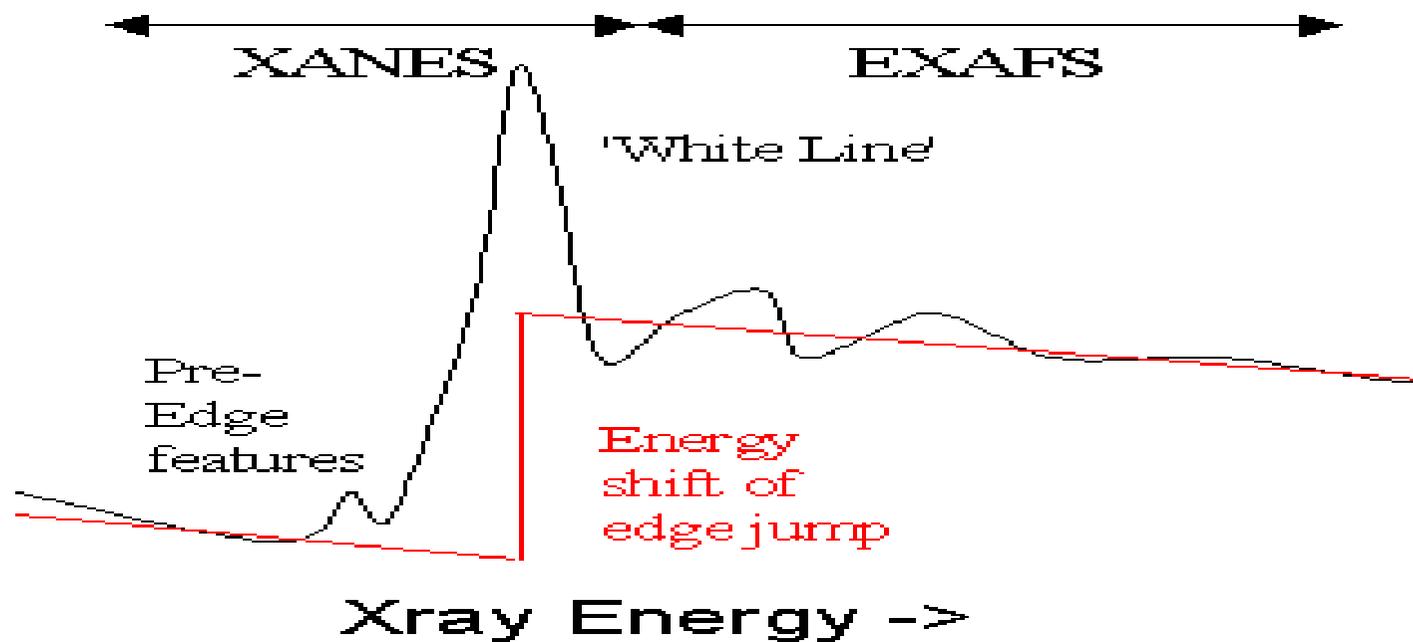
2 phase response to As toxicity

- A) Initially, at low sublethal concentrations, As is accumulated mainly in the root tip → toxicity mainly to dividing new cells
- B) At higher but still sublethal concentrations, As is accumulated in more tissues → various types of damage

