

Applications of X-ray Absorption Spectroscopy in Environmental, Health and Chemical Sciences

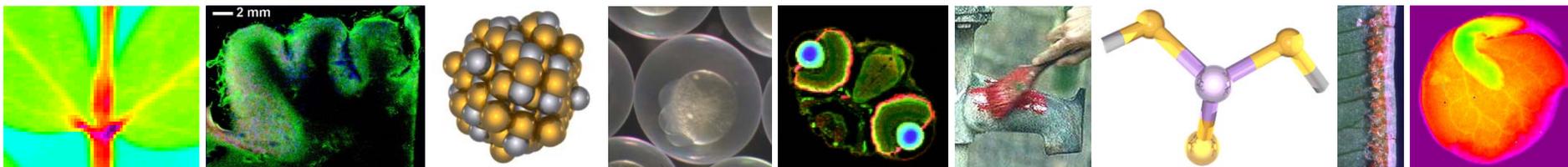
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National School on Neutron and X-ray Scattering
Argonne National Laboratory
Oak Ridge National Laboratory
August 10-24, 2013





Overview

- Why use X-ray absorption spectroscopy in environmental, health and chemical studies?
- X-ray absorption spectroscopy combined with X-ray fluorescence mapping
- Case studies:
 - Arsenic in plants
 - Mercury in zebrafish as vertebrate model
 - Mercury in human brain
 - Arsenic, selenium and Bangladesh

Synchrotron Facilities in Canada and USA

(those used in my talk!)

CLS:
Canadian Light Source
(Saskatoon, SK)



SSRL:
Stanford Synchrotron Radiation
Lightsource
(Stanford, CA)



APS:
Advanced Photon Source
(Argonne, IL)

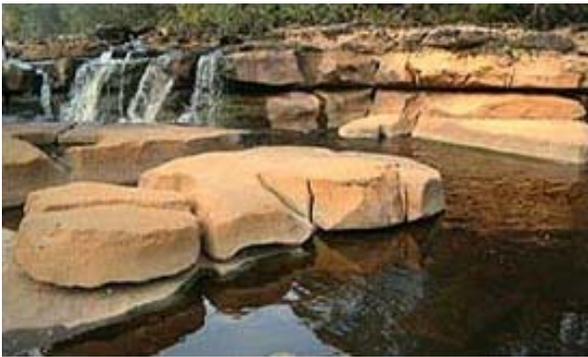


<http://johomaps.com/na/na2.html>

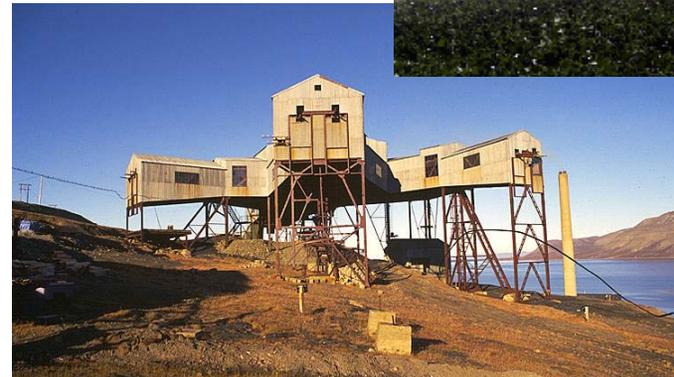
Introduction: XAS of Complex Systems

- X-ray absorption spectroscopy gives local structural information around a central absorbing atom
- It can do this for a pure system
 - e.g. crystalline solid, purified solution
- It can do this equally well for a complex system
 - Element present in more than one form
 - Sample has mixture of states and species
- XAS is very amenable to investigating complex systems, often being one of the few tools that can do so
- Ideal for looking at metals and other elements in biological and environmental samples

Sources of metals in our Environment



Natural sources



Manmade sources

Sources of Human Exposure to Metals



food



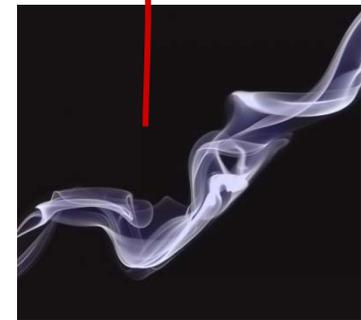
drinking
water



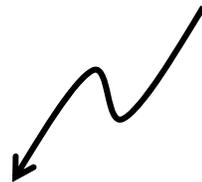
contact



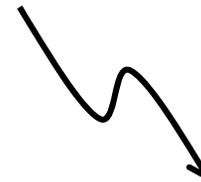
inhalation



Metals in Humans (and other organisms)



Essential elements



Toxic elements

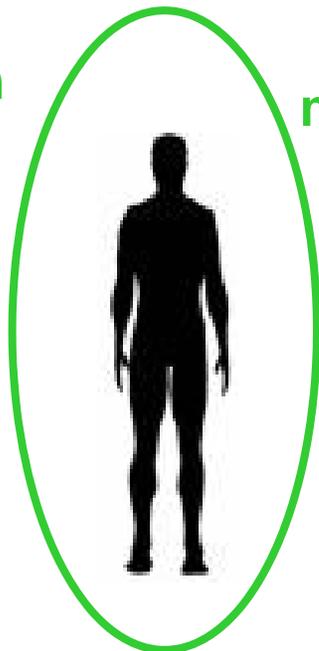
copper

selenium

molybdenum

iron

zinc

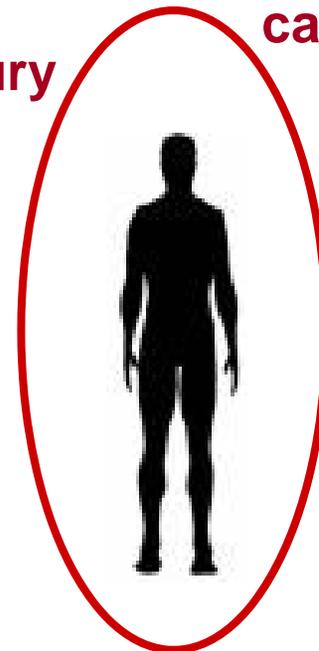


mercury

cadmium

arsenic

lead

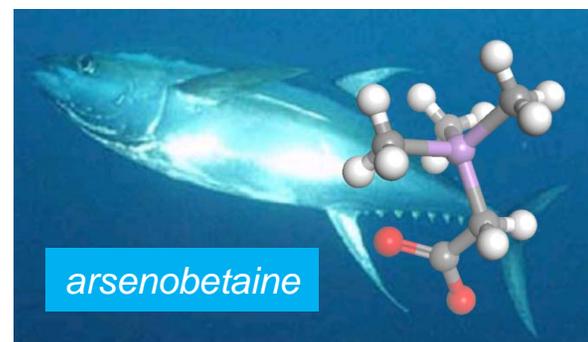
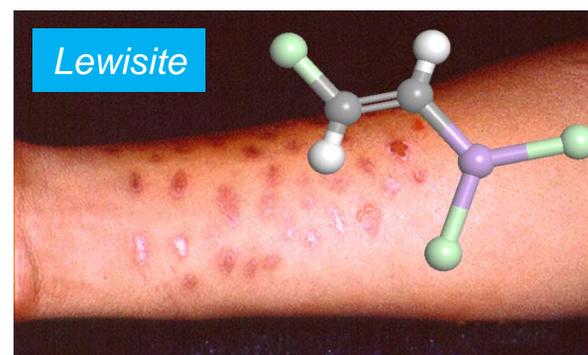


Just about all metals are toxic at high levels

Molecular Form Matters!

Example: Arsenic

- Arsenic is infamous as a poison
 - e.g. Lewisite – war gas known as “dew of death”
- However, not all arsenic is poisonous!
 - Arsenobetaine (0.02 wt% in seafood) is not toxic at all



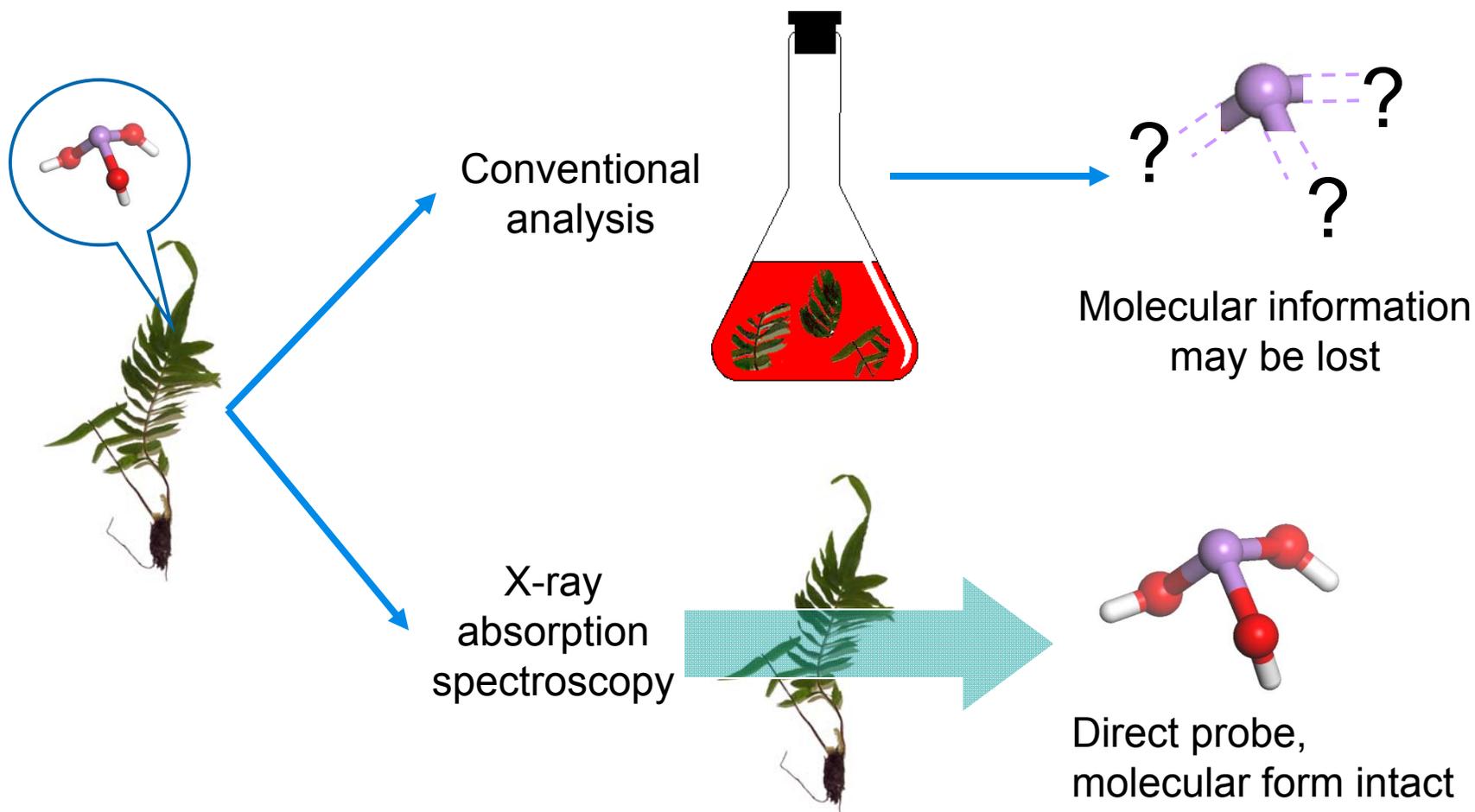
X-ray Absorption Spectroscopy in Environmental & Biological Sciences

- Chemical form affects the element's properties, e.g.
 - Solubility and mobility in groundwater
 - Bioavailability to organisms
 - Toxicity to higher organisms including humans
- Samples are often heterogeneous, e.g.
 - Organisms, tissues, cells
 - Soil, sediment, mine tailings
- Need to know the chemical form of a potentially toxic element in a complex matrix
 - X-ray absorption spectroscopy can do this

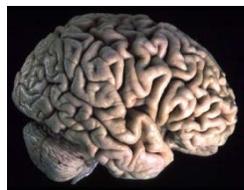
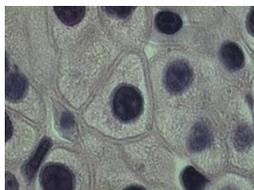
X-ray Absorption Spectroscopy in Environmental & Biological Sciences

- XAS probes the local chemical form of any element
 - Atomic property so no confusion over which element
- Due to properties of X-rays, XAS can be measured in almost any matrix
 - e.g. biological tissues, sediments, etc.
- Analyzes all forms of the element with no “hidden” phases, e.g.
 - Crystalline or amorphous phases, aqueous solutions, adsorbed species, even gases
- *In situ* probe - little pretreatment is needed
- Information on mixtures of chemical species
- Spatial information from fluorescence mapping

X-ray Absorption Spectroscopy: Direct and Potentially Non-Destructive

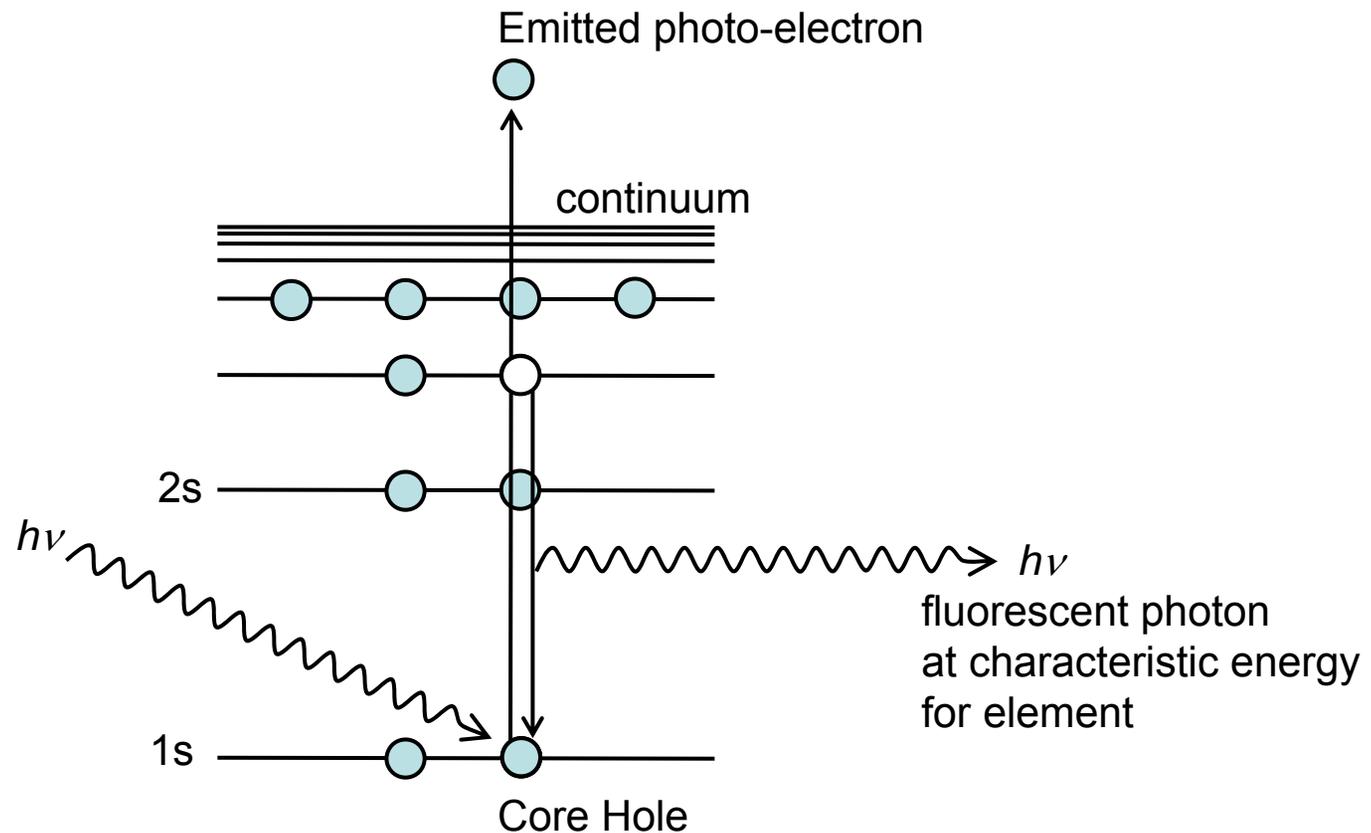


Chemically Specific Mapping of Biological Samples



- Many complex samples have spatial structure
- Questions we may want to answer:
 - How is an **element** distributed?
 - What is an element's **chemical form** in a particular location?
 - How is a **chemical species** distributed?
- We would like to do this when levels are dilute, and on intact living specimens
- We use **X-ray fluorescence mapping** in combination with **X-ray absorption spectroscopy** to answer this

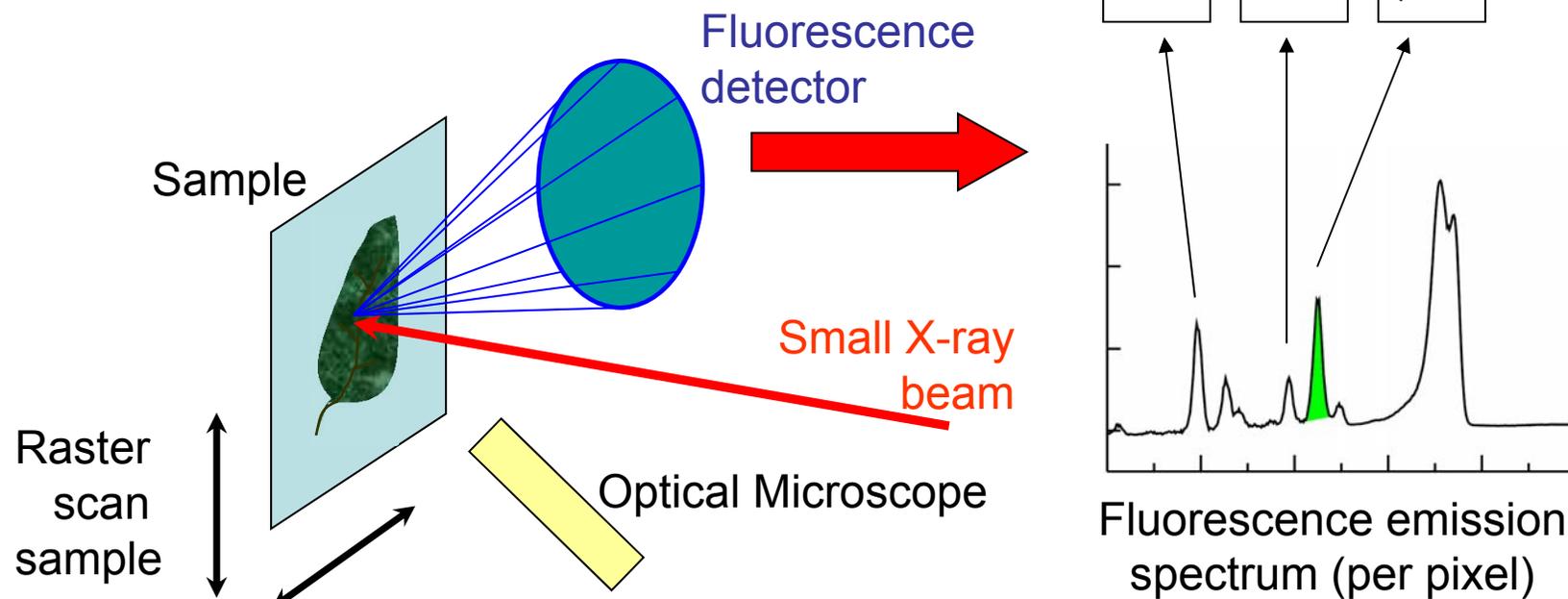
X-ray Fluorescence Mapping



X-ray Fluorescence Mapping

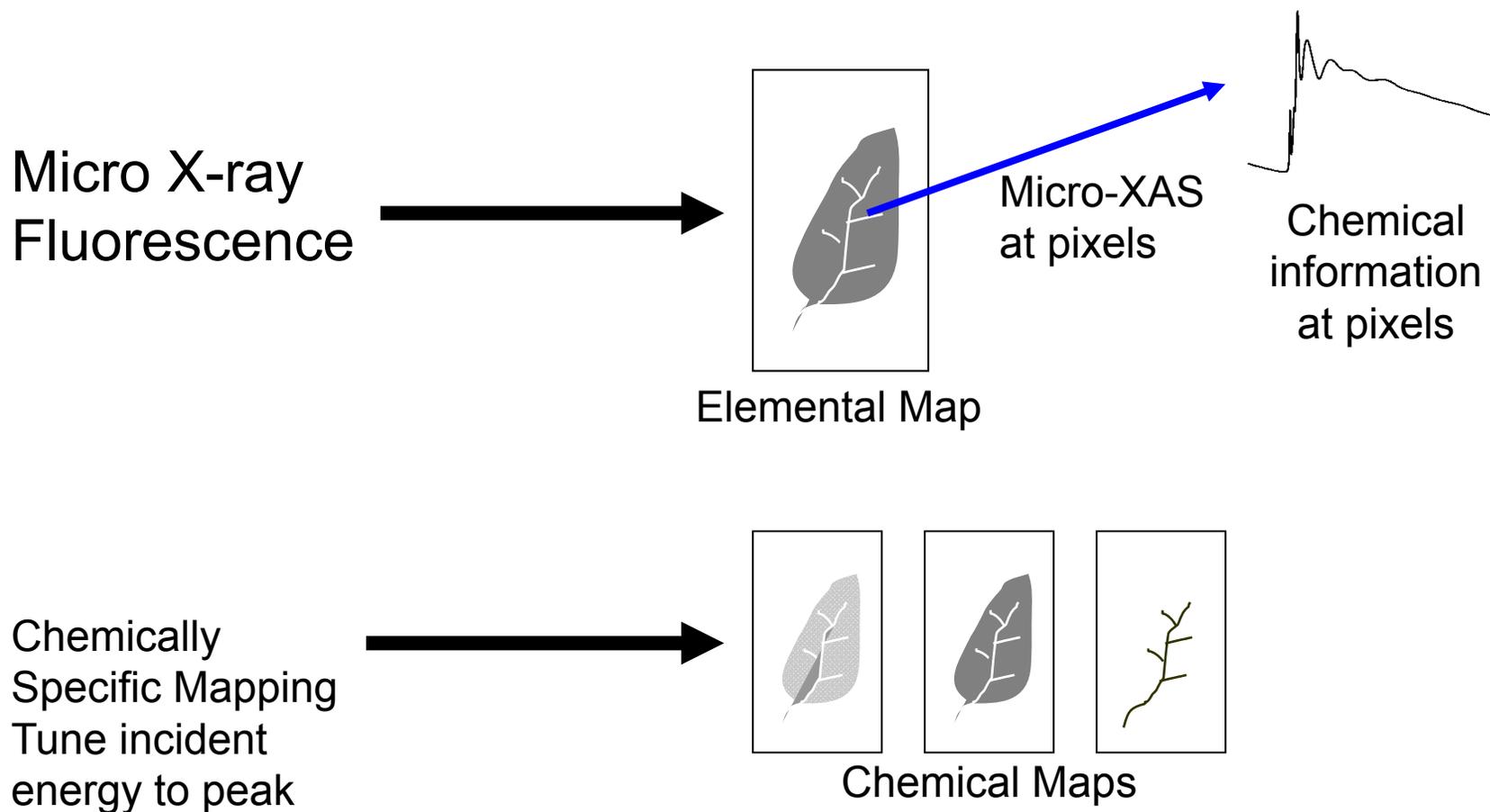
At each pixel, fluorescent line intensities yield elemental quantities

