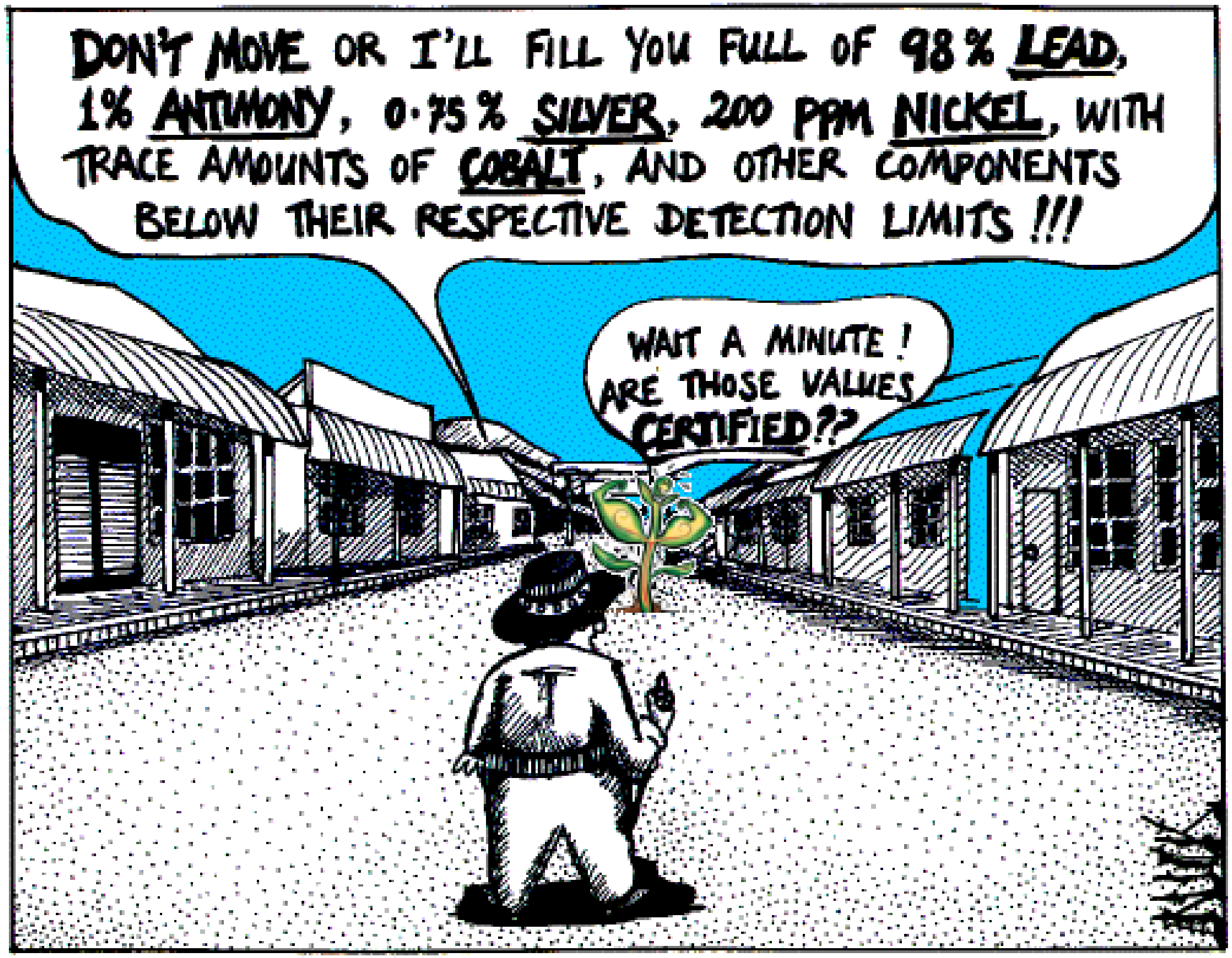
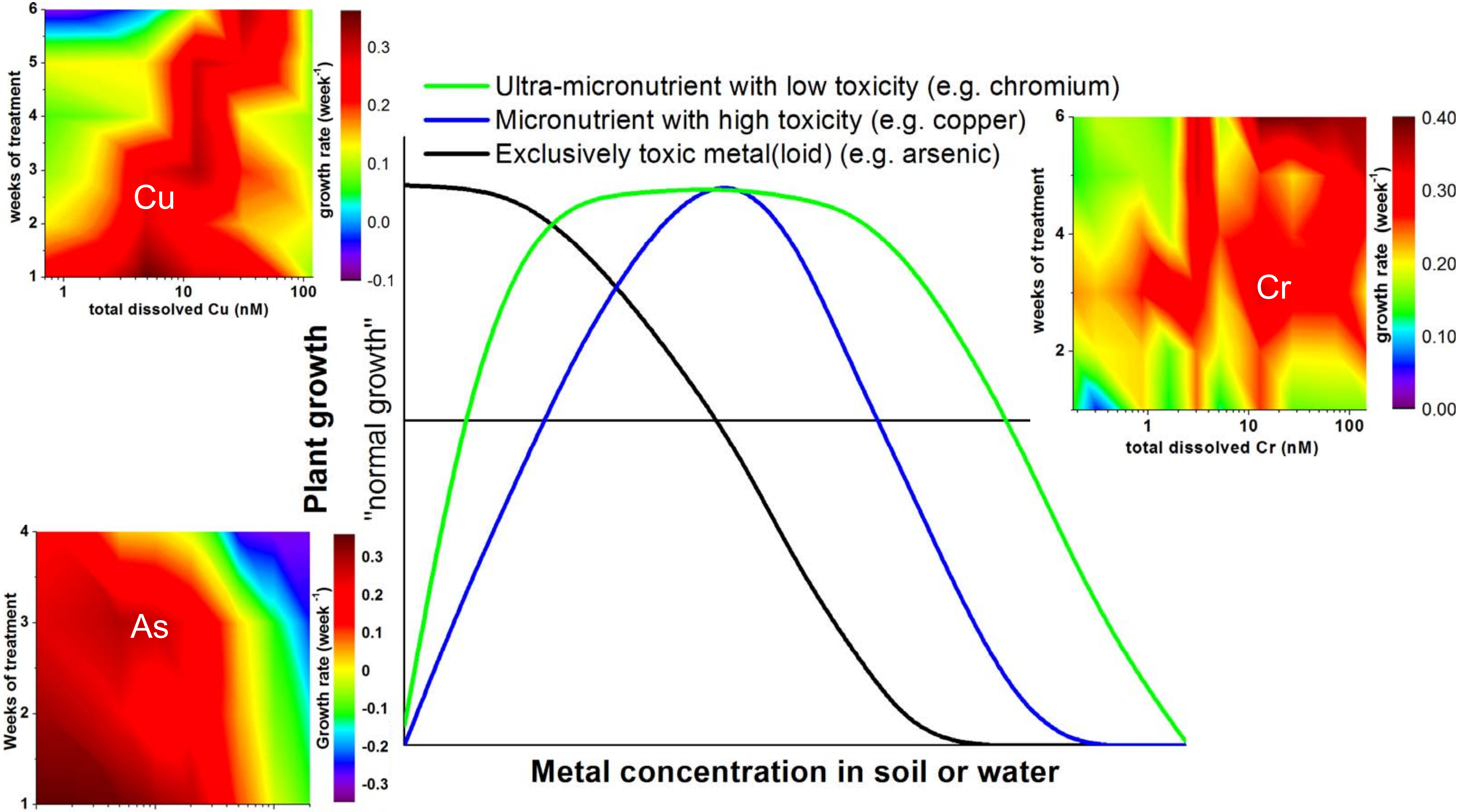
Metal(loid)s and Plants - a complicated relationship
→ Arsenic toxicity



Heavy metal-hyperaccumulation in the Wild West

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

Variations of the dose-response principle for different elements



Examples (colour maps) show the growth response of *Ceratophyllum demersum*

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Cr: Küpper H, Mohi Ud Din A, Bokhari SNH, Stärk HJ, Mattusch J, Morina F (2026) *unpublished;*
Cu: Thomas G, Stärk H-J, Wellenreuther G, Dickinson BC (2013) Aquatic toxicology 140-141, 27-36

Arsenic

As

33

74.92

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

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

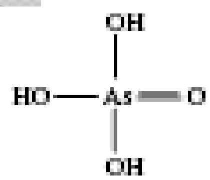
Arsenic: Applications

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

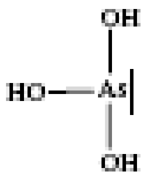
- The *Poison of Kings* and the *King of Poisons*

Arsenic trioxide

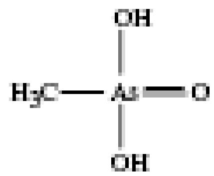
Structures of the most common As compounds



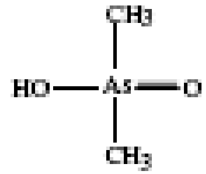
Arsenate
[As(V)]



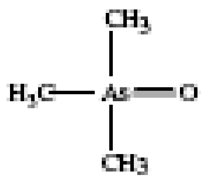
Arsenite
[As(III)]



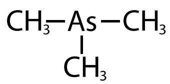
Monomethylarsonic acid
[MMA]



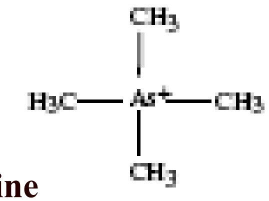
Dimethylarsinic acid
[DMA]



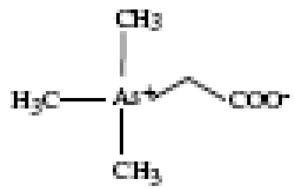
Trimethylarsine oxide
[TMAO]



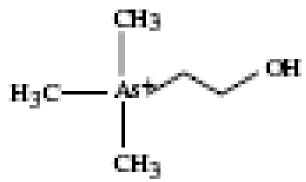
Trimethyl Arsine



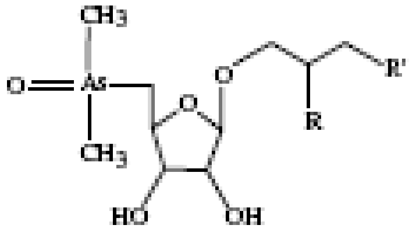
Tetramethylarsonium ion
[TeMA]



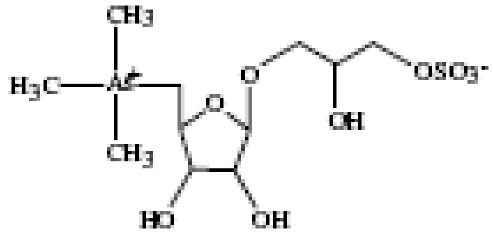
Arsenobetaine
[AB]



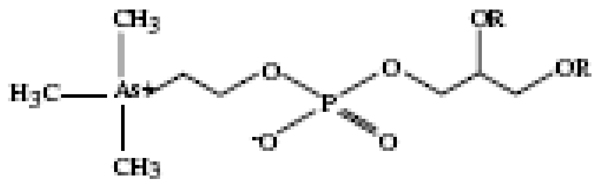
Arsenocholine
[AC]



Dimethylarsinylribosides



Trimethylarsonioribosides

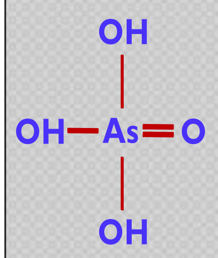


Glycerylphosphorylarsenocholine R = H

Phosphatidylarsenocholine R= $CO(CH_2)_nCH_3$

R_1 =	R_2 =
-OH	$-SO_3H$
-OH	-OH
-OH	$-OSO_3H$
-OH	$-OPO_3HCH_2CH(OH)CH_2OH$
$-NH_2$	$-SO_3H$

Sources of Arsenic Contamination

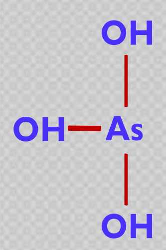


- **Through anthropogenic activities such as mining, smelting, phosphate fertilizer, and the use of As-containing pesticides, wood preservatives, and feed additives.**

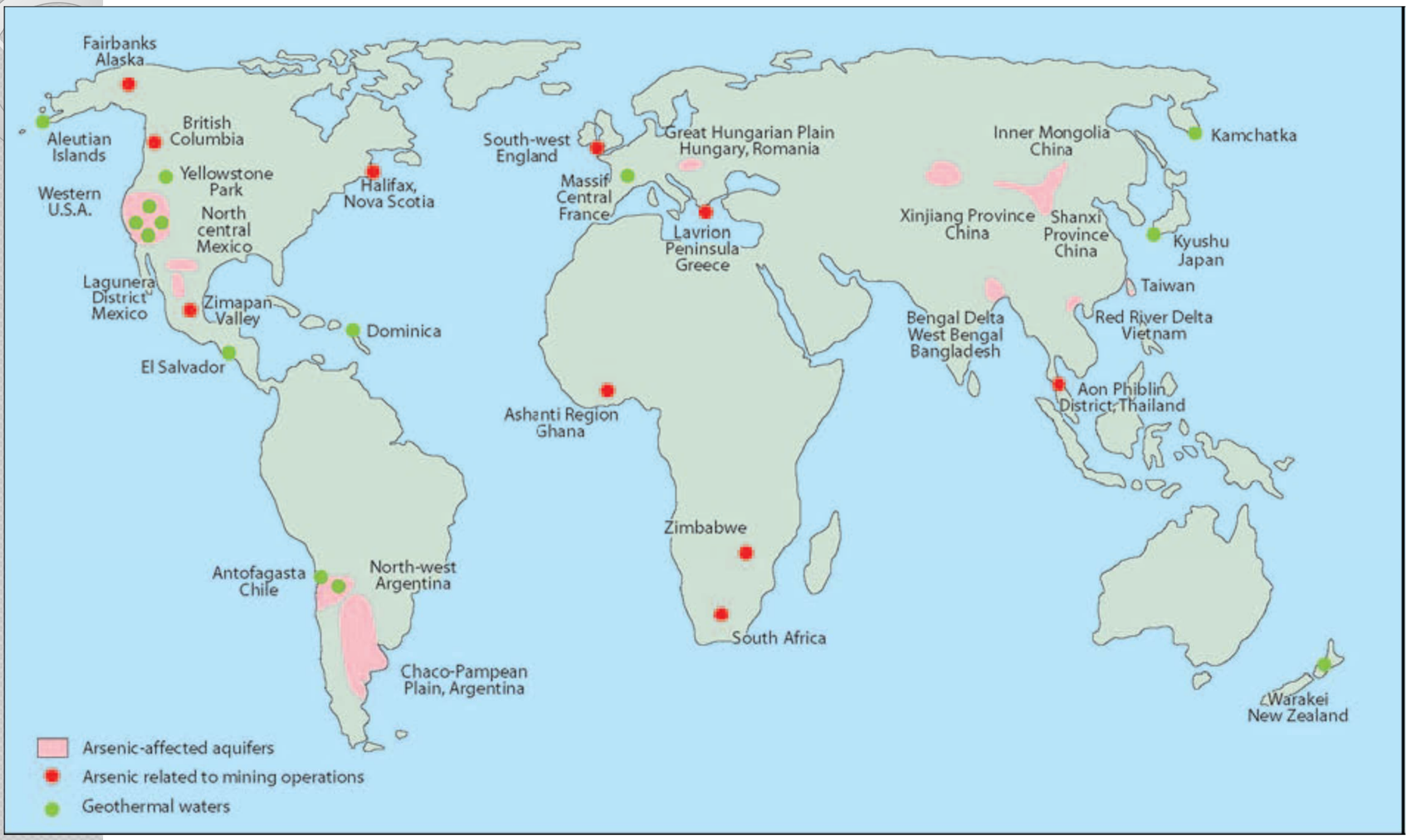
  e.g. Contamination in US fields

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

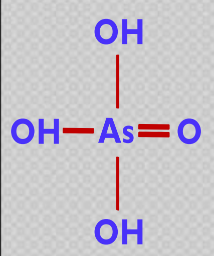
  e. g. Contamination in South east Asia + USA