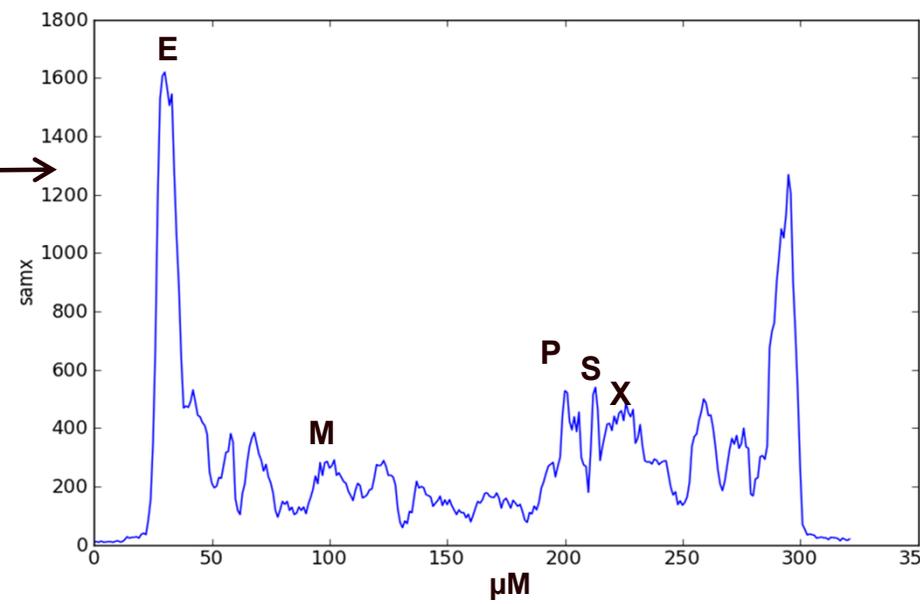
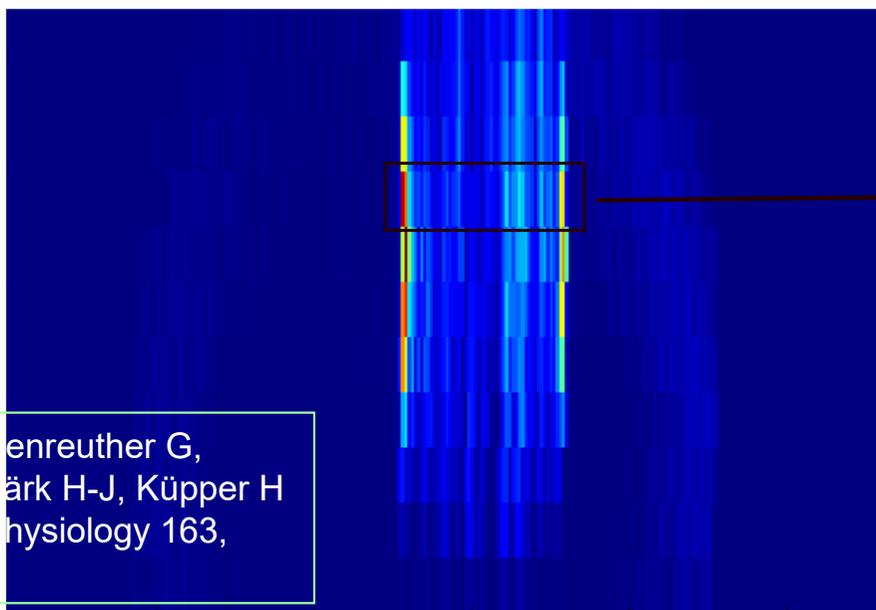
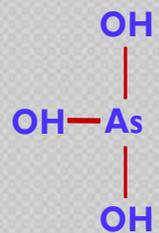