- Fixed energy above absorption edge of element(s) of interest
- Spatially raster sample in beam
- Also called: Fluorescence microprobe; μ -XRF (μ -X-ray fluorescence); SRIXE



Simultaneous Spatial and Chemical Information

Two routes to information

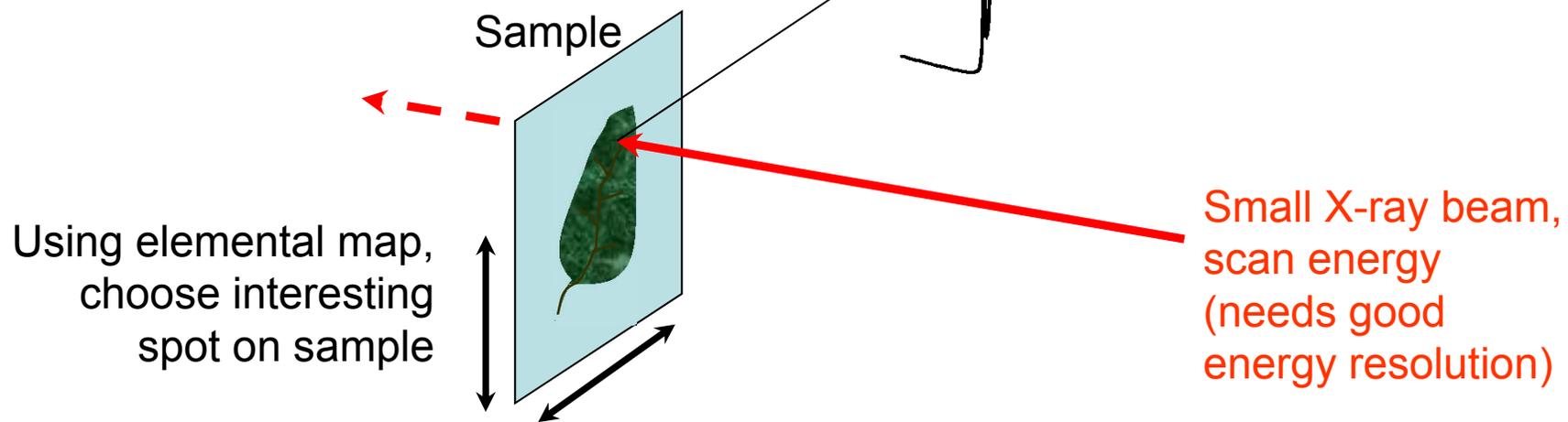


Micro-XAS

Spectrum from pixel gives chemical information

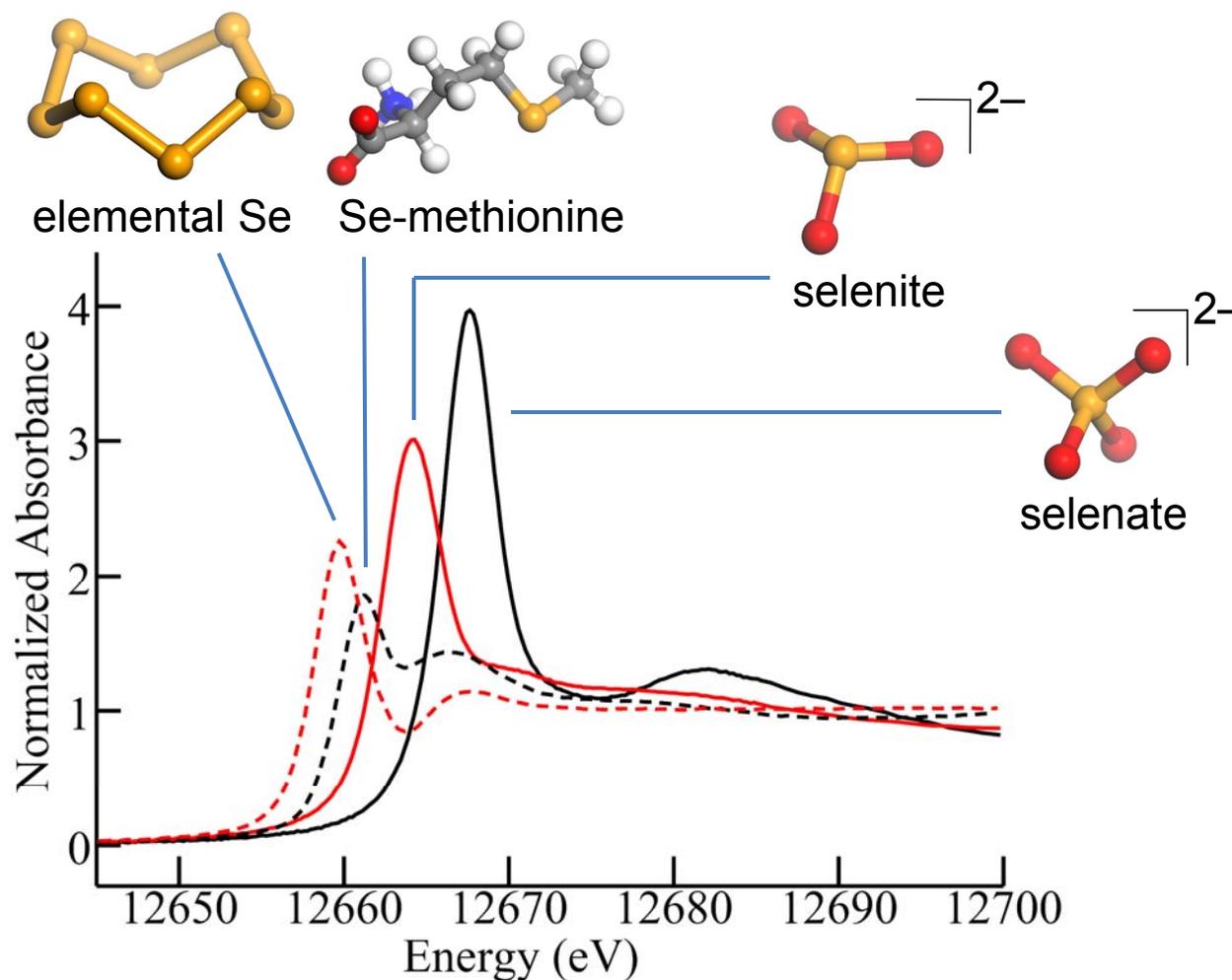
- Complete fluorescence map
- Select pixel of interest
- Scan incident energy
- Also called μ -XAS, μ -XANES, μ -XAFS

Collect spectrum by scanning incident energy, monitor fluorescence



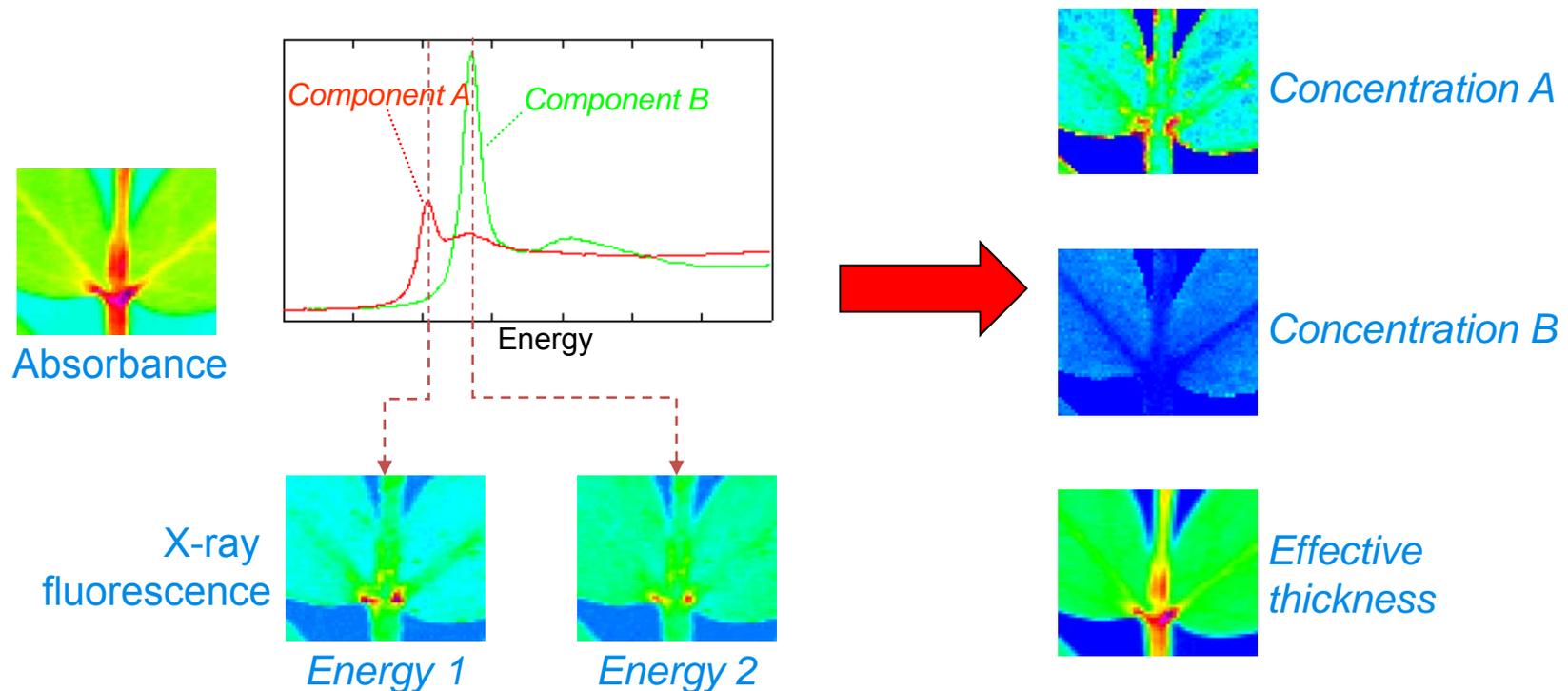
Chemically Specific Mapping

Use sensitivity of near-edge (XANES) to map different chemical species



Chemically Specific Mapping

- Map at two or more energies to generate quantitative maps of each species
- Works best for species with large contrast in the edge
- Needs excellent energy resolution and stability
- Also known as oxidation state mapping, XANES-mapping, etc.



Chemically Specific Mapping vs. XFM

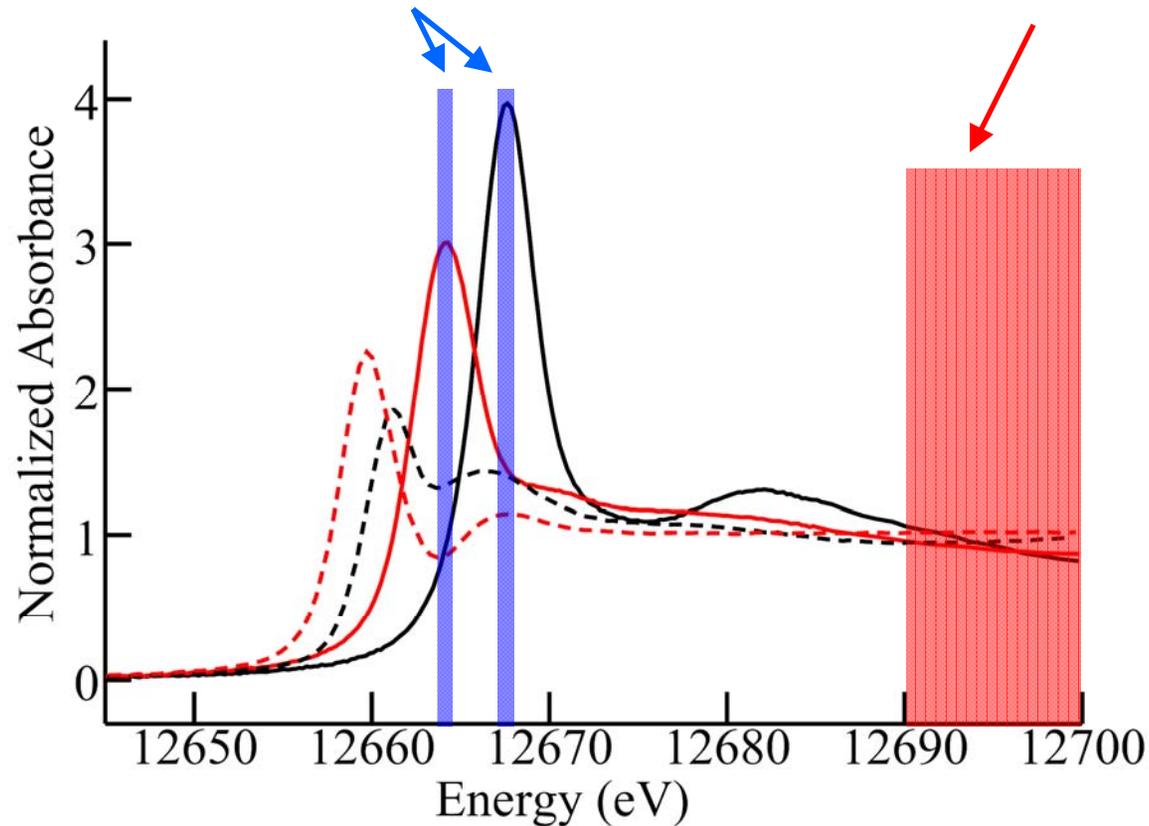
Differences lie in the incident energy

Chemical Specific Mapping

Energy at the edge:
Image *chemical species*

X-ray Fluorescence Mapping

Energy above the edge:
Image *element(s)*



Choice of Chemically Specific Mapping

- X-ray Fluorescence Mapping plus micro-XAS:
 - ✓ Gives entire spectrum at selected points
 - ✗ May miss spatial detail
 - ✗ Longer dwell time at those pixels
- Chemically Specific Mapping:
 - ✗ Need to know which species to look for
 - ✗ Need good spectral contrast
 - ✓ Gives complete spatial maps of each species
 - ✓ Shorter dwell times



Case Study: An Arsenic-Loving Fern

Pickering, I. J.; Gumaelius, L.; Harris, H. H.; Prince, R. C.;
Hirsch, G.; Banks, J. A.; Salt, D. E.; George, G. N.
Environmental Science & Technology (2006) 40, 5010-5014.



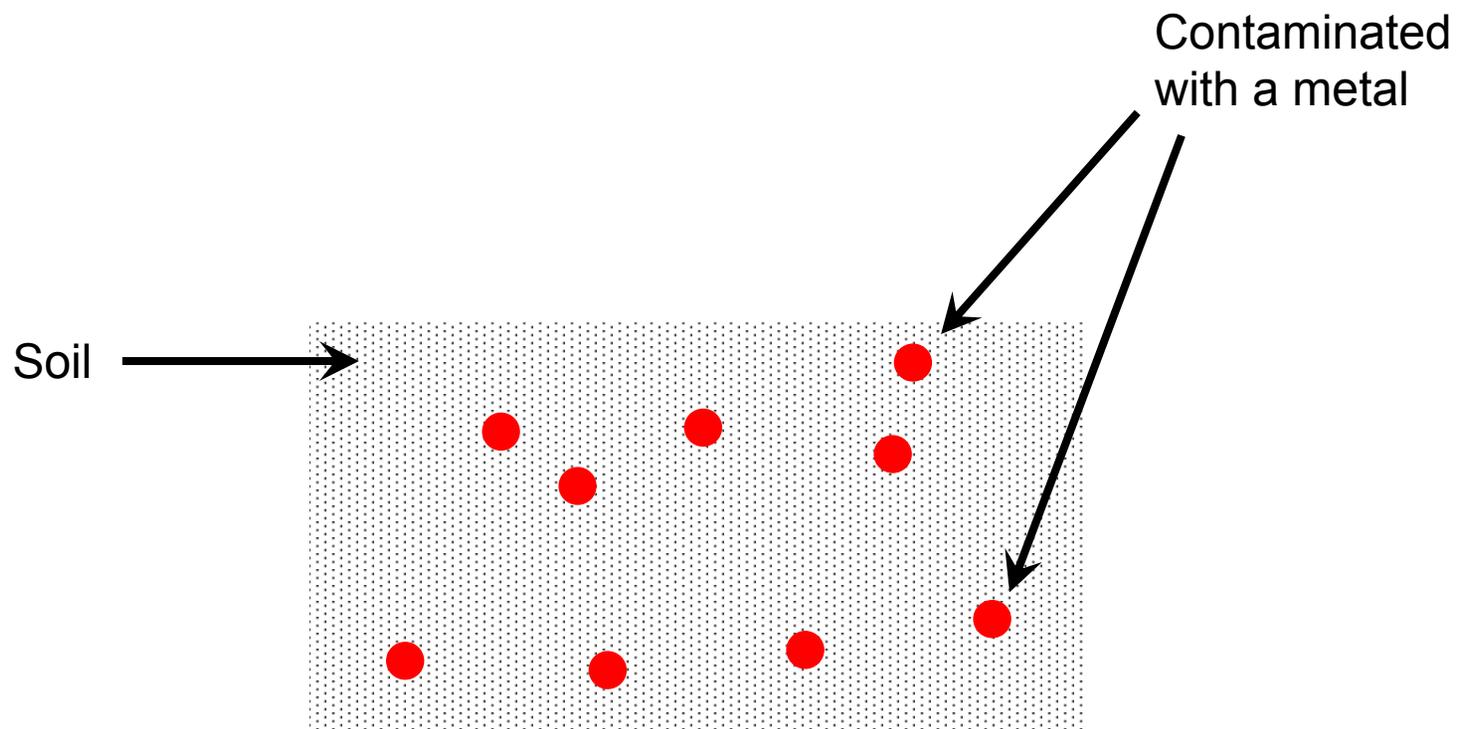
Pteris vittata

- A hyperaccumulator of arsenic
 - Takes up, transports, stores and tolerates arsenic within its tissues
 - Tissue concentration is highly magnified compared with the soil
 - Can store >2% dry weight As
- Arsenic is a major environmental problem in many countries
- *Pteris vittata* shows potential in arsenic **phytoremediation**
 - Use of plants to remove arsenic from contaminated areas
 - (Either *Pteris vittata* itself or its pathways in engineered plants)