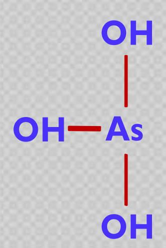


Arsenic Contamination



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

$$H_3AsO_3 + H_2O + O_2 \longrightarrow H_3AsO_4 + H_2O_2$$

Arsenic Toxicity to Plants: Proteomics

Available online at www.sciencedirect.com

SCIENCE DIRECT®

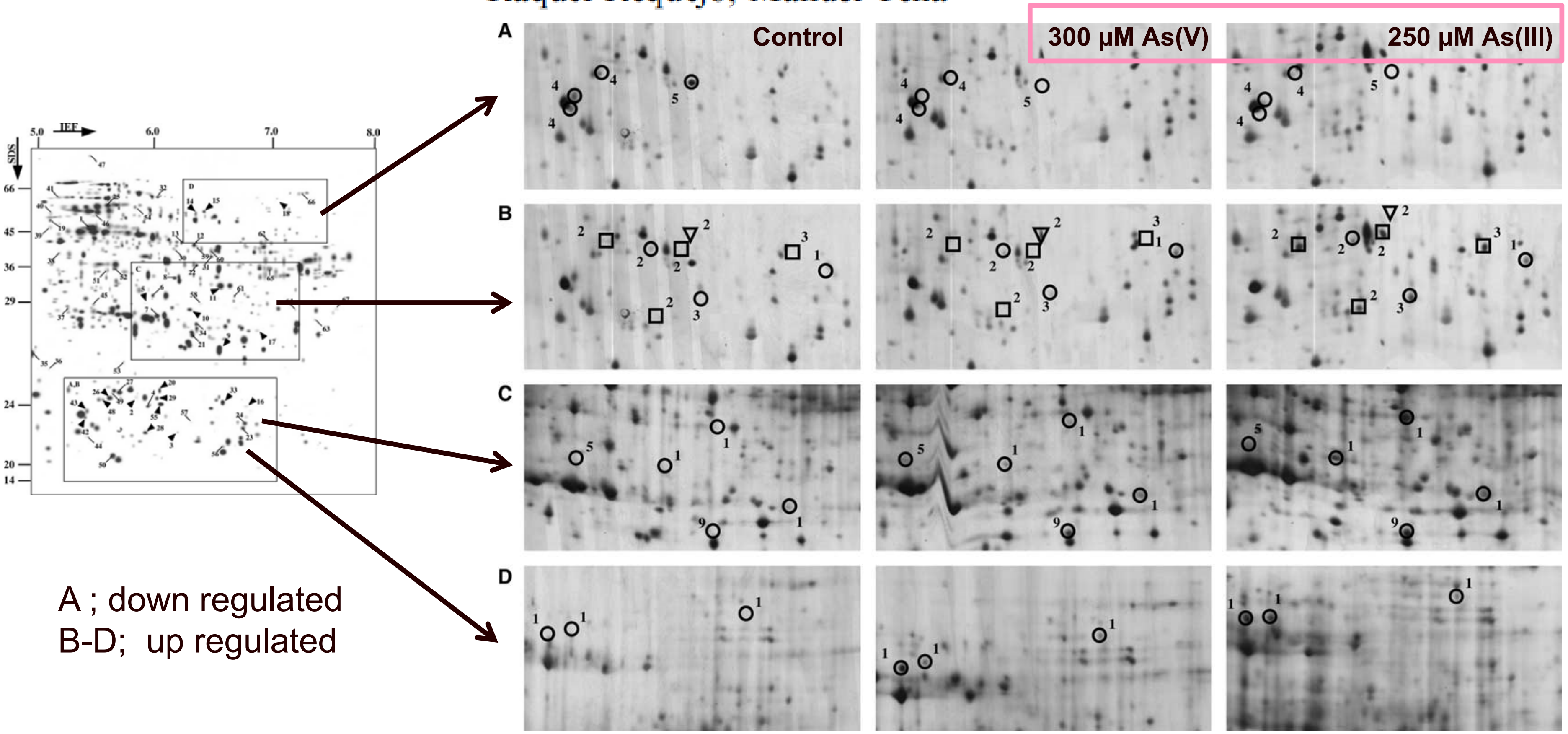
PHYTOCHEMISTRY

Phytochemistry 66 (2005) 1519–1528

www.elsevier.com/locate/phytochem

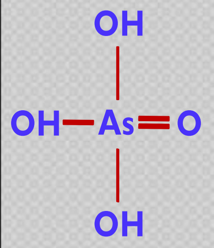
Proteome analysis of maize roots reveals that oxidative stress is a main contributing factor to plant arsenic toxicity

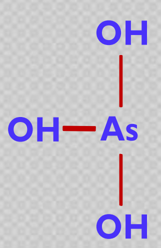
Raquel Requejo, Manuel Tena *



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

Ceratophyllum demersum L.

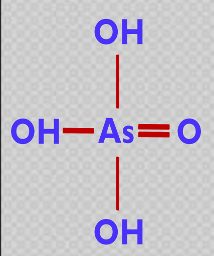


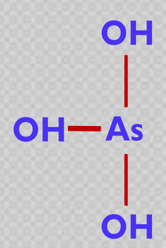


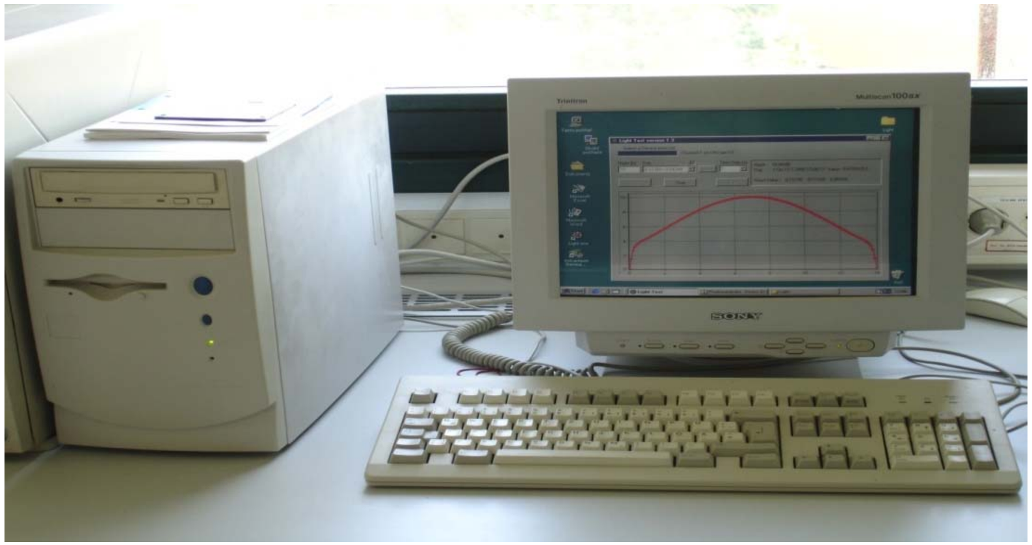
Ceratophyllum demersum is a rootless aquatic weed
Shows rapid growth, has worldwide distribution and can be easily harvested.

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

Simulation of environmental conditions

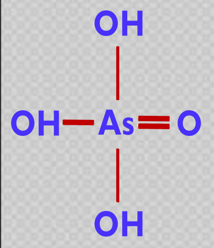


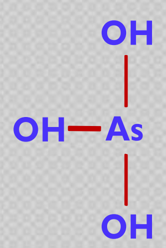


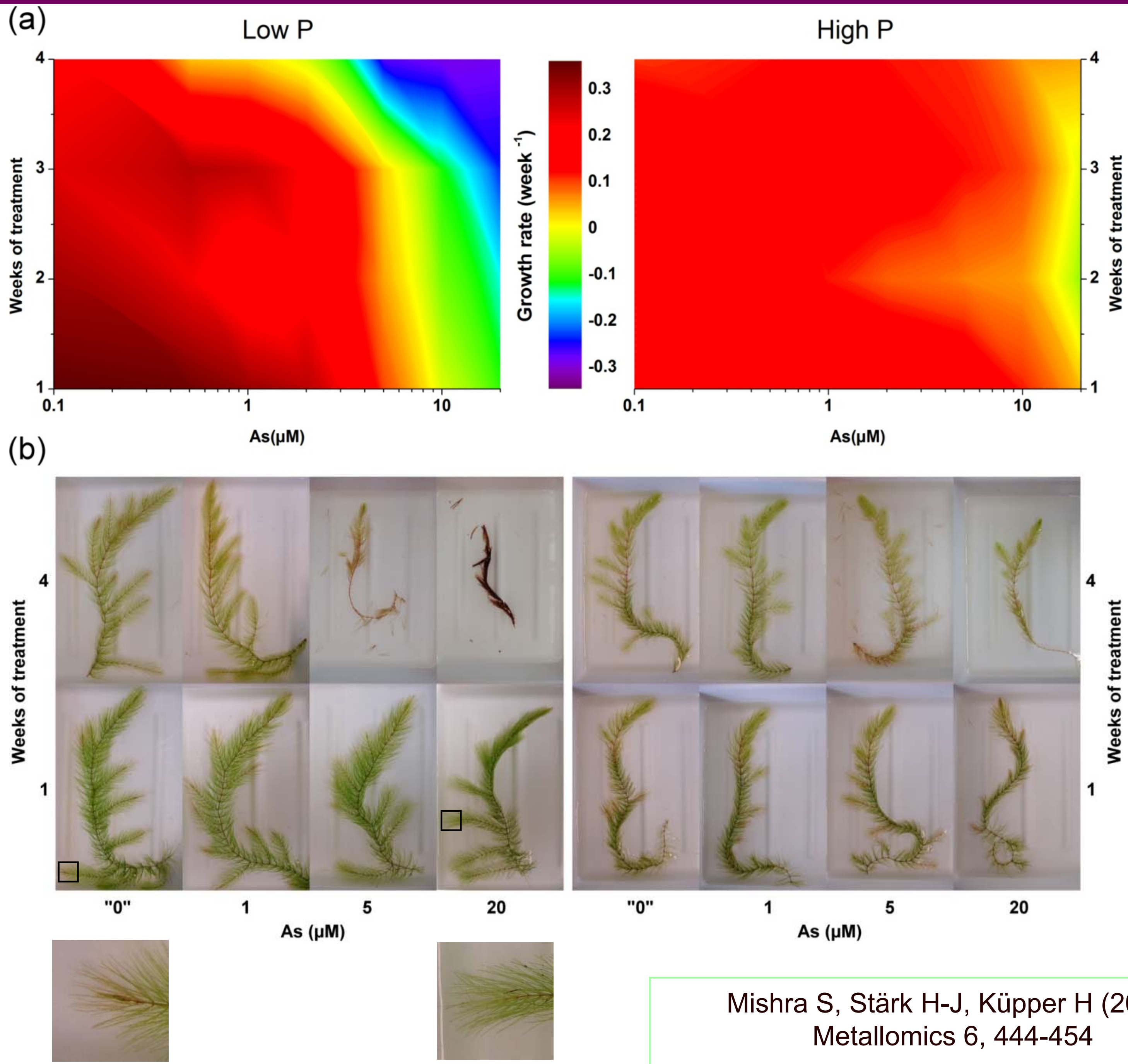


- Exposure to environmentally relevant arsenic concentrations (0.5 to 20 μM; within the range of contaminated areas e.g. in India)
- Long exposure time of 4 weeks
- Sinusoidal light and temperature (18-24 ^{o}C)cycles
- Low plant biomass to liquid ratio
- Continuous flow to achieve constant concentrations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