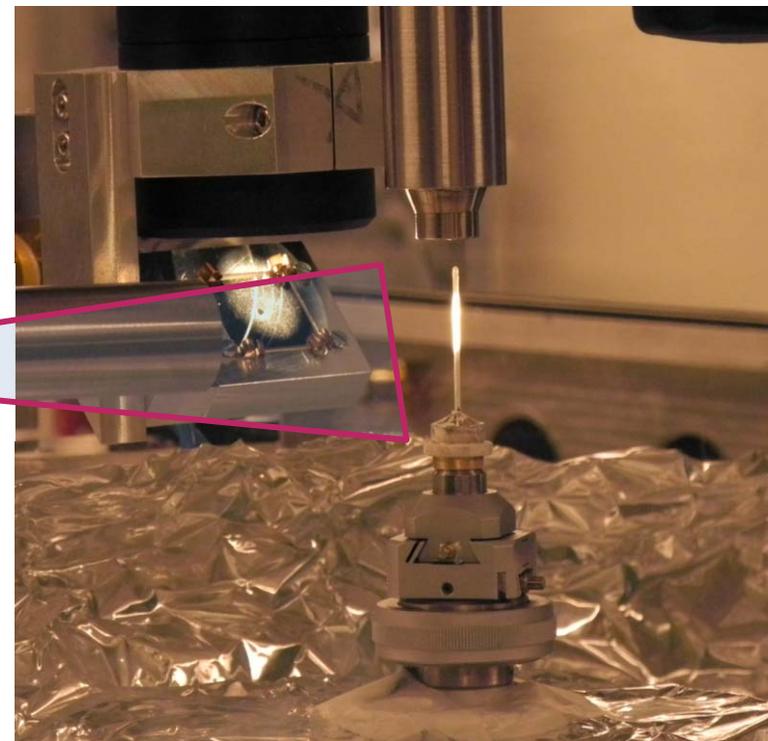
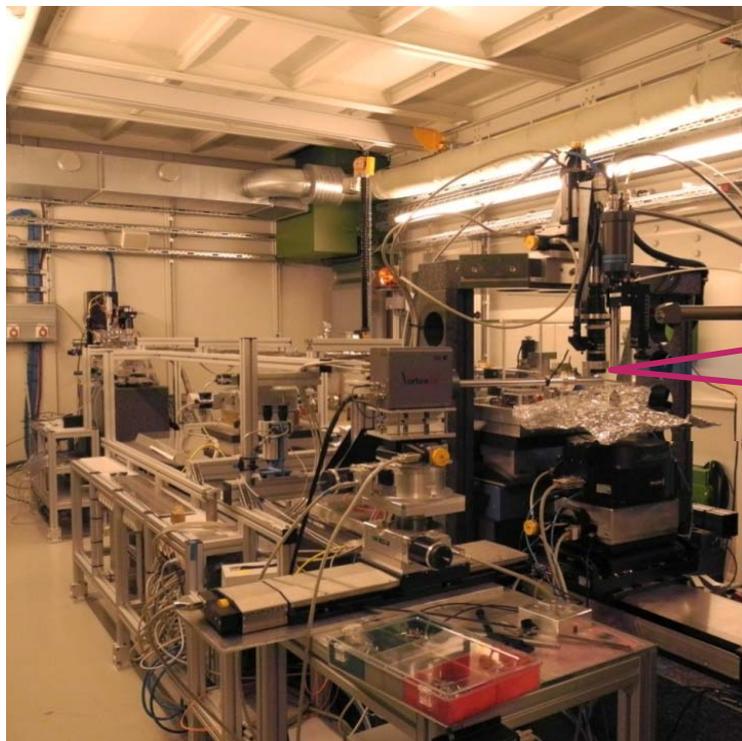
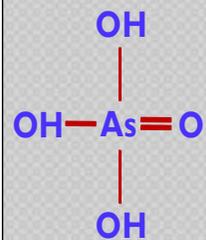
Comparison with Ni distribution