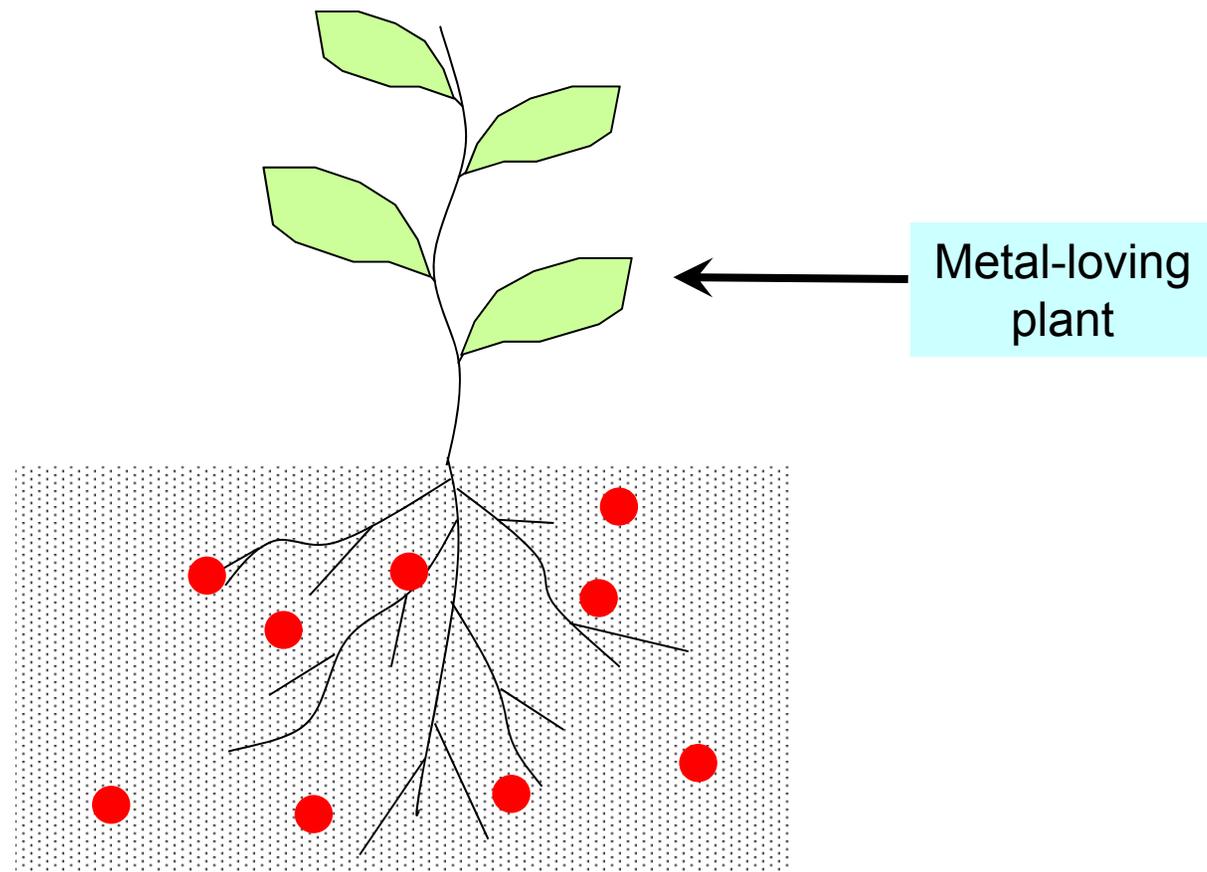
Phytoremediation

Soil contaminated with a metal – how do we clean this up?



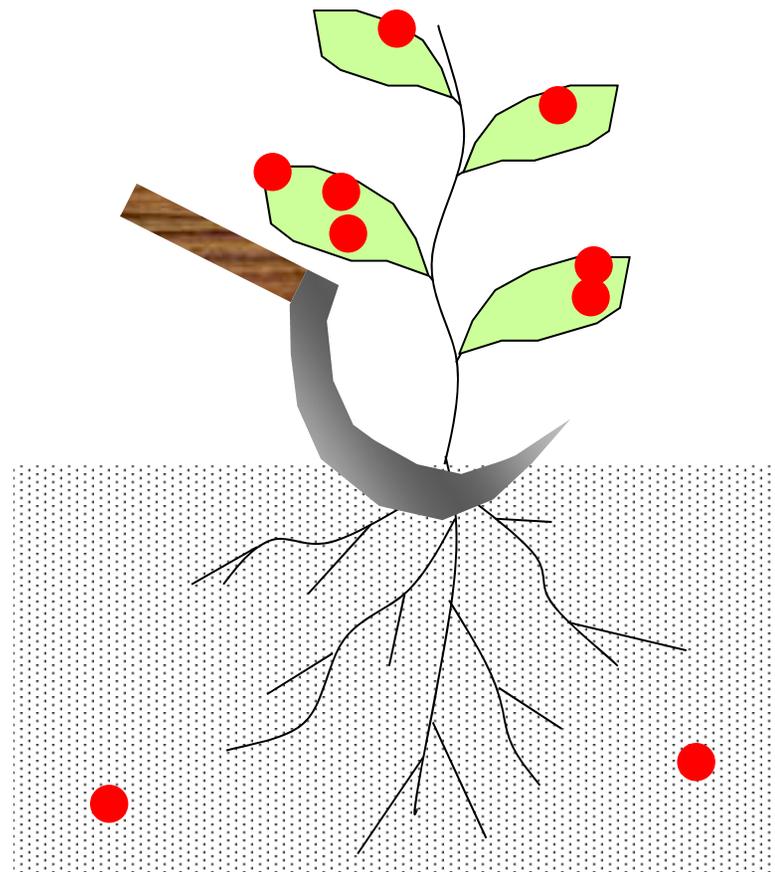
Phytoremediation

Grow plants which take up the metal...



Phytoremediation

Harvest the plants and remove the contamination!

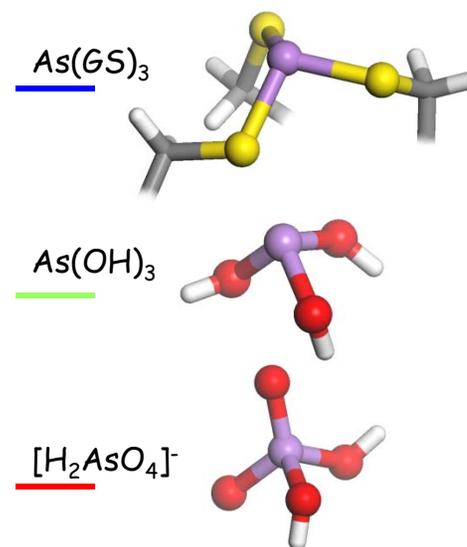
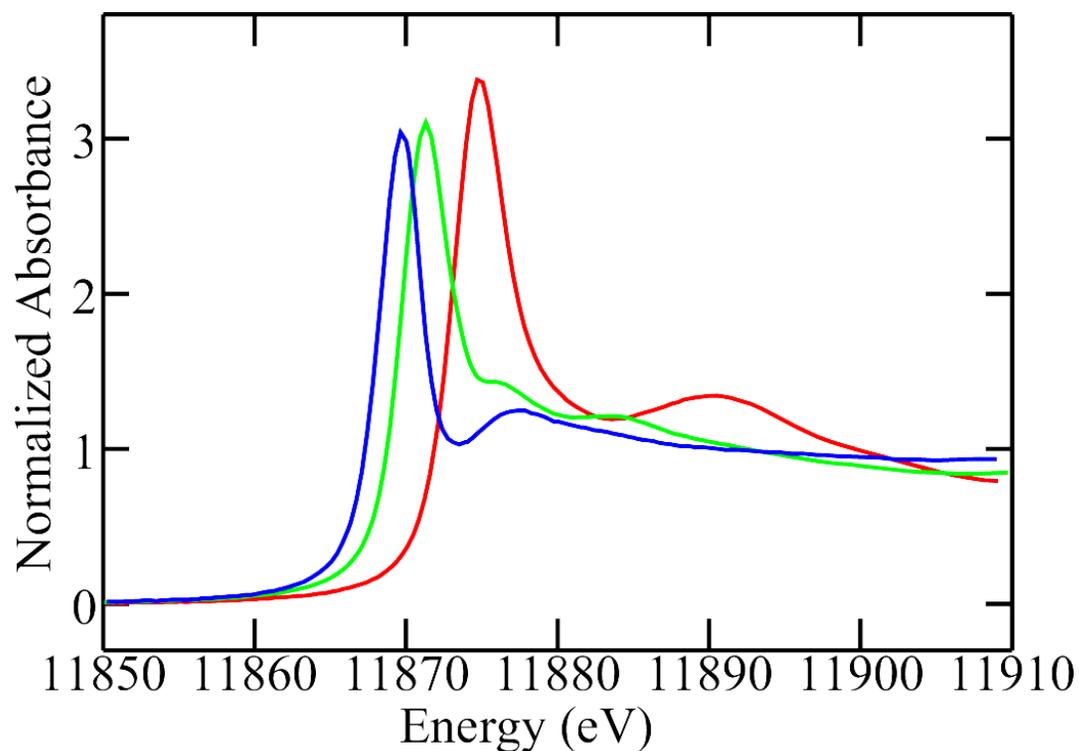


Pteris vittata - Questions

- Unanswered Questions –
 - What chemical forms of Arsenic are present?
 - What biotransformations of Arsenic are taking place?
 - Where does biotransformation occur?
 - How does the plant avoid poisoning itself?
- Need a direct probe of arsenic chemical form within living plant tissues
 - X-ray absorption spectroscopy to determine speciation
 - Chemically specific mapping to determine localization

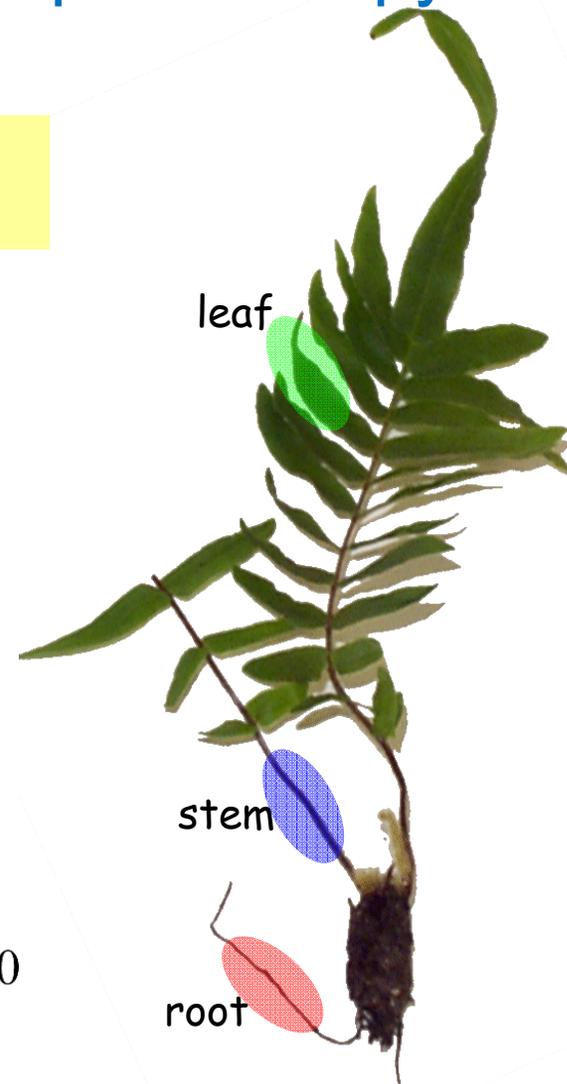
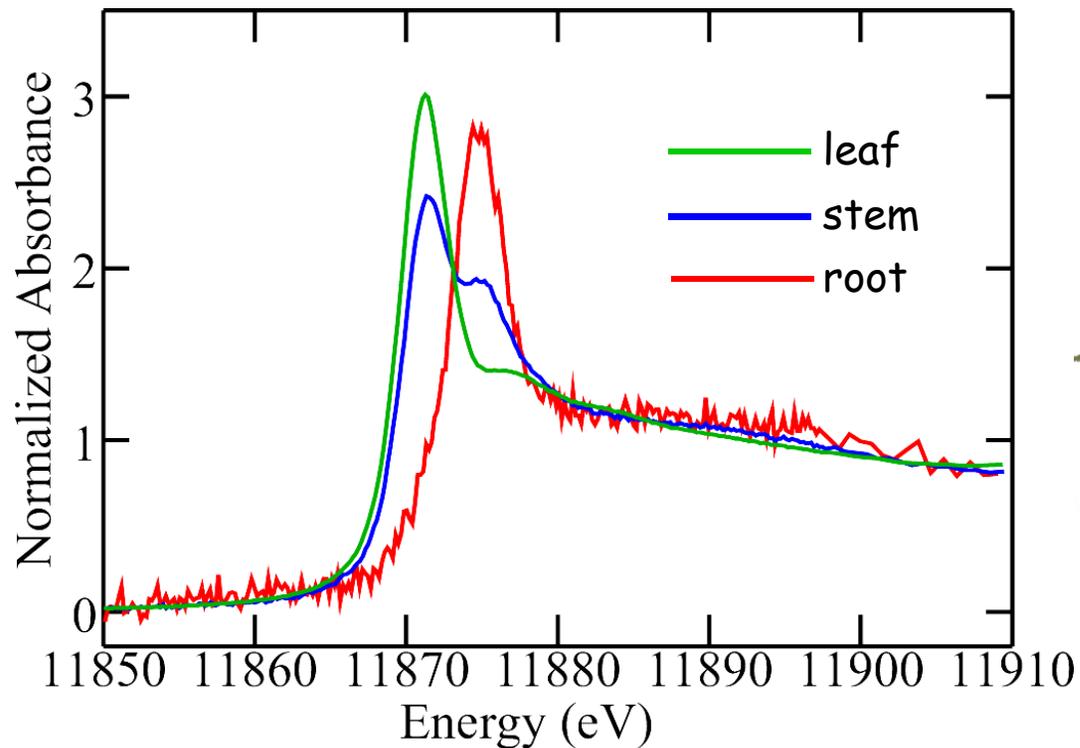
Pteris vittata – X-ray absorption spectroscopy

Use As K-edge XAS as a fingerprint of chemical form.



Pteris vittata – X-ray absorption spectroscopy

As K-edge XAS indicates chemical difference in different parts of the plant.



Pteris vittata – X-ray absorption spectroscopy

Leaves - Predominantly arsenite

Stems - Mixture of arsenite and arsenate

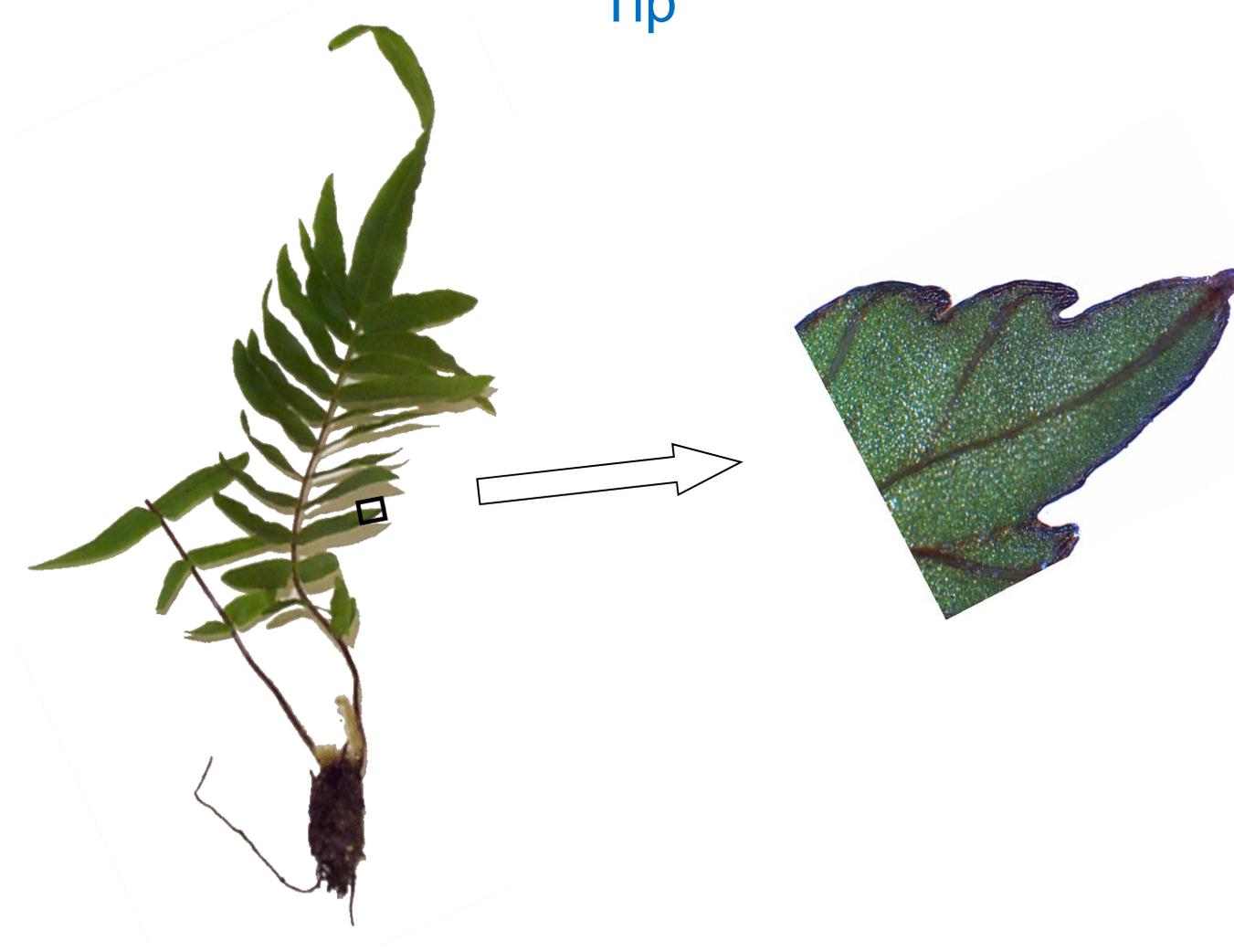
Roots - Predominantly arsenate.