Growth rate of As exposed plants

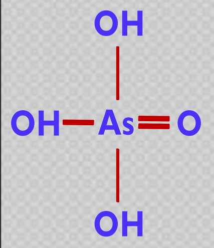


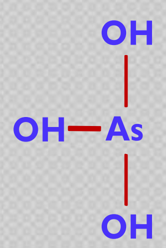


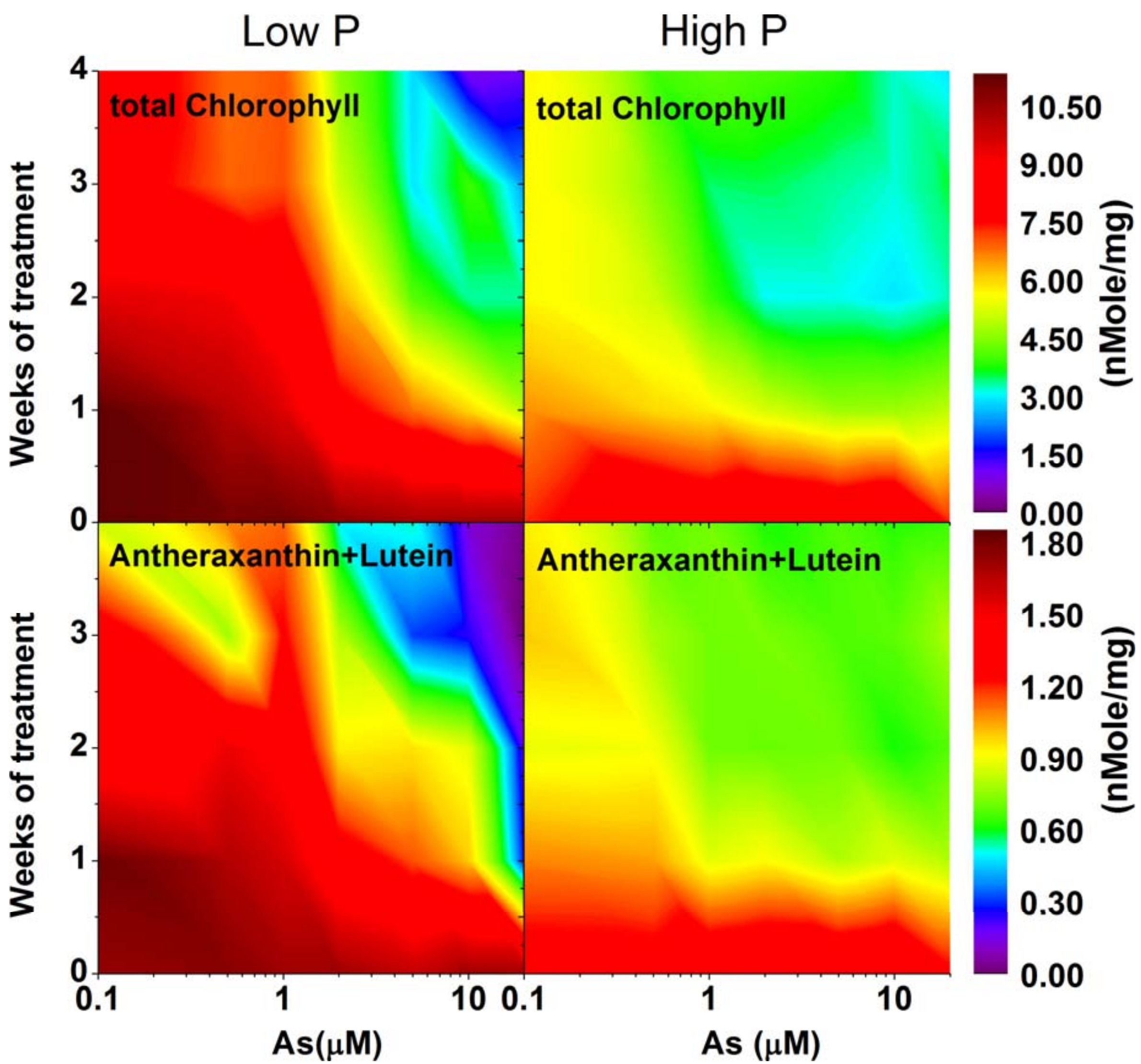


Mishra S, Stärk H-J, Küpper H (2014)
Metallomics 6, 444-454

Effect of As on pigments

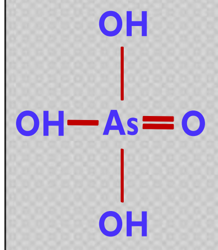


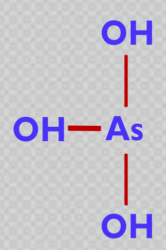


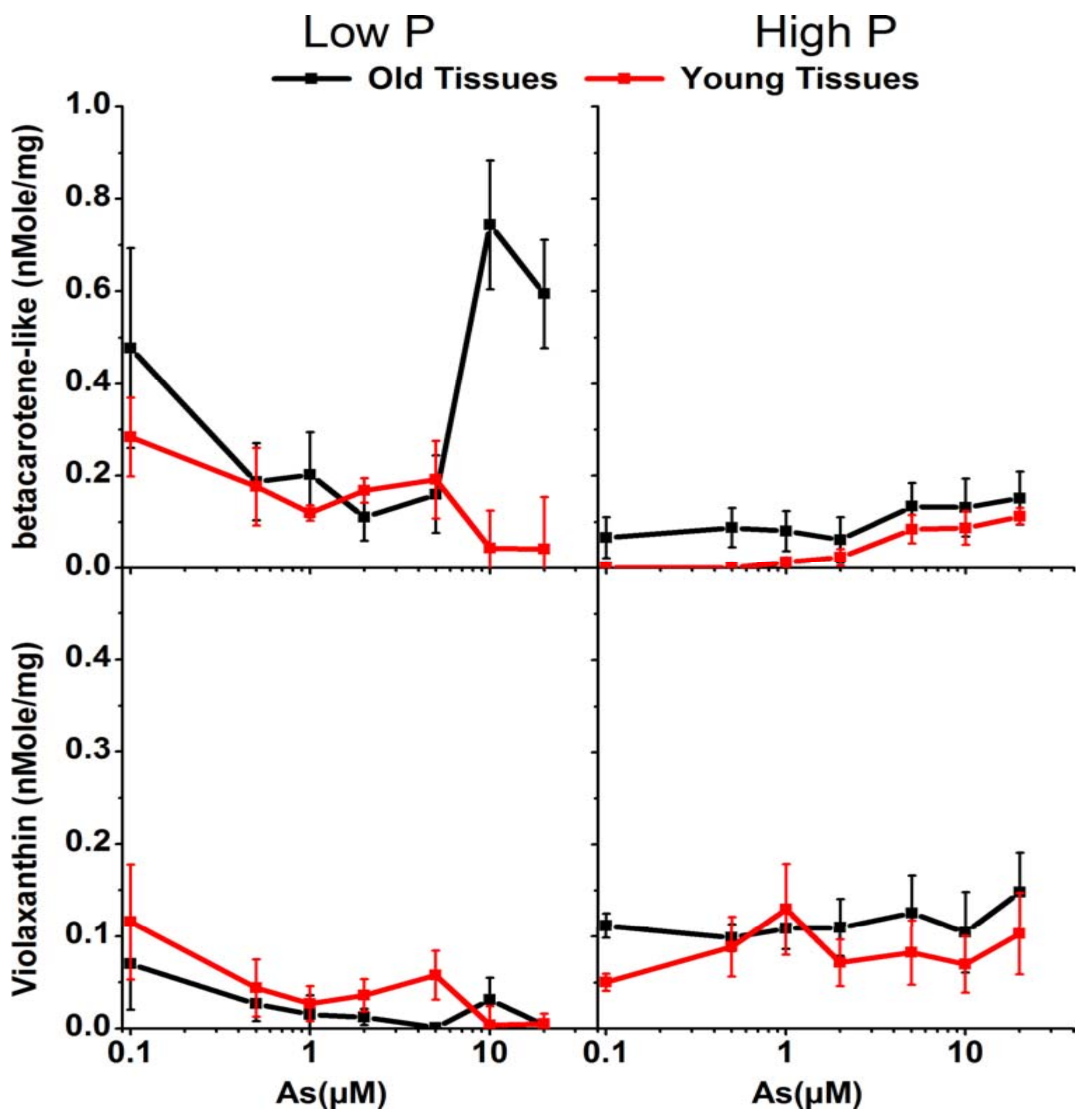


Mishra S, Stärk H-J, Küpper H (2014) Metallomics 6, 444-454

Effect of As on pigments

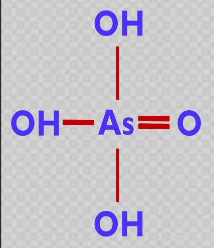


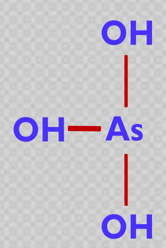


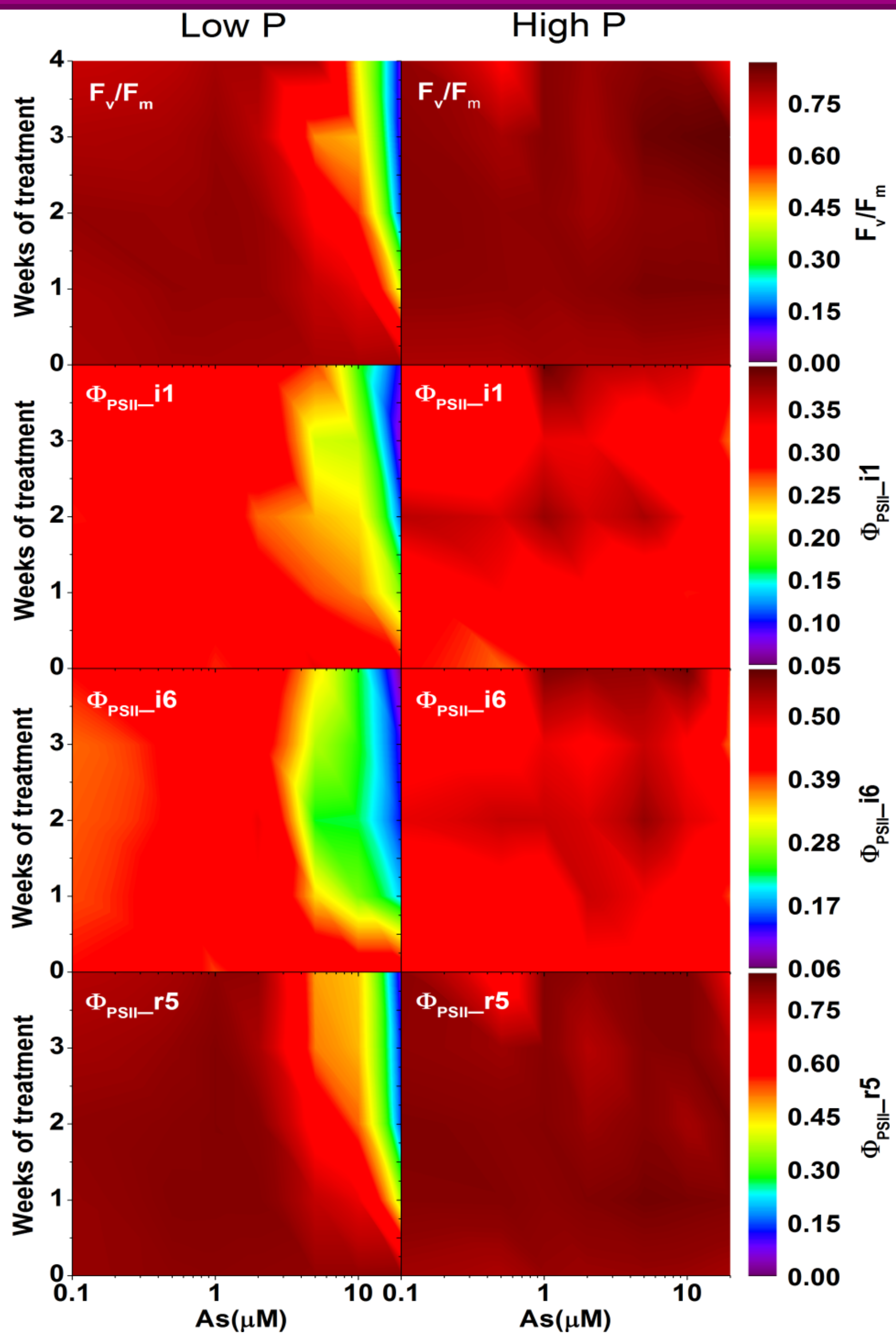


Mishra S, Stärk H-J, Küpper H (2014) Metallomics 6, 444-454

Effect of As on Photosynthetic Parameters



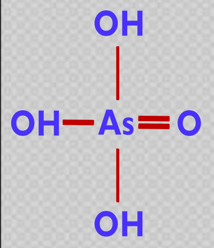


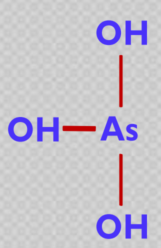


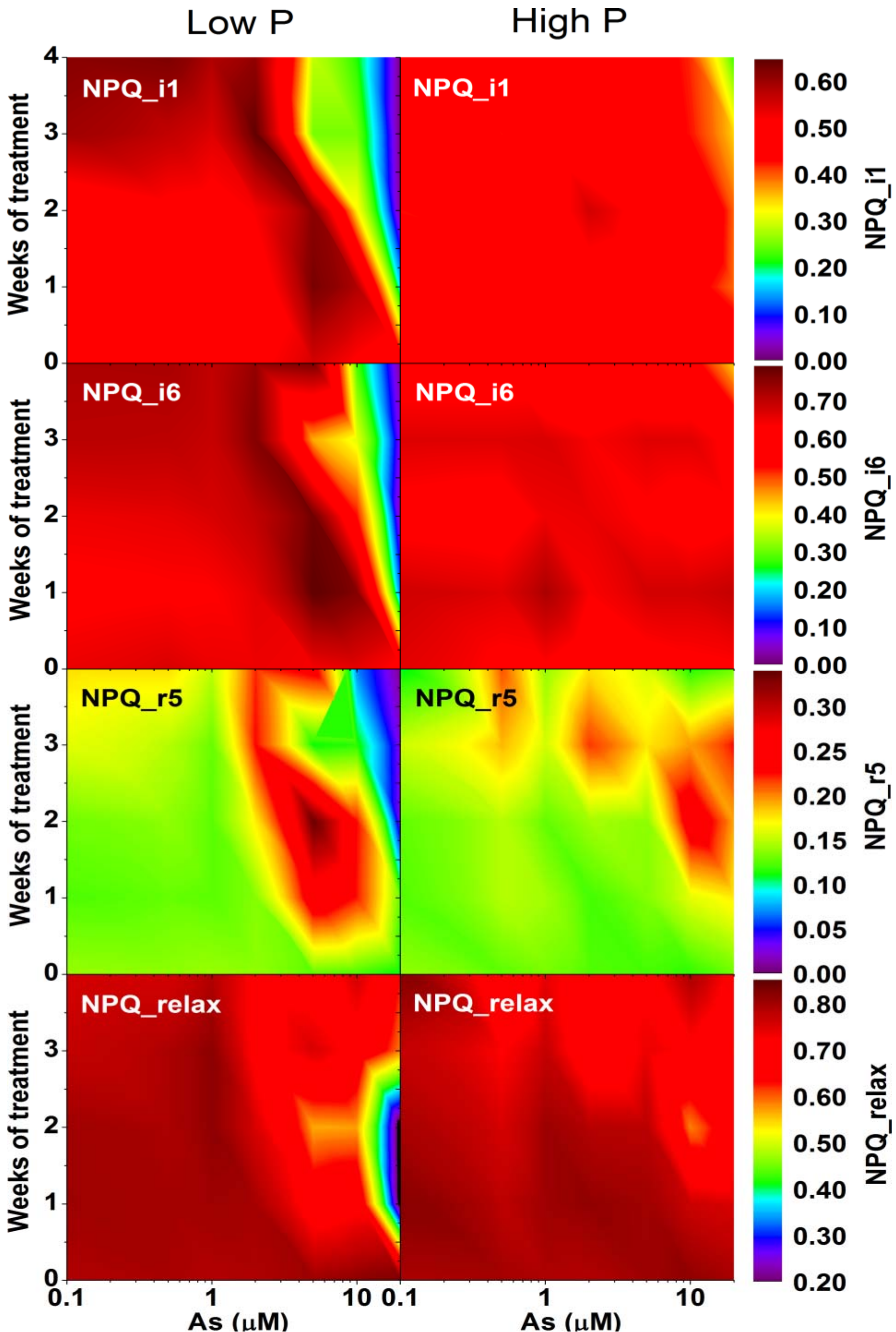
Effect of Arsenic on photochemical parameters

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

Effect of As on Photosynthetic Parameters



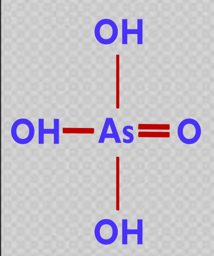


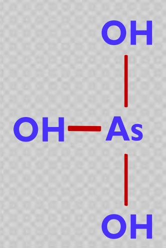


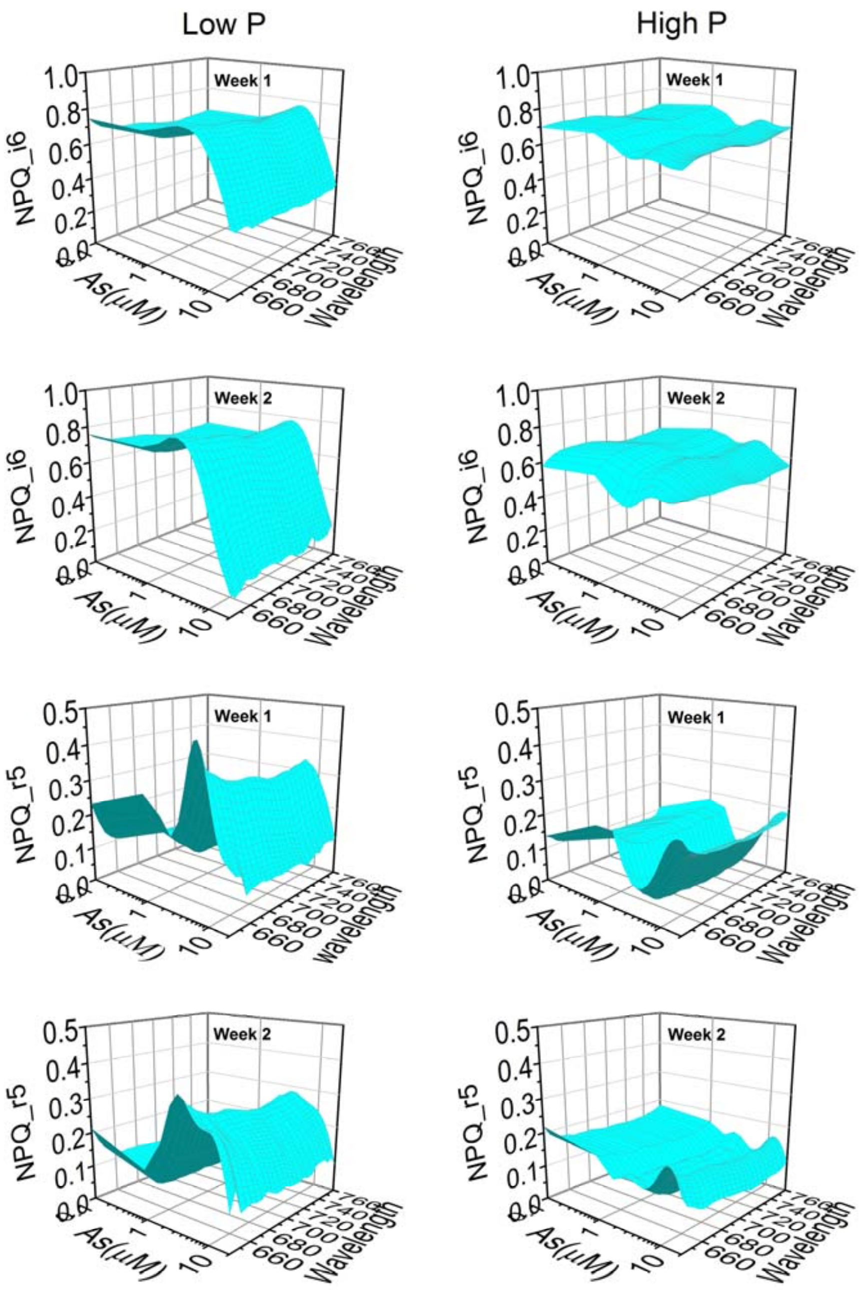
Effect of Arsenic on
non- photochemical parameters

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

Effect of As on Photosynthetic Parameters



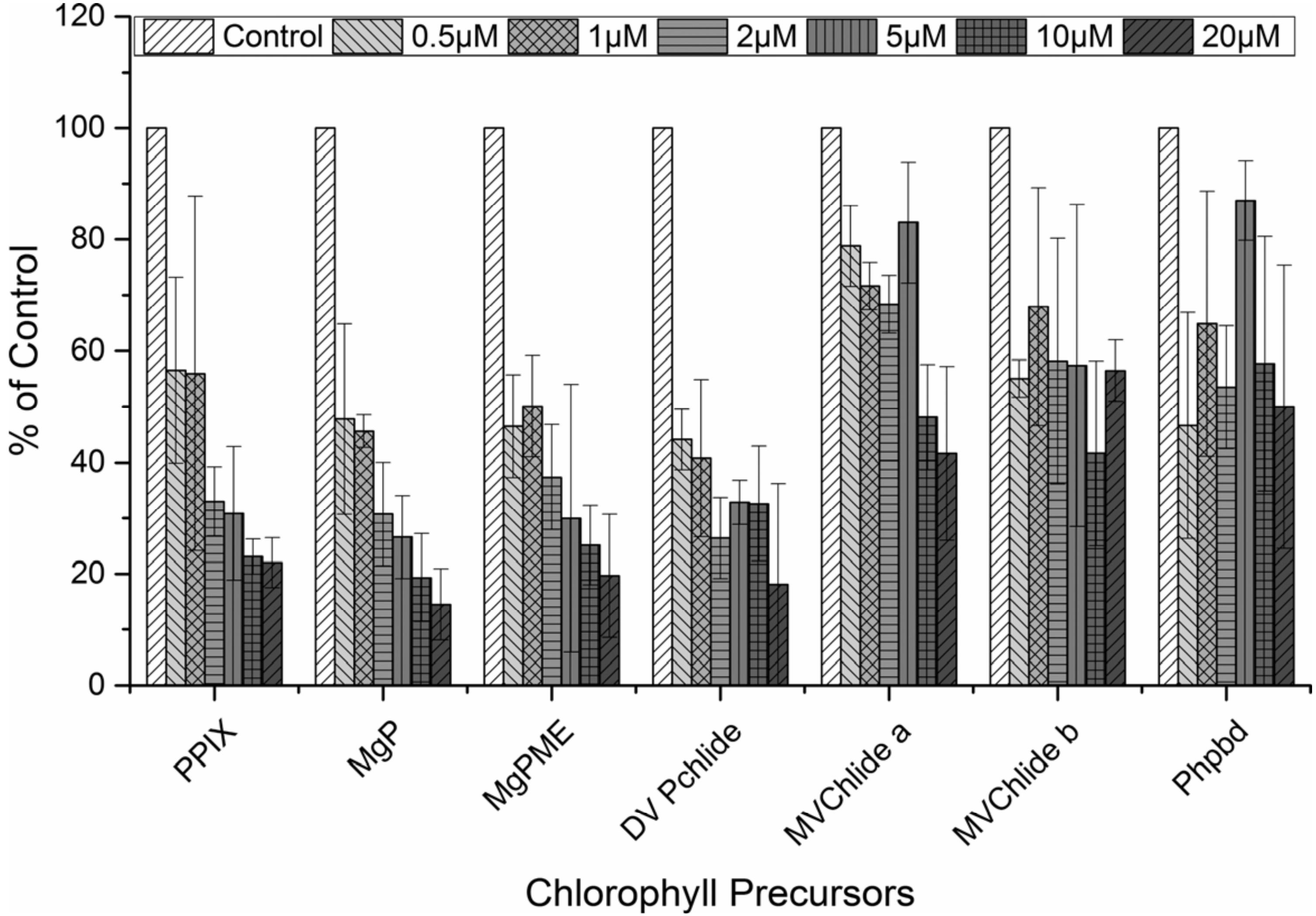




Effect of As on non-photochemical quenching measured in a spectrally resolved way

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

Effect of As on Chlorophyll Biosynthesis

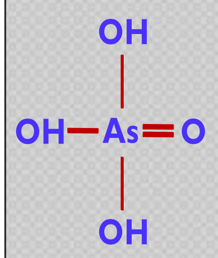


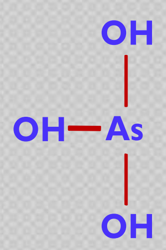
A) Analysis of precursors of chlorophyll and degradation metabolites revealed that the observed decrease in chlorophyll concentration was associated with hindered biosynthesis, and was not due to degradation

