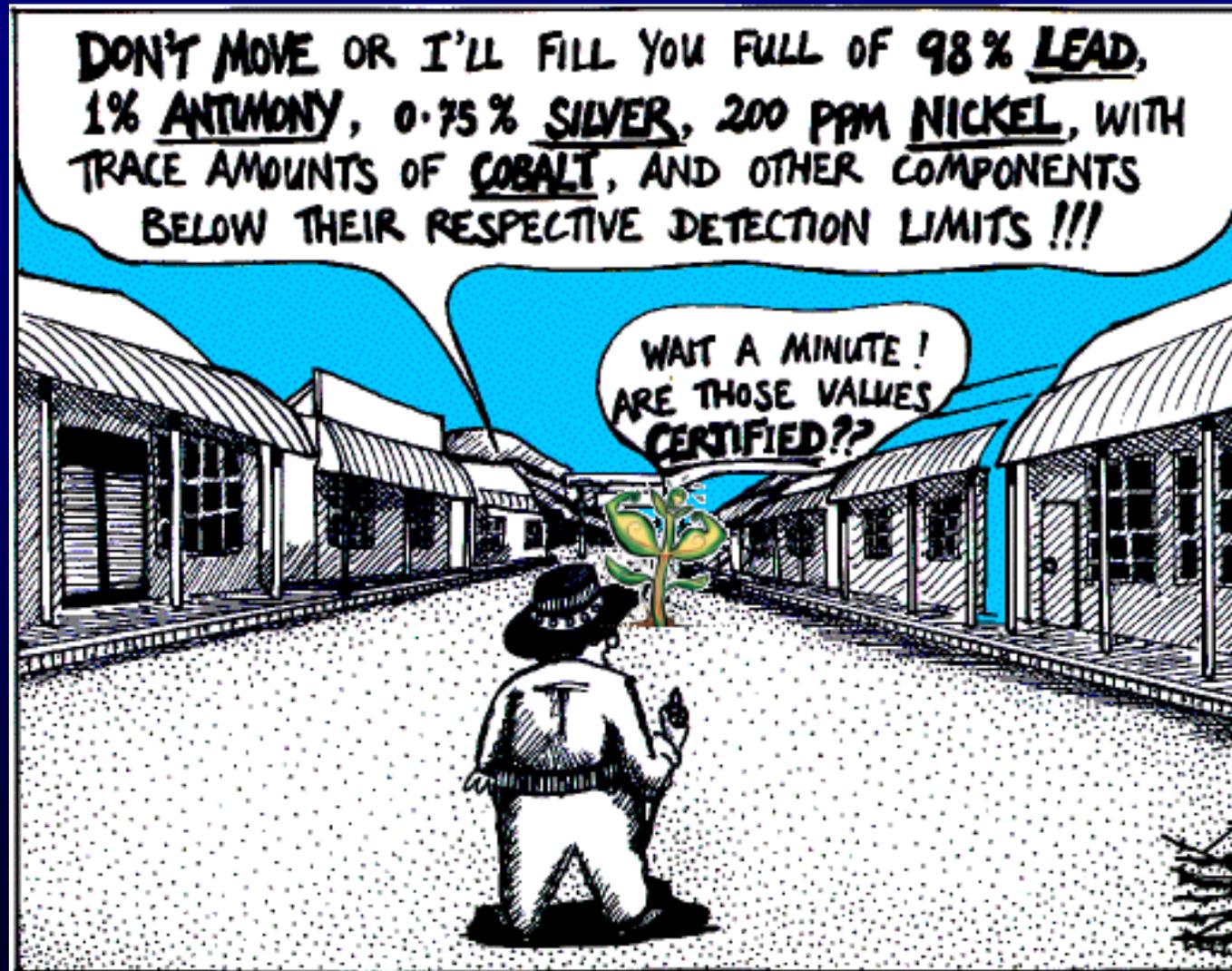


Advanced Course on Bioinorganic Chemistry & Biophysics of Plants – Introduction

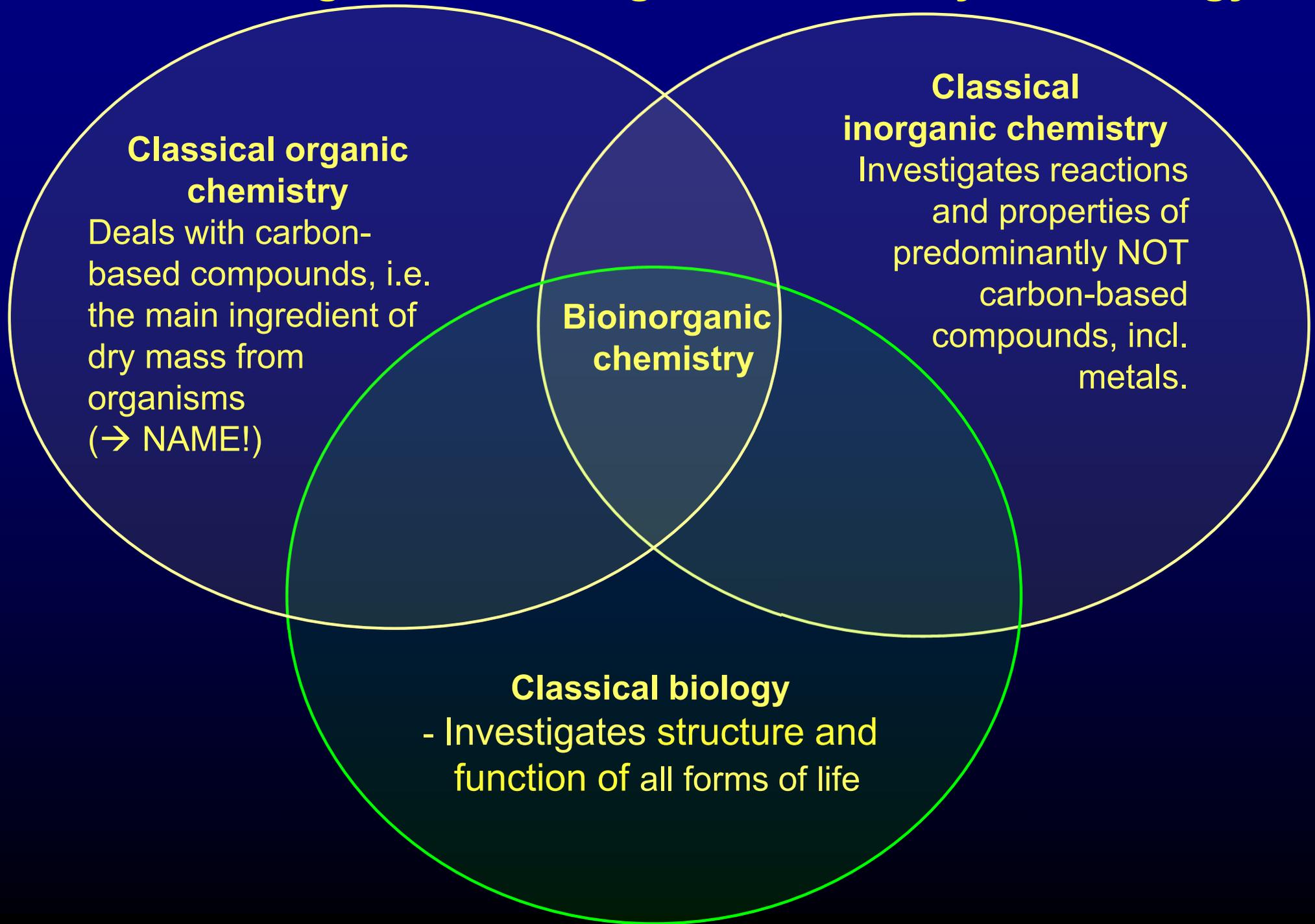


Schwermetall-Hyperakkumulation im Wilden Westen

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

I.
Basics of Bioinorganic Chemistry and Biophysics

Bioinorganic Chemistry versus Classical organic and inorganic Chemistry and Biology



Themes of bioinorganic chemistry research

Metal coordination in biological ligands

- Metal(loid) transport
- Metal(loid) storage
- Metal-based catalysis in biology, usually via metal-based active sites in enzymes
- Metals as structural elements in proteins
- Metal(loid) deficiency and toxicity
- Metal(loid) detoxification

Methods used for investigating these questions include for example (in solutions, in model systems, but also in living cells)

- UV/VIS absorption and fluorescence spectroscopy (→ electronic transitions to/from excited states)
- X-ray absorption and emission spectroscopy (→ ionisation energies = X-ray absorption edges and emission bands, their element-specific characteristics and their modification by redox state and neighbouring atoms)
- EPR spectroscopy (→ analysis of the ligand environment of paramagnetic metal ions)
- NMR spectroscopy (→ analysis of the environment of NMR-active nuclei)

Biophysics versus Classical Experimental Physics and Classical Biology

Classical Experimental Physics

Deals with interactions (e.g. energetics, speeds and forces) between particles, explains the basic principles of matter

Biophysics

Investigates e.g. electrostatic interactions between biological macromolecules, energy transfer between and within biologically relevant molecules

Classical Biology

Investigates interactions between organisms (individuals, groups, species) and between organisms and abiotic factors

Themes of biophysical research

Energetics and kinetics of biological processes

- transport (e.g. of metals)
- catalysis in biology, usually via metal-based active sites in enzymes
- reversible coupling of biologically relevant molecules without bond formation/breakage
- protein folding

Methods used for investigating these questions include for example (in solutions, in model systems, but also in living cells)

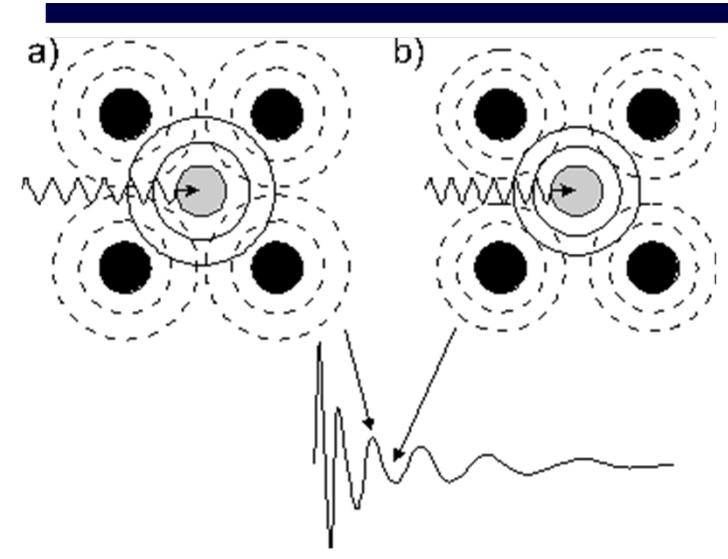
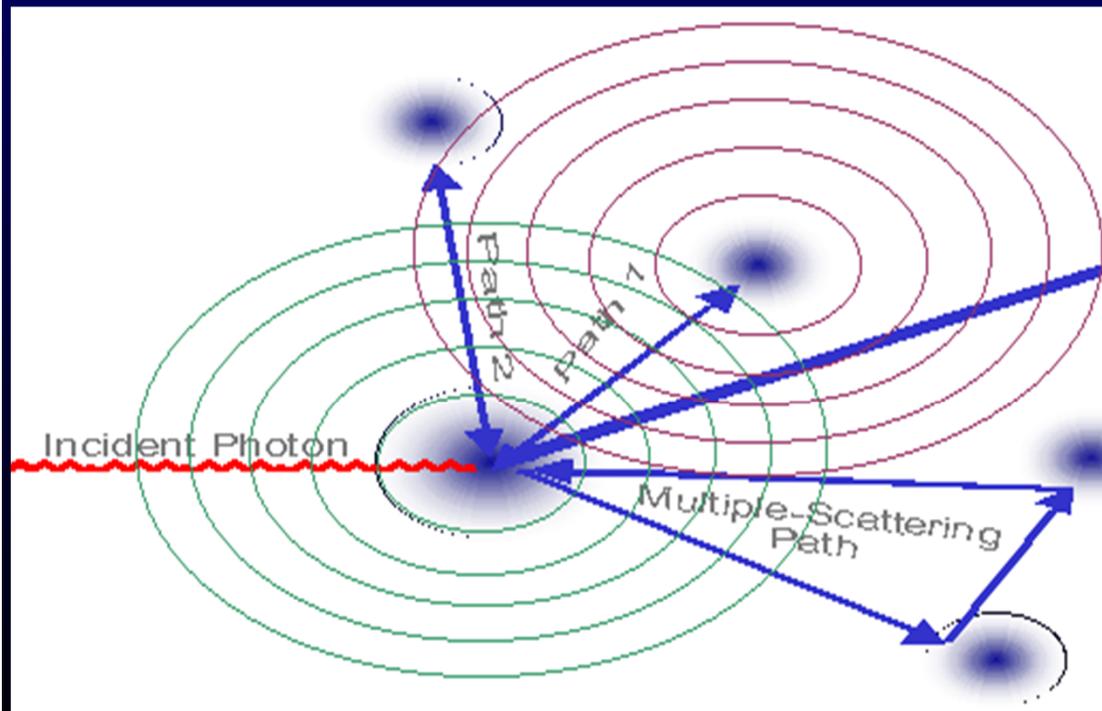
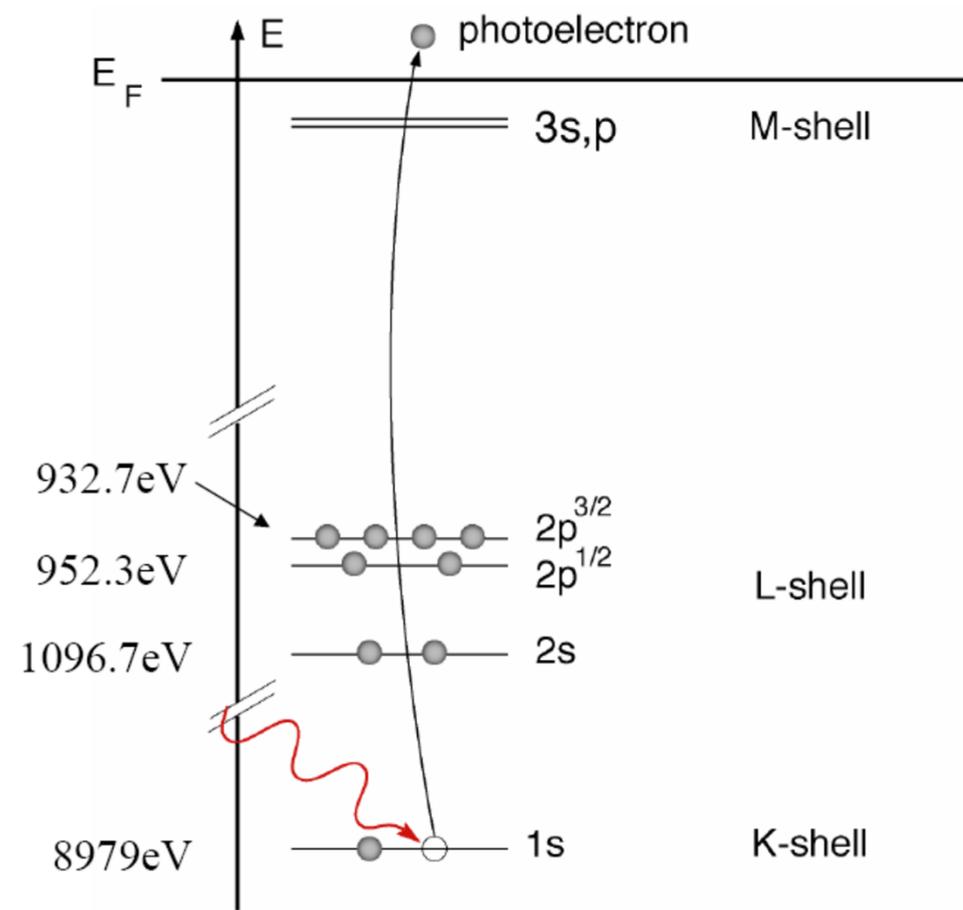
- UV/VIS absorption and fluorescence spectroscopy (→ electronic transitions to/from excited states → e.g. analysis of chromophore coupling)
- X-ray absorption spectroscopy (→ ionisation energies = X-ray absorption edges and emission bands, their element-specific characteristics and their modification by redox state and neighbouring atoms)
- EPR spectroscopy (→ e.g. spin labelling for analysis of protein folding)
- NMR spectroscopy (→ e.g. analysis of kinetics of protein (re-/un-)folding)