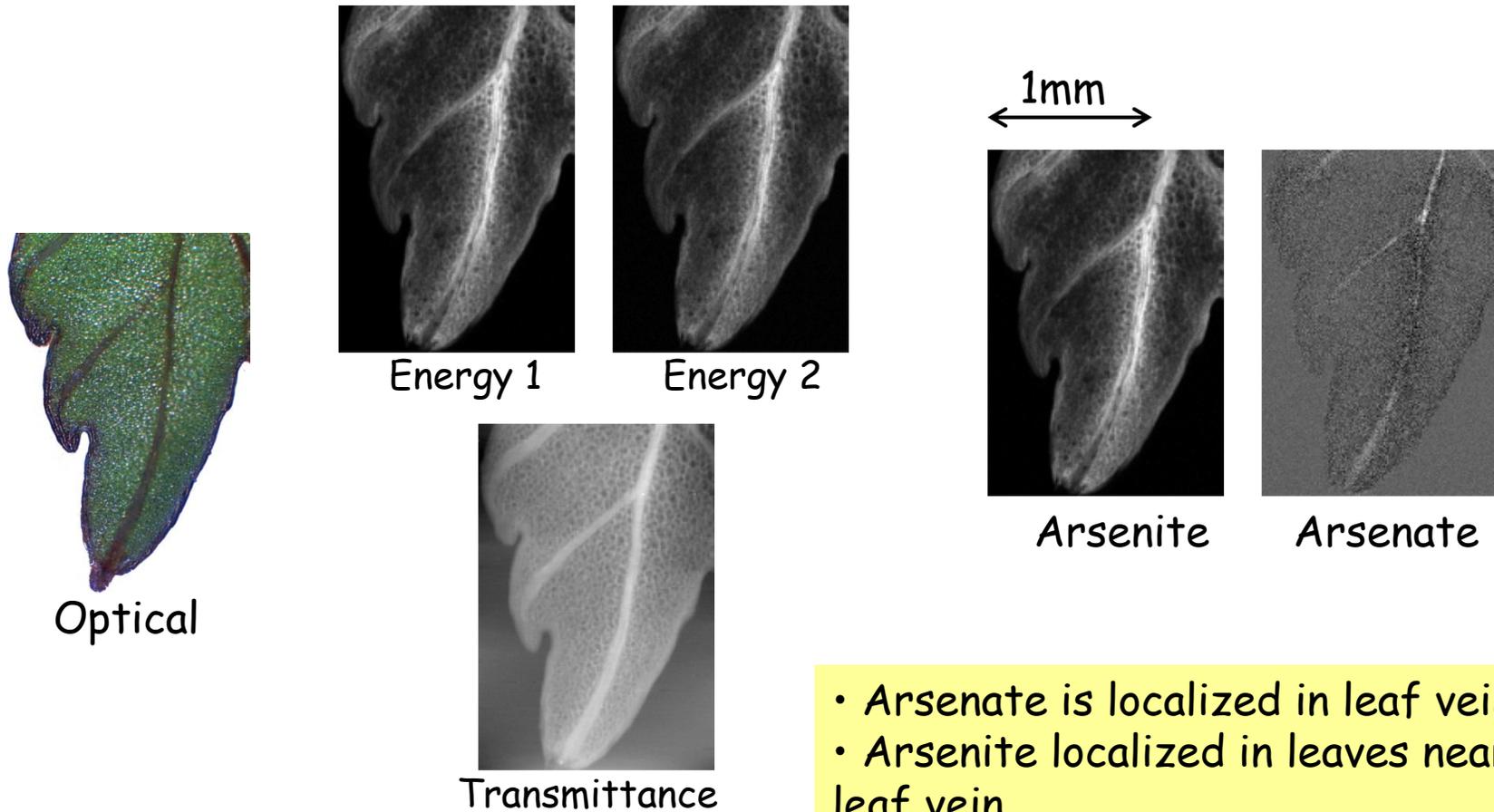
Hypothesis: arsenate is taken up from soil - transported via xylem to photosynthetic tissues and there converted to arsenite.

Use Chemically Specific Mapping to provide more detail.

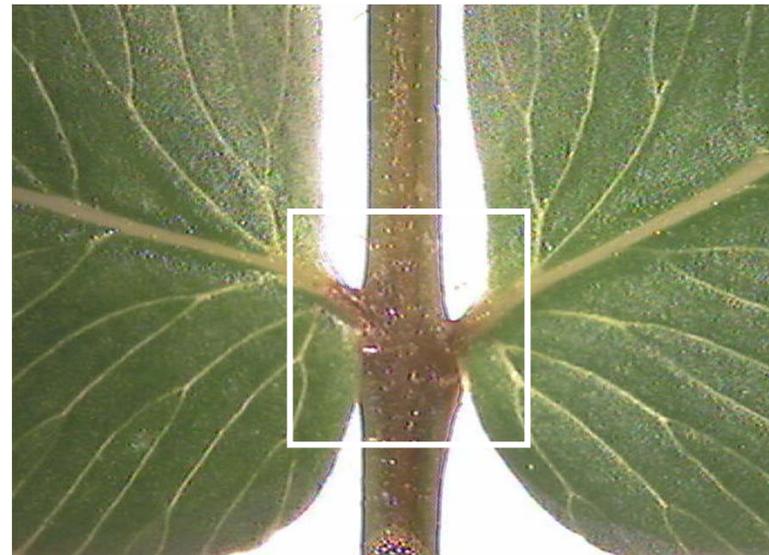
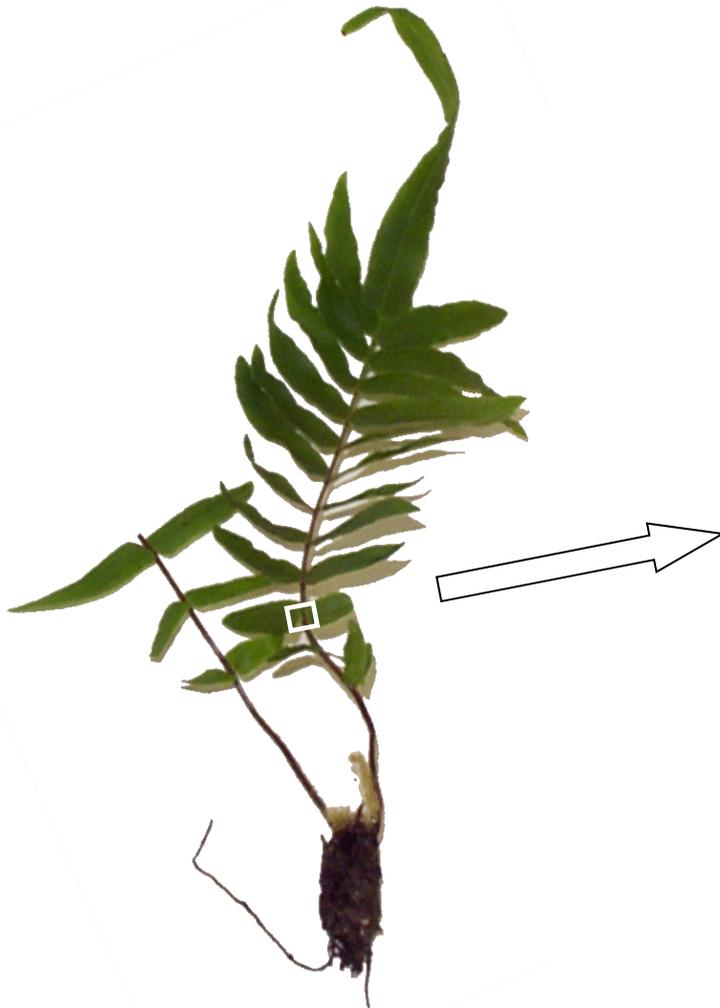
Pteris vittata: Chemically Specific Mapping of Leaflet (Pinna) Tip



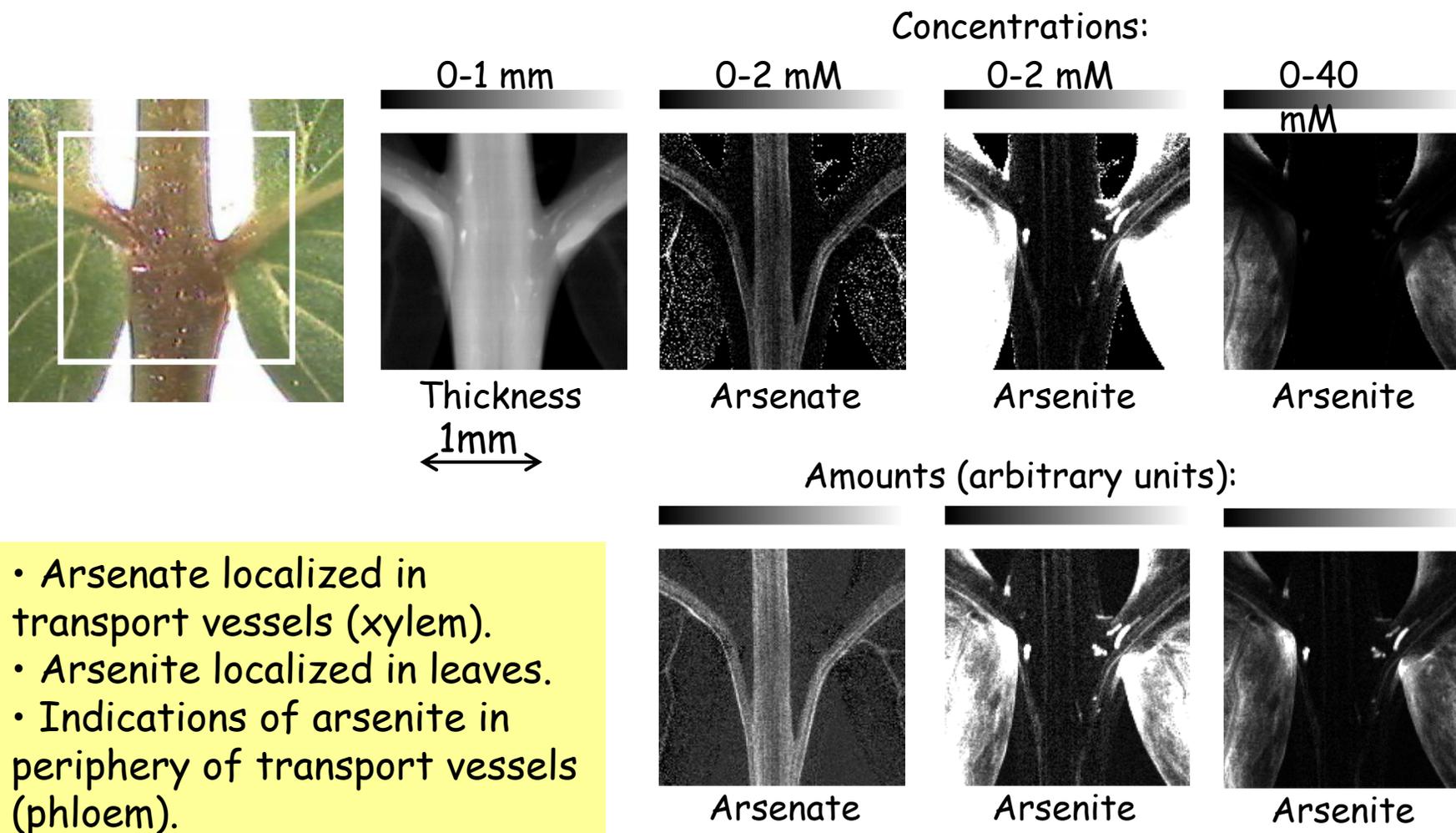
Leaflet Shows Strong Arsenite, With Arsenate in Vein



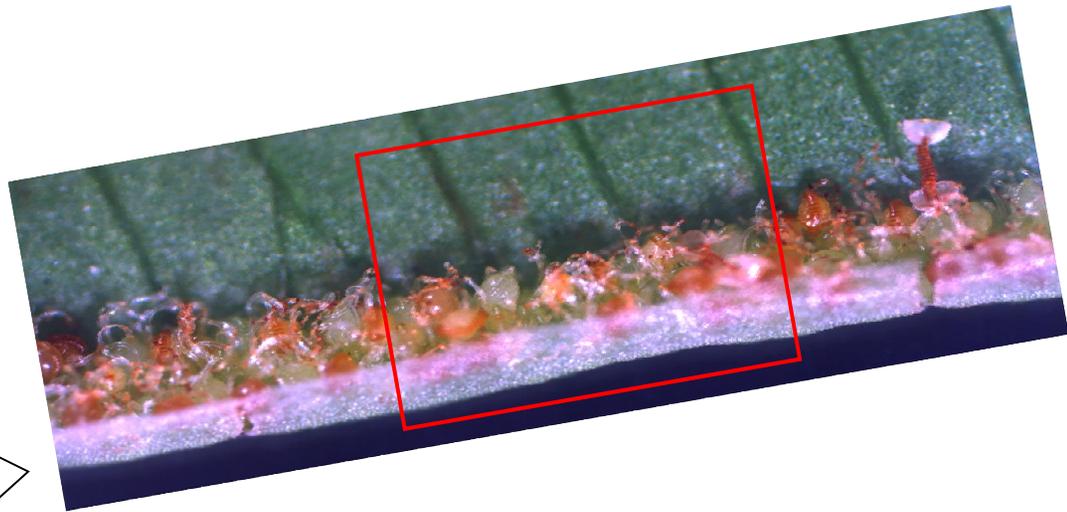
Pteris vittata – Chemically Specific Mapping of Stem (Rachis)



Transport Vessels Reveal Arsenate and Arsenite



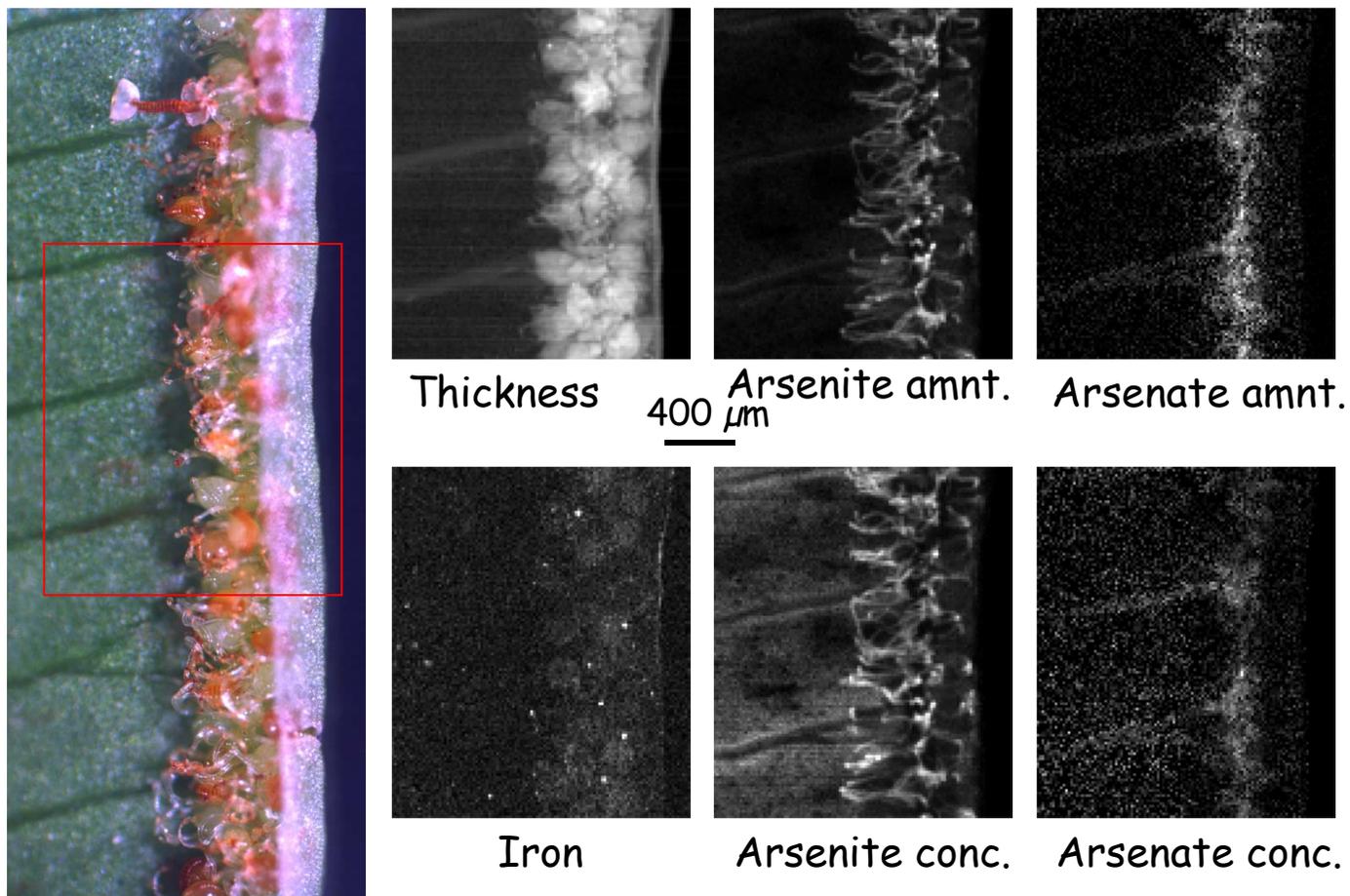
Pteris vittata – Chemically Specific Mapping of Sporangia



Spores contained in pouch-like sporangia or sori at edge of leaflet



Sporangia Show Highly Localized Arsenite



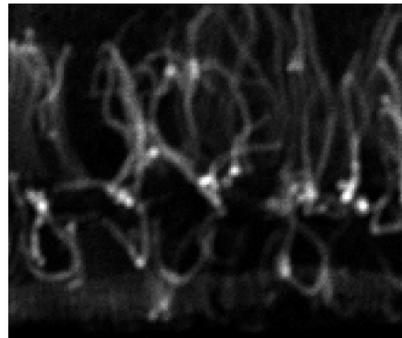
Thickness 0-0.9mm; arsenite amnt. 0-1.5 $\mu\text{mol.cm}^{-2}$; arsenate amnt. 0-0.075; arsenite conc. 0-25 mM; arsenate conc. 0-2 mM

Sporangia at High Resolution: Arsenite in Filaments

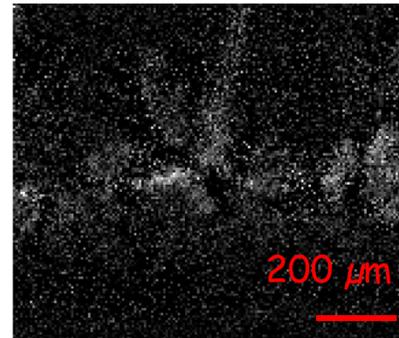
Filaments are the water glands associated with the sporangia



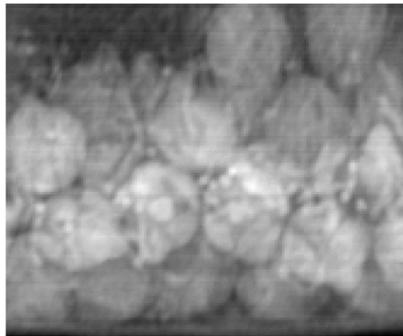
Optical



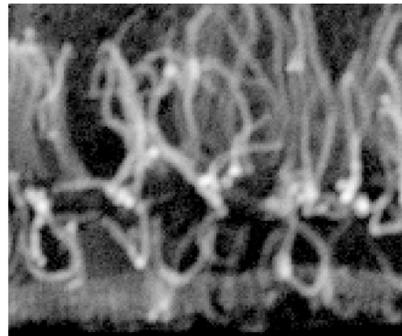
Arsenite



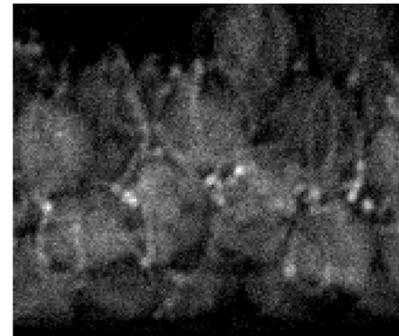
Arsenate (x20)



Thickness



Arsenite (log)



Scatter



Optical micrograph of isolated sporangium

Step size = 6 μm

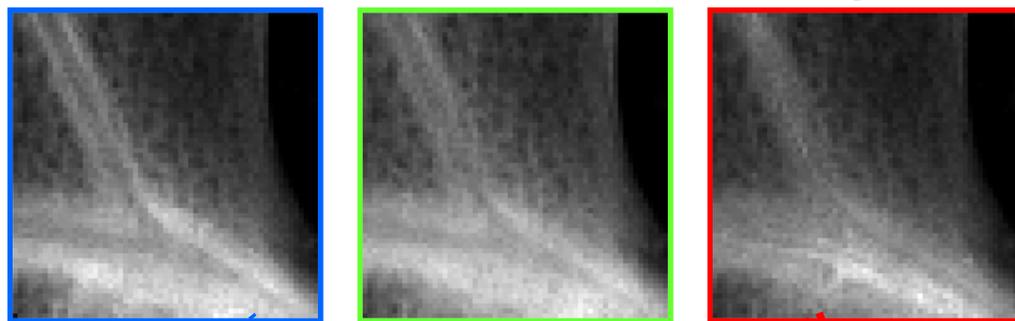
What About Sulfur ?

- Arsenic loves sulfur coordination
- Cells are full of available sulfur
- Why isn't arsenic coordinated by sulfur???