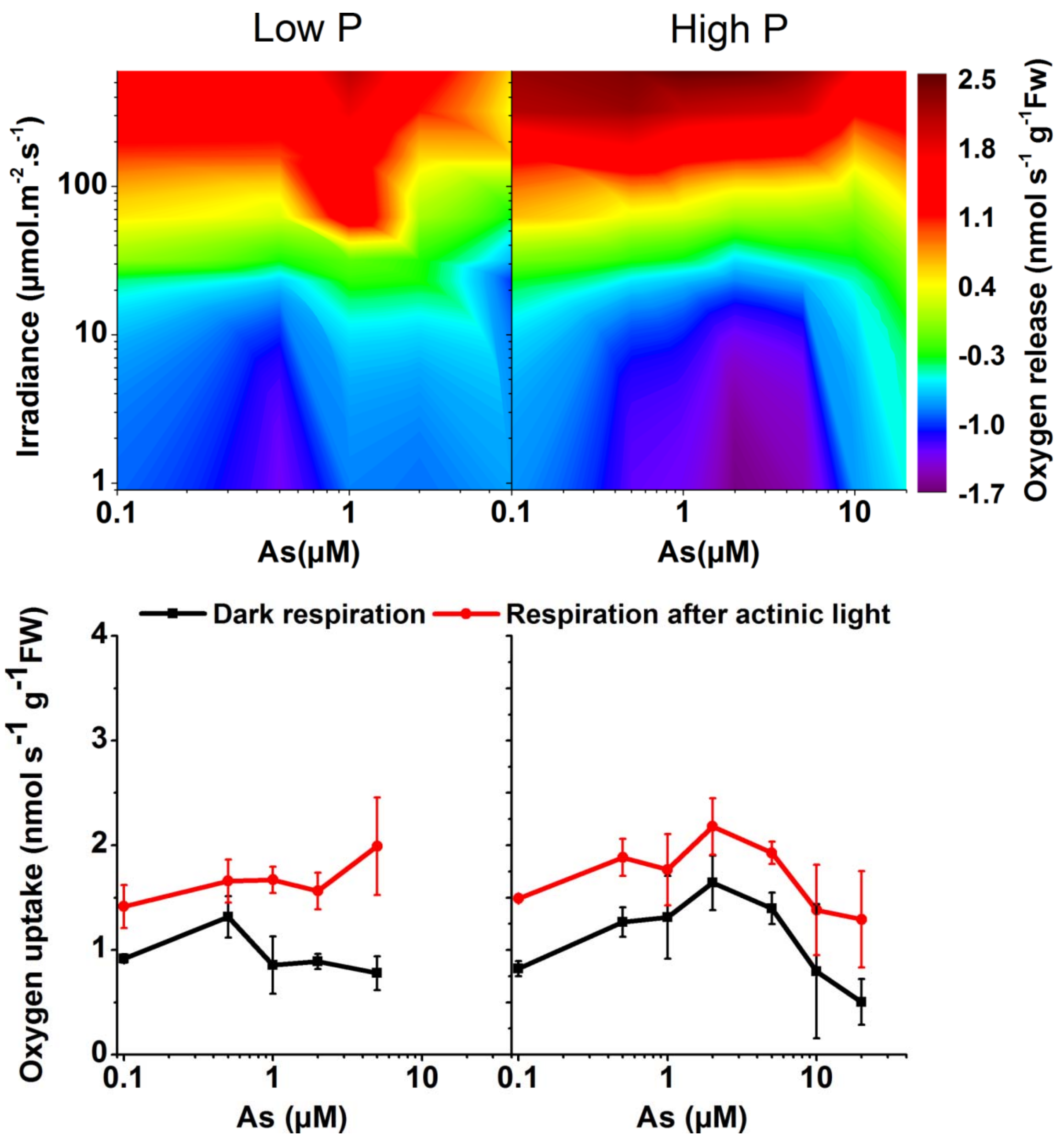
B) The results indicate that the pathway was blocked upstream of tetrapyrrole synthesis.

Mishra S, Alfeld M, Sobotka R, Andresen E, Falkenberg G, Küpper H (2016) Journal of Experimental Botany 67, 4639-4646

Effect of As on photosynthesis and respiration

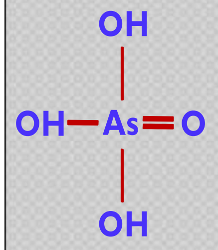


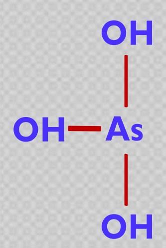


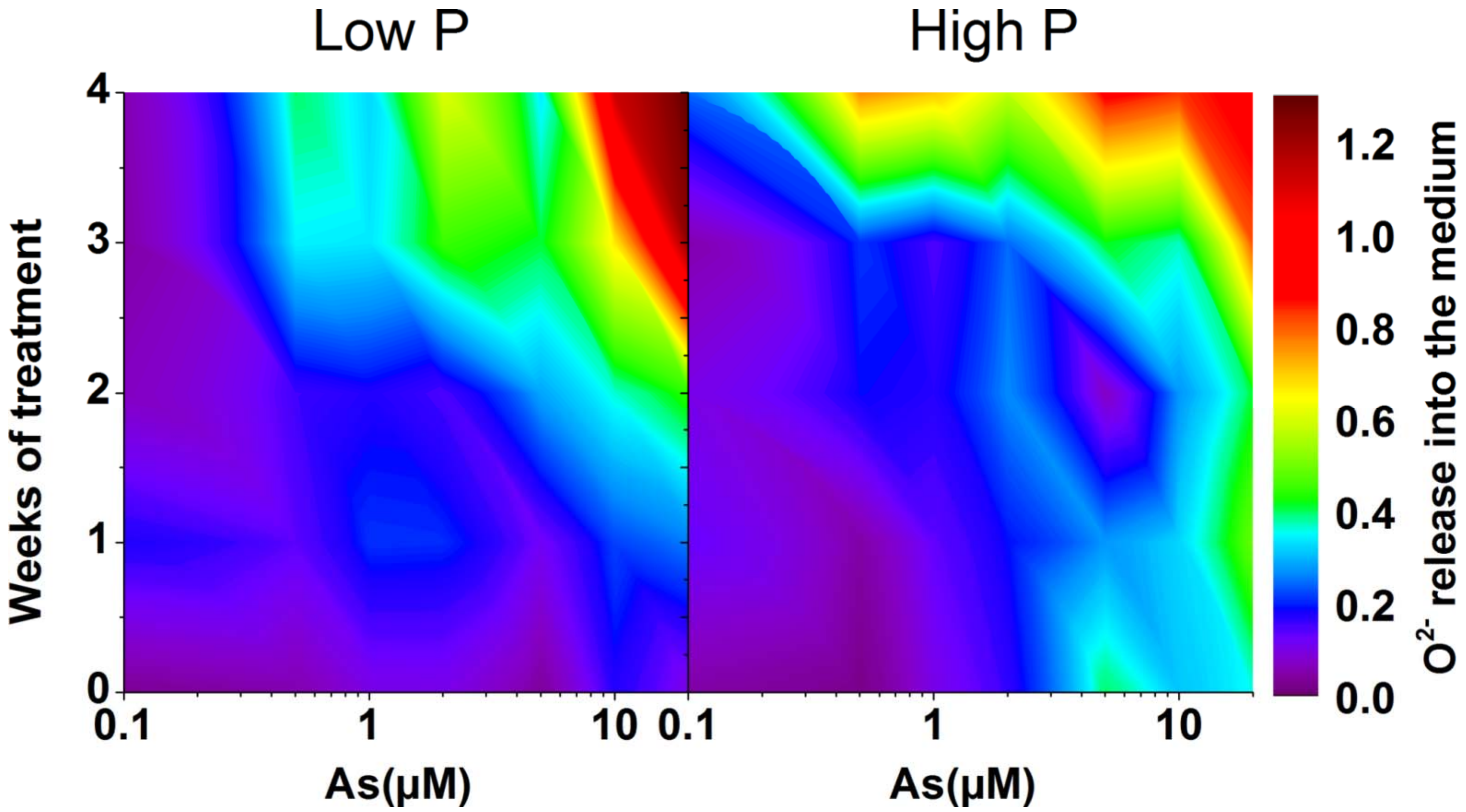


Mishra S, Stärk H-J, Küpper H (2014) Metallomics 6, 444-454

Production of Superoxide upon As exposure

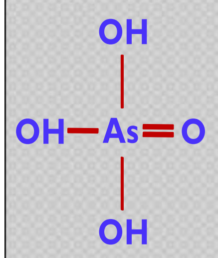


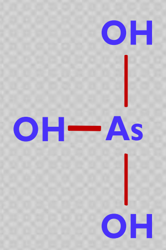


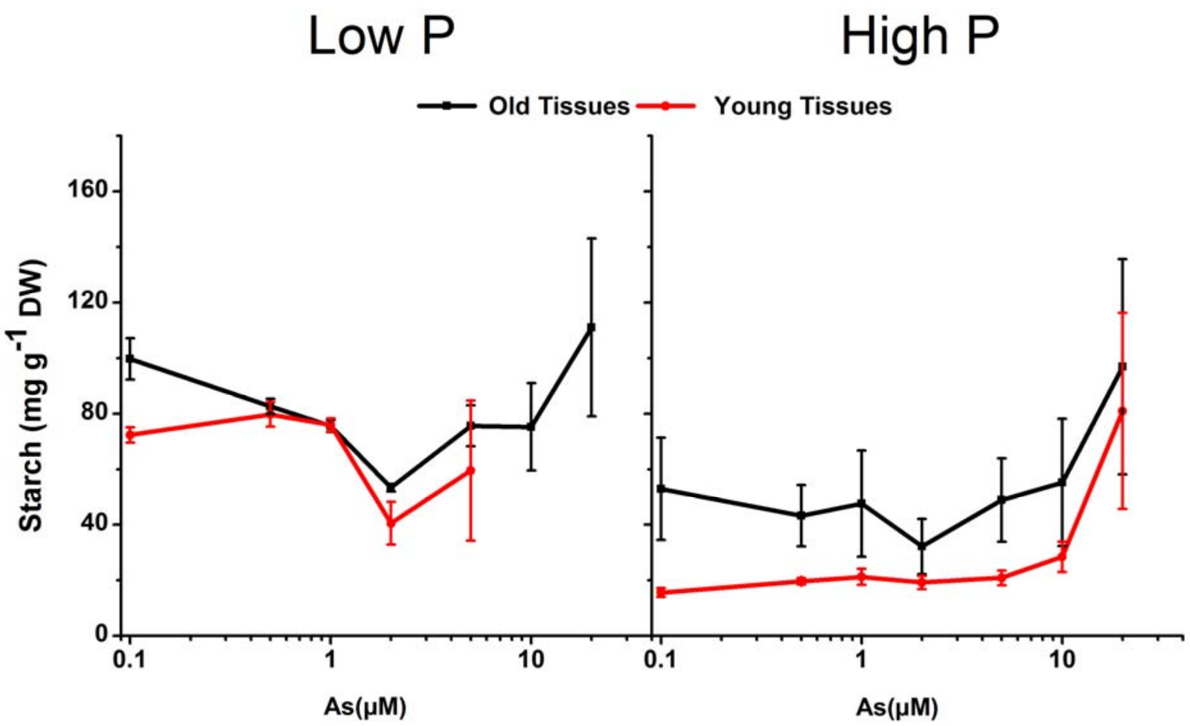


Mishra S, Stärk H-J, Küpper H (2014) Metallomics 6, 444-454

Effect on level of starch





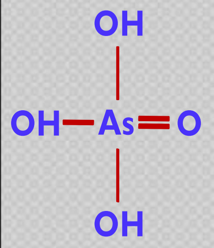


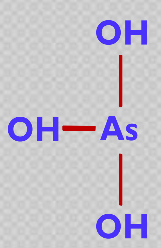
Mishra S, Stärk H-J, Küpper H (2014) Metallomics 6, 444-454

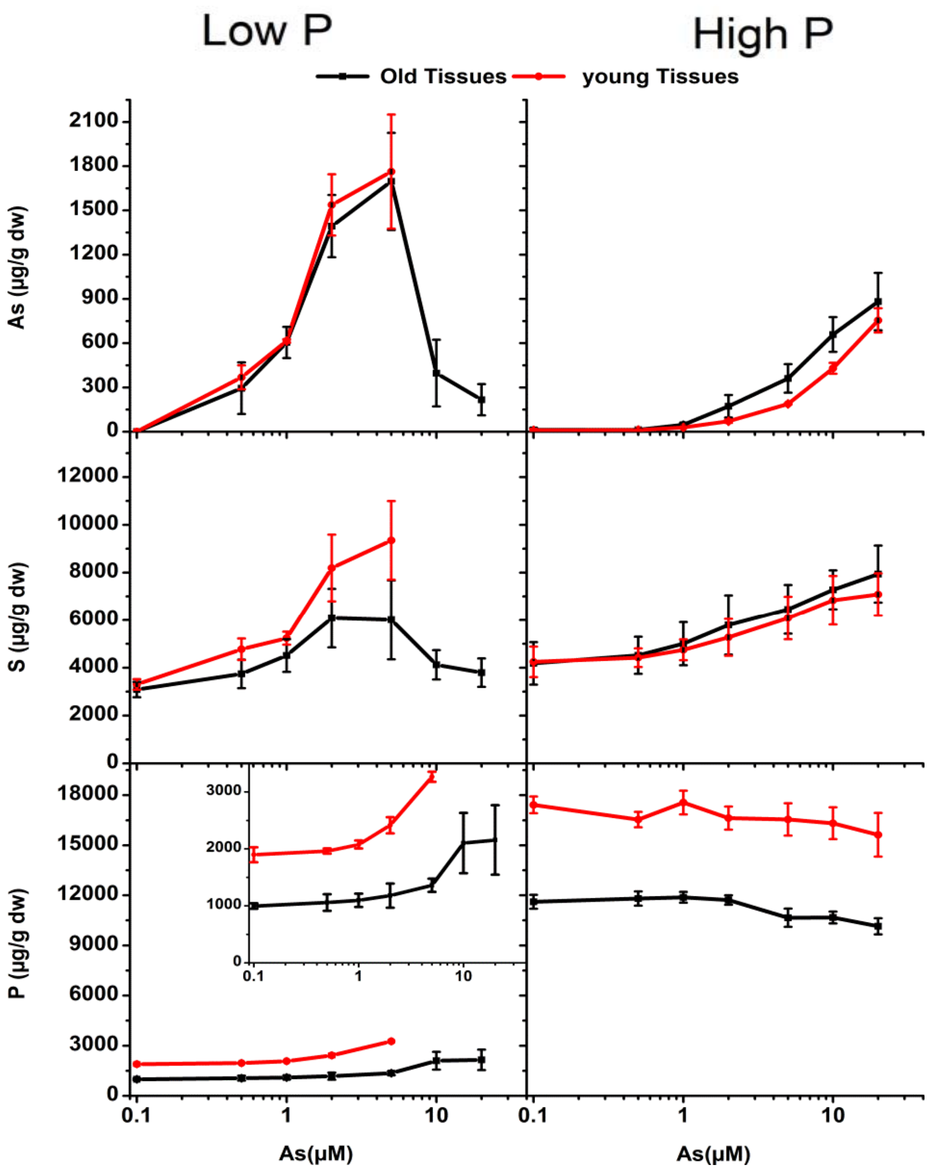
Accumulation, Distribution & Speciation of Arsenic in *C. demersum*

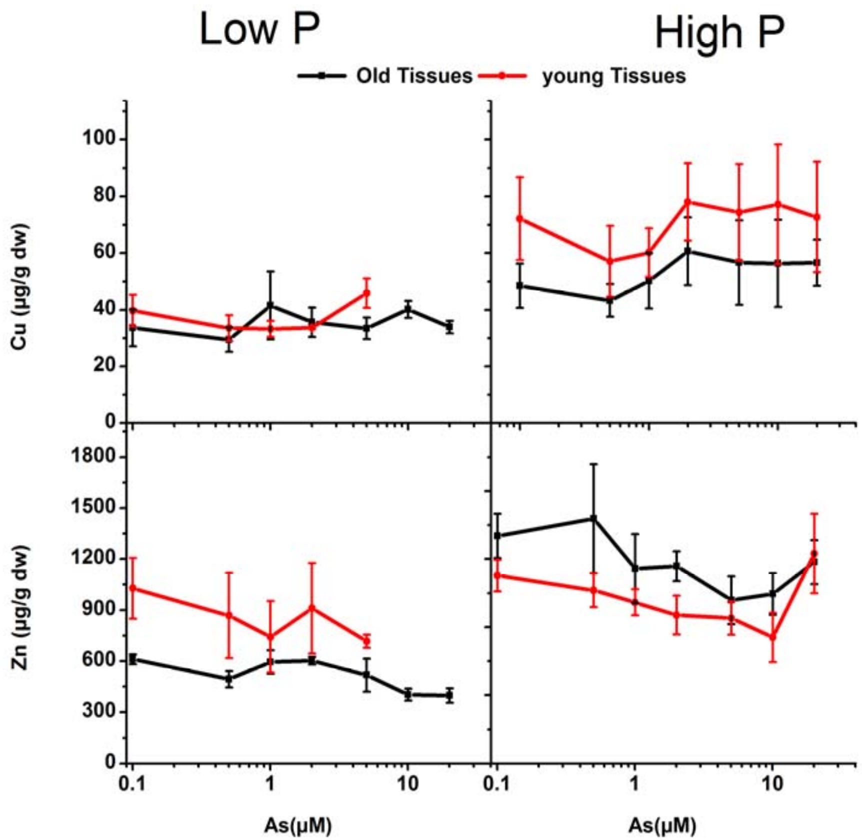
(HPLC-ICP-MS-ESI-MS, µ-XRF, µ-XANES)