II.

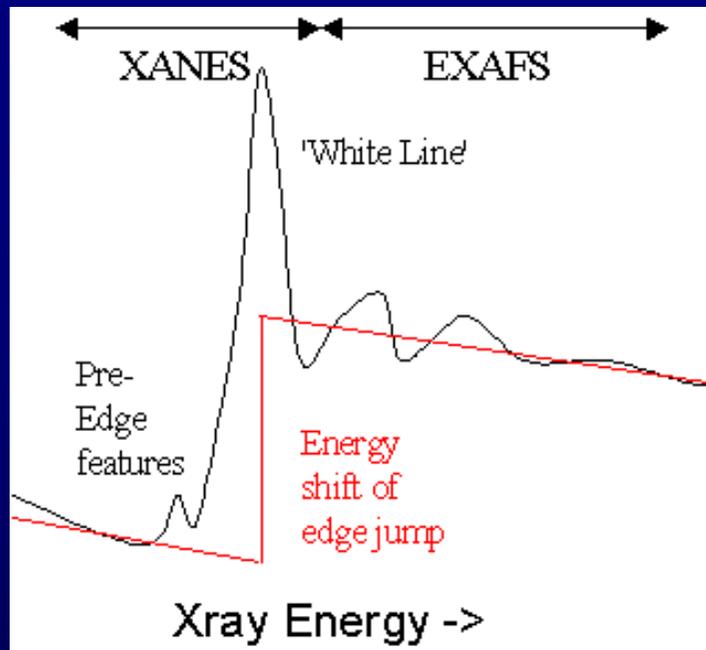
What will we show you?

Theoretical Background of important biochemical and biophysical methods...

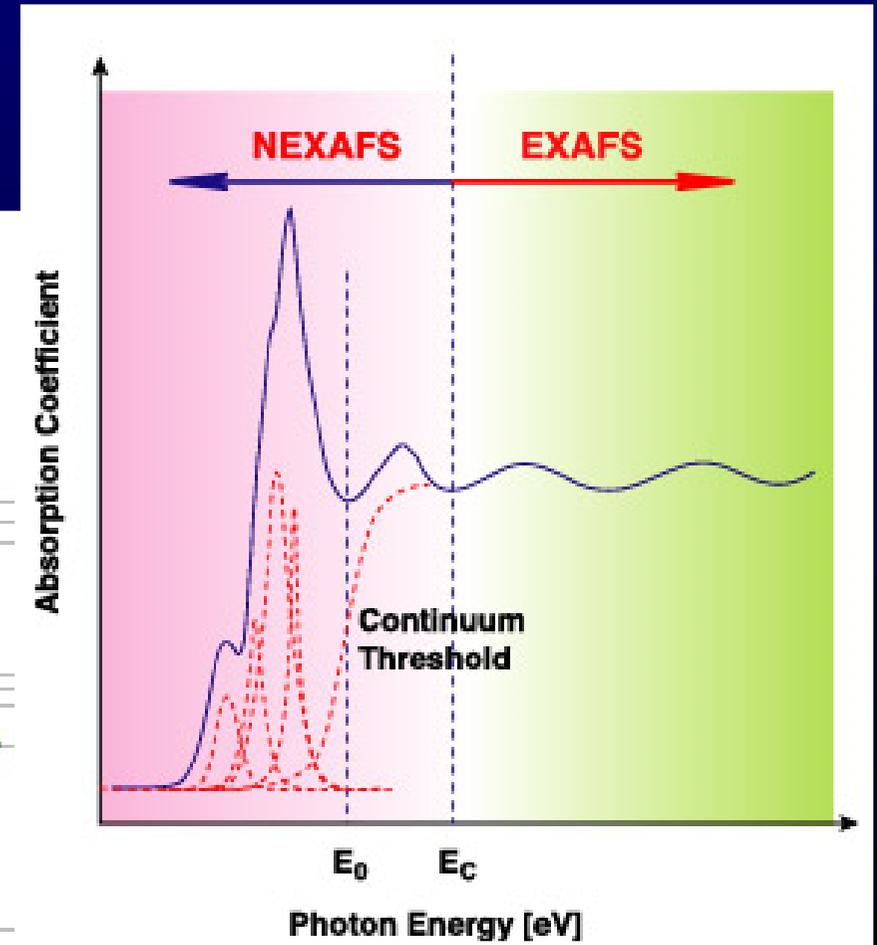
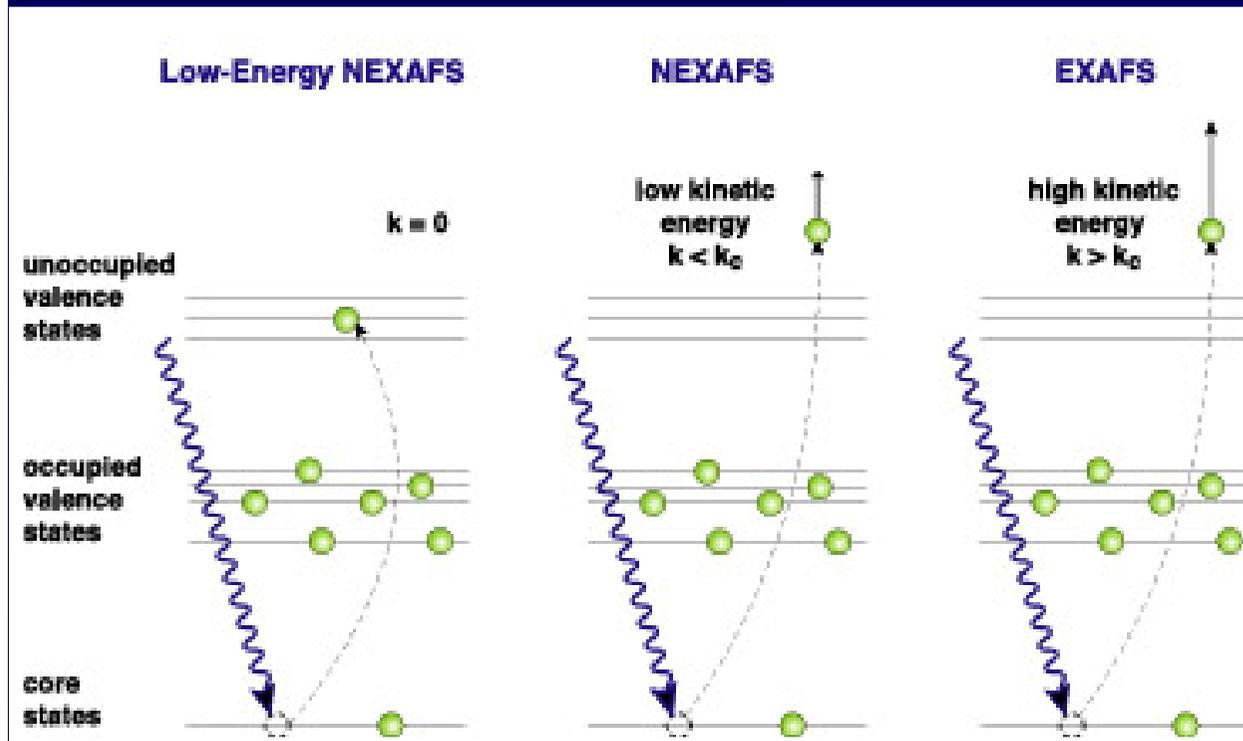
Cu