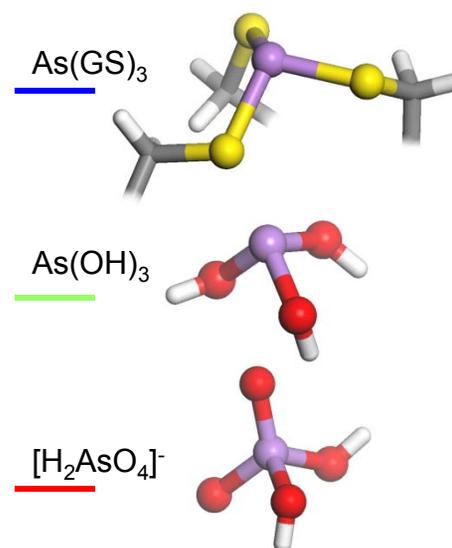
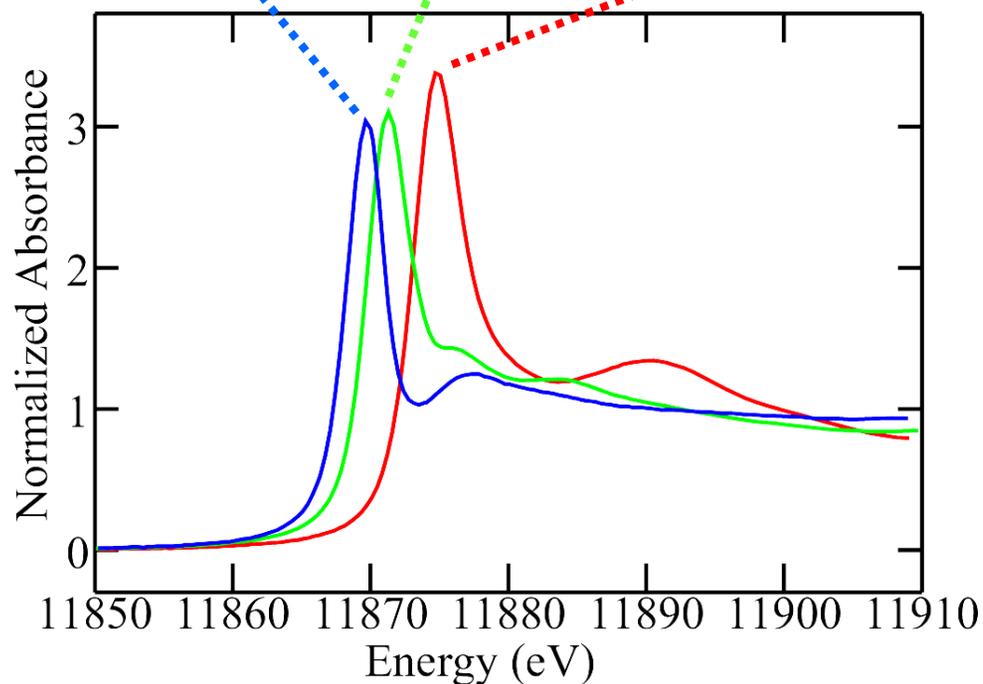
Well actually,
maybe it is...

- Bulk near-edge and EXAFS shows borderline evidence for thiolate-coordinated species
- Use 3-component Chemically Specific Mapping to attempt to localize As-thiolate species

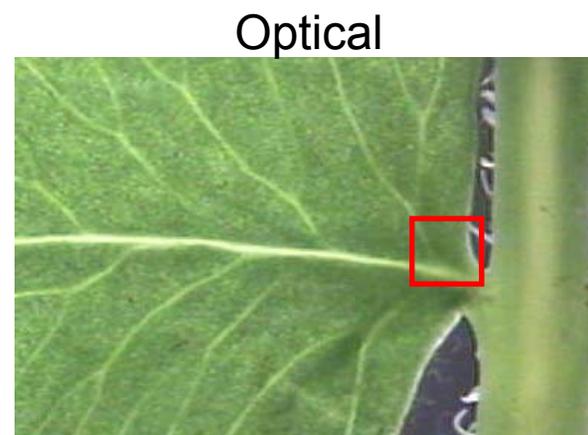
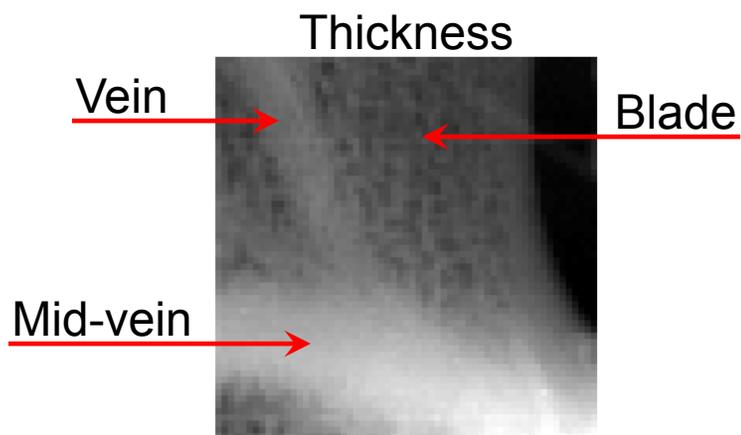
Use 3-Energy Chemically Specific Mapping to Find Sulfur Species



Collect images at energies sensitive to arsenic chemical species



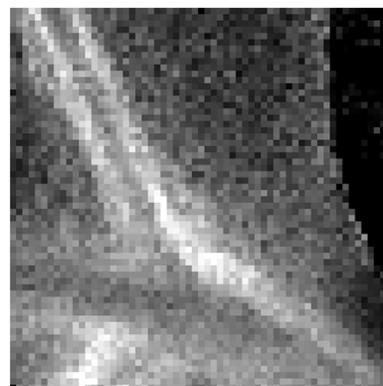
Arsenic-Thiolate Revealed Near Vein



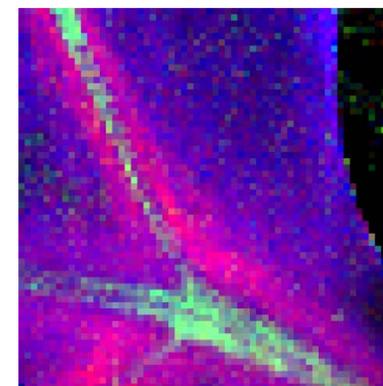
Arsenite:
stored in blade



Arsenate:
in veins (xylem)



Arsenic-thiolate:
surrounds veins



As-thiolate
Arsenate
Arsenite

Arsenic in Fern - Summary

- Sporophytes:
 - Arsenate is transported in the xylem to the leaves
 - In leaves, arsenite is stored at high levels
 - Thiolate-coordination may be implicated in reduction
- Reproductive tissues:
 - Arsenic is excluded

Pickering et al., *Environ. Sci. Technol.* (2006)
40:5010-5014

Mercury

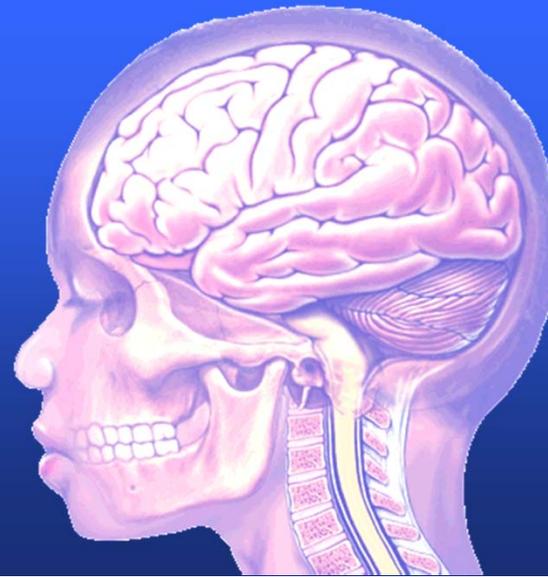


In collaboration with Graham George, University of Saskatchewan

Mercury

- Mercury is one of the most toxic elements to which humans are commonly exposed
- Acute exposure of organomercury leads to severe consequences, especially in children or fetuses

- microcephaly
- cerebropalsy
- seizures
- mental retardation
- blindness
- quadriparesis



Minamata Japan

- Caused by dumping of mercury-containing industrial waste by Chisso Corporation* into Minamata Bay in the 1950's and 60's
- Local fish became heavily contaminated with methylmercury compounds
- First victim was a 5-year old girl in 1956
- Final death toll approached 2,000 people
- Discovery that organo-mercury compounds affect foetal development



* <http://www.chisso.co.jp/english/index.asp>
(<http://www.jnc-corp.co.jp/english/>)

Mercury in our food



Fish are a significant source of mercury in our diets and a major source of potentially neurotoxic methylmercury species.

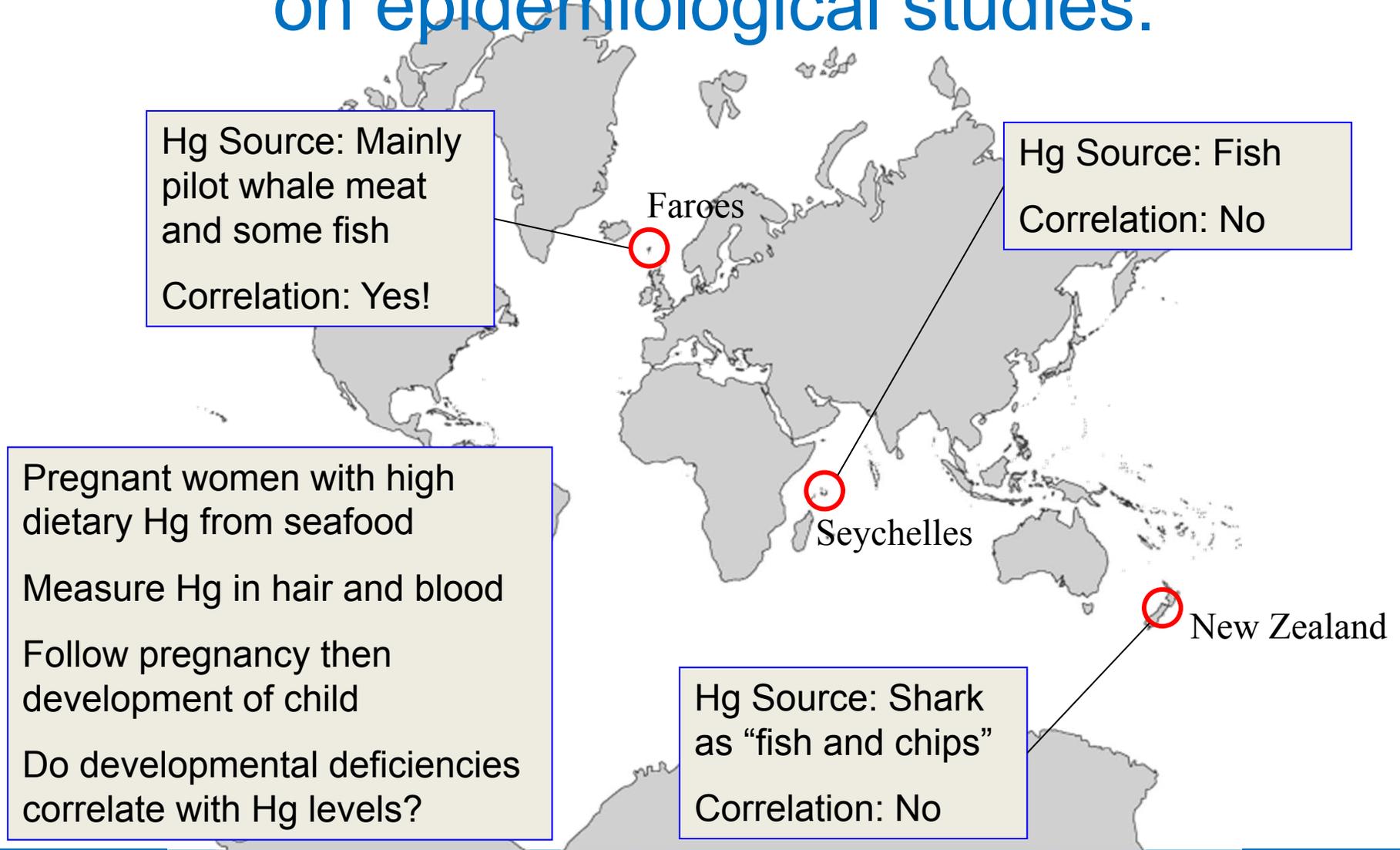


How much mercury in your diet is safe?

In the USA, the FDA, the AHA and the EPA disagree

- EPA: “eat no more than two fish meals a week” “do not eat fish that are high in mercury”
- FDA: “nutritional benefits of eating fish outweigh the risks from mercury”
- AHA: “healthy people should eat fish at least twice a week”

Recommended limits are mainly based on epidemiological studies.



Banning Fish will Impact World Health

The United Nations Food and Agriculture Organization estimates that over one billion people depend on marine fish as primary daily nutrition

If the West passes legislation declaring fish unsafe to eat then other countries may follow suit

This could significantly and negatively impact world health

Research Goals

- Despite mercury's importance, mechanisms by which it exerts its toxic effects remain unknown
- Understand effects of mercury at the molecular level
 - How is it transported?
 - Where is it localized and is it mobile?
 - How does molecular form affect these properties?
- Use X-ray absorption spectroscopy and X-ray fluorescence mapping:
 - Developing vertebrates (zebrafish)
 - Human tissues



Mercury in Zebrafish Larvae

M. Korbas, S. R. Blechinger, P. H. Krone, I. J. Pickering and G. N. George. Localizing organomercury uptake and accumulation in zebrafish larvae at the tissue and cellular level. *PNAS* **105**(34), 12108-12112 (2008).

M. Korbas, T. C. MacDonald, I. J. Pickering, G. N. George and P. H. Krone. Chemical form matters: differential accumulation of mercury following inorganic and organic mercury exposures in zebrafish larvae. *ACS Chemical Biology*, **7**(2) 410-419 (2012)



15th National School on Neutron and X-Ray
Scattering: August 14, 2013

Advanced
Photon Source
(20-ID)



Argonne
NATIONAL
LABORATORY

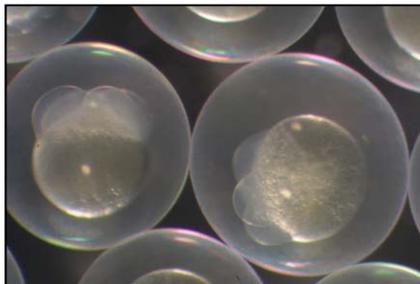


UNIVERSITY OF
SASKATCHEWAN

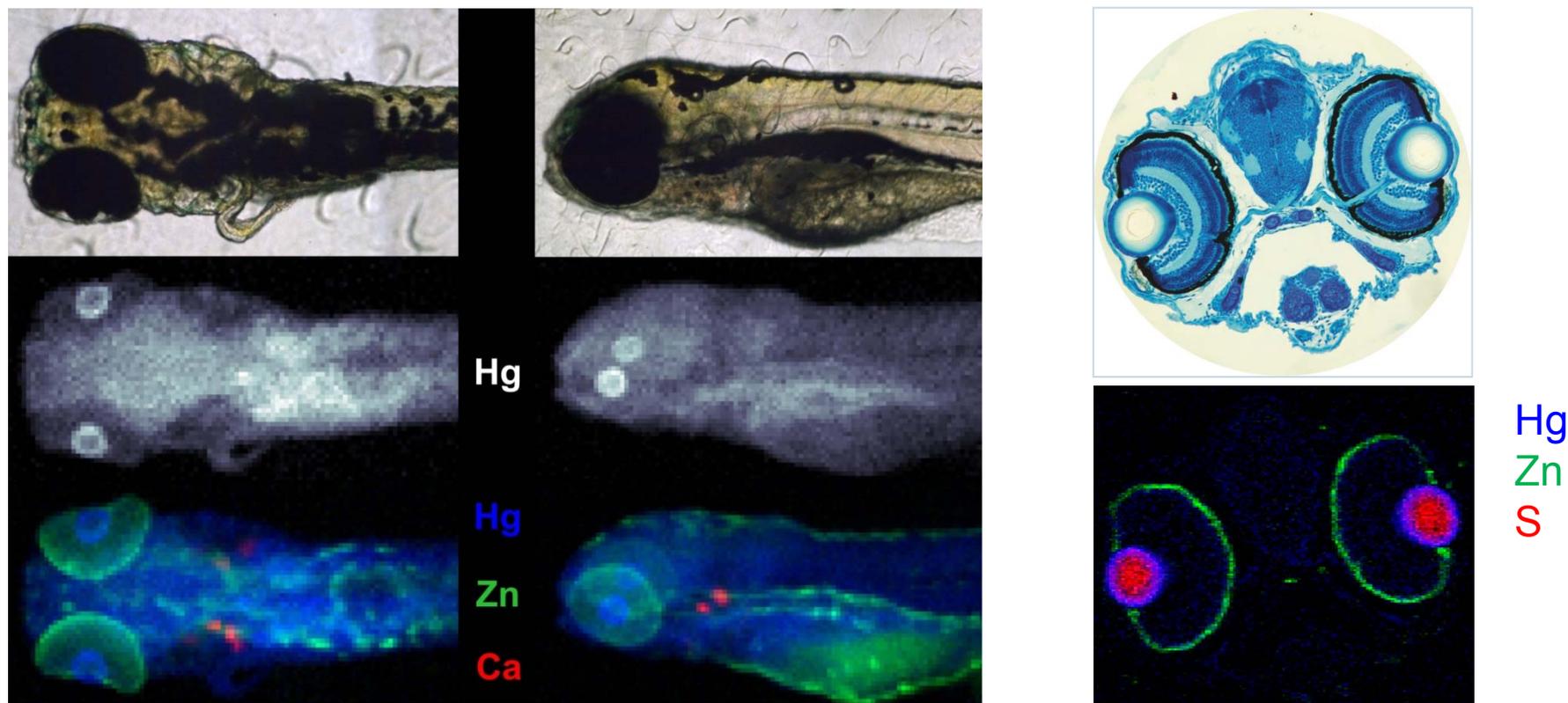


Mercury in Zebrafish

- Mercury is well known as a toxic element but different forms show widely different toxic effects
 - Methyl mercury species considered neurotoxic
- Use zebrafish to study how different mercury forms accumulate
 - Zebrafish are a well-established vertebrate model
 - Easy to maintain, quick growth, well characterized staging series



Zebrafish and Methylmercury L-Cysteine



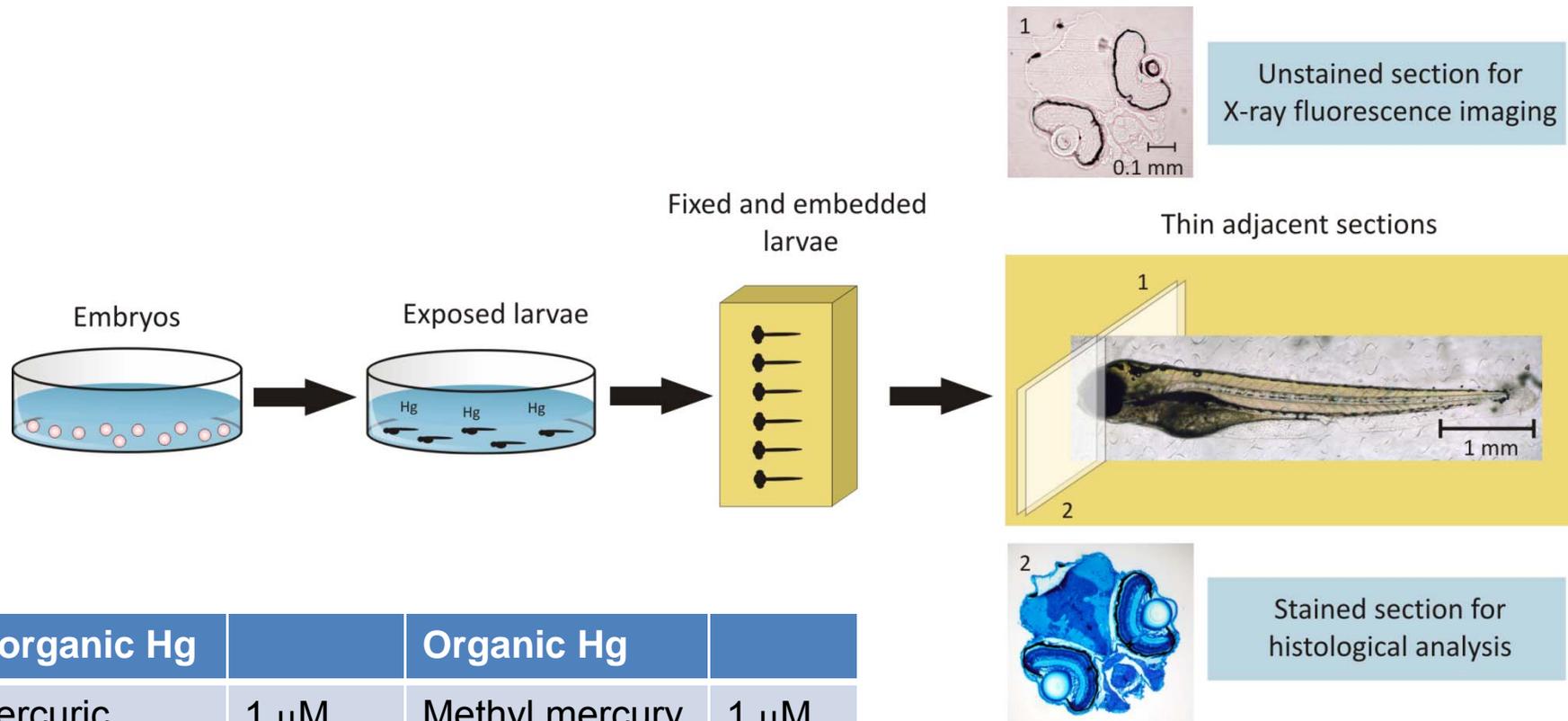
3.5 dpf

24-hour exposure

CH₃Hg(L-Cys): 100 μM

Organic mercury accumulates preferentially in actively dividing lens epithelial cells