Elemental analysis of As exposed plants



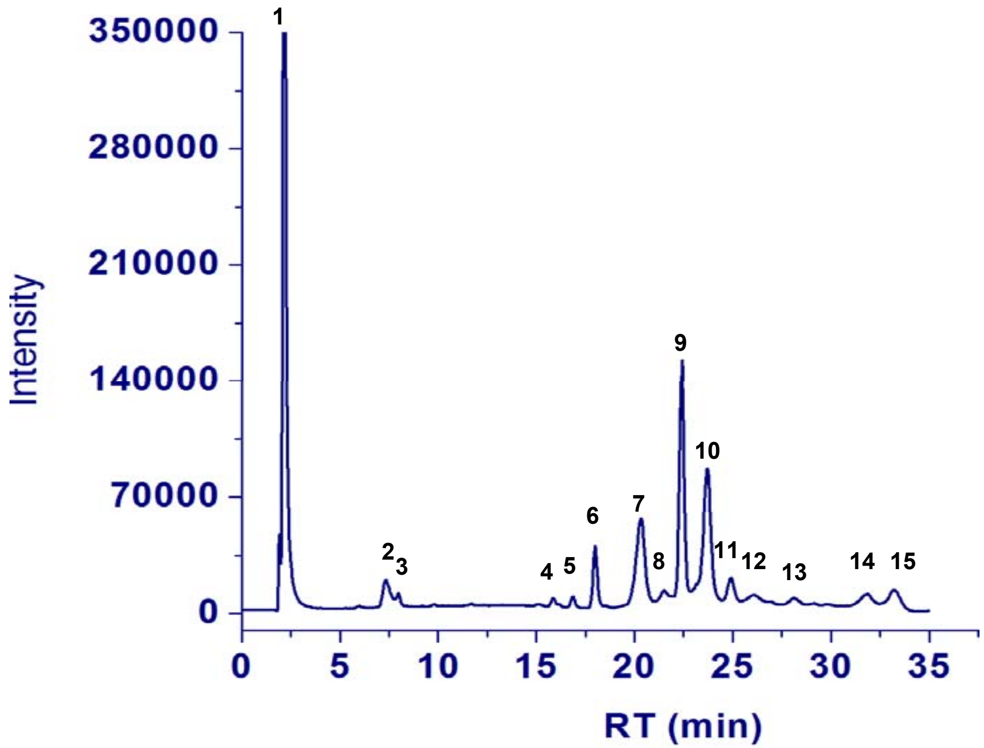






Mishra S, Stärk H-J, Küpper H (2014) Metallomics 6, 444-454

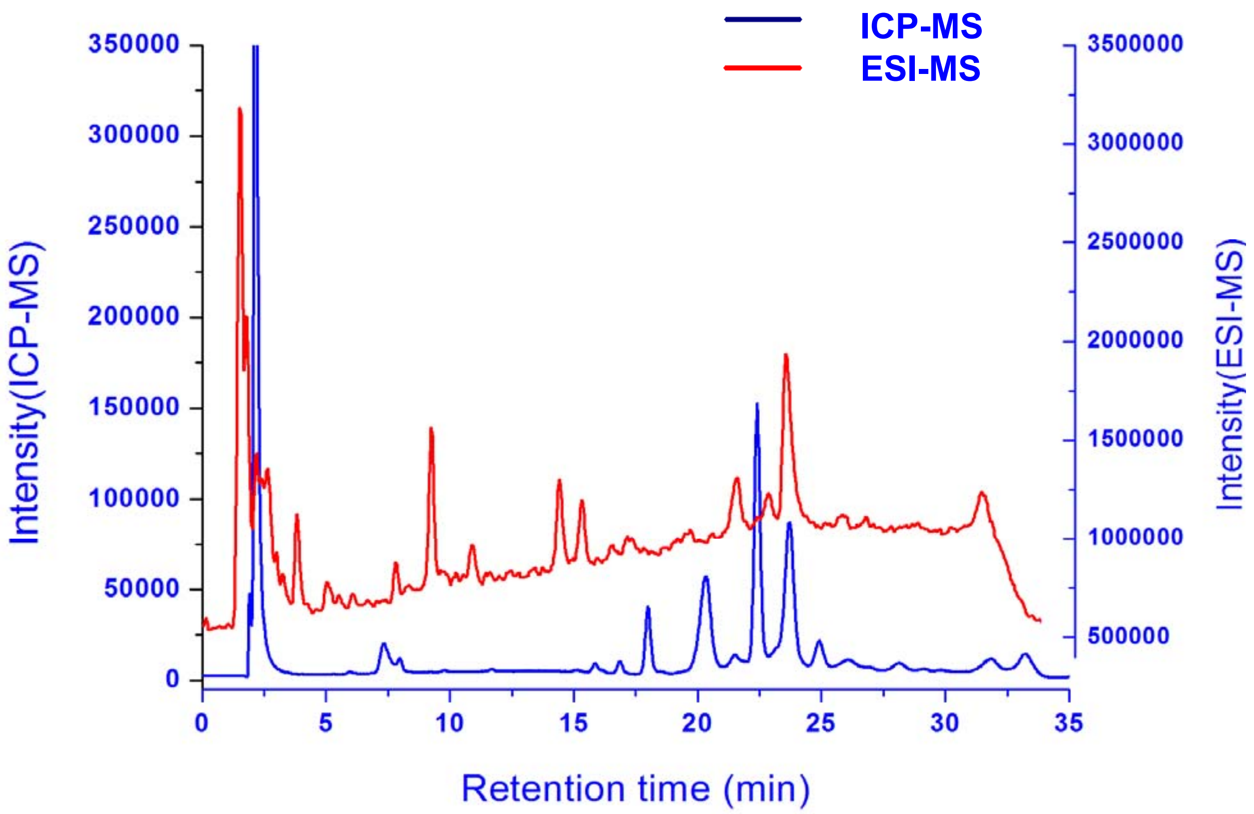
As Speciation Analysis



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

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

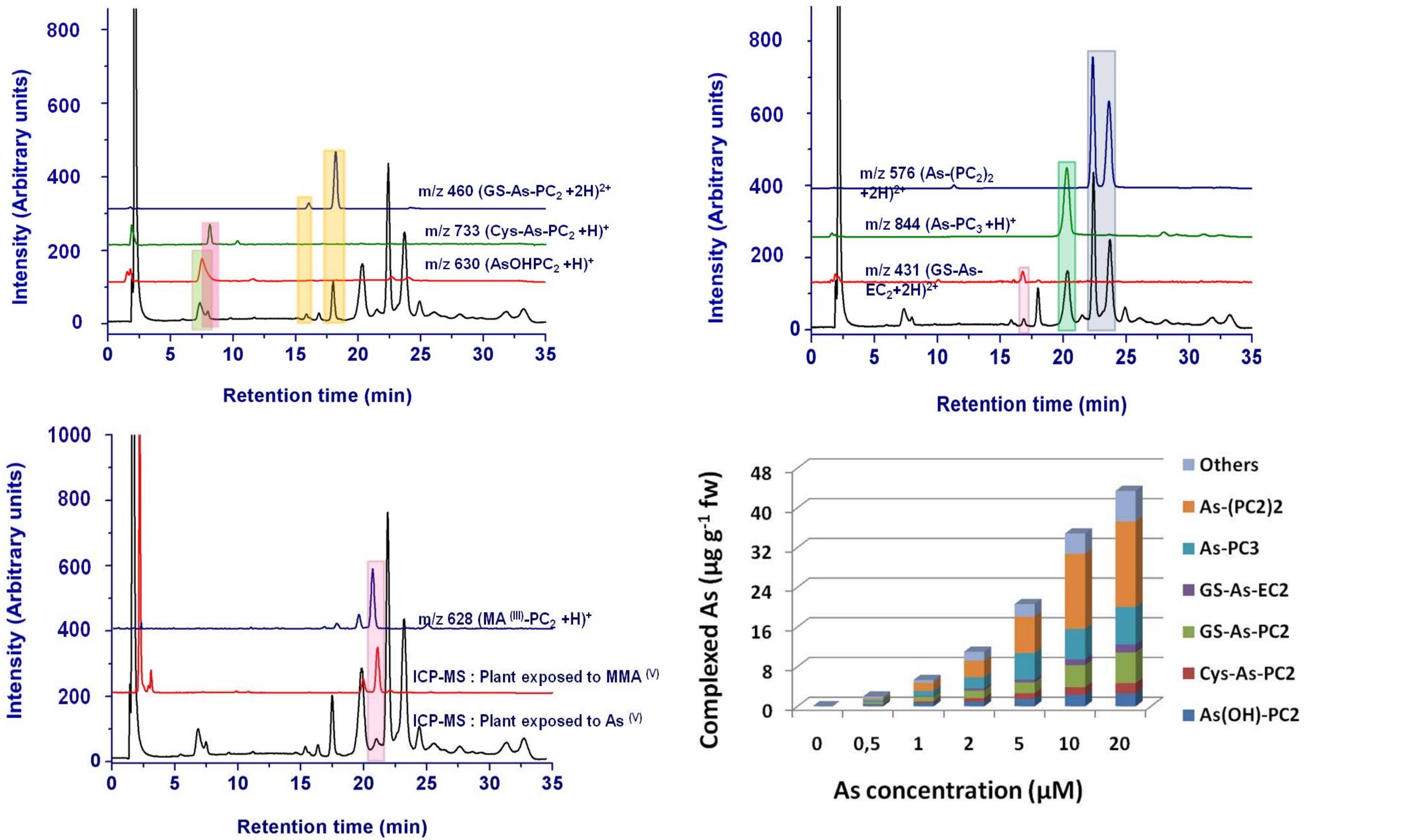
As Speciation Analysis



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

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

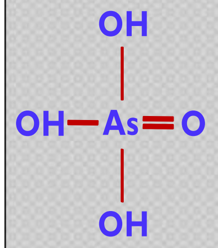
As Speciation Analysis

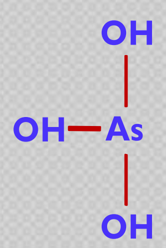


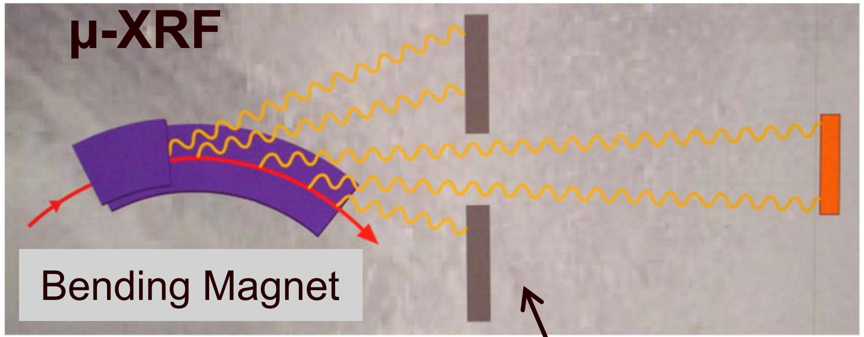
Separation of As-PC complexes; *m/z* traces of As-PC species measured by ESI-MS and *m/z* 75 (As) measured by ICP-MS
Quantification of various As species (based on ICP-MS data)

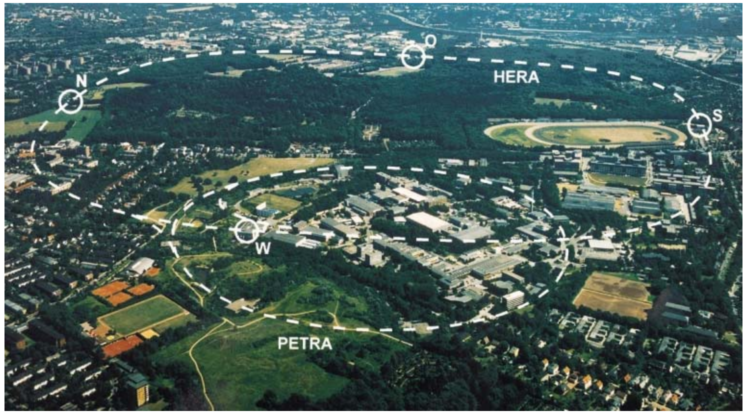
Mishra S, Wellenreuther G, Mattusch J, Stärk H-J, Küpper H (2013) Plant Physiology 163, 1396-1408