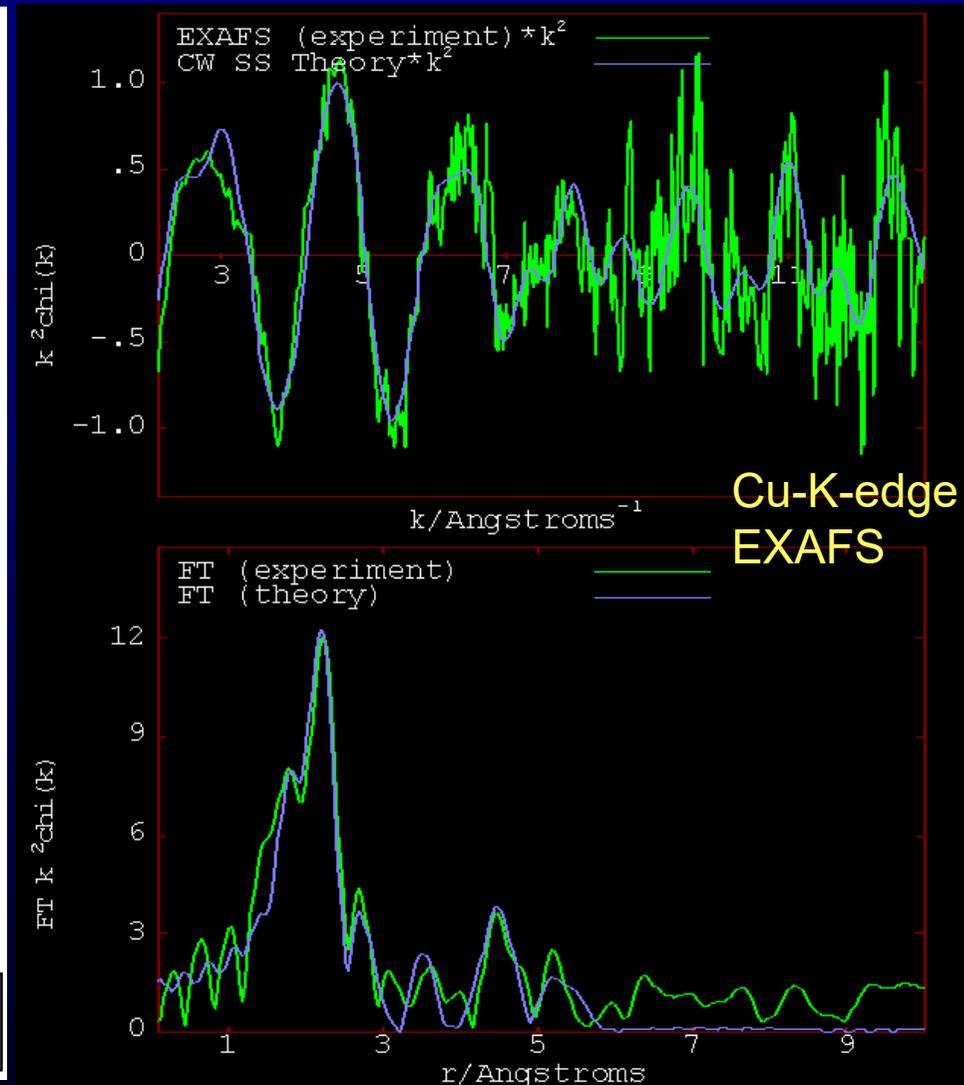
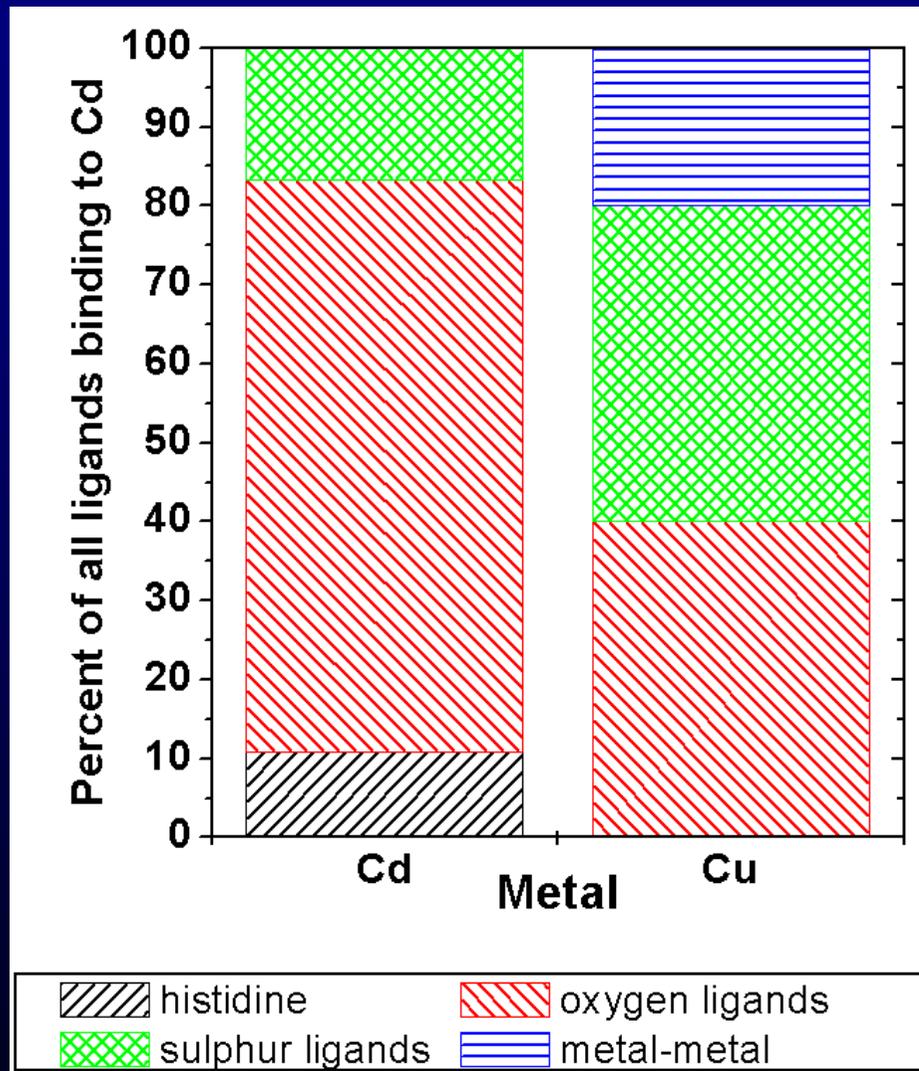
... differences in the basics and applications between related methods...



methods...



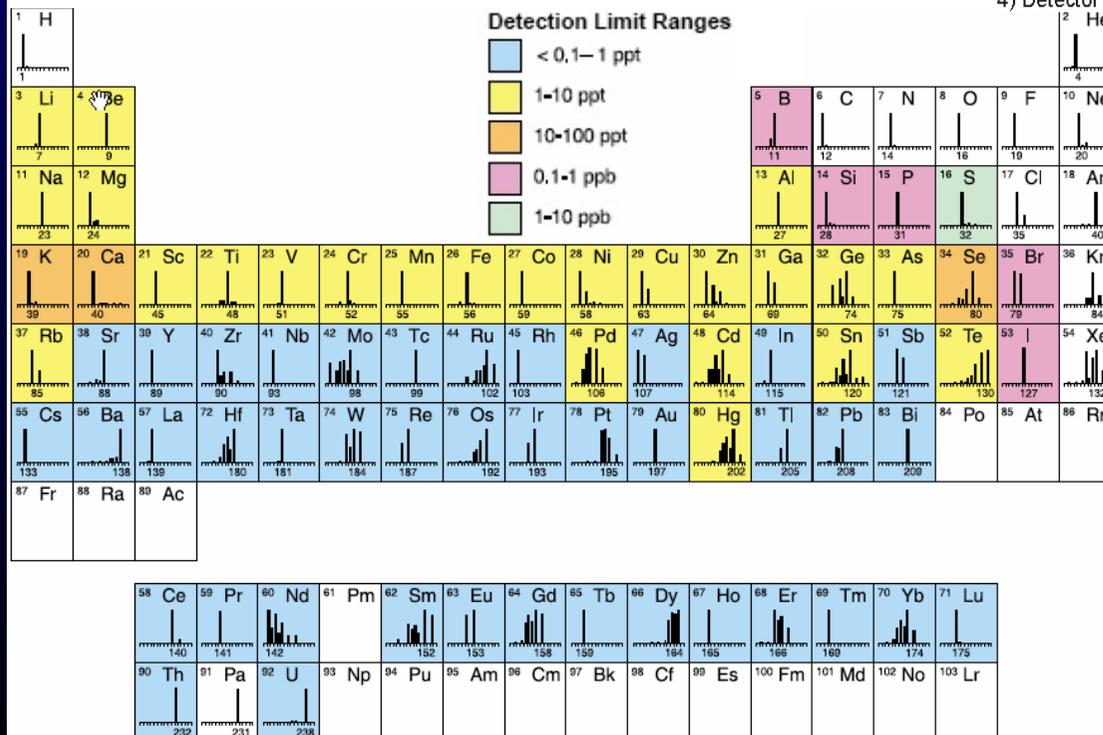
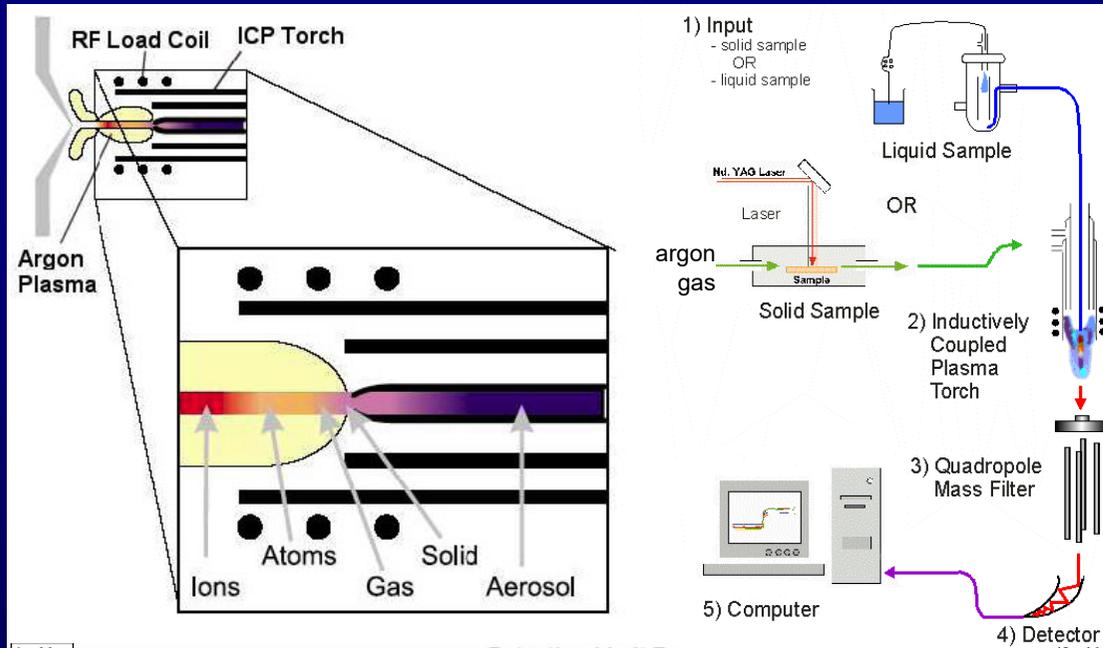
...and the use of these methods for answering questions in bioinorganic chemistry and biophysics.



Cd: Küpper H, Mijovilovich A, Meyer-Klaucke W, Kroneck PMH (2004) Plant Physiology 134 (2), 748-757

Cu: Mijovilovich A, Leitenmaier B, Meyer-Klaucke W, Kroneck PMH, Götz B, Küpper H (2009) Plant Physiology 151, 715-31

Construction principles of measuring instruments as well as advantages and disadvantages resulting from it.



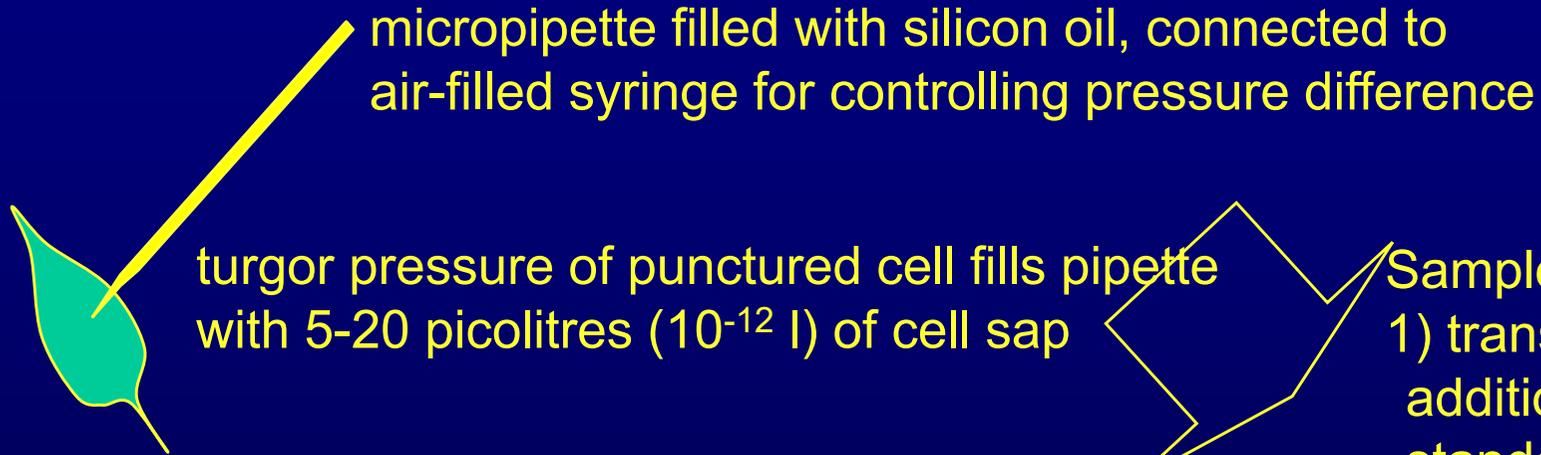
Advantages:

- Detection limits for most elements equal to or better than those obtained by Graphite Furnace –AAS (GFAAS)
- Higher throughput than GFAAS
- minimum of matrix interferences due to the high-temperature of the ICP source
- Superior detection capability to ICP-AES with the same throughput
- Ability to obtain isotopic information.

Disadvantages:

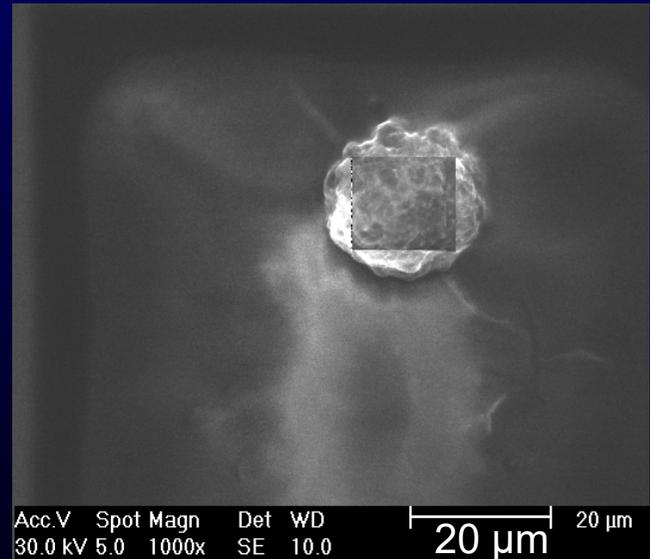
- more complicated technique than AAS
- much more expensive than AAS
- elements that prefer to form negative ions, such as Cl, I, F, etc. are very difficult to determine via ICP-MS because ions formed by the ICP discharge are typically positive ions.

Principles of sample preparation for specific methods...