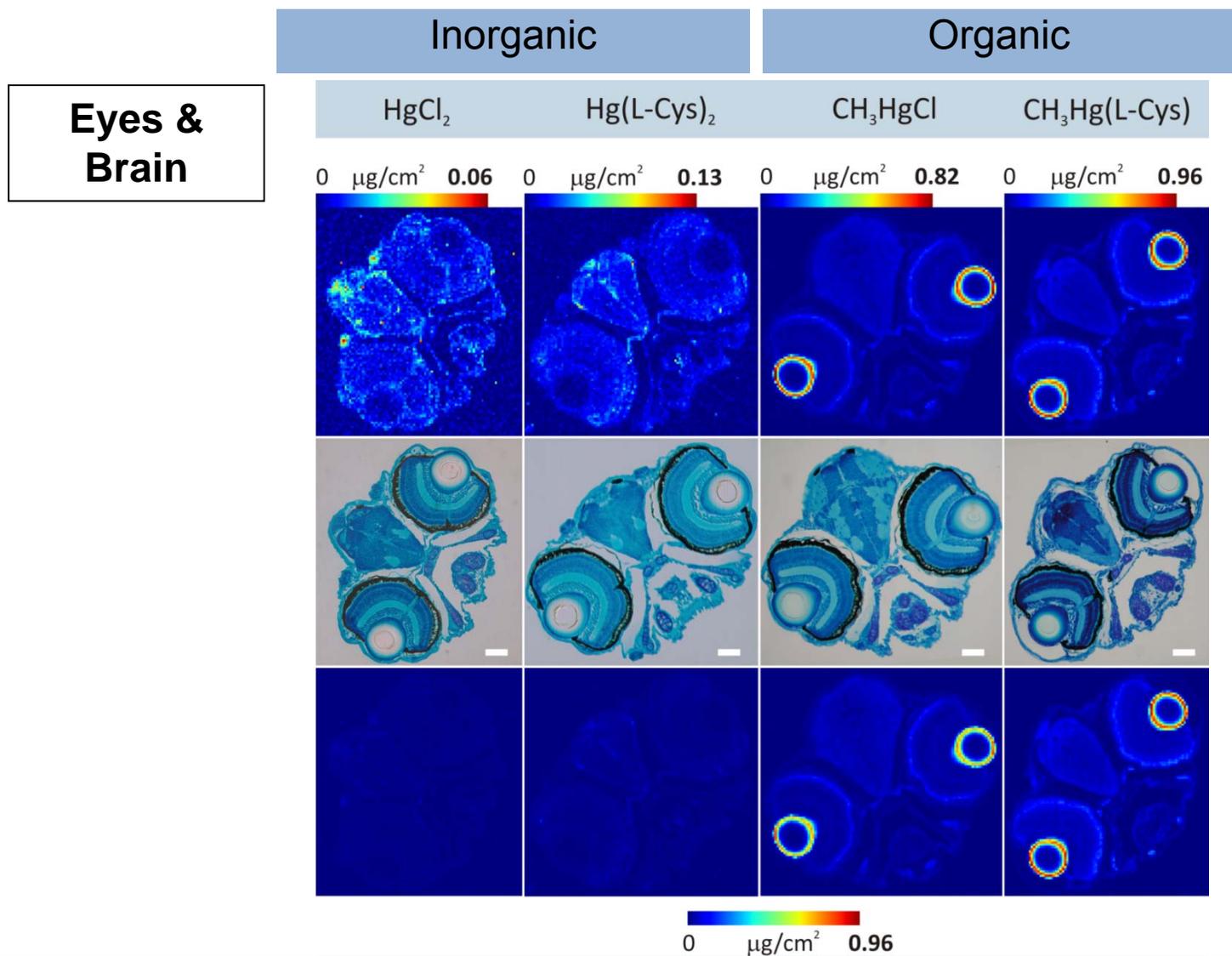
Mercury in Zebrafish: Exposure



Inorganic Hg		Organic Hg	
Mercuric chloride	1 μ M	Methyl mercury chloride	1 μ M
Mercury bis-L-cysteinate	100 μ M	Methyl mercury L-cysteinate	2 μ M

Korbas M et al (2008) PNAS 105:12108–12112
 Korbas M, MacDonald TC et al (2012) ACS Chem Biol 7:410-419

Chemical Form Results



Advanced Photon
Source (20-ID)

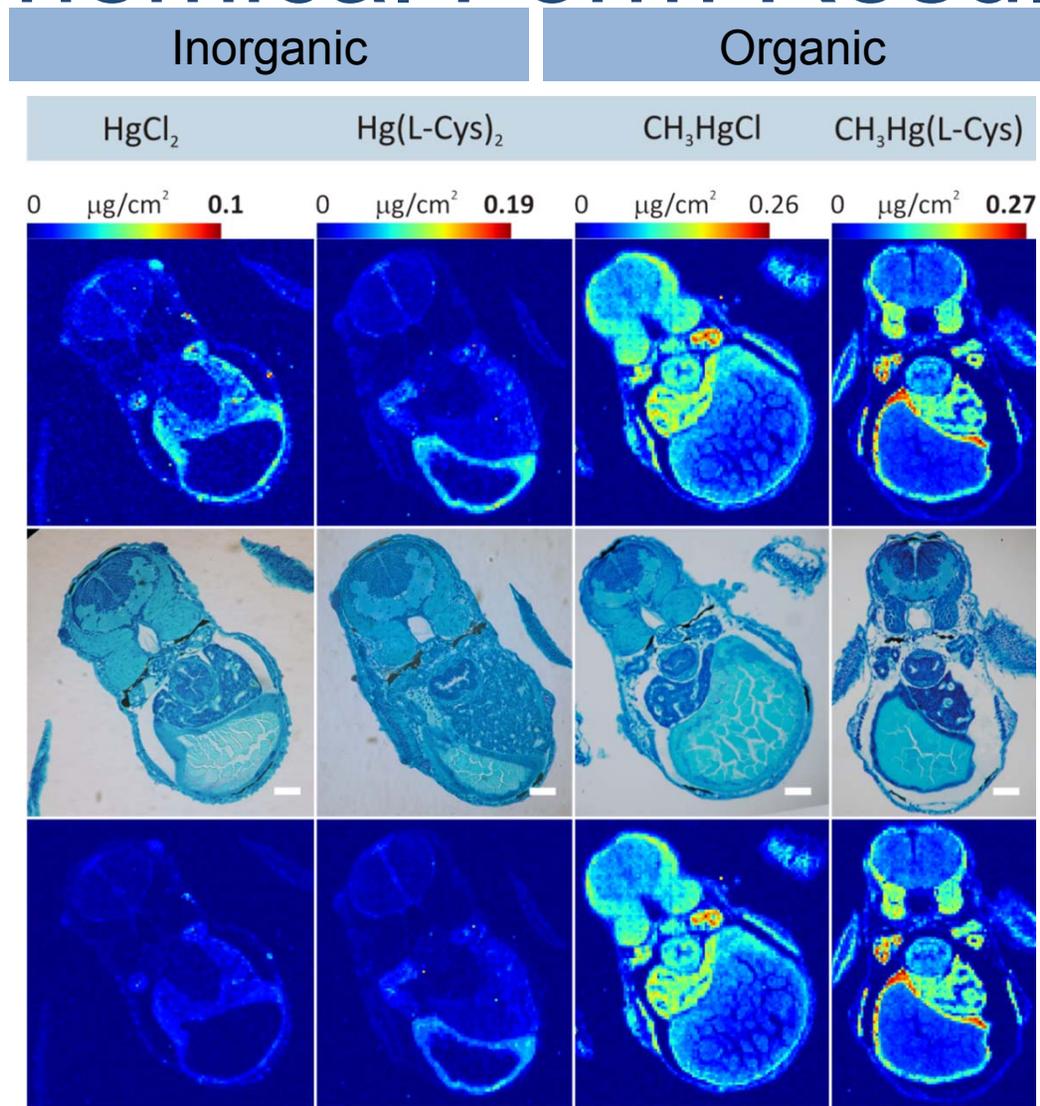


Korbass, M. & MacDonald, T.C. et al.
ACS Chemical Biology, 7(2) 410-419 (2012)



Chemical Form Results

Liver &
Kidney



Advanced Photon
Source (20-ID)

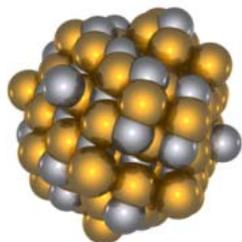
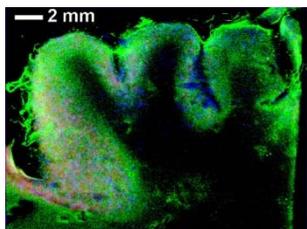


Korbass, M. & MacDonald, T.C. et al. 0 $\mu\text{g}/\text{cm}^2$ 0.27
ACS Chemical Biology, 7(2) 410-419 (2012)



Conclusions from Zebrafish

- Chemical form plays an important role in toxicity
- Preferential accumulation:
 - *Organomercury* in eye lens epithelium, skeletal muscle and gut tube
 - *Inorganic mercury* in brain ventricular region
 - *Both* accumulate in sensory organs (olfactory epithelium and neuromasts) and brain
- Organomercury mostly accumulates to higher levels
- Zebrafish: model system to study toxic metals



Mercury, Selenium and Human Brain

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ACS Chem. Neurosci., 2010, 1 (12), 810-818

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Gosia Korbas



Stanford Synchrotron
Radiation Lightsource



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Objectives

To investigate the molecular nature of **mercury** and **selenium** in human brain samples

Case	1	2	3	4	5
Gender	F	F	M	F	F
Age (yrs)	29	48	60	76	67
Cortex	frontal	occipital	occipital	occipital	occipital
Mercury Exposure	acute poisoning at age 8 yrs	acute poisoning, 10 months to death	fish consumption	fish consumption	none known
Toxicant	CH ₃ Hg-X	(CH ₃) ₂ Hg	CH ₃ HgS(thiol)	CH ₃ HgS(thiol)	n/a
Hg(ppb)	1179	2670	324	120	0.06
Pathology	severe atrophy	severe atrophy	normal	normal	normal



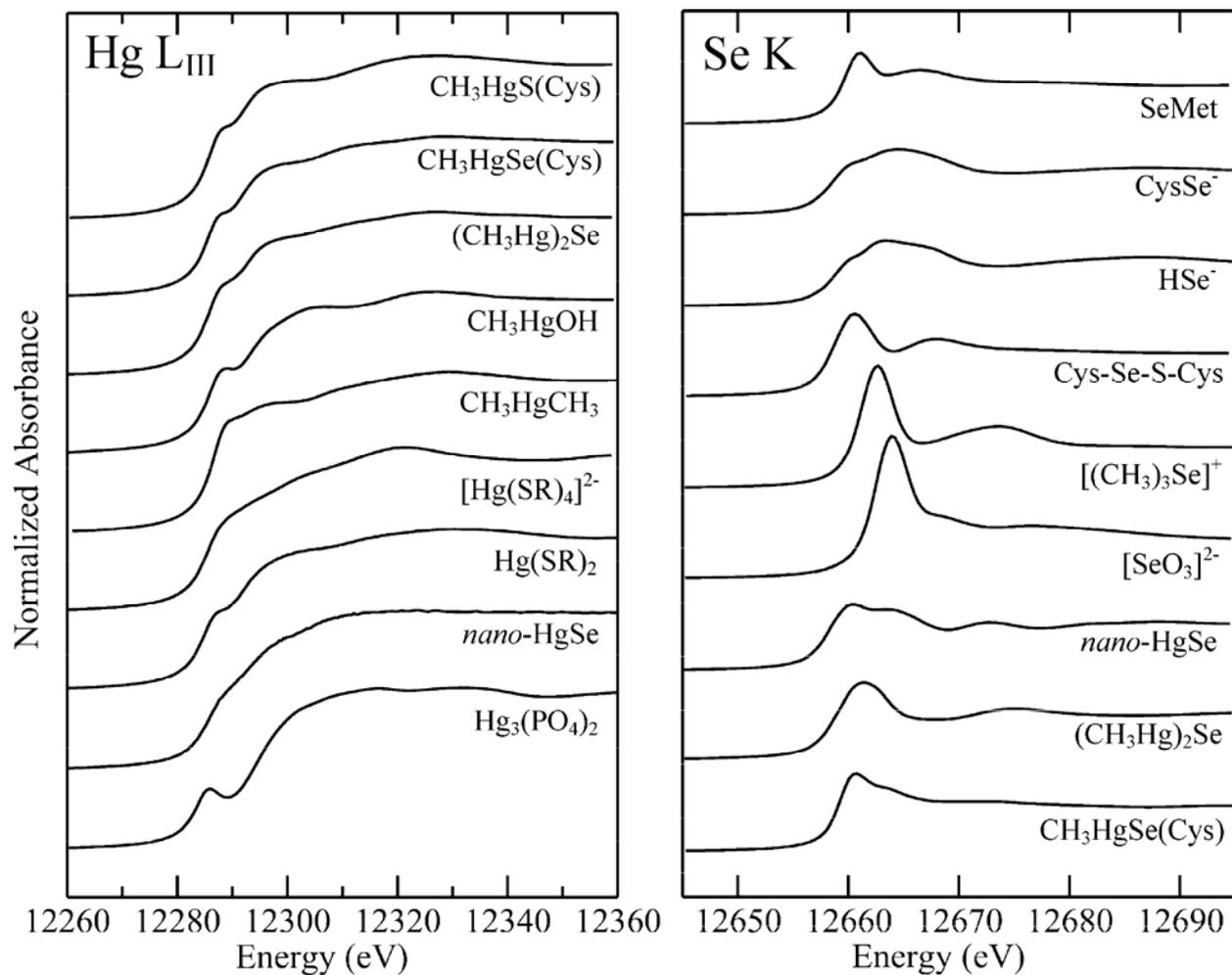
Korbas et al. *ACS Chem. Neurosci.* (2010) 1:810-818
Slide courtesy of Gosia Korbas



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Near-edge spectra as chemical fingerprints



Korbass et al. *ACS Chem. Neurosci.* (2010) 1:810-818

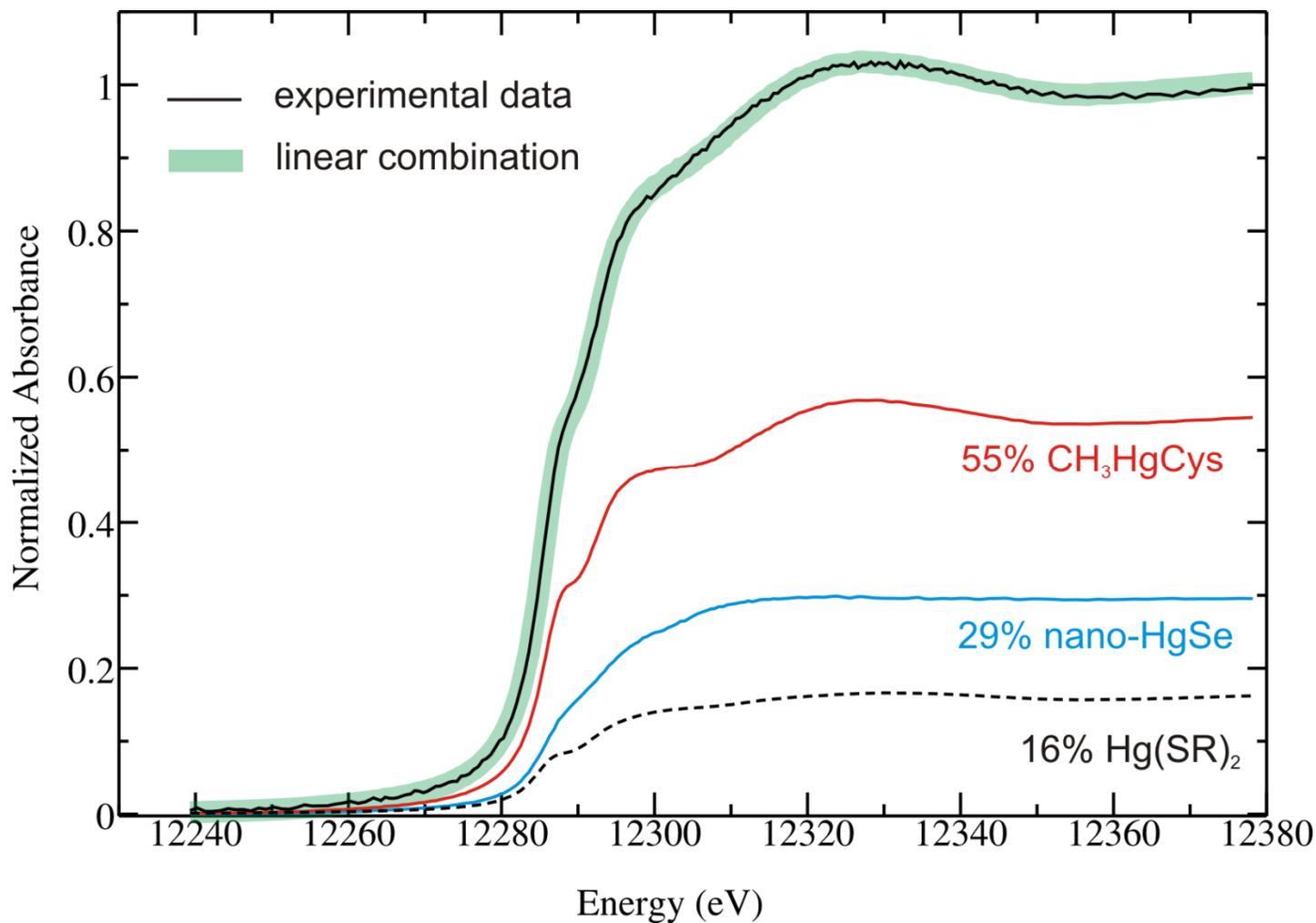


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Chemical speciation - Mercury

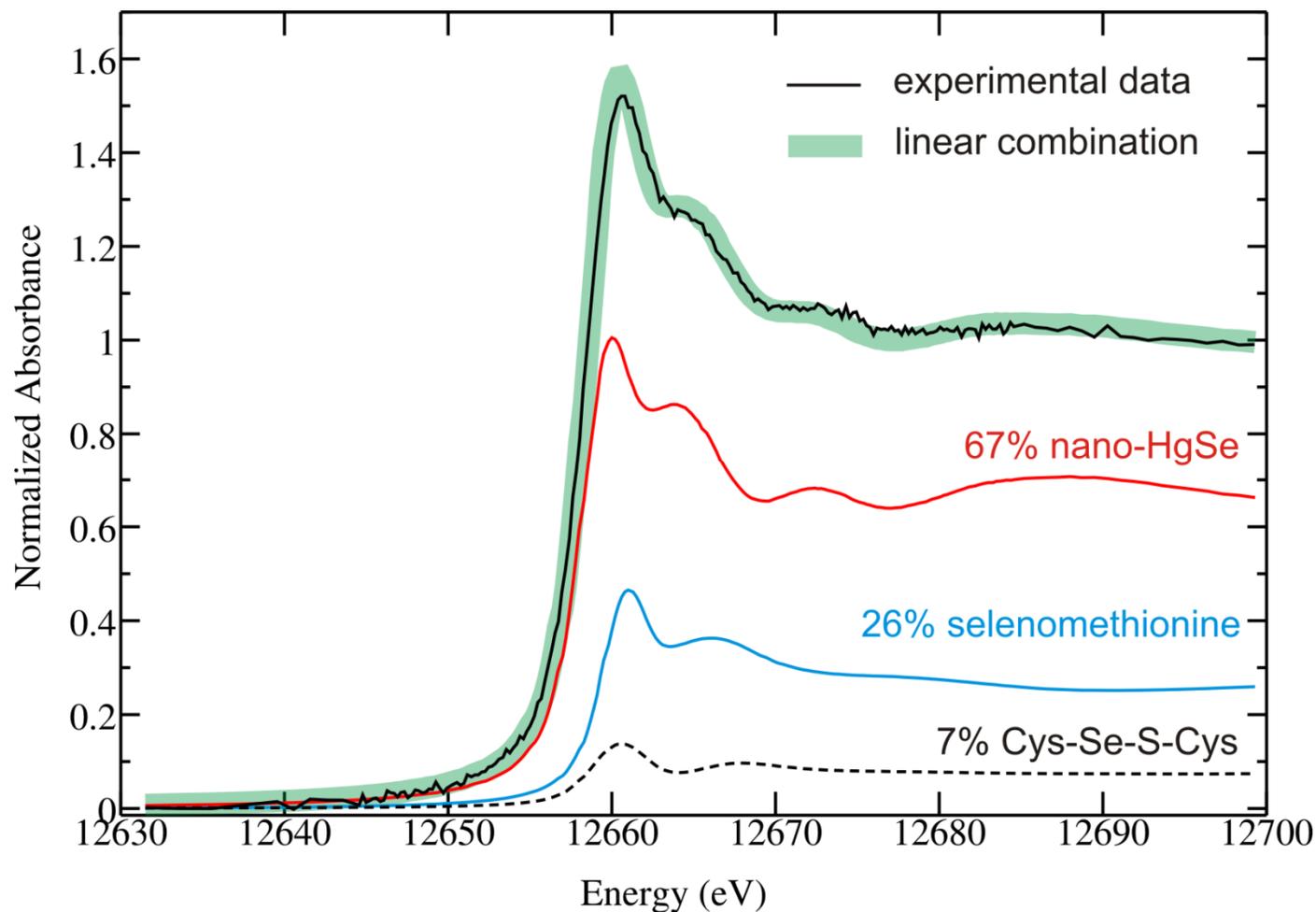
CASE 2



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Chemical speciation - Selenium