- A) Ni is accumulated more in the mature parts of the roots
- B) Interaction between As and Ni distribution?

μ -XANES

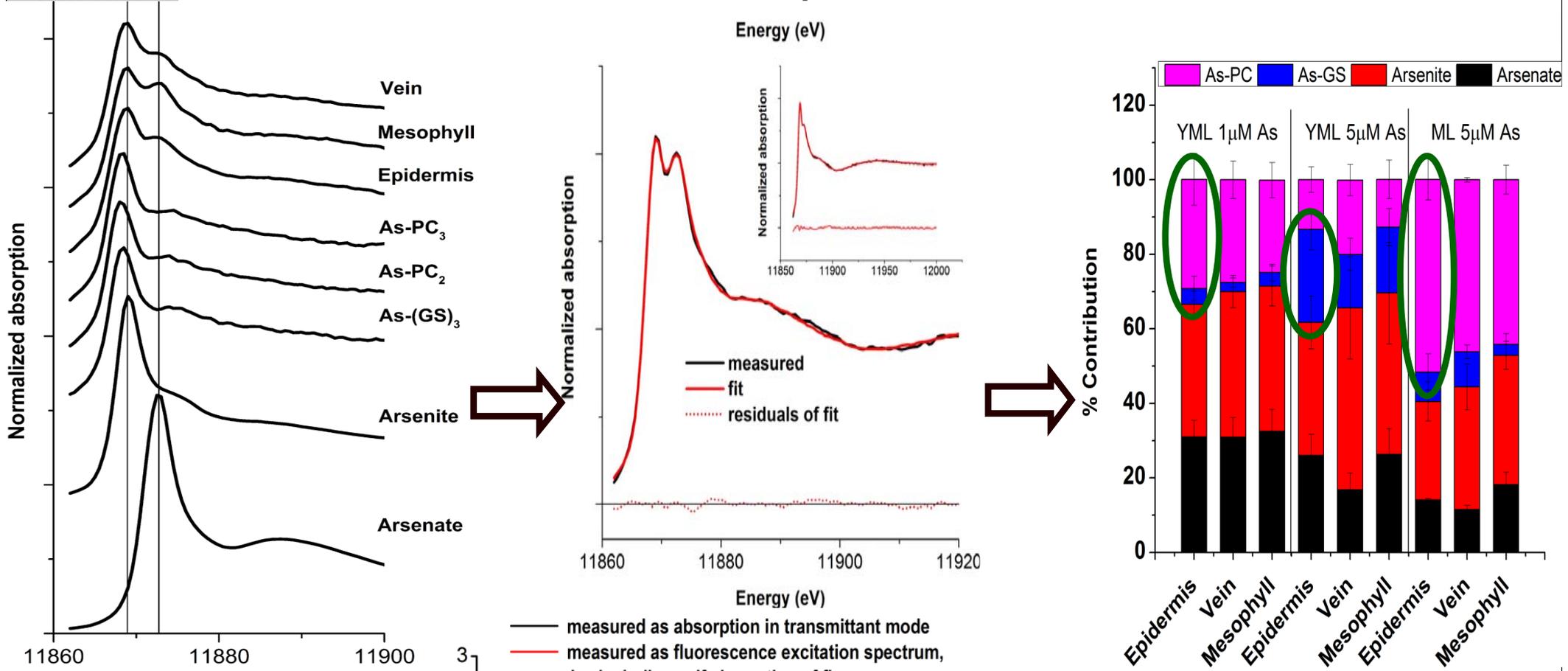


Speciation of As in different tissues

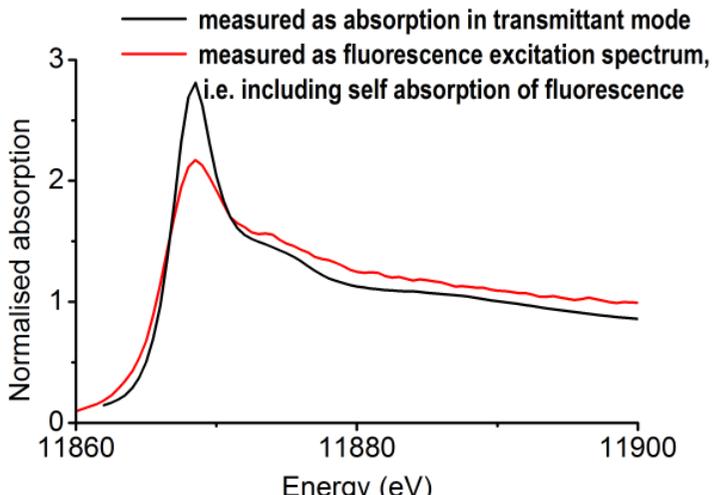


Mishra S, Weisenreuther G,
Mattusch J, Stärk H-J, Küpper H
(2013) Plant Physiology 163,
1396-1408