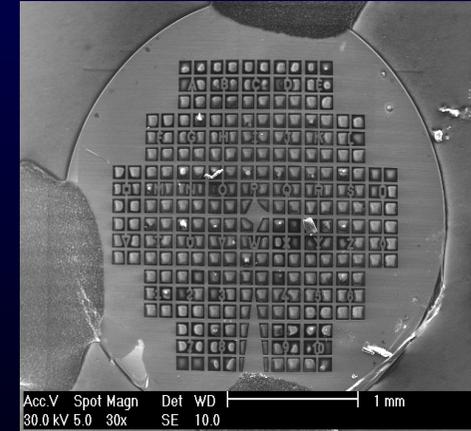


- Sample preparation:
- 1) transfer to storage grid, addition of internal standard (e.g. RbF) and matrix (e.g. mannitol)
 - 2) transfer to analysis grid, drying with isopentane

- Analysis:
- 1) recording of EDXA spectra in SEM
 - 2) data processing

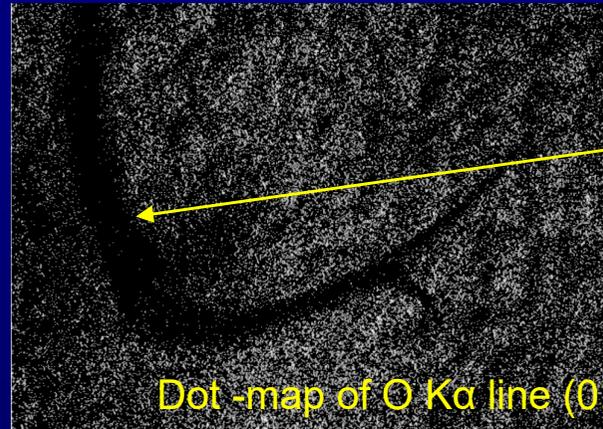


typical dried sample



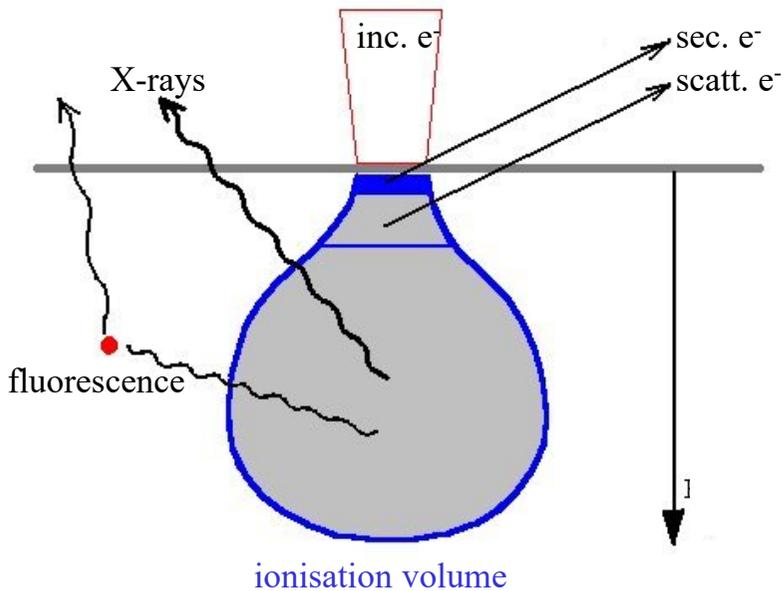
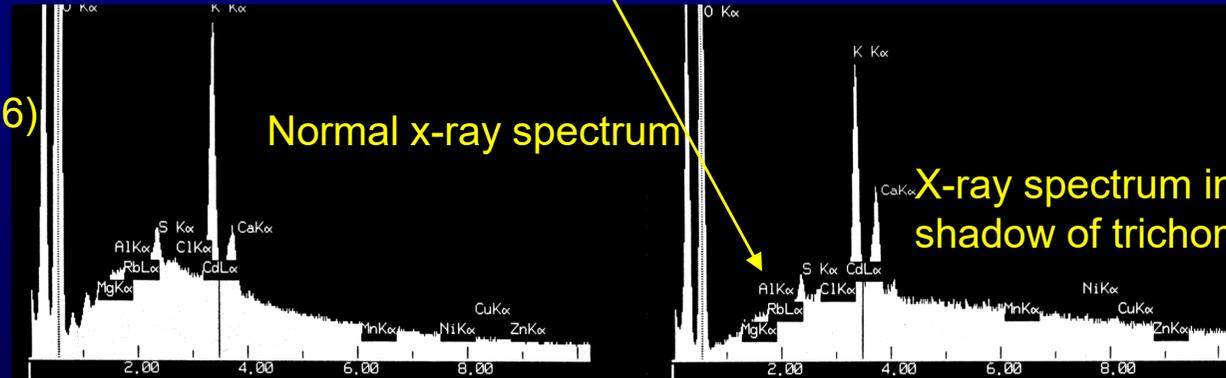
analysis grid

...and problems associated with these samples.



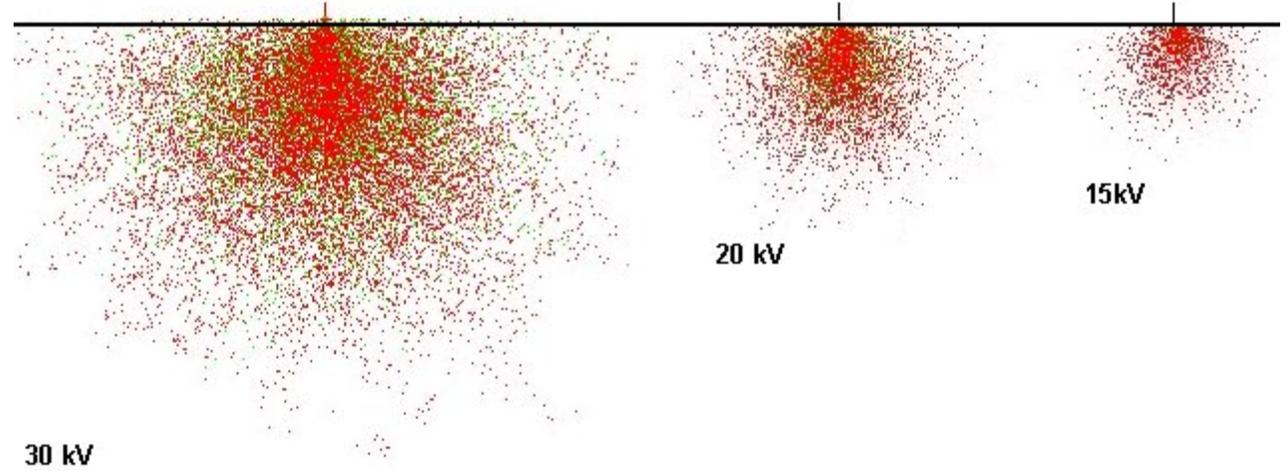
Effect of shading

shading inside a sample leads to absorption of low-energy x-rays

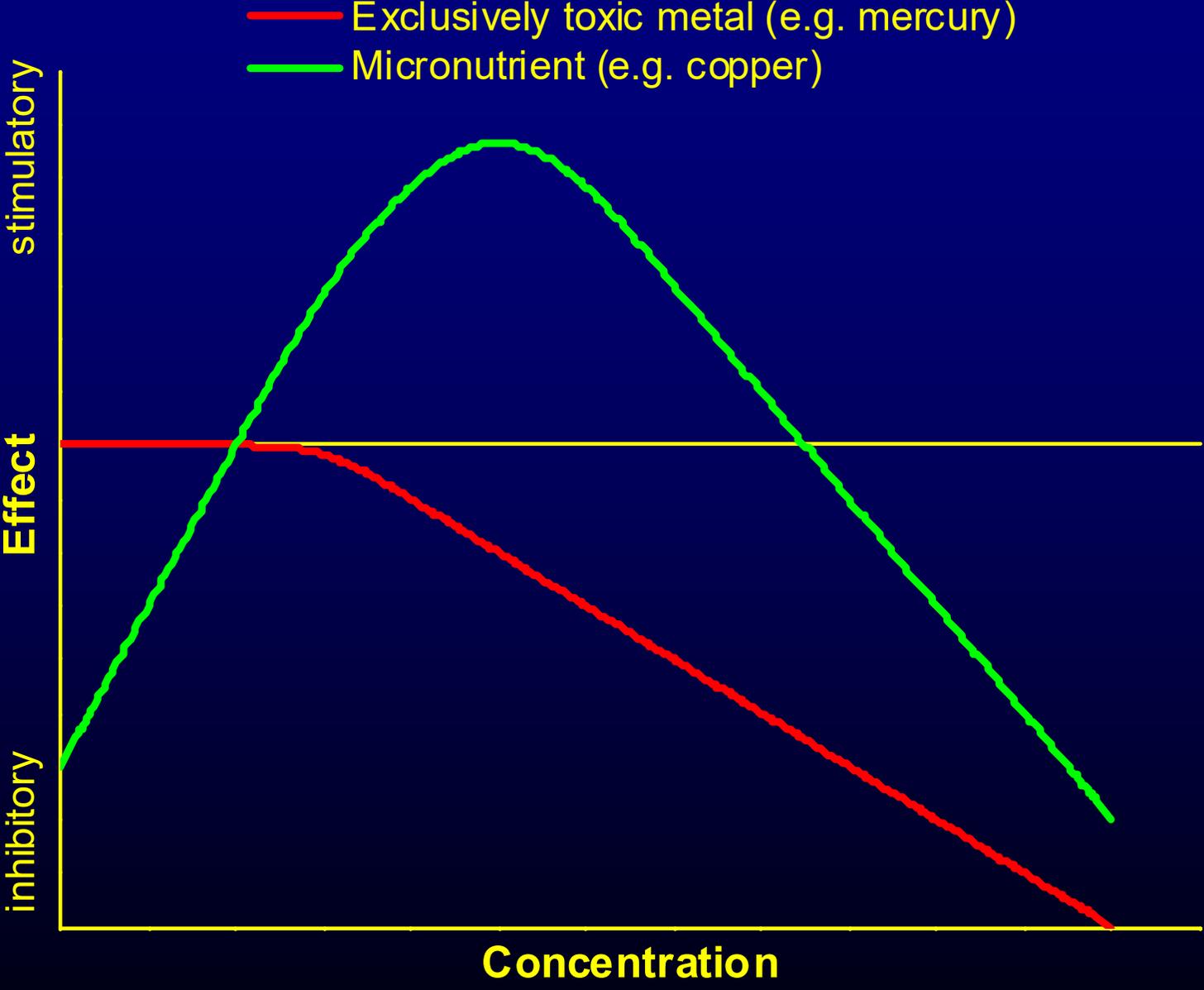


Effect of acceleration voltage

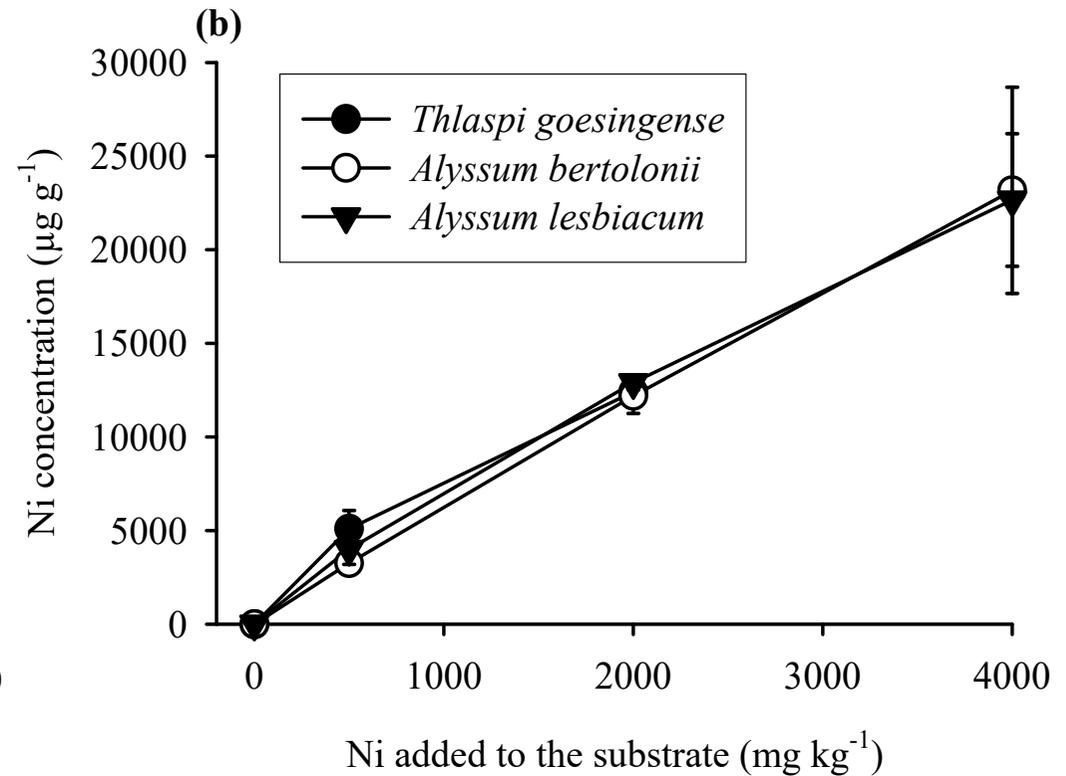
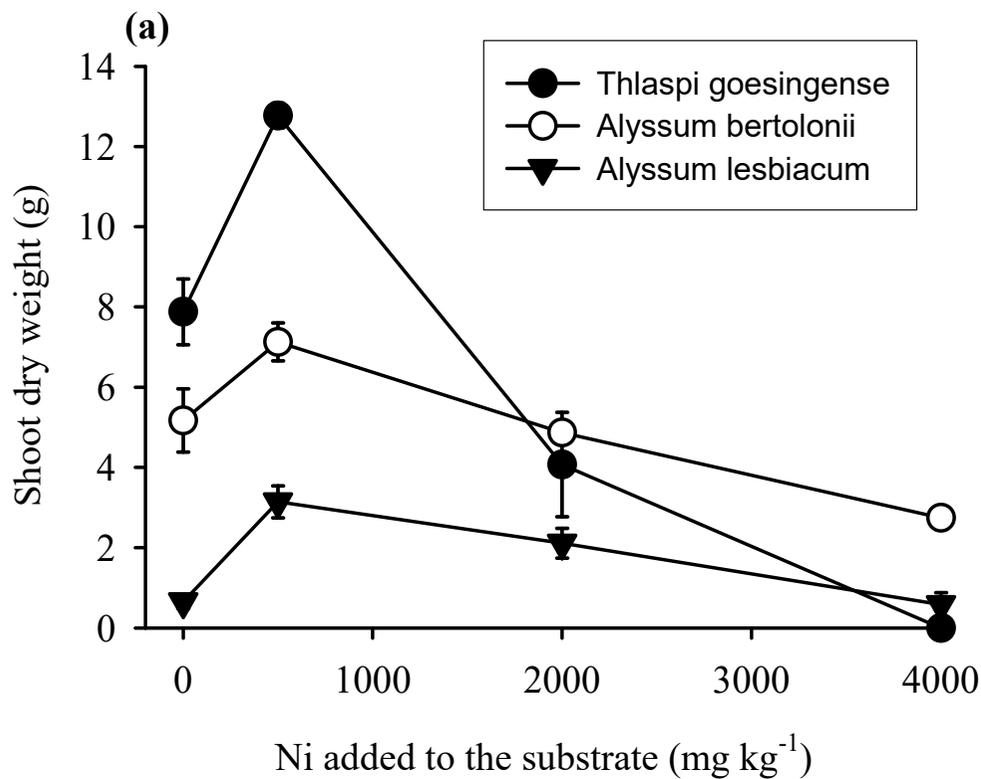
high acceleration voltage leads to deeper penetration into the sample!