CASE 2

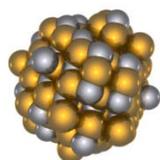


Korbass et al. *ACS Chem. Neurosci.* (2010) 1:810-818
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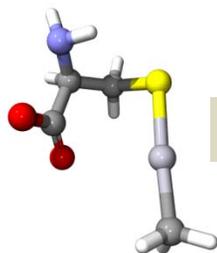
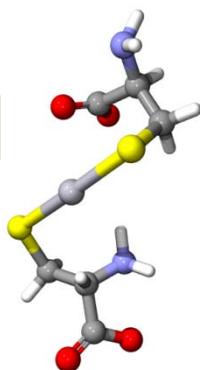
Mercury in brain

Three main Hg species



nano-HgSe

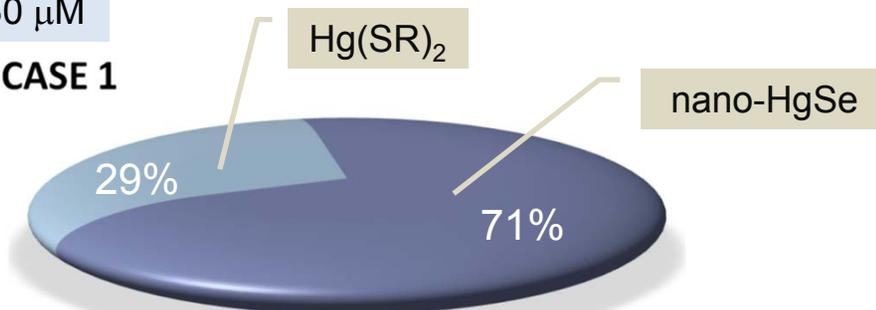
Hg(SR)₂



CH₃HgCys

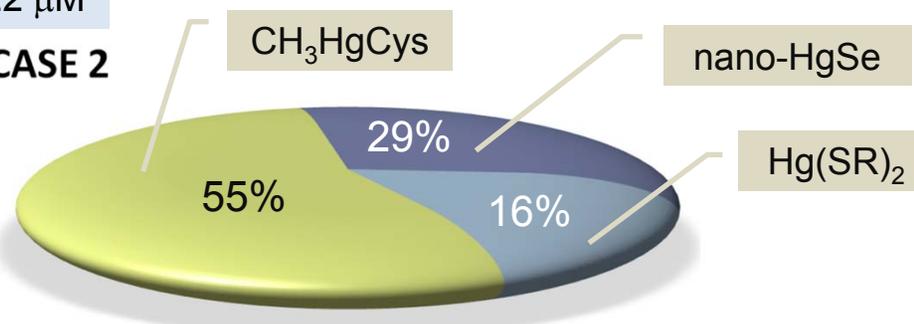
Total Hg: 30 μ M

CASE 1



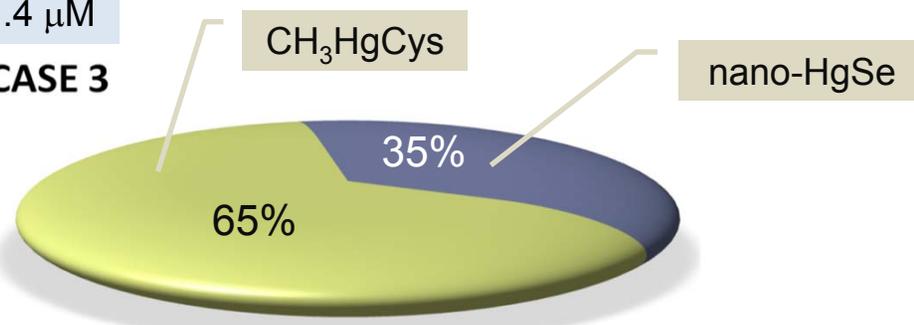
Total Hg: 22 μ M

CASE 2



Total Hg: 1.4 μ M

CASE 3



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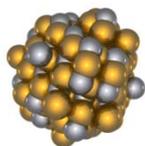


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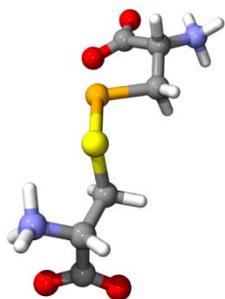


Selenium in brain

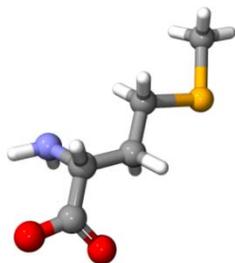
Three main Se species



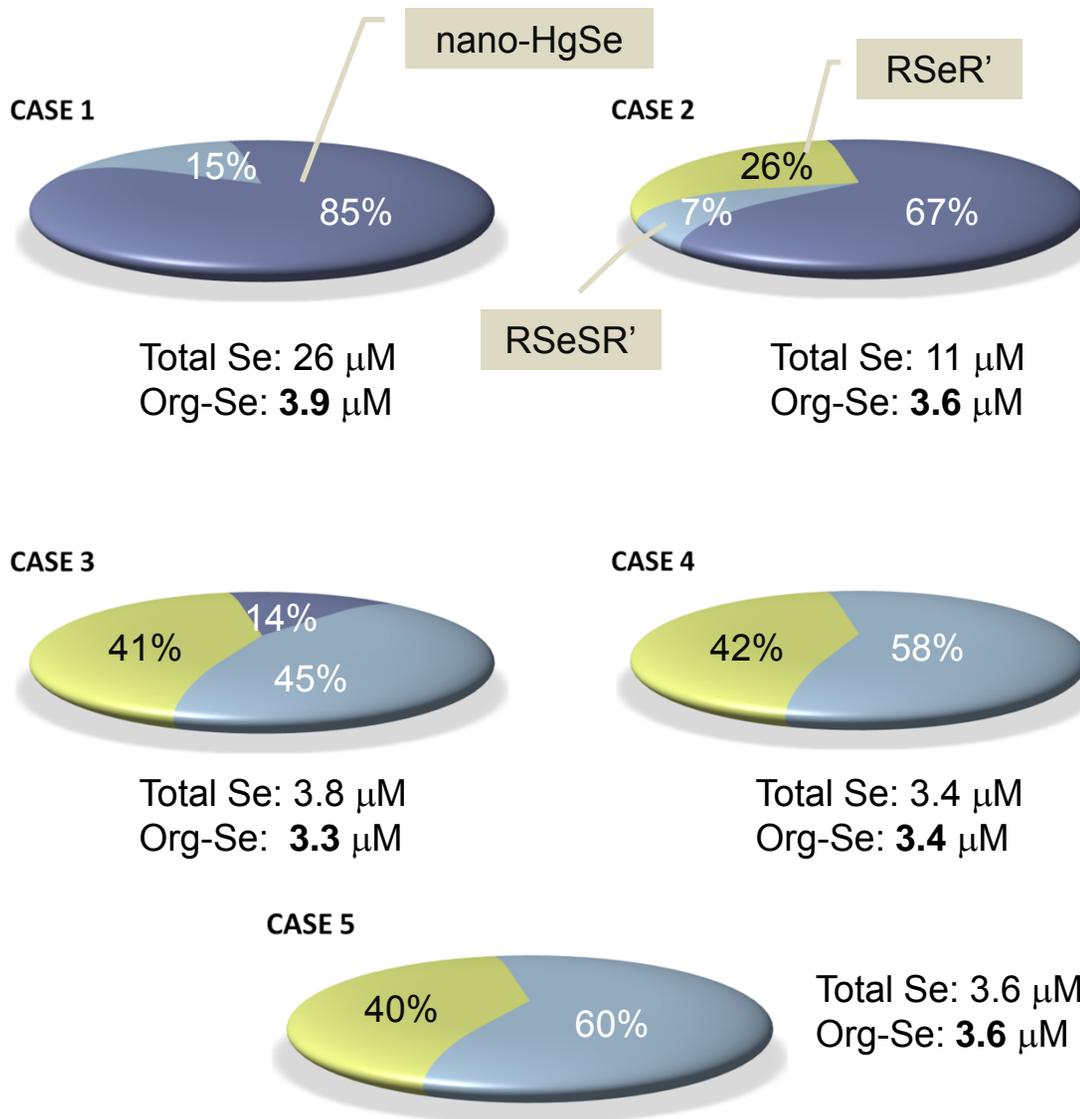
nano-HgSe



RSeSR'



RSeR'



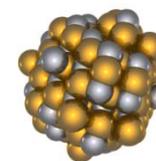
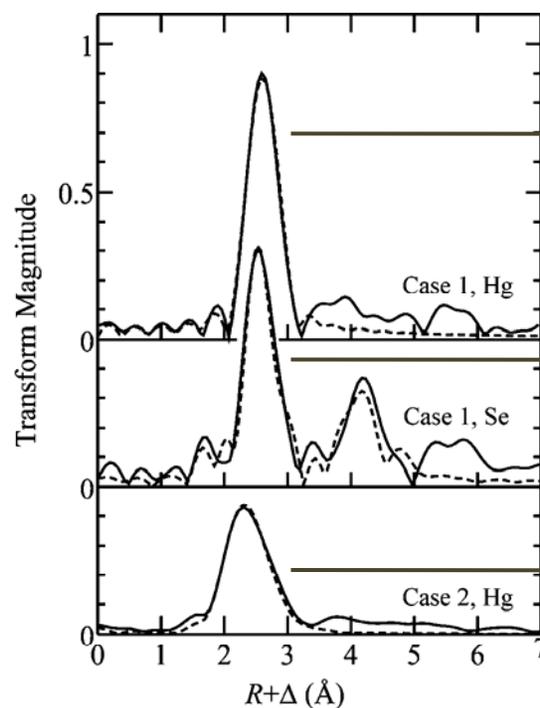
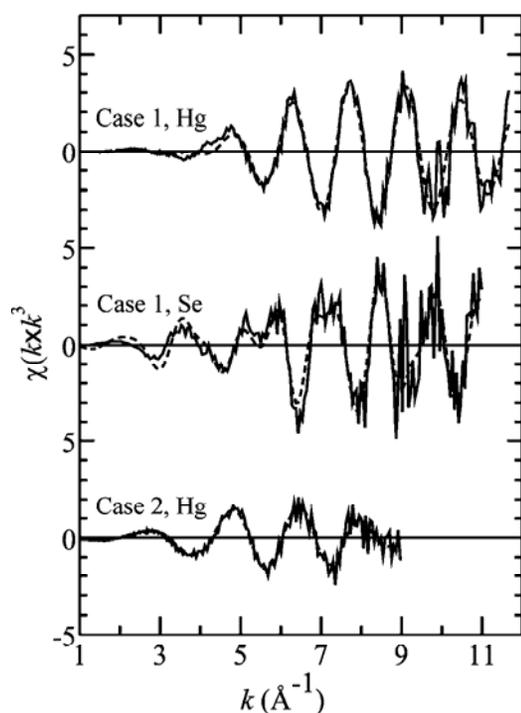
Korbas et al. *ACS Chem. Neurosci.* (2010) 1:810-818
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EXAFS Shows Evidence for HgSe Species



3.5 Hg-Se @ 2.63 \AA

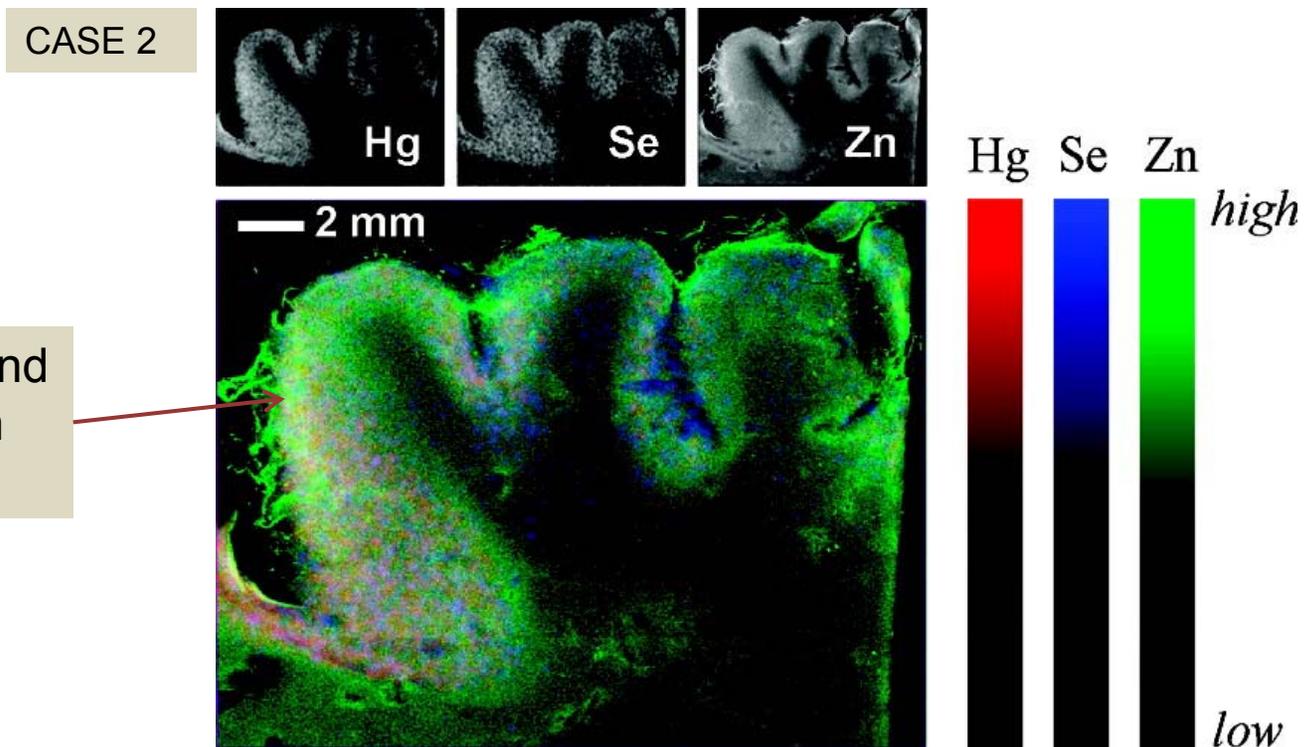
3.5 Se-Hg @ 2.61 \AA

1.4 Hg-S @ 2.32 \AA
1.0 Hg-Se @ 2.61 \AA



Korbas et al. *ACS Chem. Neurosci.* (2010) 1:810-818

XFM Shows Co-localized Hg and Se



Majority of Hg and Se co-located in grey matter



Korbass et al. *ACS Chem. Neurosci.* (2010) 1:810-818



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Selenium and Mercury in Brain: Summary

- Nano-HgSe seen in all higher exposures
 - Likely an inert species involved in detoxification
- Total brain Hg is not good measure of Hg toxic potential
- Se distributed between
 - organic selenium: remarkably constant
 - nano-HgSe: increases with Hg levels
- “Organic” brain average Se level is not depleted
- Se may be redistributed from other structures to bind with Hg



Korbass et al. *ACS Chem. Neurosci.* (2010) 1:810-818

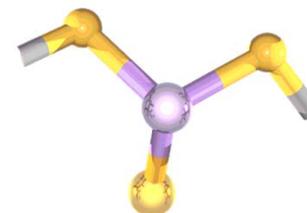


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Case Study: Arsenic and Selenium in Bangladesh

- Graham George
- As-Se Molecule
 - Jürgen Gailer (U. Calgary)
 - Roger Prince (ExxonMobil)
- Bangladesh Clinical Trial
 - Julian Spallholtz (Texas Tech)
 - Paul LaPorte (U. Chicago)
 - Selim Ahmed (Bangladesh)
 - www.bangladesh-selenium.org



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Gailer et al., *J. Am. Chem. Soc.* **122**, 4637-4639 (2000).



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Arsenic and Selenium



Arsenic has no confirmed biological function
Selenium is an essential trace element

H																He	
Li	Be										B	C	N	O	F	Ne	
Na	Mg										Al	Si	P	S	Cl	Ar	
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac															

Lanthanides

Actinides



Metals

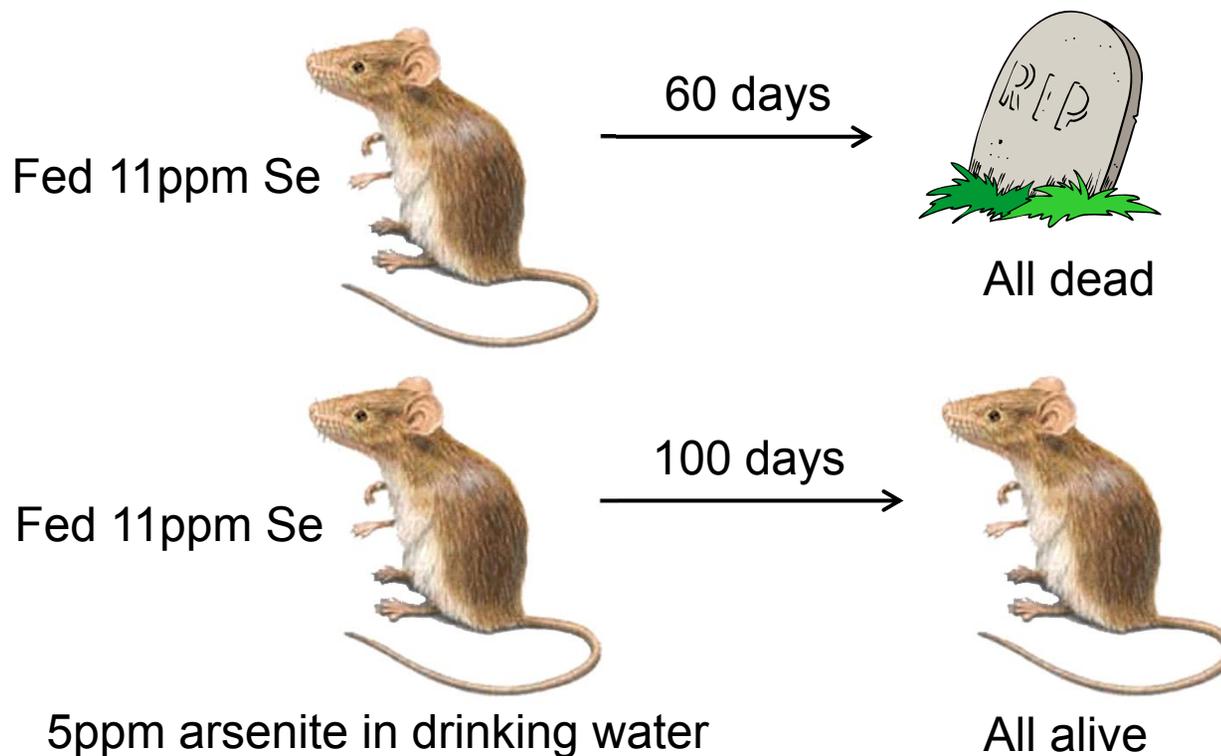


Non-metals



Two Wrongs that **do** Make a Right – Arsenic and Selenium

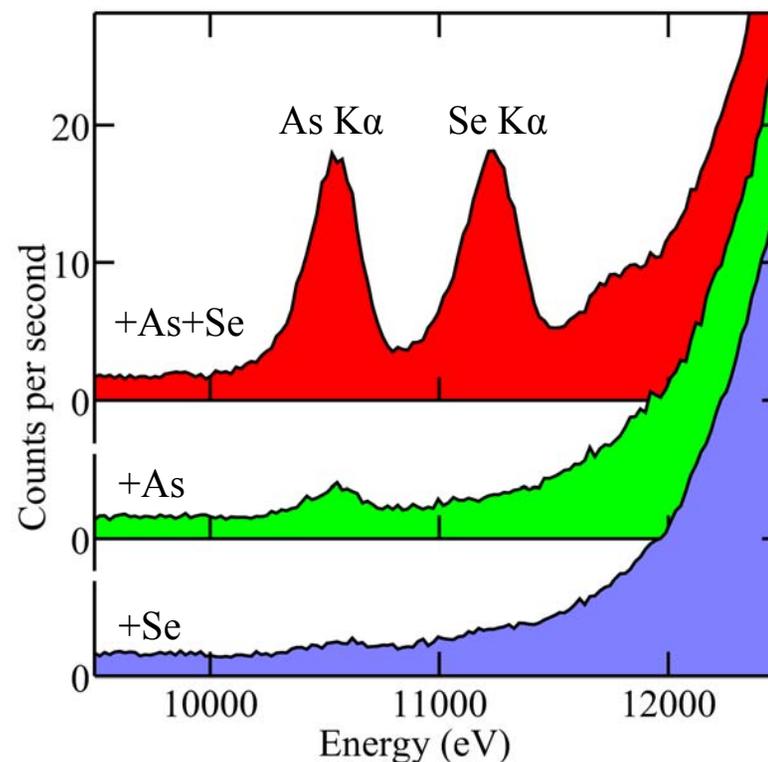
1938 – Arsenite completely protected rats fed upon selenized wheat.