Tissue specific As speciation through confocal μ -XANES: linear combination fitting with correction for absorption and baseline drift



Mishra S, Wellenreuther G,
Mattusch J, Stärk H-J, Küpper H
(2013) Plant Physiology 163,
1396-1408



→ highest As-PC in the epidermis of young-mature leaves at 1 μ M As and mature leaves at 5 μ M
→ highest As-GS in epidermis of young-mature leaves at 5 μ M As

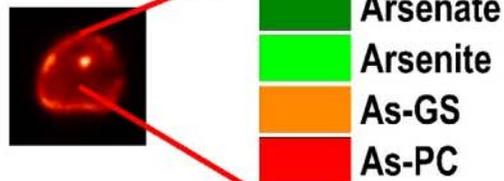
Speciation of arsenic in a non-accumulator plant



Young leaves

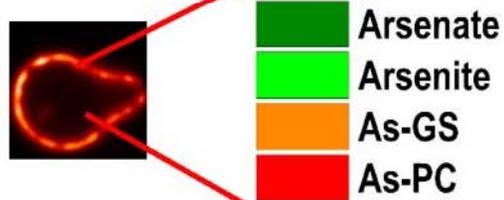
Mature leaves

Epidermis



Mesophyll

Epidermis

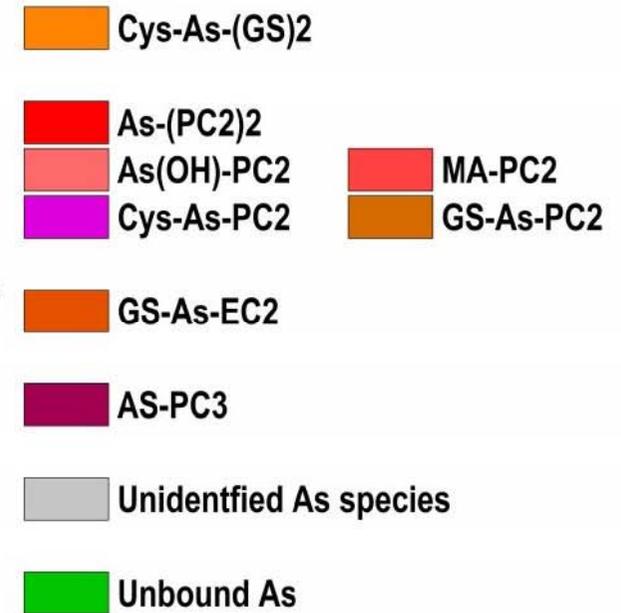
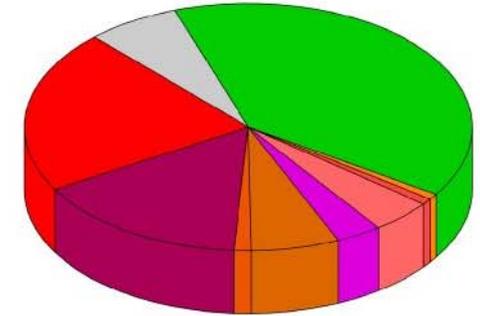