Principles

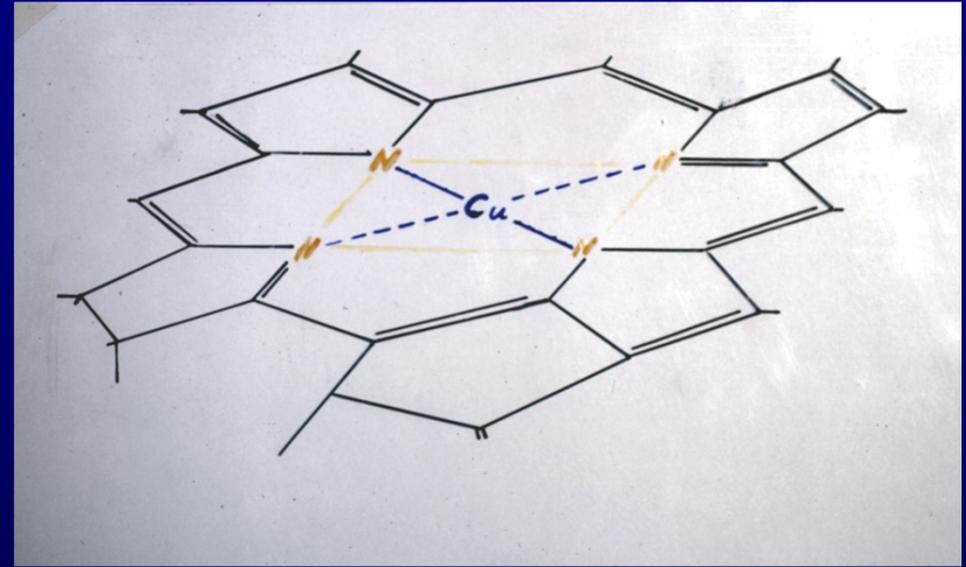
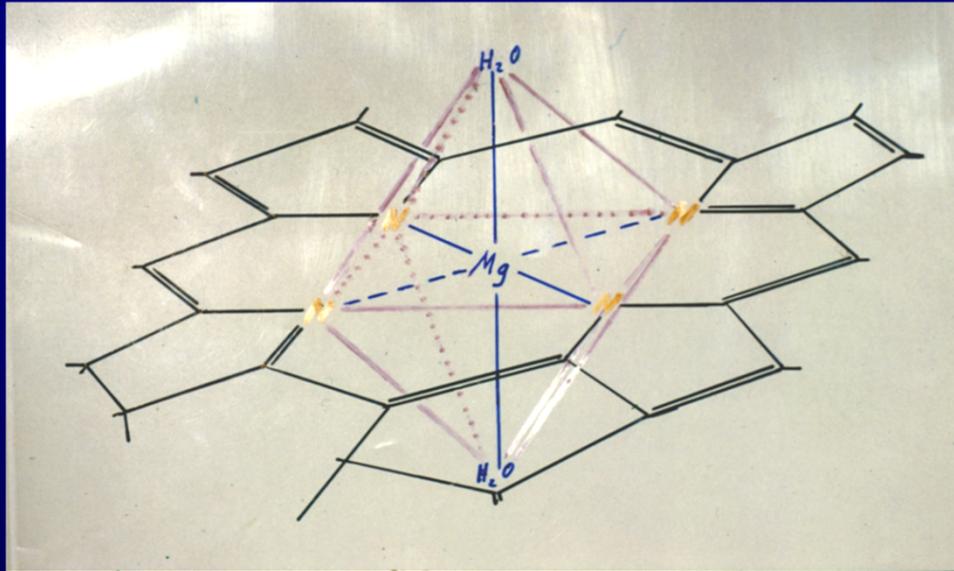


We show you plants that strongly like potentially toxic metals...



Effects of Ni^{2+} addition on hyperaccumulator plant growth and Ni^{2+} concentration in shoots

...why trace metals can become toxic for plants...



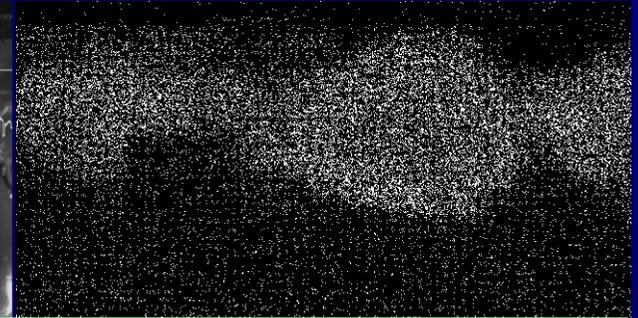
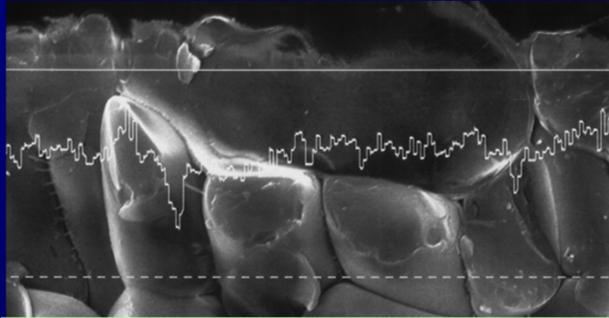
- shift of absorbance/fluorescence bands --> less energy transfer
- different structure --> proteins denature
- do not readily perform charge separation when in reaction centre
- unstable singlet excited state --> “black holes” for excitons

...and how plants defend themselves against that toxicity.

Mechanisms

- Generally: aktive transport processes against the concentration gradient
→ transport proteins involved.

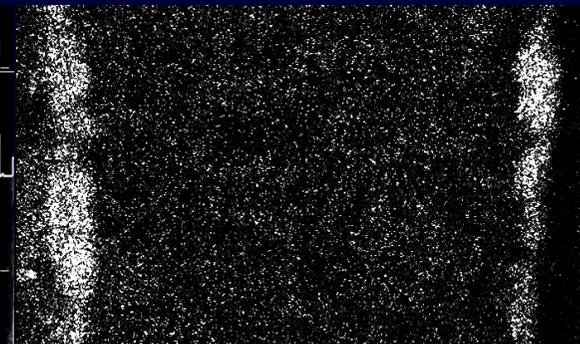
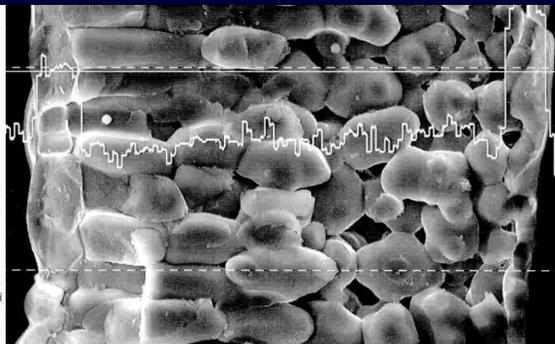
- Exclusion from cells:
 - observed in brown algae
 - in roots



Küpper H et al., 2001, J Exp Bot 52 (365), 2291-2300

- Sequestration in the vacuole:
 - plant-specific mechanism (animals+bacteria usually don't have vacuoles...)
 - very efficient, because the vacuole does not contain sensitive enzymes
 - saves the investment into the synthesis of strong ligands like phytochelatins
 - main mechanism in hyperaccumulators