A lethal dose of arsenite (or selenite) can be completely counteracted by an equal and otherwise lethal dose of selenite (or arsenite).

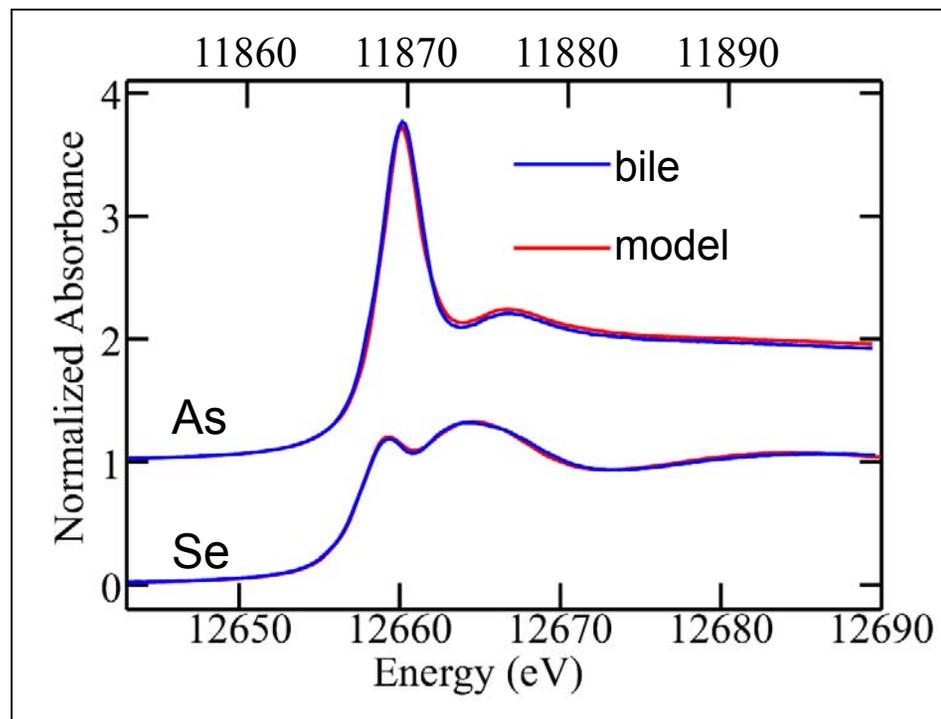
Arsenic and Selenium in Bile

- As and Se:
excreted in bile only
when both are present
- As:Se 1:1 in bile
- Suggests an arsenic-selenium molecule
responsible for mutual detoxification



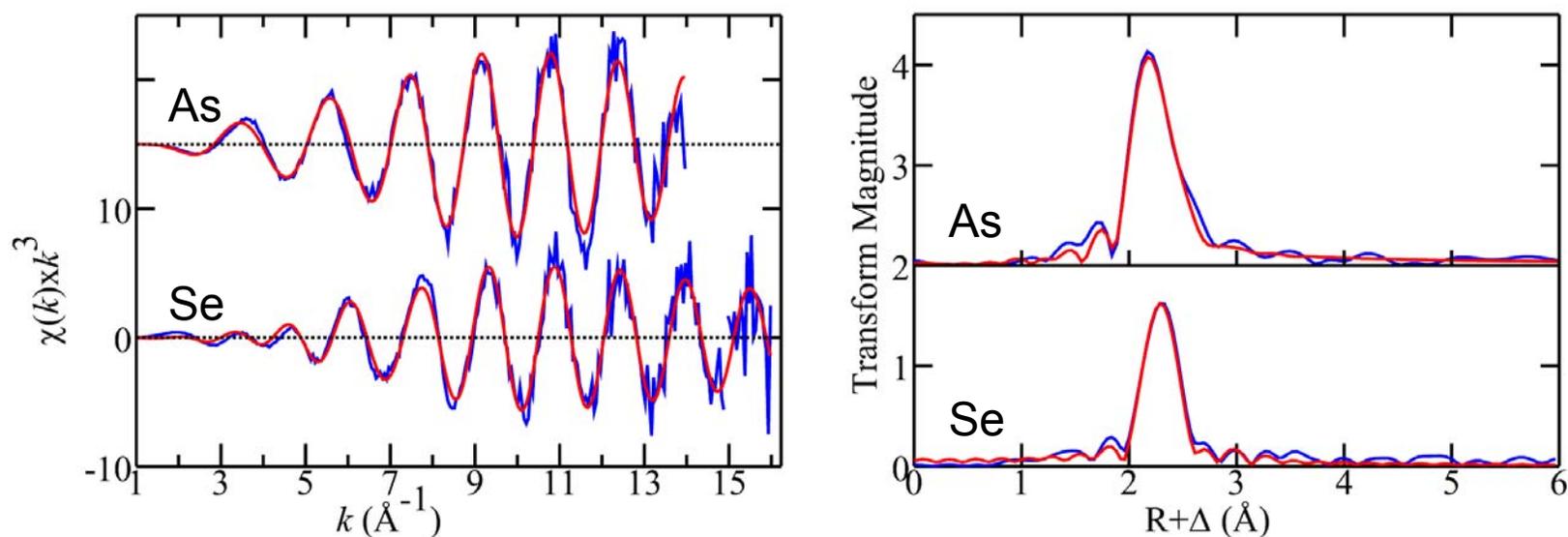
Arsenic-Selenium Antagonism

- Bile As and Se K near-edge spectra identical with model synthesized from arsenite, selenite and glutathione



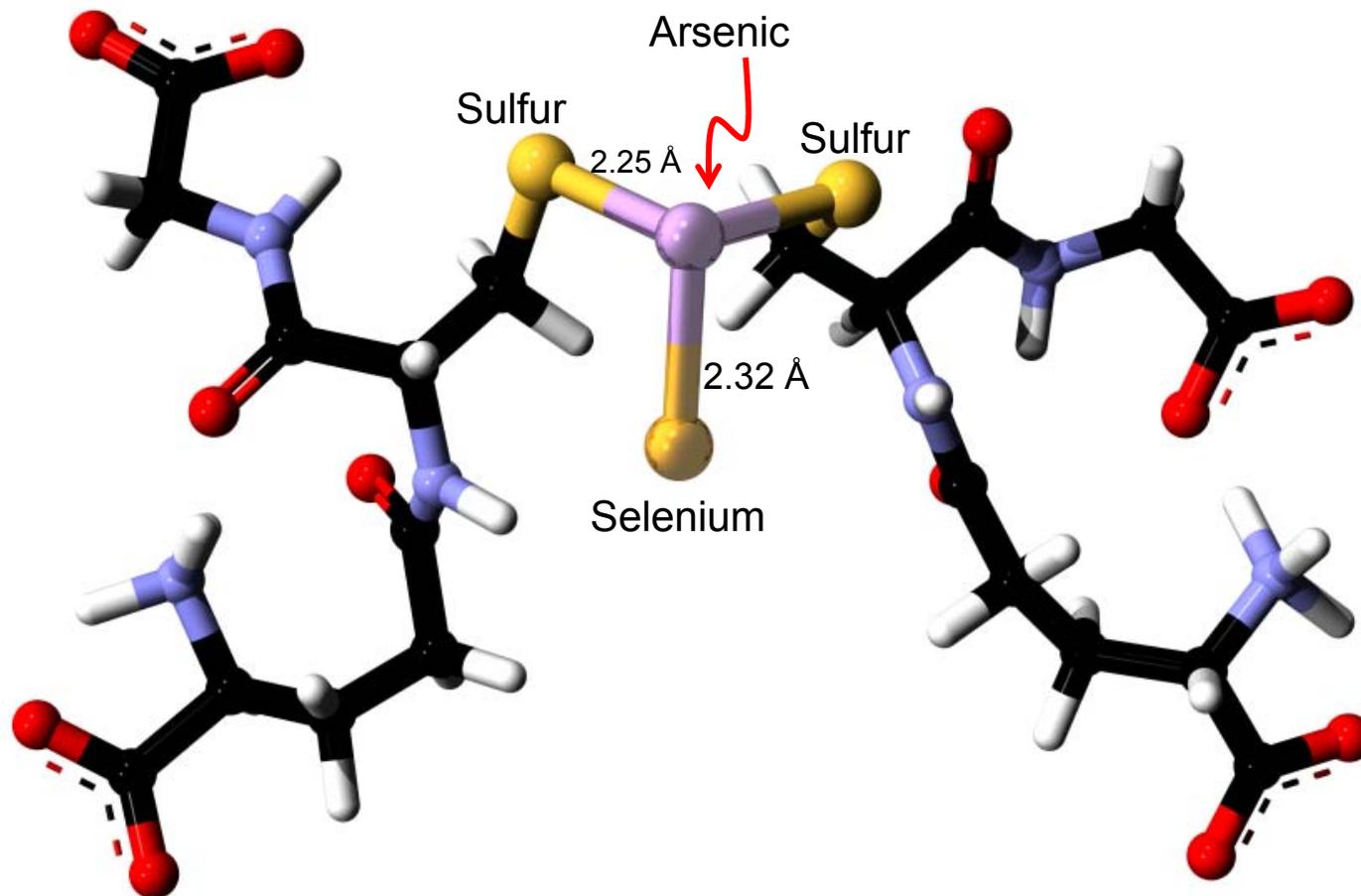
Arsenic-Selenium Antagonism

- Bile As and Se EXAFS used to structurally characterize species in bile



As-Se Species Identified in Bile

- An unusual molecule with 1:1 Se:As - the seleno-bis(S-glutathionyl) arsinium ion



Arsenic Poisoning in Bangladesh

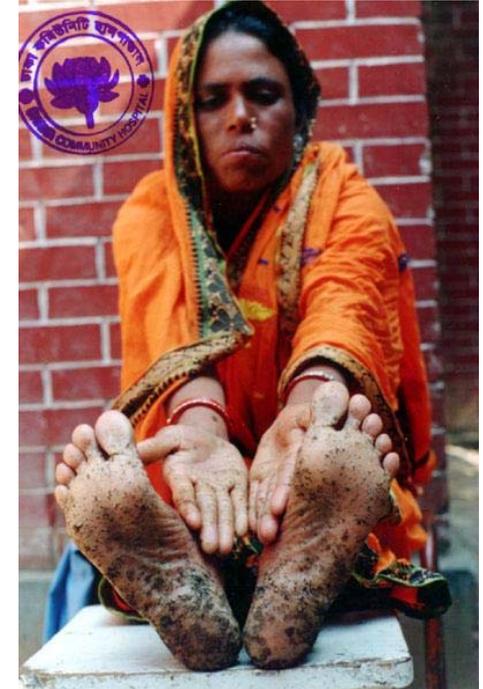
- In Bangladesh & parts of India, tube wells are contaminated with arsenic
- Called “arsenicosis”
 - chronic low-level arsenic poisoning
- Between 35 and 85 million people are affected*
- Prognosis for existing sufferers is poor



* Estimated by World Health Organization

The World's Worst Mass Poisoning

- Progression of symptoms
 - dermatitis and skin disorders
 - malignant tumors
 - death
- 25% of all deaths in affected areas are now from arsenicosis
- Anomalies:
 - Strikes only some in a given population
 - Other places (e.g. Chile) have high arsenic but no arsenicosis

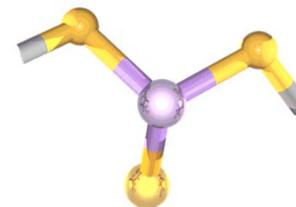


Lesions on hands and feet of arsenicosis sufferer

* Estimated by World Health Organization

A Selenium-Arsenic Hypothesis

- Selenium is an essential trace element
 - Deficiency results in dermatitis, skin disorders, malignant tumors, cardiomyopathies, and death
 - For every arsenic eliminated by formation of the arsenic-selenium molecule, one essential selenium is lost too
- Our hypothesis:
 - People are not being directly poisoned by arsenic but may be becoming selenium deficient
- Proposed solution:
 - Add selenium to diet



Clinical Trial of Selenium Supplementation

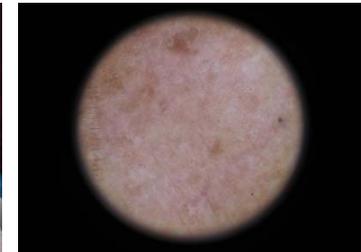
www.bangladesh-selenium.org

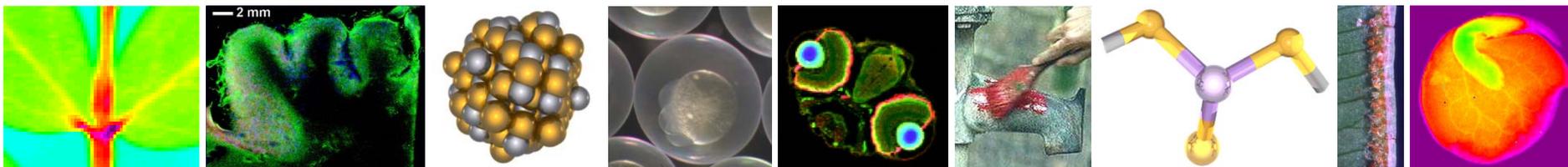
TEXAS TECH UNIVERSITY THE UNIVERSITY OF CHICAGO শিশু-মাতৃ স্বাস্থ্য ইনস্টিটিউট INSTITUTE OF CHILD AND MOTHER HEALTH Apollo Hospitals CHAKRA FORMING LIVES

Selenium Treatment of Arsenic Toxicity & Cancers in Bangladesh [SETAC]
Phase III, Double-Blind, Randomized, Placebo-Controlled Trial on the Use of Long-term, Dietary Selenium in Countering Arsenic Toxicity | Sponsors: NIH/NCI, American Cancer Society



- Project Leaders:
 - Julian Spallholz (Texas Tech)
 - Paul LaPorte (U. Chicago)
 - Selim Ahmed (Institute of Child & Mother Health, Bangladesh)





Closing Remarks

- X-ray absorption spectroscopy can yield valuable information on elements in environmental, health and chemical sciences
 - Often complex, heterogeneous systems
- Combined with X-ray fluorescence mapping, can derive spatial resolution of chemical information



Acknowledgements

- National School on Neutron and X-ray Scattering (organizers, sponsors)



Canada Research
Chairs



Canada Research Chairs Program
University of Saskatchewan
Province of Saskatchewan
CFI, NSERC, NIH, CIHR, SHRF
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Synchrotron Techniques
Heart and Stroke Foundation of Canada/CIHR
Team Grant in Synchrotron Medical Imaging



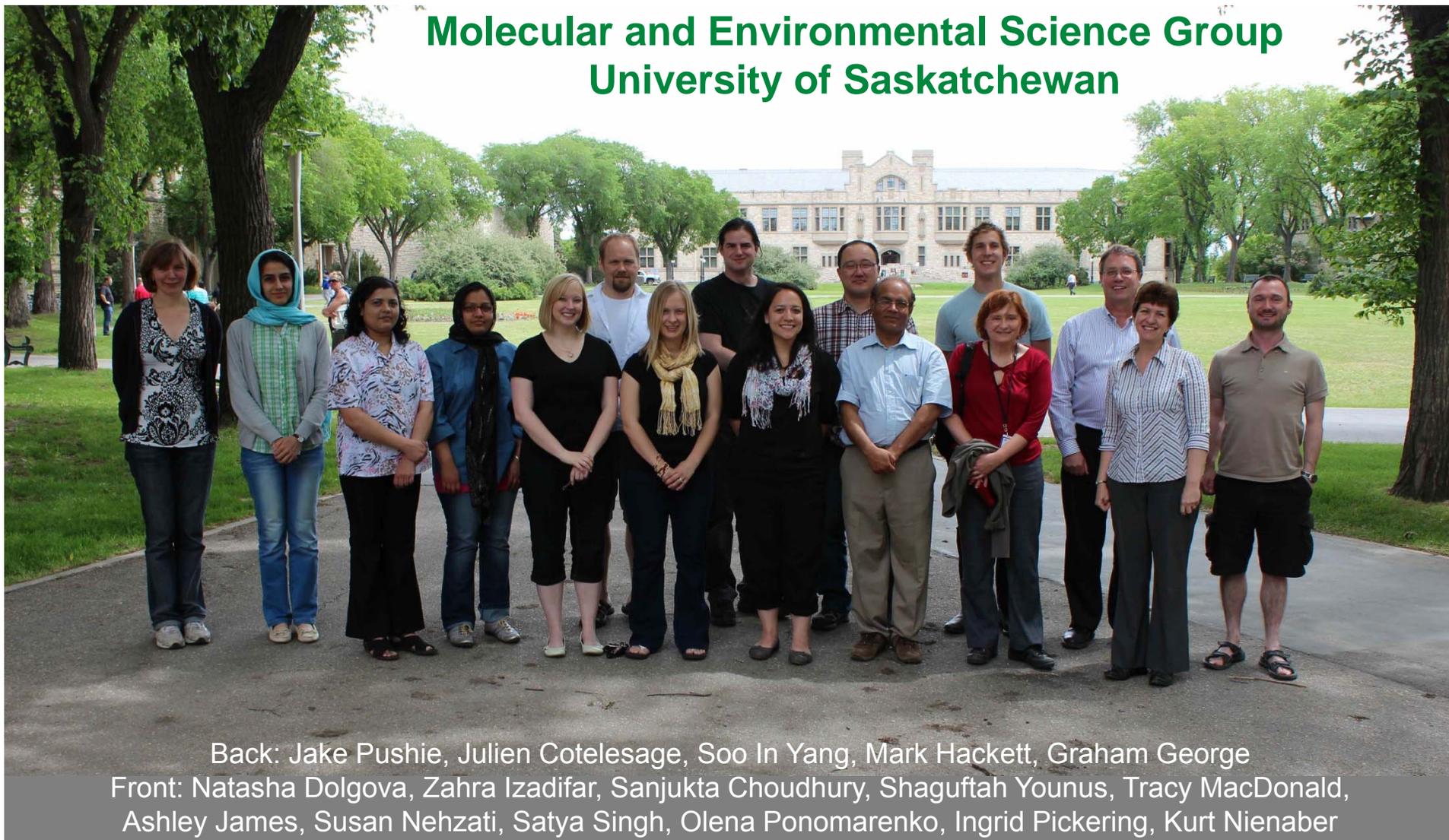
HEART &
STROKE
FOUNDATION
OF CANADA



Beamlines and their personnel



Molecular and Environmental Science Group University of Saskatchewan



Back: Jake Pushie, Julien Cotelesage, Soo In Yang, Mark Hackett, Graham George
Front: Natasha Dolgova, Zahra Izadifar, Sanjukta Choudhury, Shaguftah Younus, Tracy MacDonald,
Ashley James, Susan Nehzati, Satya Singh, Olena Ponomarenko, Ingrid Pickering, Kurt Nienaber