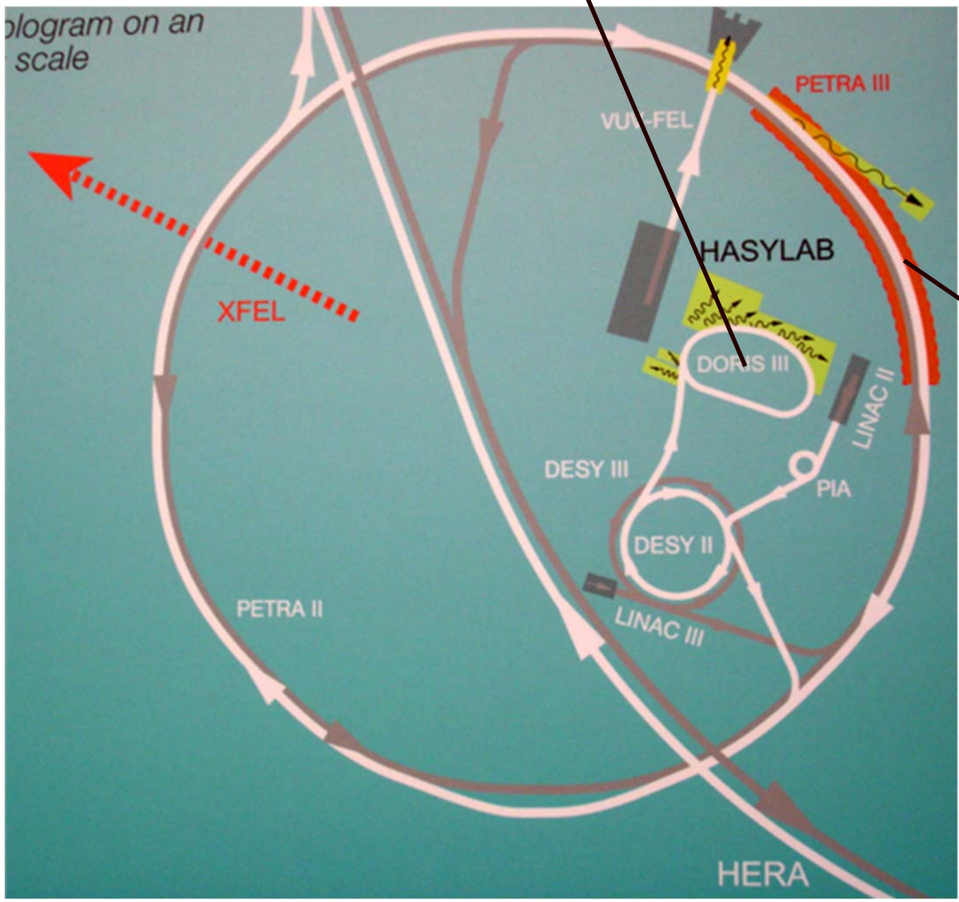
μ-XRF and μ-XANES

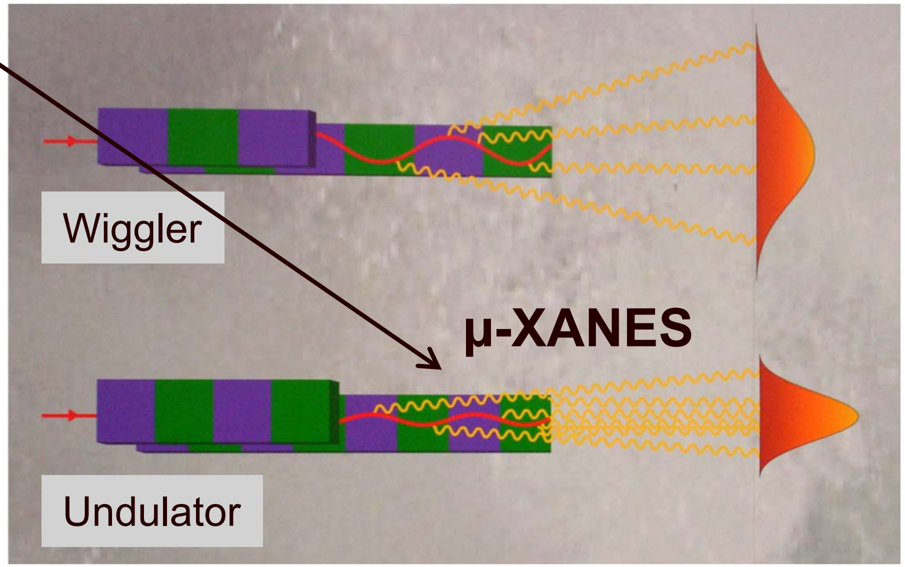




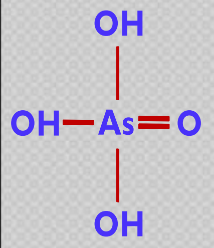


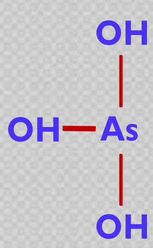


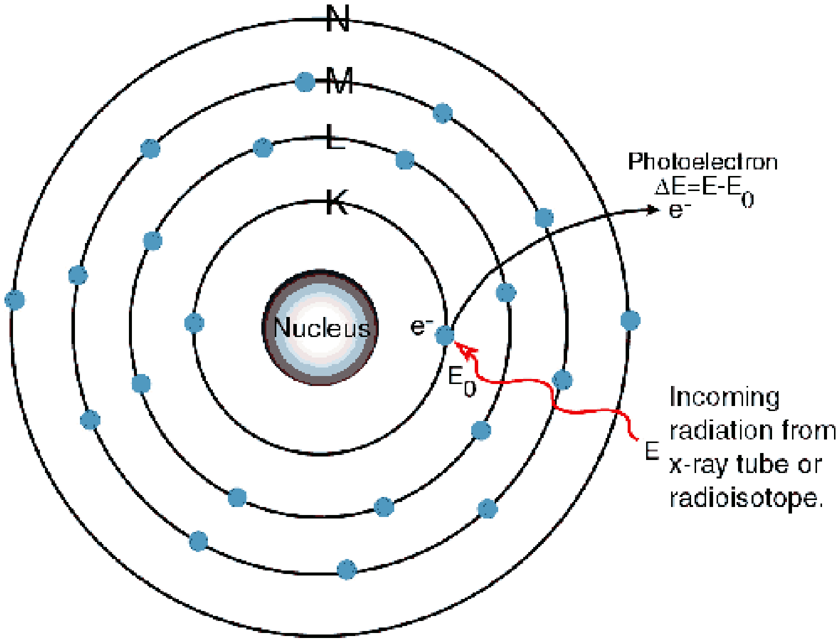


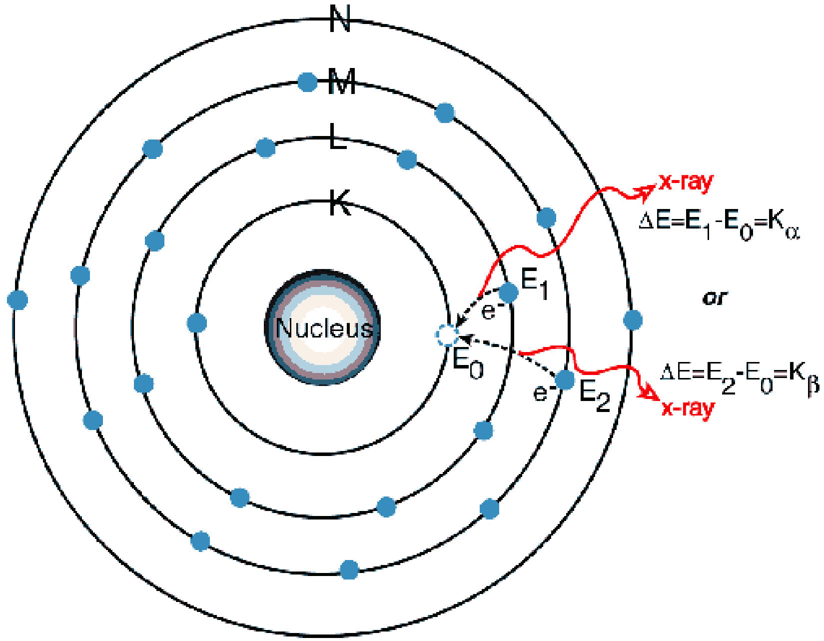


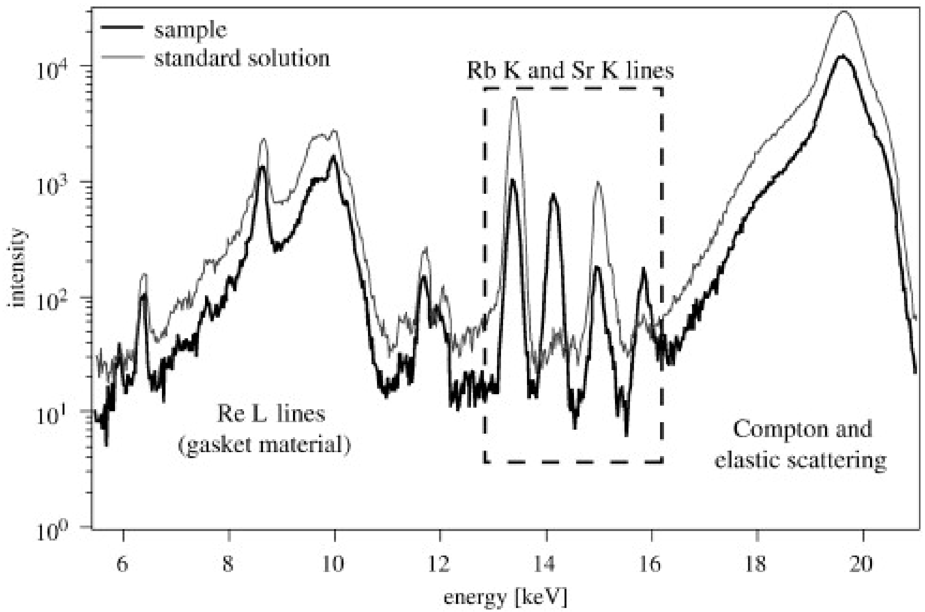
μ-XRF

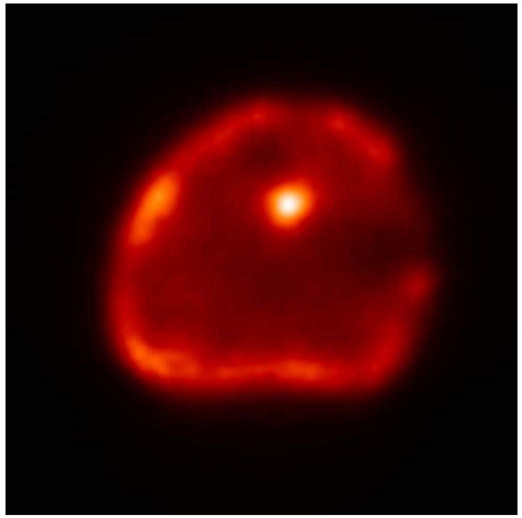

OH
OH—As=O
OH


OH
OH—As
OH

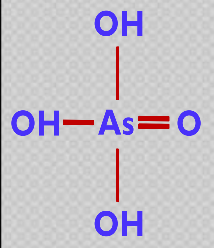

N
M
L
K
Nucleus
e⁻
E0
Photoelectron
ΔE=E-E0
e⁻
Incoming radiation from x-ray tube or radioisotope.
E

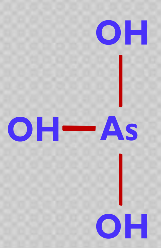

N
M
L
K
Nucleus
e⁻
E0
E1
E2
x-ray
ΔE=E1-E0=Kα
or
ΔE=E2-E0=Kβ
x-ray

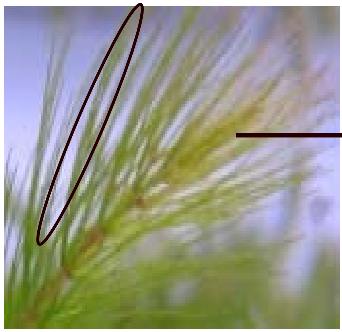

sample
standard solution
Rb K and Sr K lines
Re L lines
(gasket material)
Compton and
elastic scattering
intensity
energy [keV]

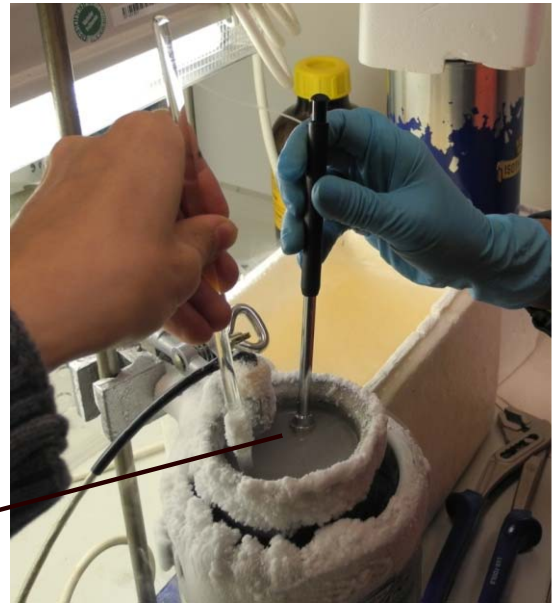


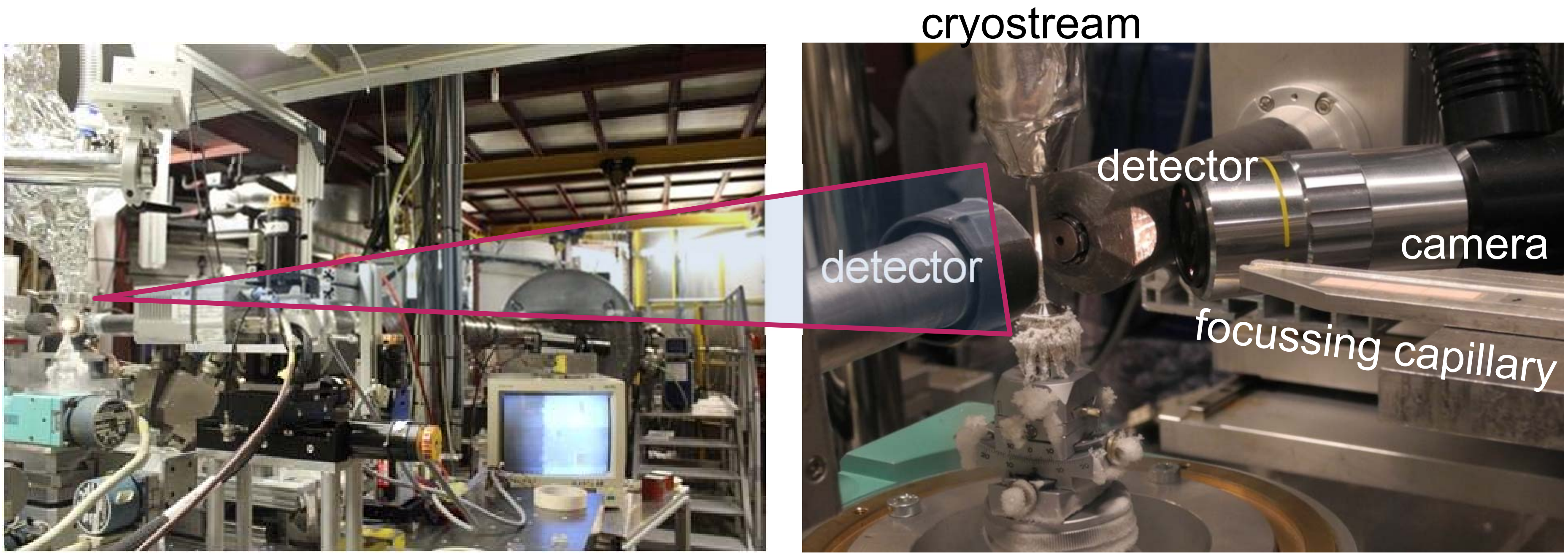
Sample preparation and instrumental setup for µ-XRF and µ-XANES