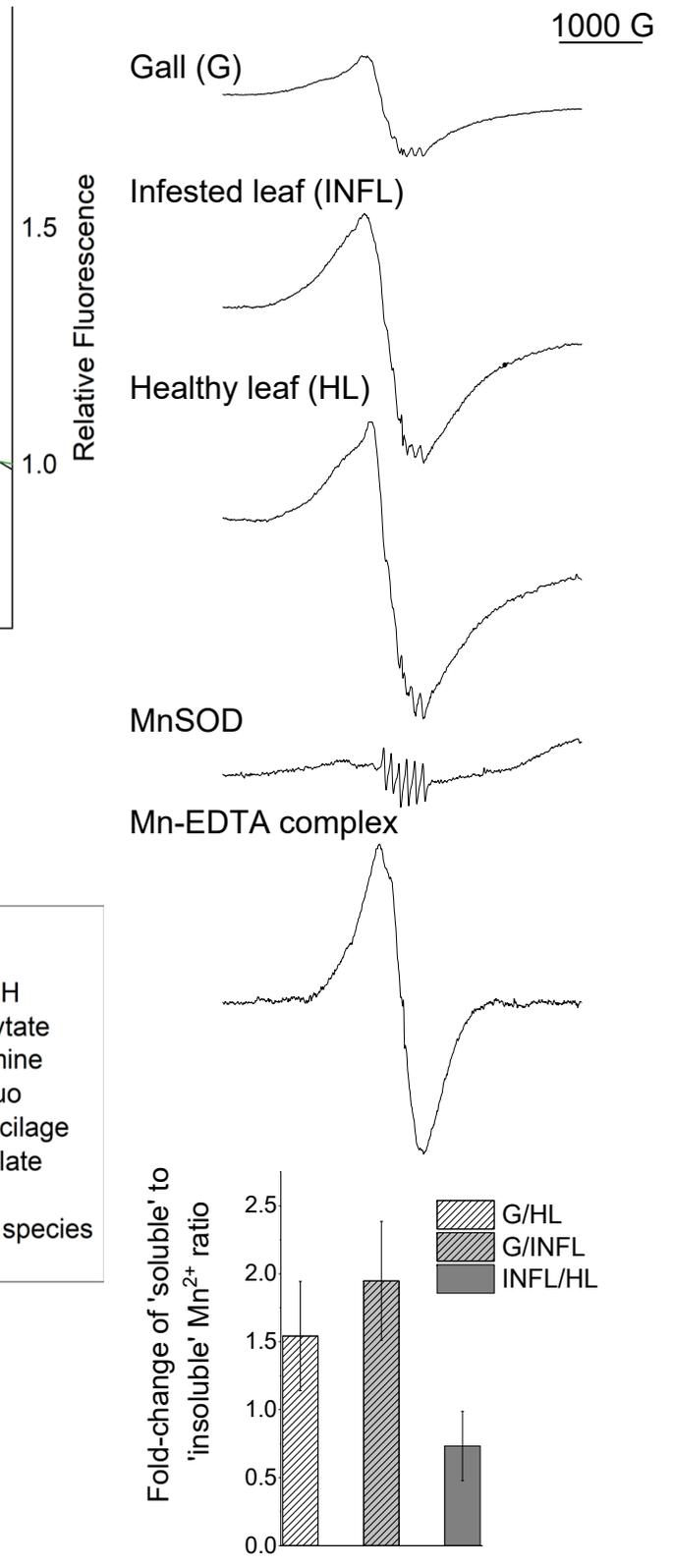
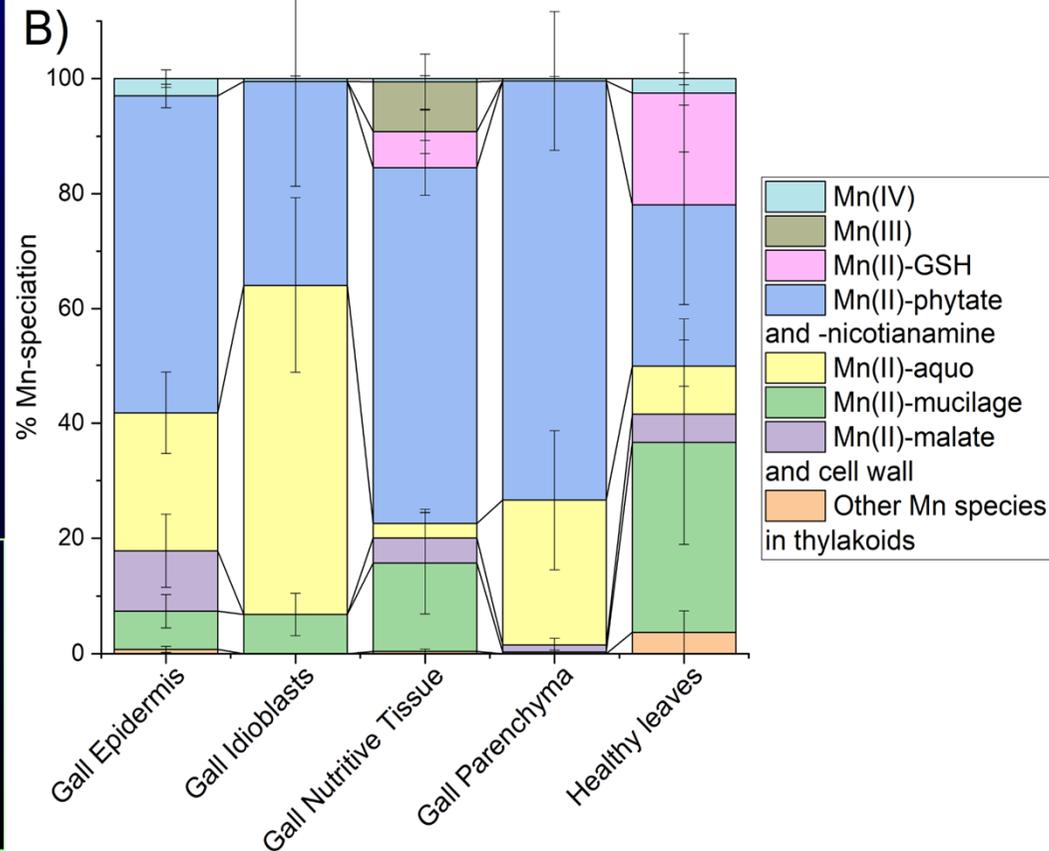
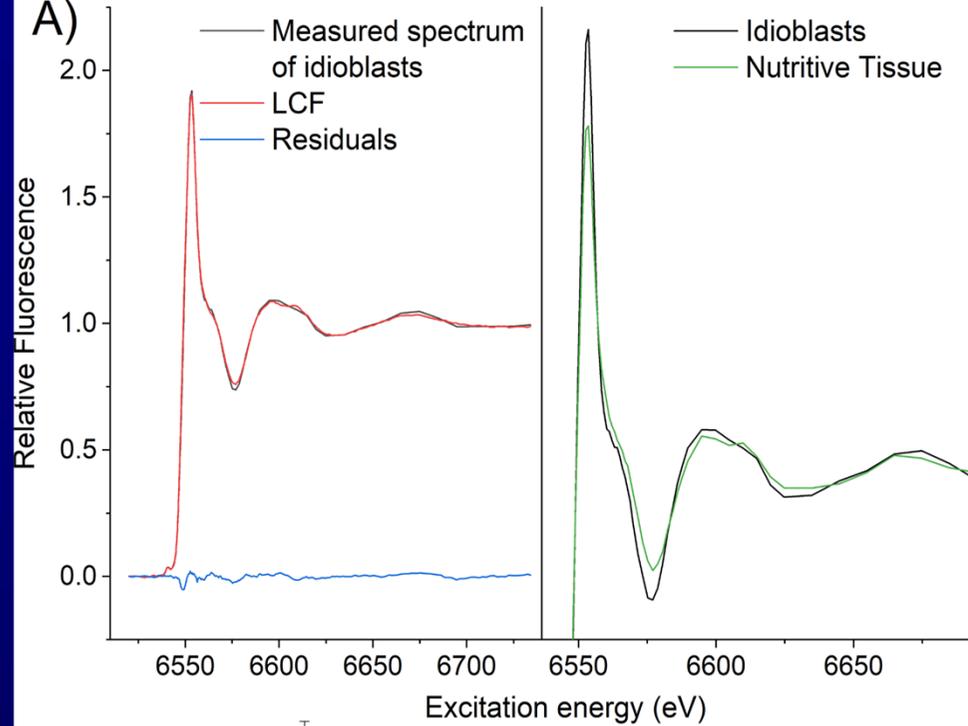
- Sequestration in least sensitive tissues, e.g. the epidermis instead of the photosynthetically active mesophyll



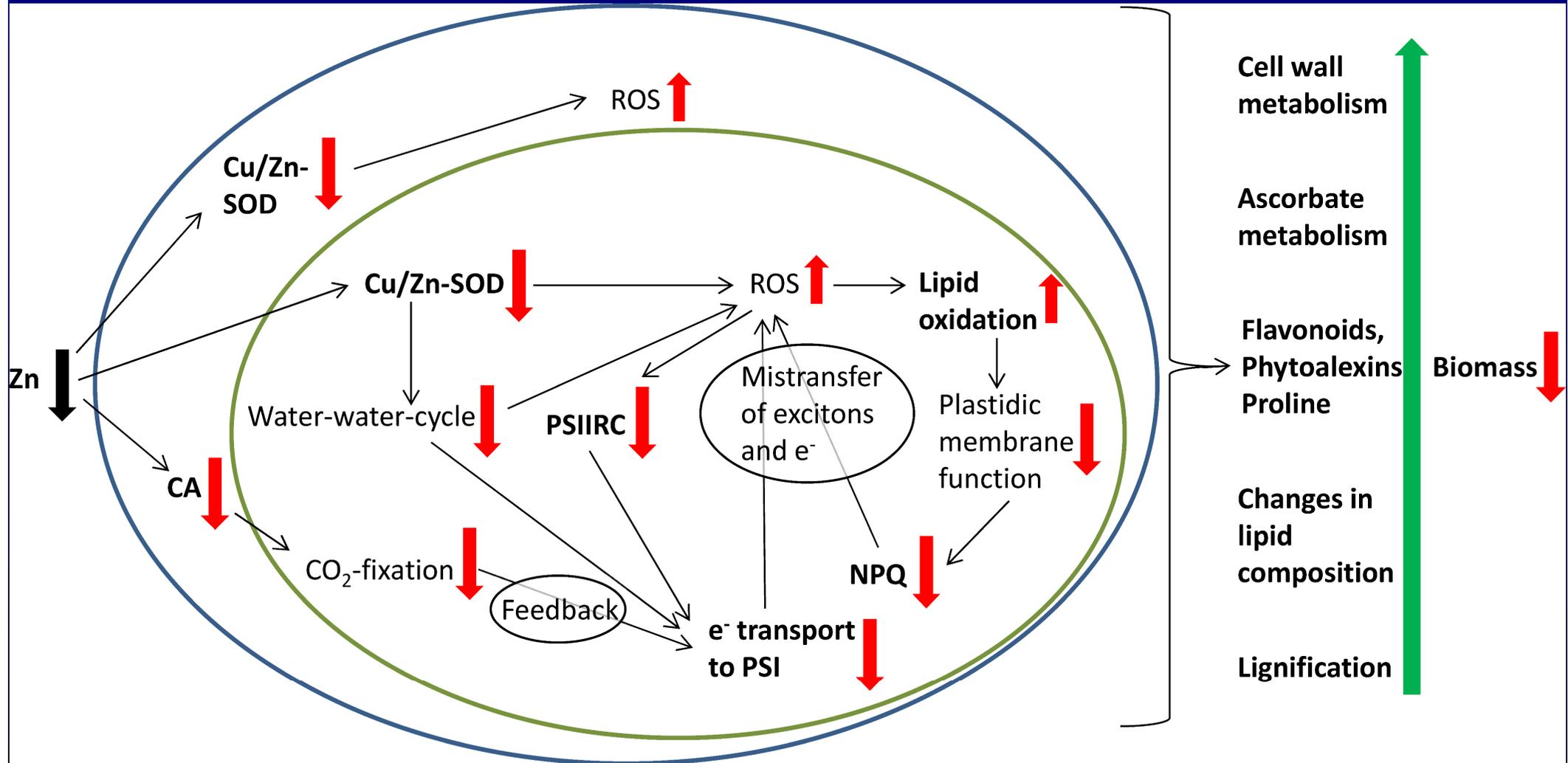
Küpper H, Zhao F, McGrath SP (1999) Plant Physiol 119, 305-11

We will show you original data from recent research...

Morina F*, Kuvelja A, Brückner D, Mojović M, Nakarada Đ, Bokhari SNH, Vujčić B, Falkenberg G, Küpper H* (2025) *New Phytologist* 246, 2222-2242.



And conclusions that can be drawn from the analysis of measured data.



Andresen A, Morina F, Bokhari SNH, Koník P, Küpper H* (2024) Disturbed electron transport beyond PSI changes metabolome and transcriptome in Zn-deficient soybean. *Biochimica et Biophysica Acta (BBA) Bioenergetics* 1865, 149018

<https://doi.org/10.1016/j.bbabi.2023.149018>

How to compare your results with previous studies...

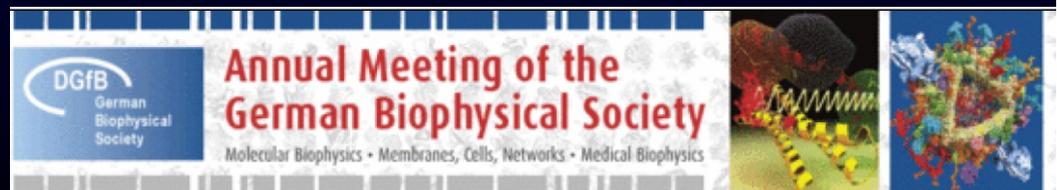
- Commercial scientific databases like Web of Science or Scopus
- Free scientific databases like medline/pubmed
- Advertisement-based “free” commercial search engines like Google

... and how to publish them



Plant, Cell & Environment

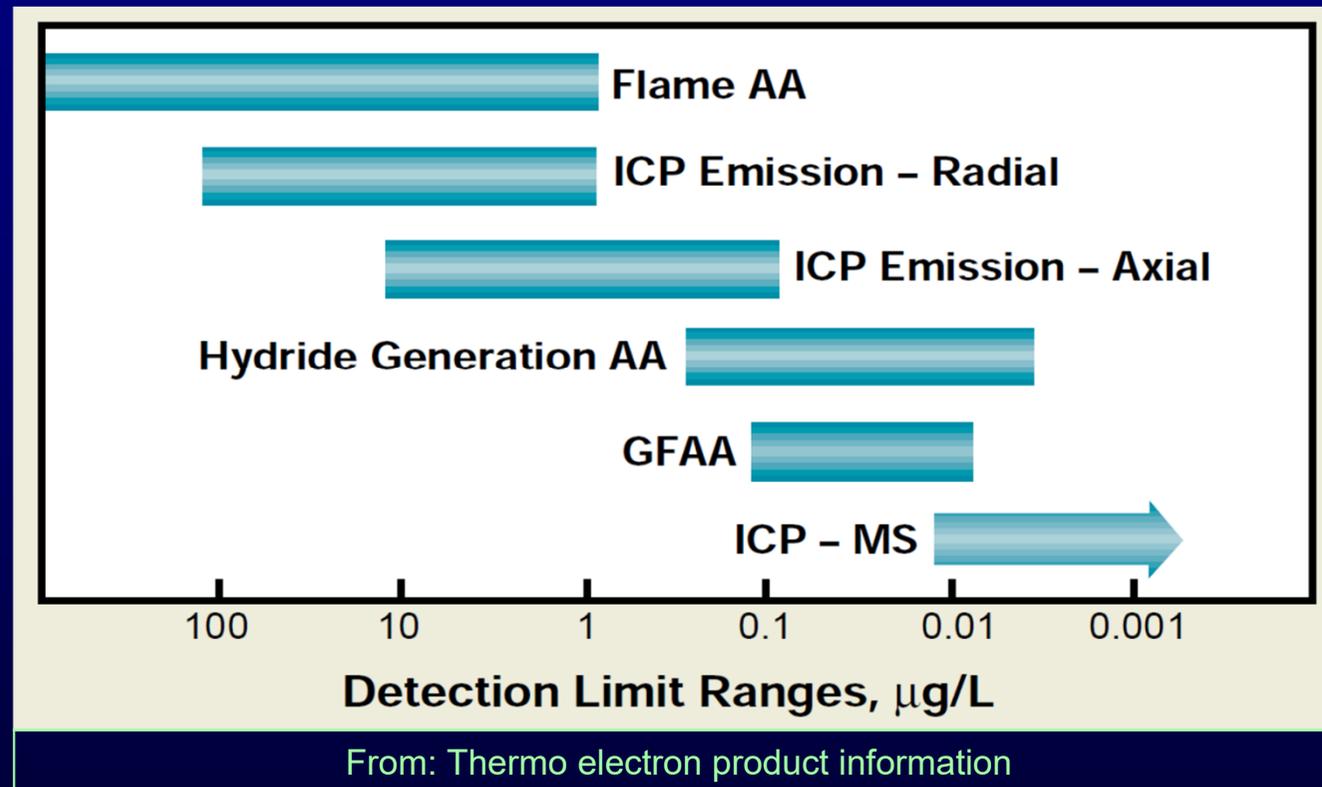
Plant, Cell and Environment (2011) 34, 208–219



III.