Mesophyll

μ -XRF

μ -XANES

Intact frozen-hydrated leaves



Chromatography

Whole plant extracts

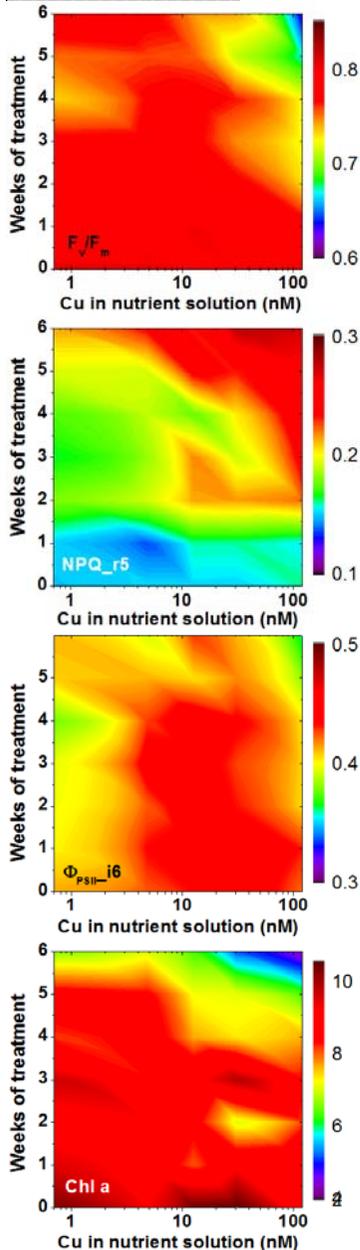
Comparison of Cu vs. As Toxicity Mechanisms

Copper toxicity at high irradiance

>10nM Cu: Damage to the PSII reaction centre
 → decreased photochemical quantum yield (F_v/F_m)

- Up-regulation of the dissipation of excitons as heat (NPQ)
- Electron transport (Φ_{PSII}) inhibited in addition to PSIIRC damage

Decrease of Chl during death of cells

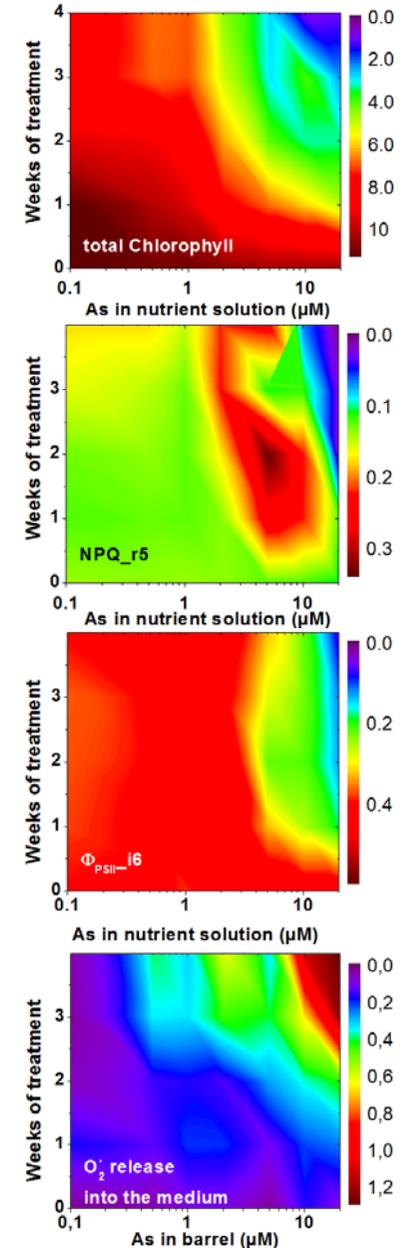


Arsenic toxicity

>0.5 μ M As: inhibition of Chl biosynthesis
 → decreased light harvesting

- > 1 μ M As: (1) As binding in nucleus (2) decreased exciton transfer from the antenna to the RC
 → up-regulation of thermal exciton dissipation (NPQ)
- >2 μ M As: Electron transport (Φ_{PSII}) inhibited
- >5 μ M As: NPQ inhibition