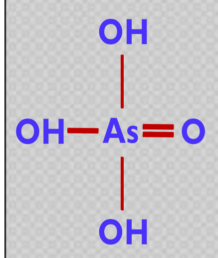


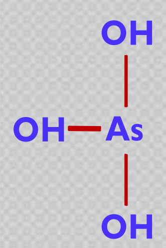


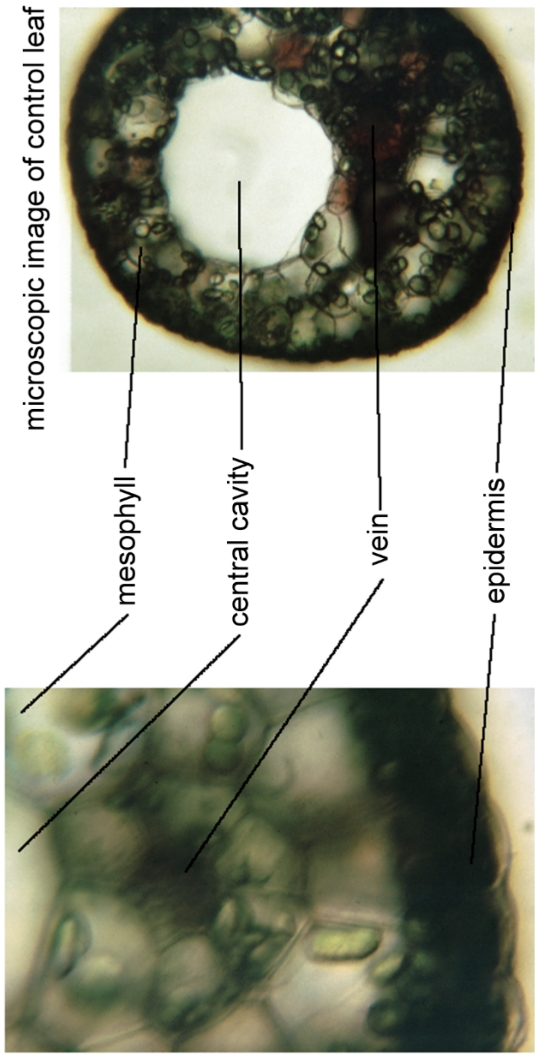


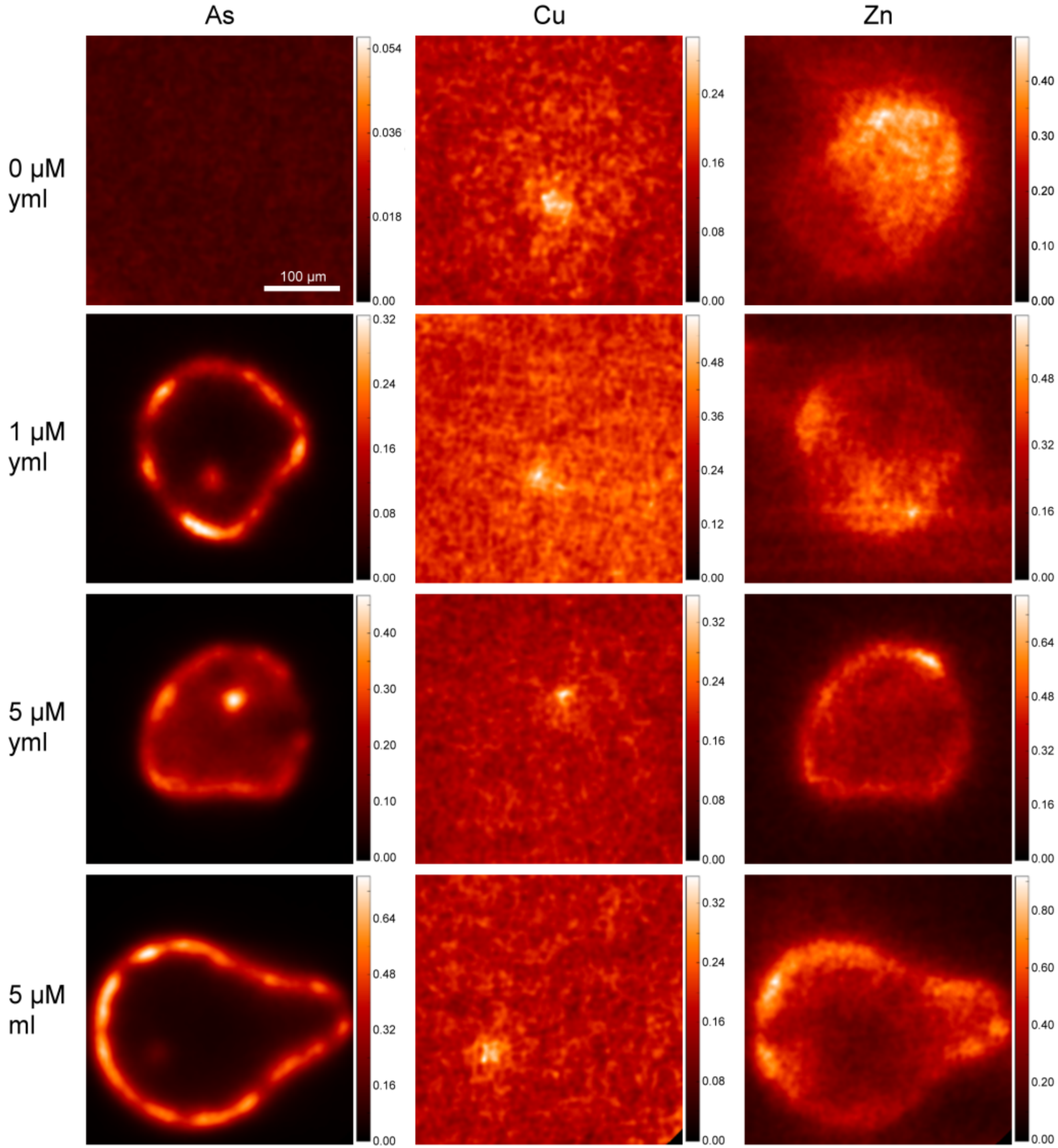


Distribution of As and its effect on Copper and Zinc

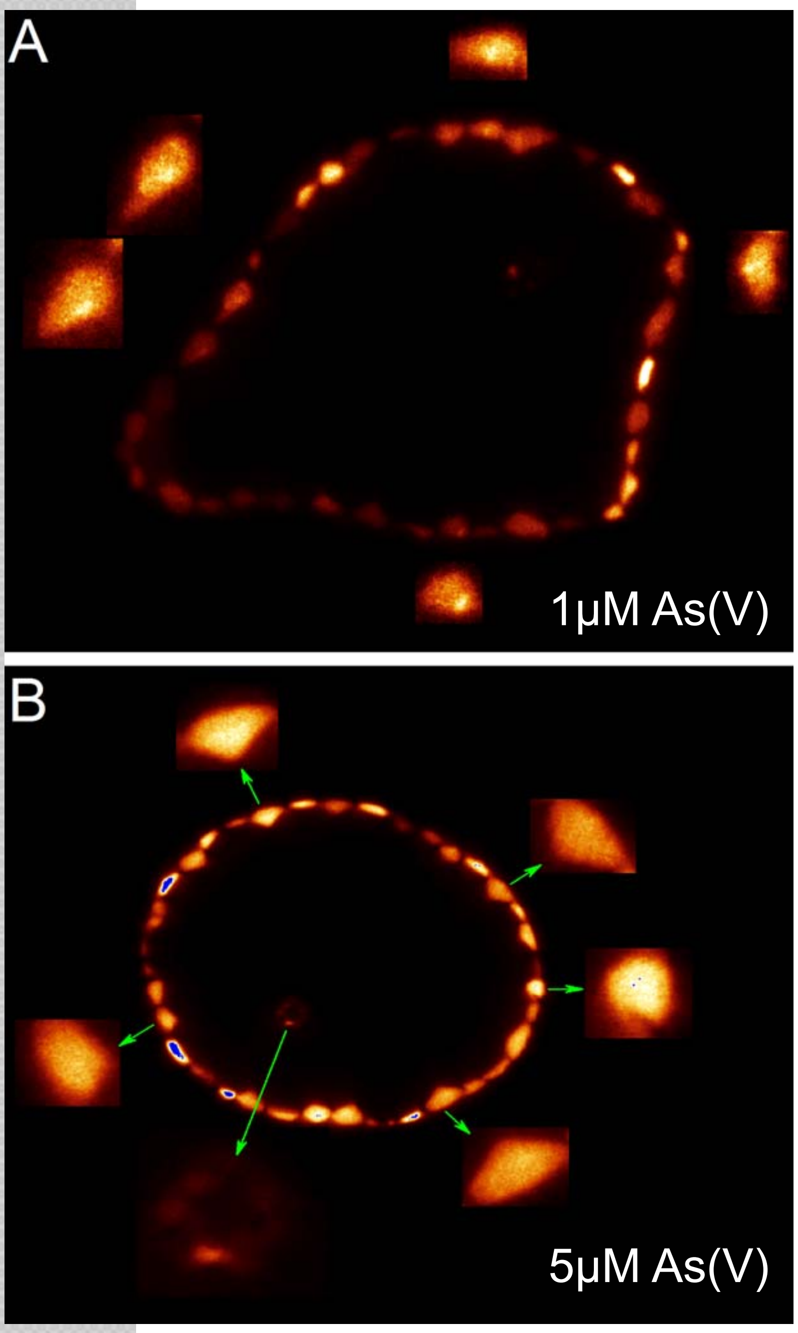








Sub-cellular distribution of As in *C. demersum* leaves



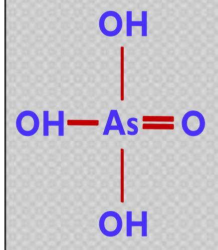
2 phase response to As toxicity

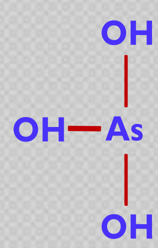
A) Initially, at sublethal concentrations (1µM As), As is accumulated mainly in the nucleus
→ genotoxicity (besides inhibition of Chl biosynthesis)

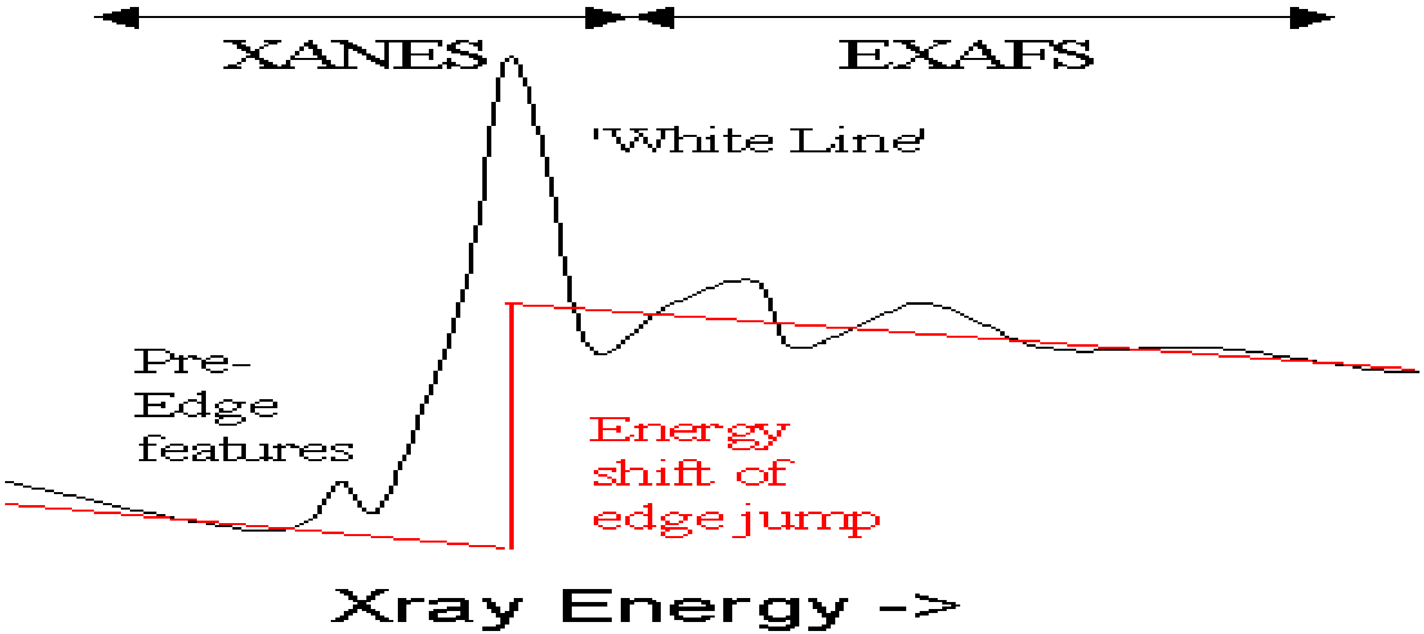
B) At lethal concentration (5 µM As), As fills the whole cell
→ various types of damage

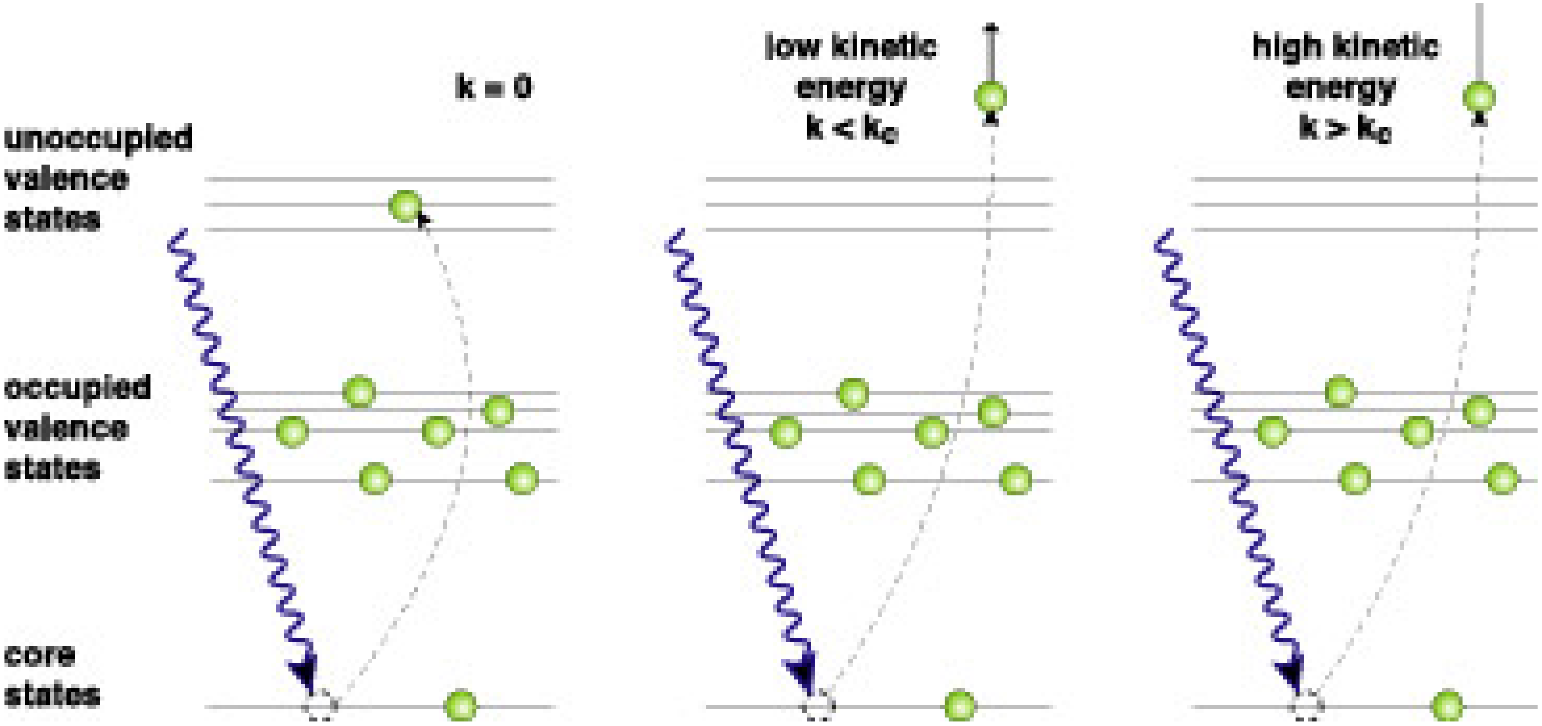
Mishra S, Alfeld M, Sobotka R, Andresen E, Falkenberg G, Küpper H (2016) Journal of Experimental Botany 67, 4639-4646

μ-XANES

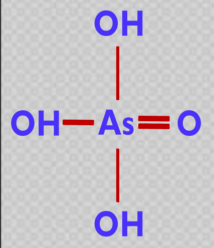

OH
OH—As=O
OH

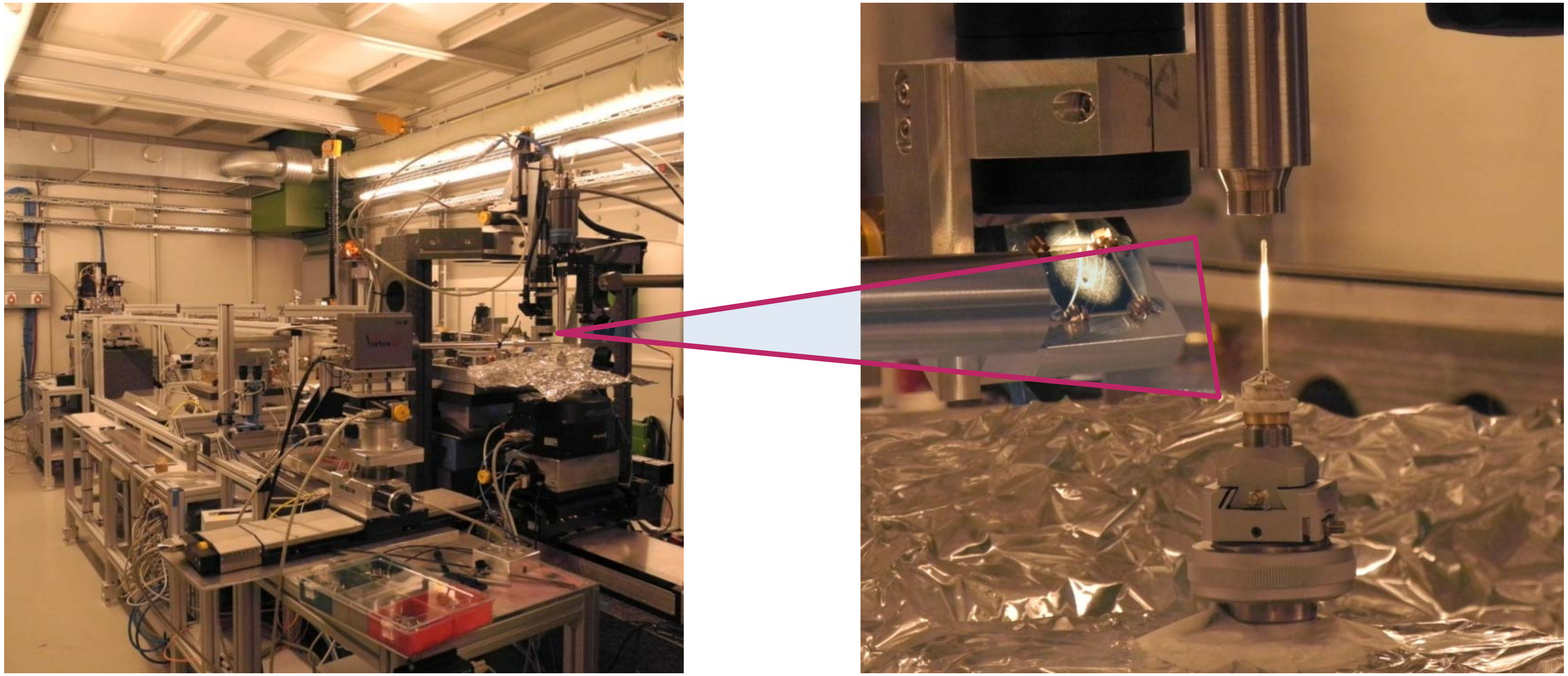

OH
OH—As
OH

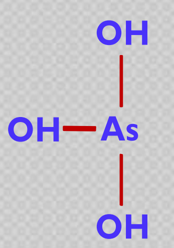

XANES
EXAFS
'White Line'
Pre-
Edge
features
Energy
shift of
edge jump
Xray Energy ->

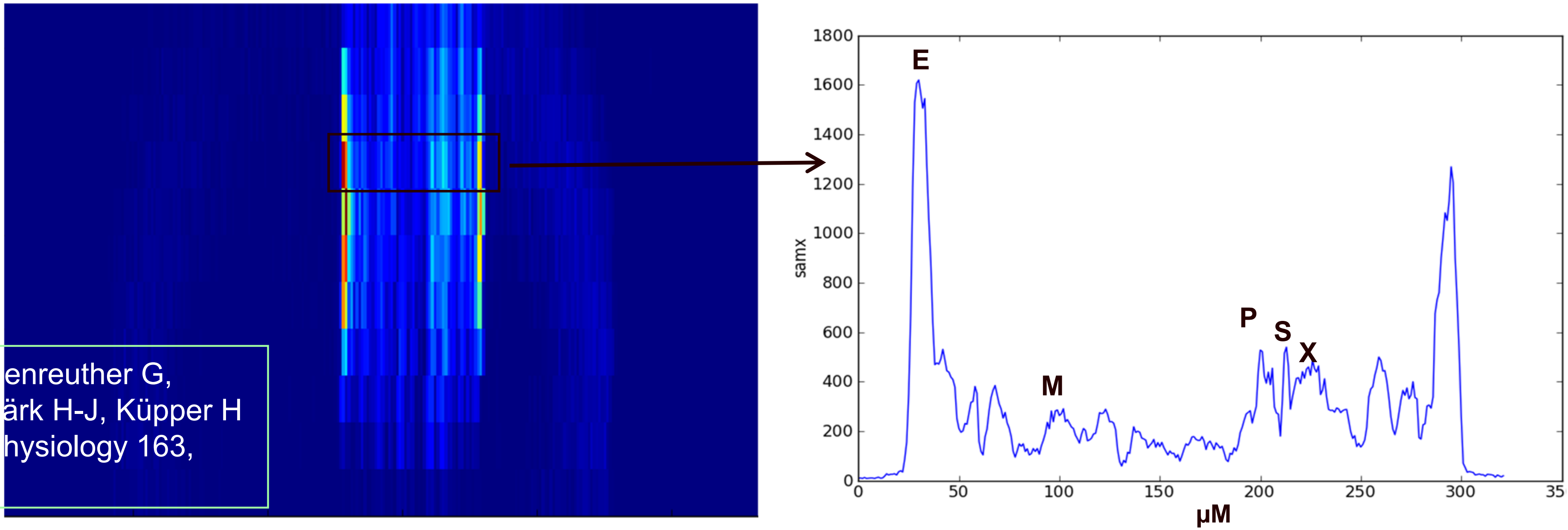

k = 0
low kinetic
energy
k < k₀
high kinetic
energy
k > k₀
unoccupied
valence
states
occupied
valence
states
core
states