**Let's start with having a look at methods of metal analysis
in liquid samples**

Detection limits of different metal analysis methods



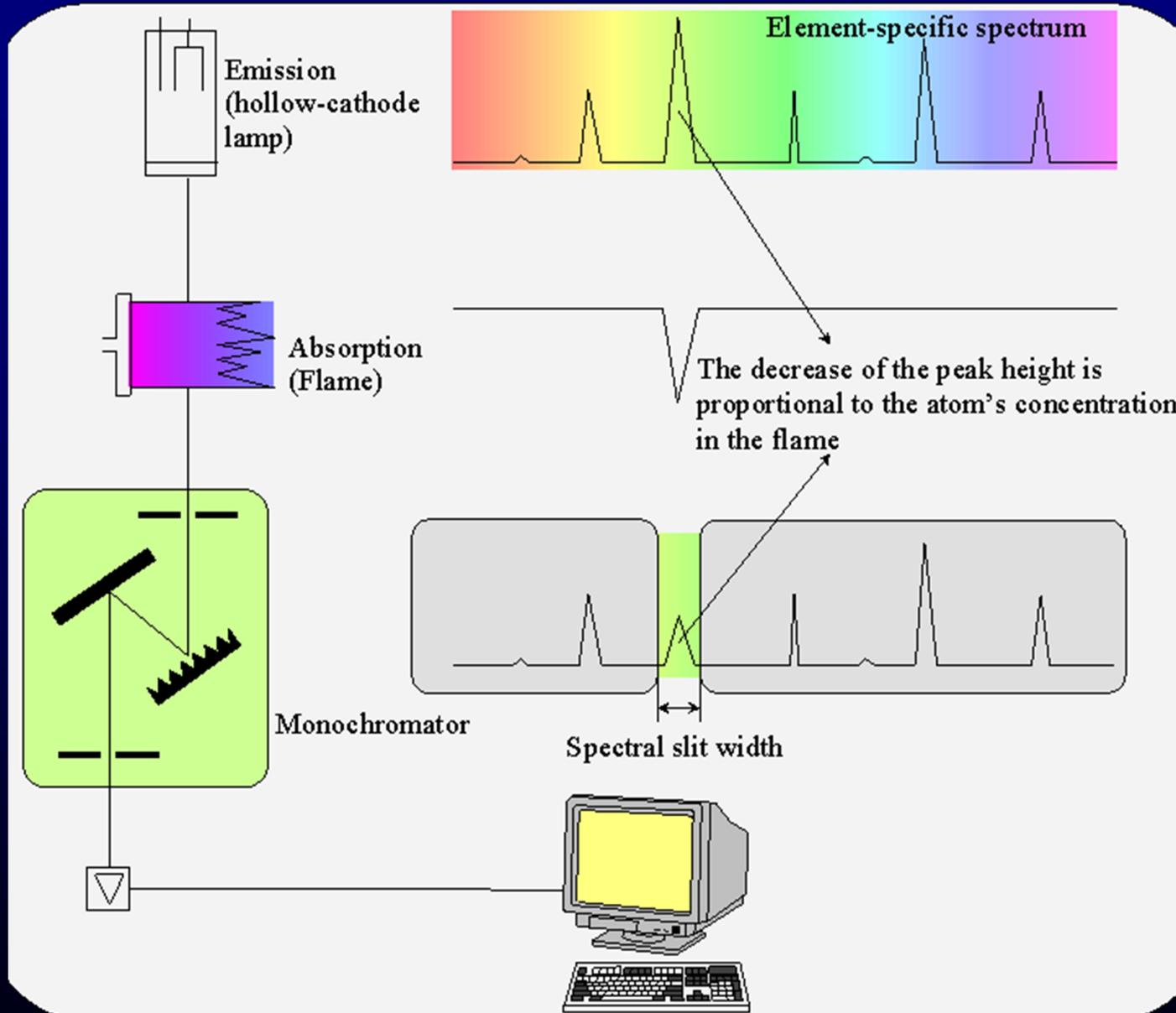
1 ppm = 1000ppb = 1 mg/L = 1 g/m³, i.e. approximately 1/10 of a sugar cube in a bath tub

1 ppb = 1 $\mu\text{g/L}$ = 1 g/1000m³, i.e. approximately a sugar cube in a swimming pool

1 ppt = 0.001 ppb = 1 ng/L = 1 g/1,000,000m³, i.e. approximately a sugar cube in Lake Constance

Metal content – methods of Measurement (I)

Atomic Absorption Spectroscopy (AAS)



Advantages:

- easy to use,
- fast if only 1 element is needed
- affordable

Disadvantages:

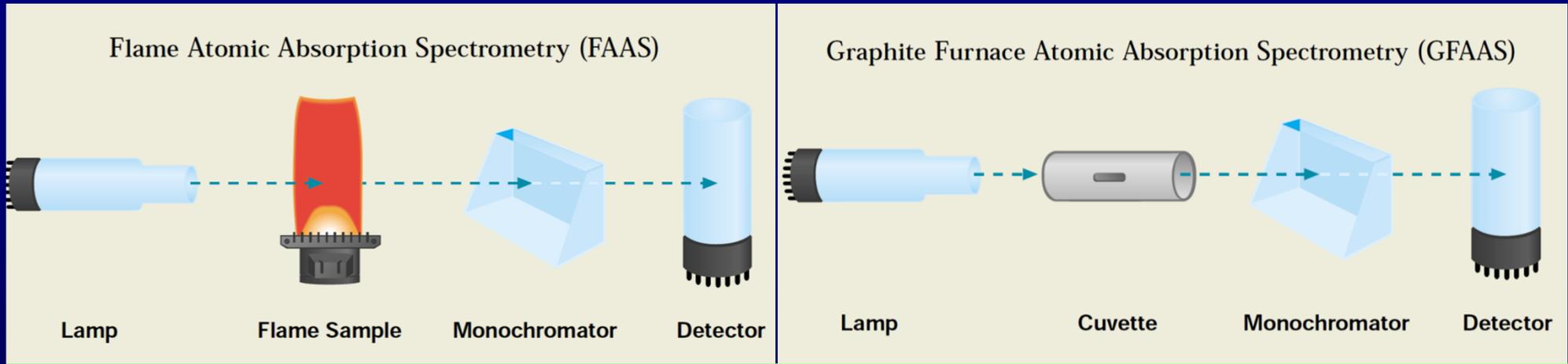
- insensitive for some elements (e.g. sulphur)
- slow if many elements are needed



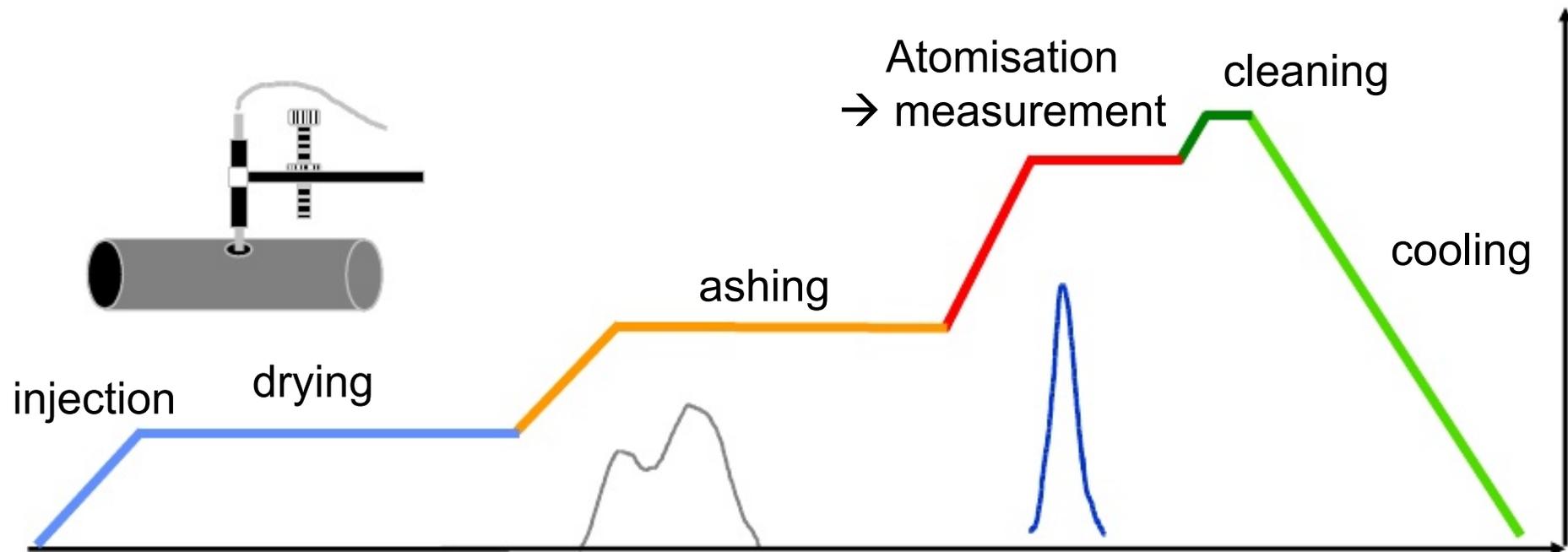
Metal content – methods of Measurement (II)

Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS)

Principle



From: <http://www.scribd.com/doc/15784148/Graphite-Furnace-Analysis> (bottom); Thermo electron teaching file (top)



Metal content – methods of Measurement (II)

Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS)

Criteria	Flame	Furnace
Elements	67	48
Sensitivity	ppm - %	ppt – ppb
Precision	Good	Fair
Interferences	Few	Many
Speed	Rapid	Slow
Simplicity	Easy	More complex
Flame Hazards	Yes	No
Automation	Yes	Yes (unattended)
Operating Cost	Low	Medium

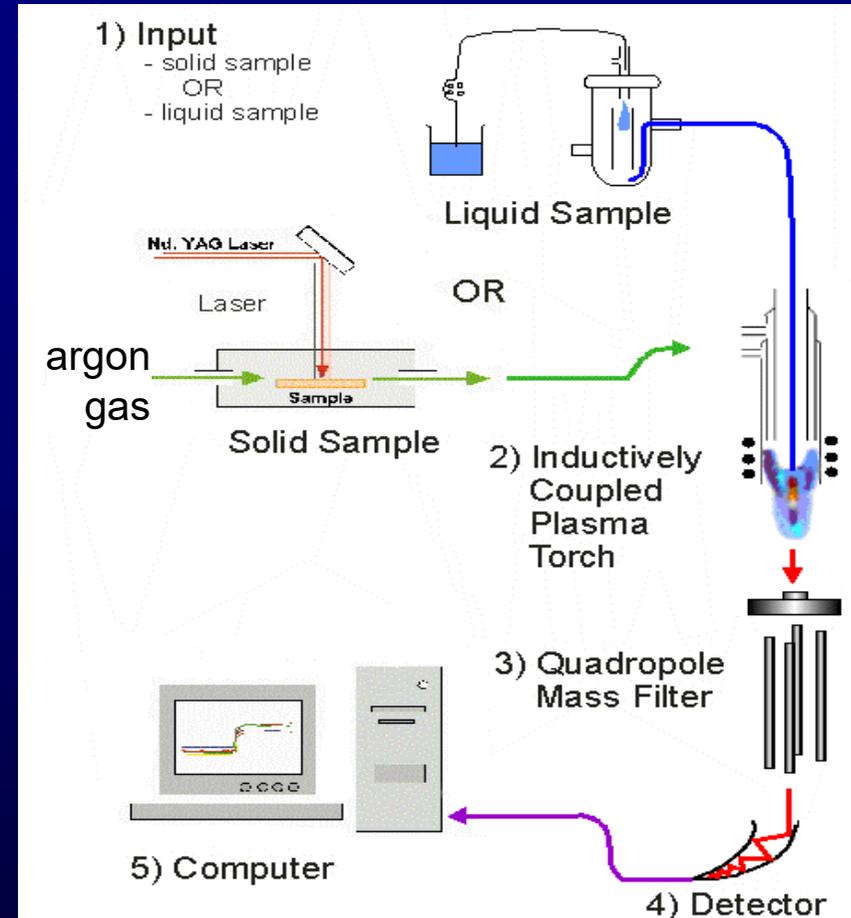
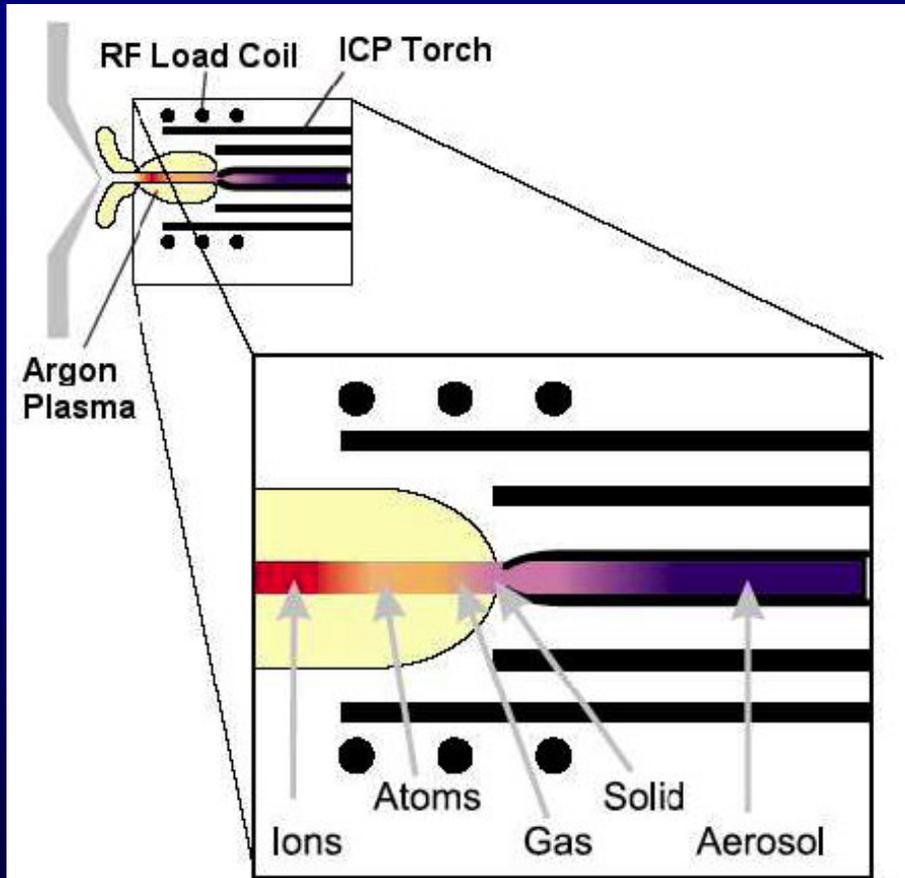
From: <http://www.scribd.com/doc/15784148/Graphite-Furnace-Analysis>