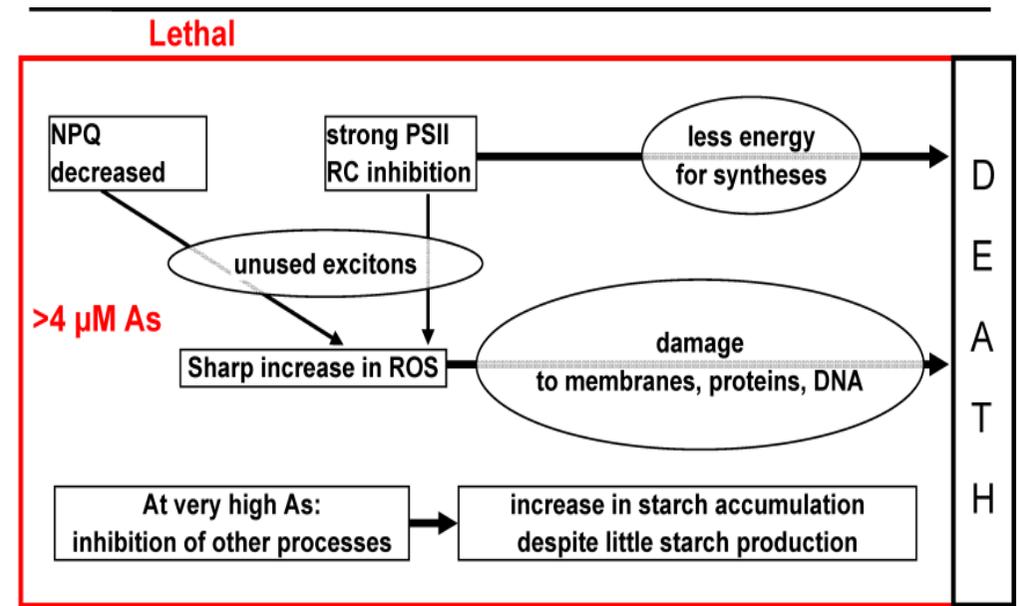
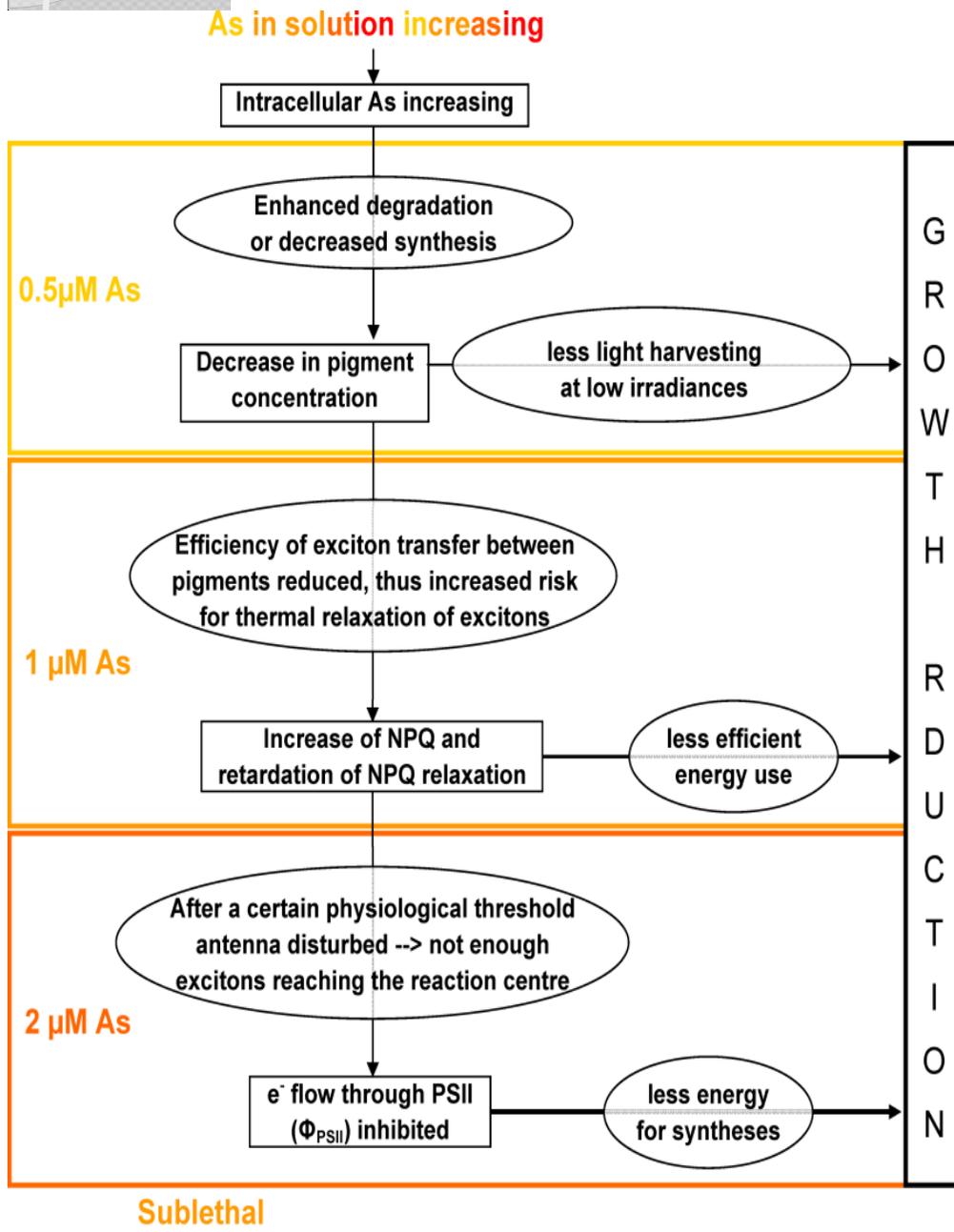
Malfunctioning of photosynthesis leads to generation of ROS in addition to increased inhibitions