Speciation of As in different tissues



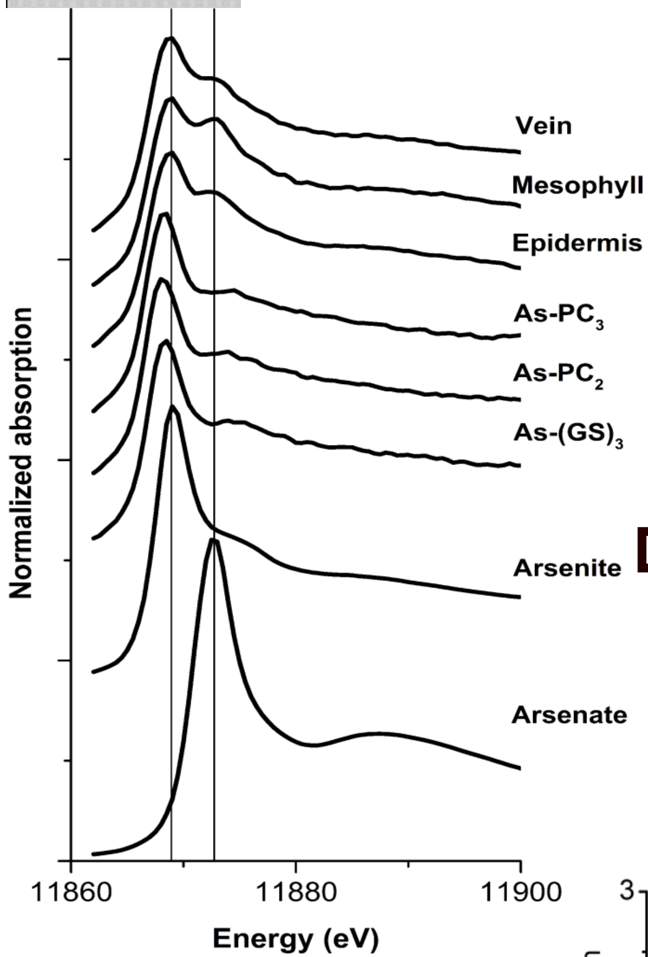


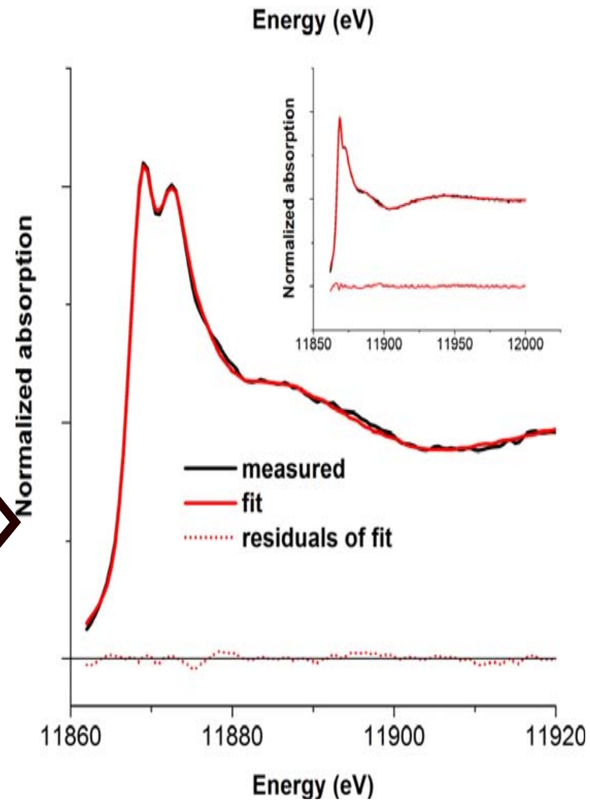


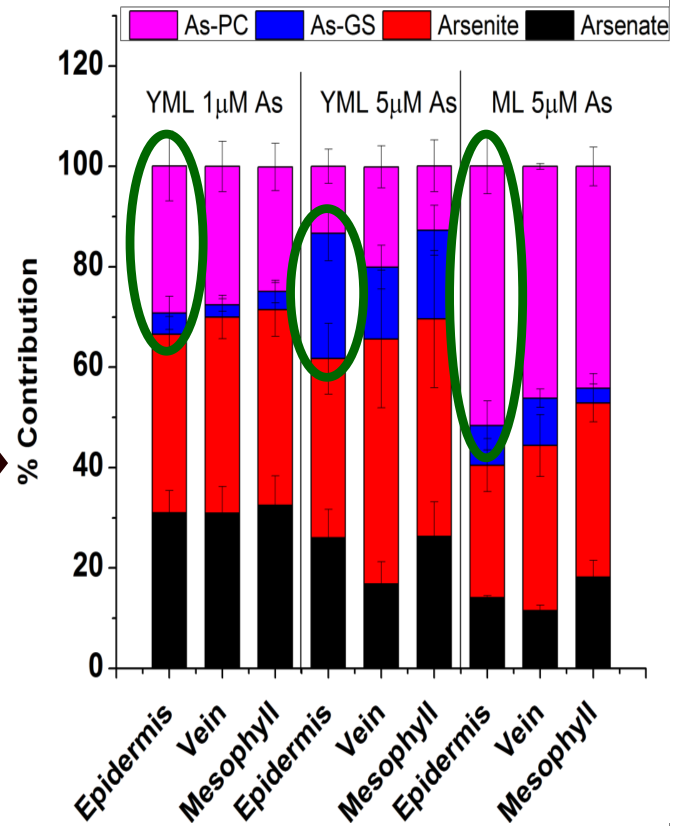


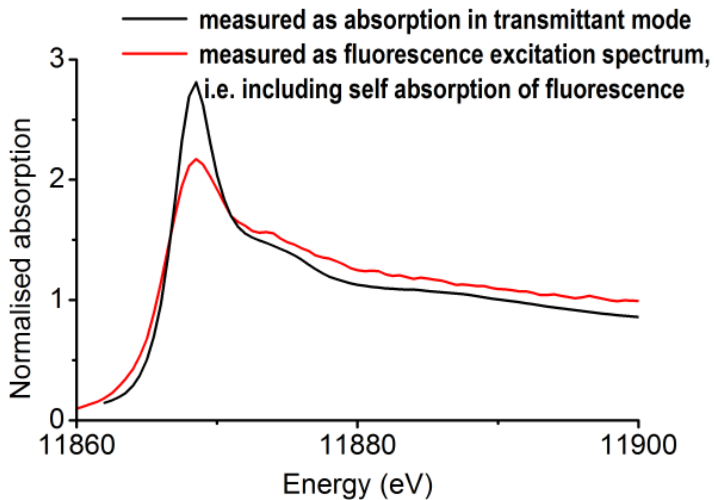
Mishra S, Wellenreuther G, Mattusch J, Stärk H-J, Küpper H (2013) Plant Physiology 163, 1396-1408

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







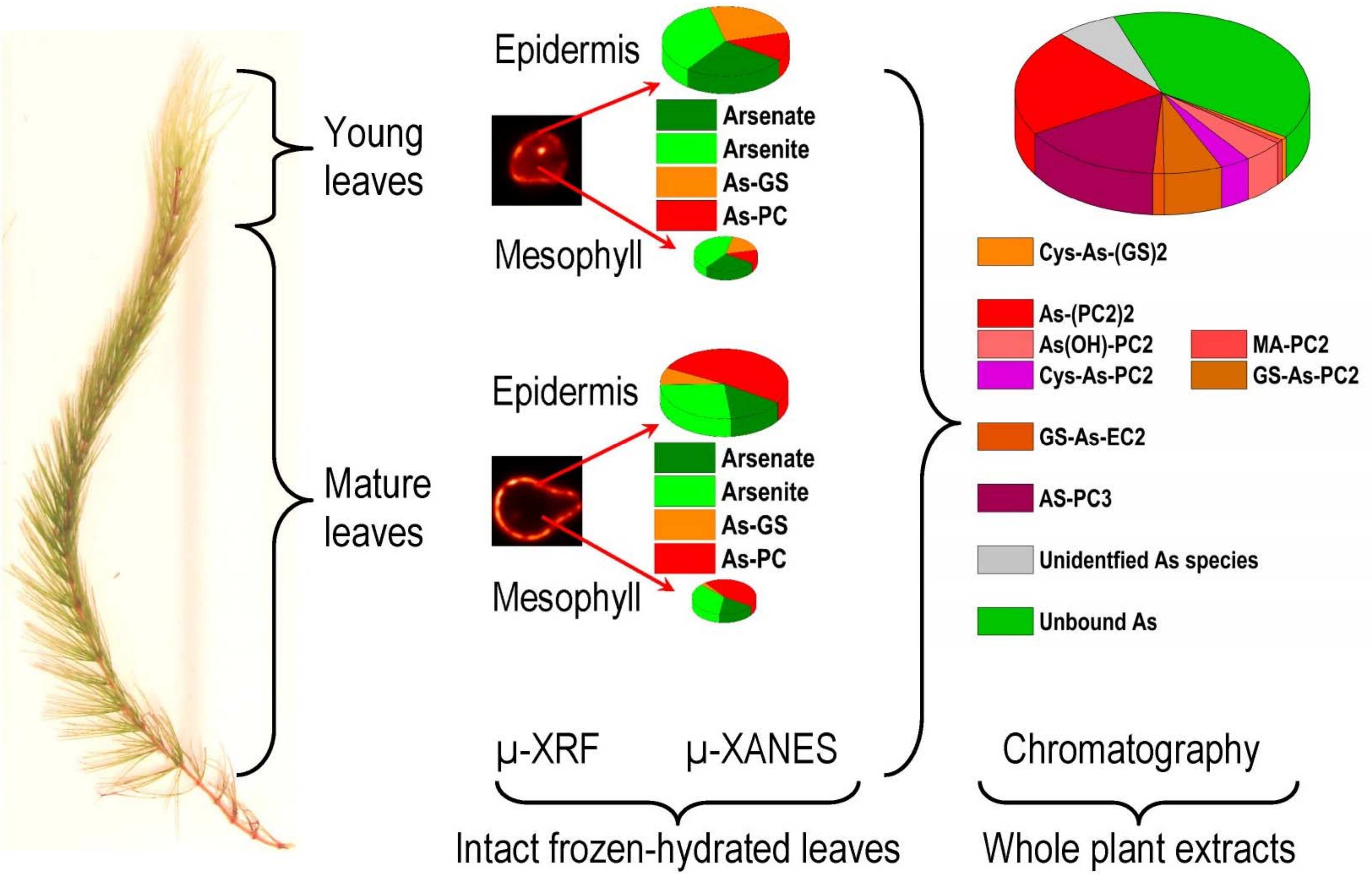


→highest As-PC in the epidermis of young-mature leaves at 1µM As and mature leaves at 5µM

→ highest As-GS in epidermis of young-mature leaves at 5µM As

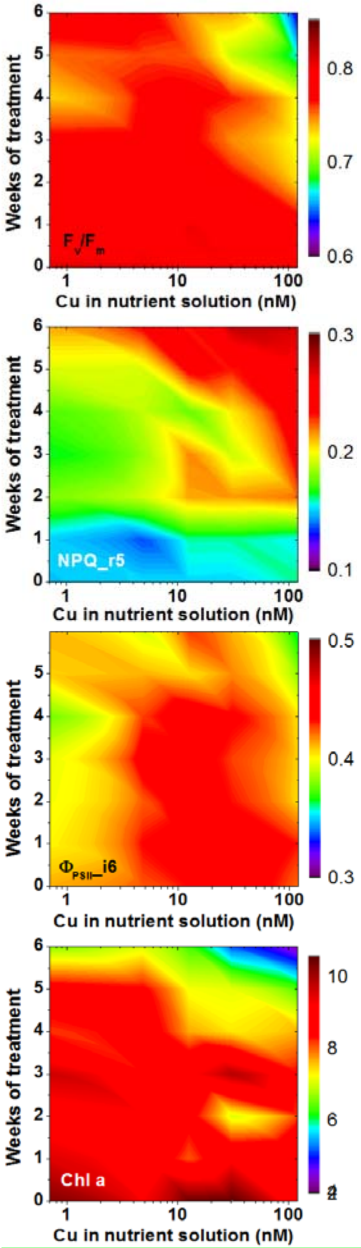
Mishra S, Wellenreuther G, Mattusch J, Stärk H-J, Küpper H (2013) Plant Physiology 163, 1396-1408

Speciation of arsenic in a non-accumulator plant



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

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

>0.5µM As: inhibition of Chl biosynthesis

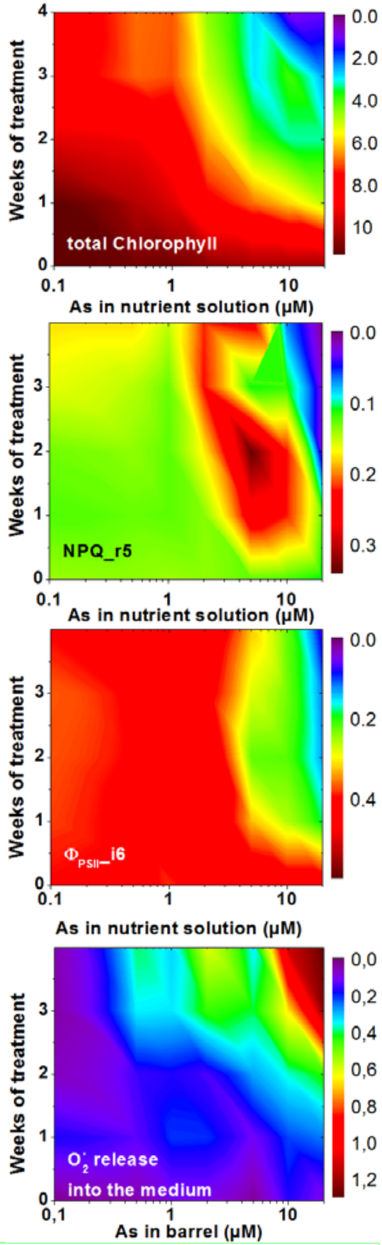
→ decreased light harvesting

> 1µM As: (1) As binding in nucleus
(2) decreased exciton transfer from the antenna to the RC
→ up-regulation of thermal exciton dissipation (NPQ)

>2µM As: Electron transport (Φ_{PSII}) inhibited

>5µM As: NPQ inhibition

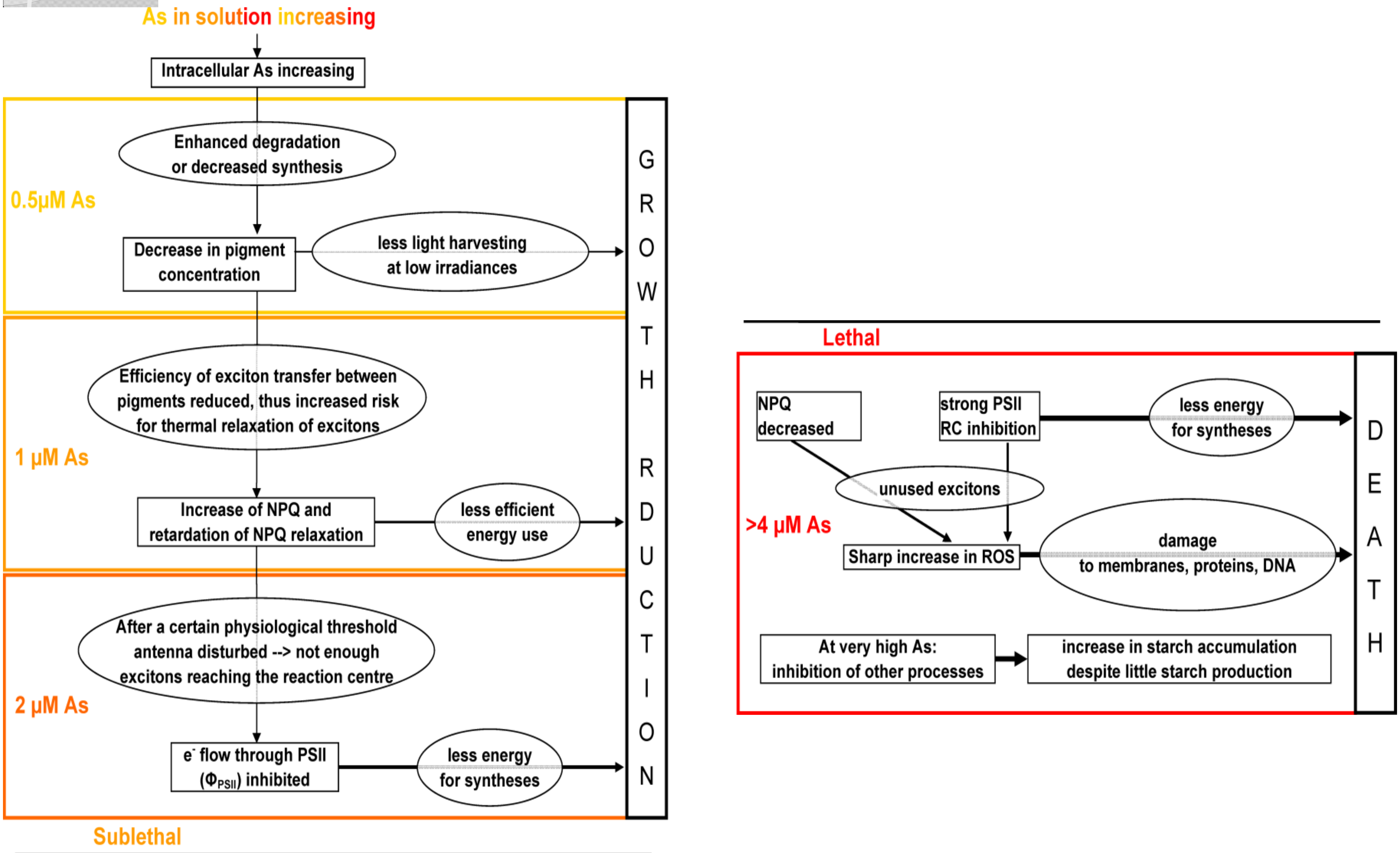
Malfunctioning of photosynthesis leads to generation of ROS in addition to increased inhibitions



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



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

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