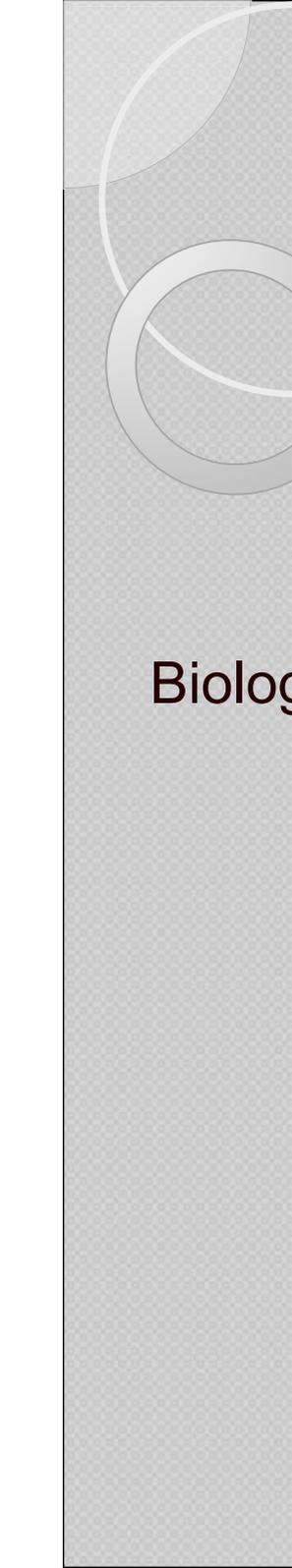


As: Mishra S, Stärk H-J, Küpper H (2014) Metallomics 6, 444-454

Cu: Thomas G, Stärk H-J, Wellenreuther G, Dickinson BC (2013) Aquatic toxicology 140-141, 27-36

Scheme of pathways of arsenic toxicity in plants





**All slides of my lectures can be downloaded
from my workgroup homepage**

Biology Centre CAS → Institute of Plant Molecular Biology → Departments
→ Department of Plant Biophysics and Biochemistry,
or directly

http://webserver.umbr.cas.cz/~kupper/AG_Kuepper_Homepage.html