From: GBC product information

Metal content – methods of Measurement (III)

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)



From: Perkin Elmer teaching file (top); LC-ICP-MS experiment at UFZ Leipzig (bottom)



Metal content – methods of Measurement (III)

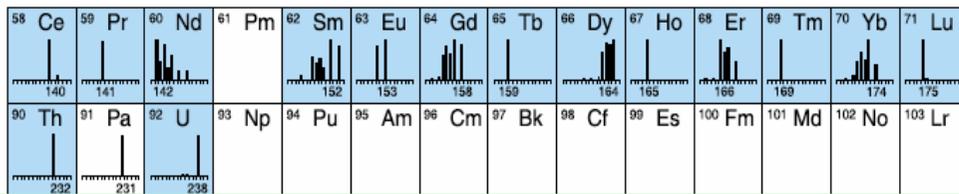
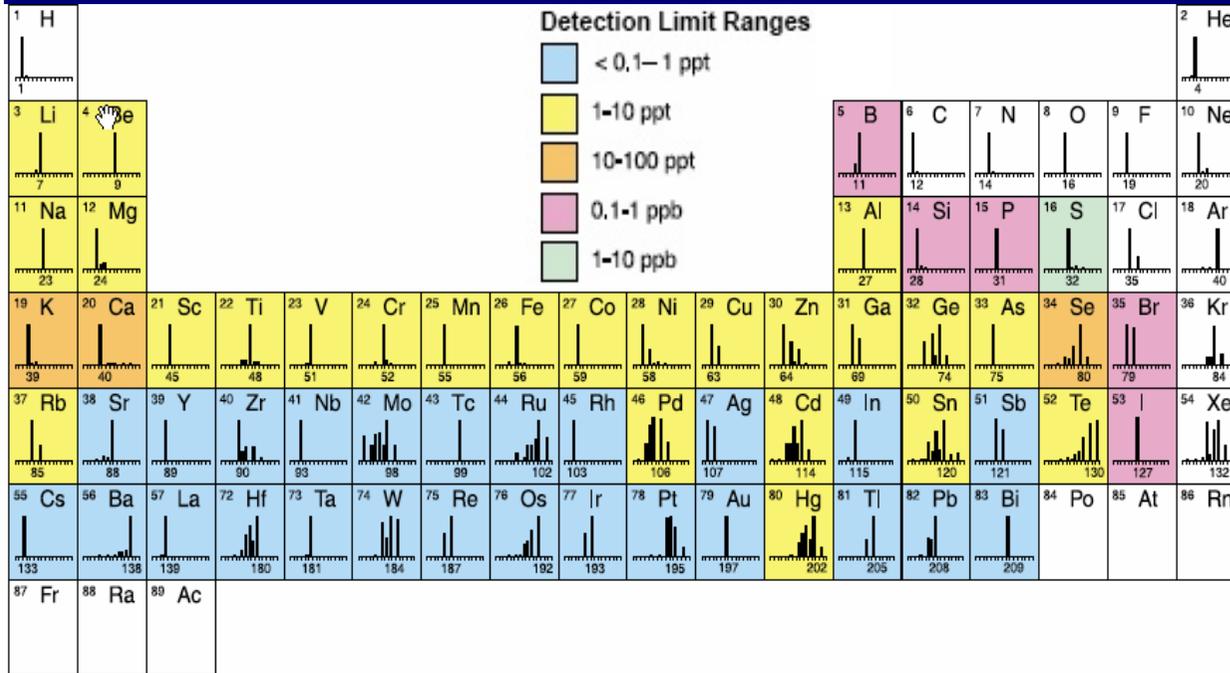
Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Advantages:

- Detection limits for most elements equal to or better than those obtained by Graphite Furnace –AAS (GFAAS)
- Higher throughput than GFAAS
- minimum of matrix interferences due to the high-temperature of the ICP source
- Superior detection capability to ICP-AES with the same sample throughput
- Ability to obtain isotopic information.

Disadvantages:

- more complicated technique than AAS
- much more expensive than AAS
- elements that prefer to form negative ions, such as Cl, I, F, etc. are very difficult to determine via ICP-MS because ions formed by the ICP discharge are typically positive ions.

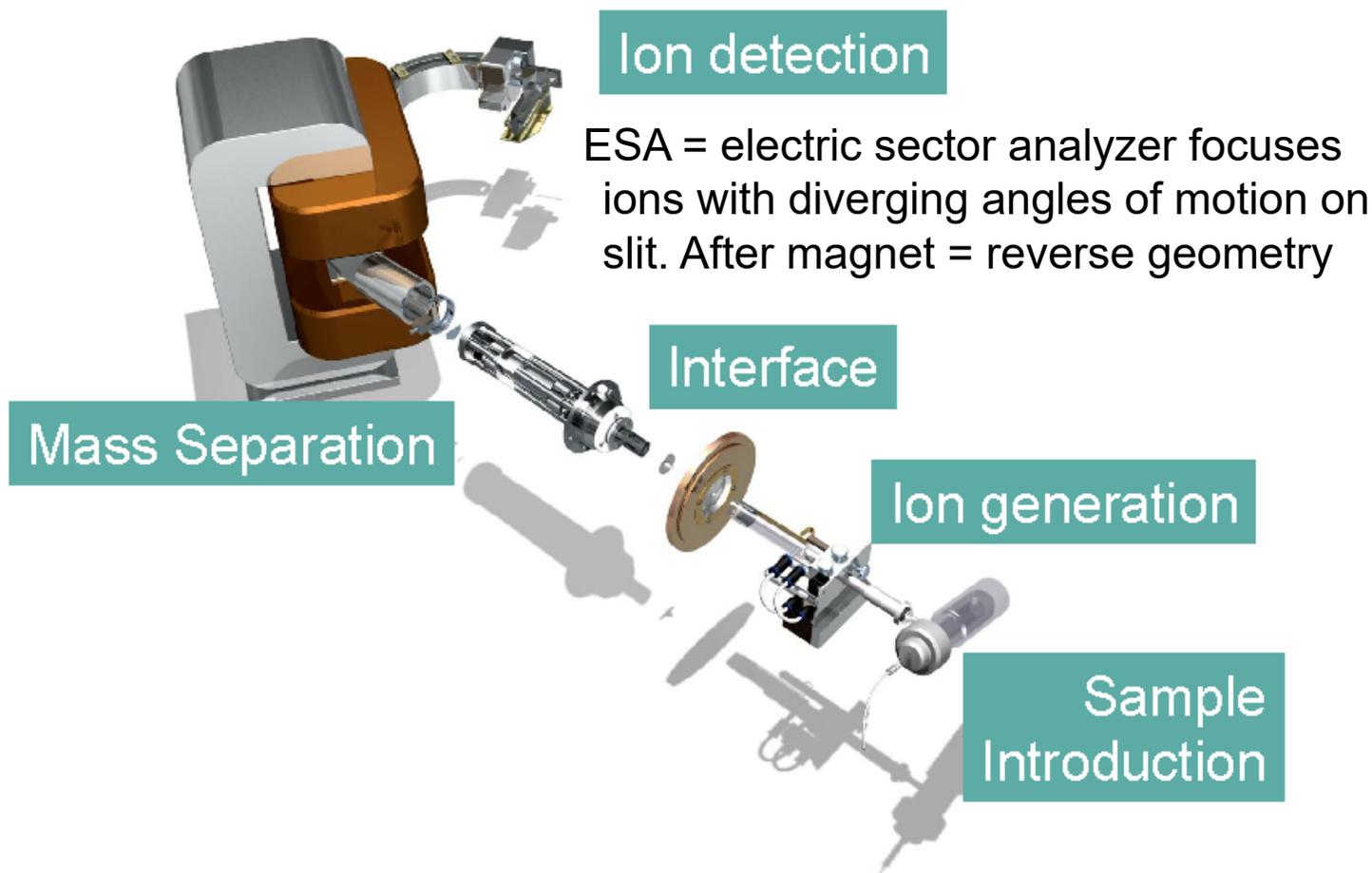


From: Perkin Elmer product information

Metal content – methods of Measurement

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Principle components of an ICP-SFMS

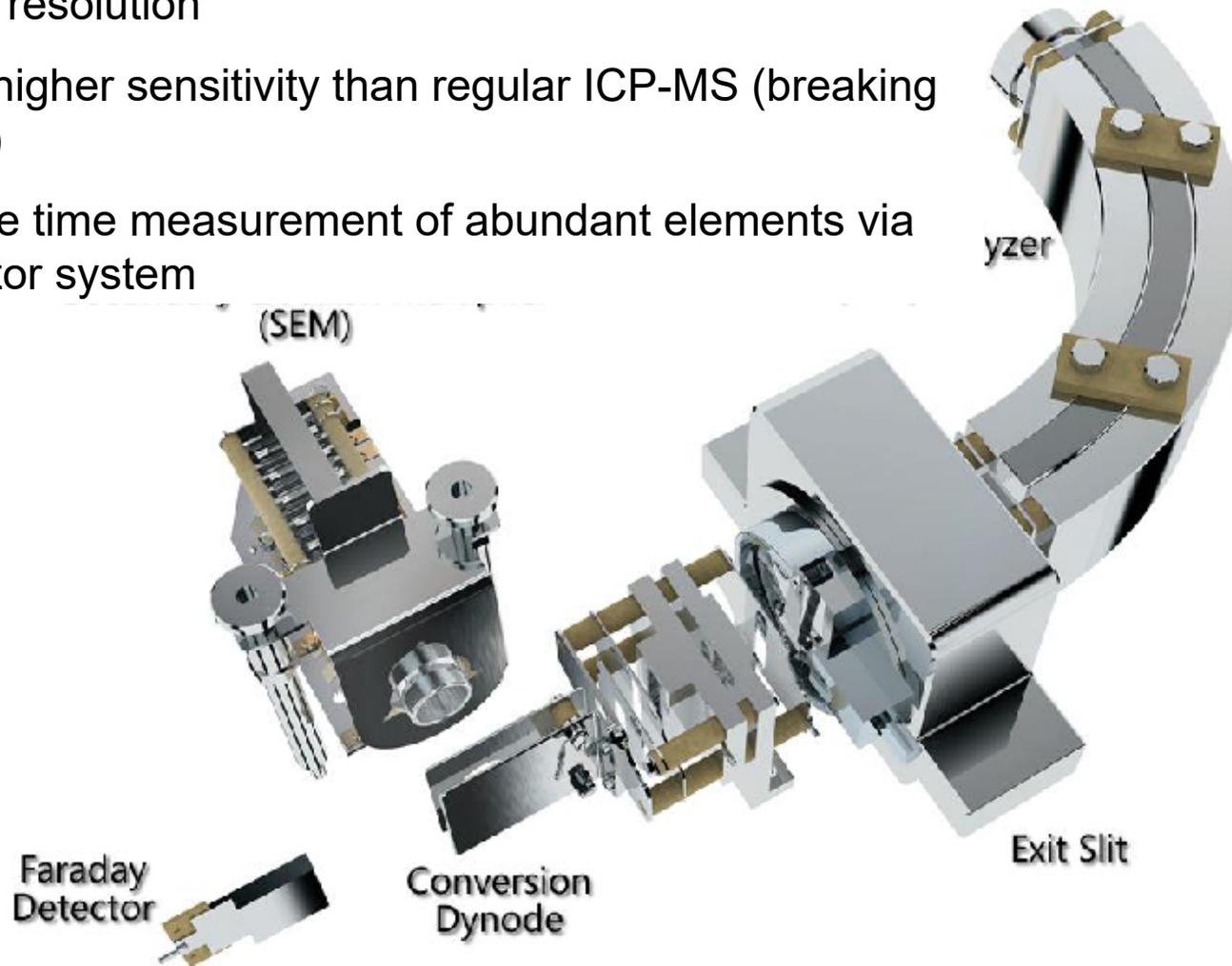


Metal content – methods of Measurement Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

ELEMENT XR Ion Detection System

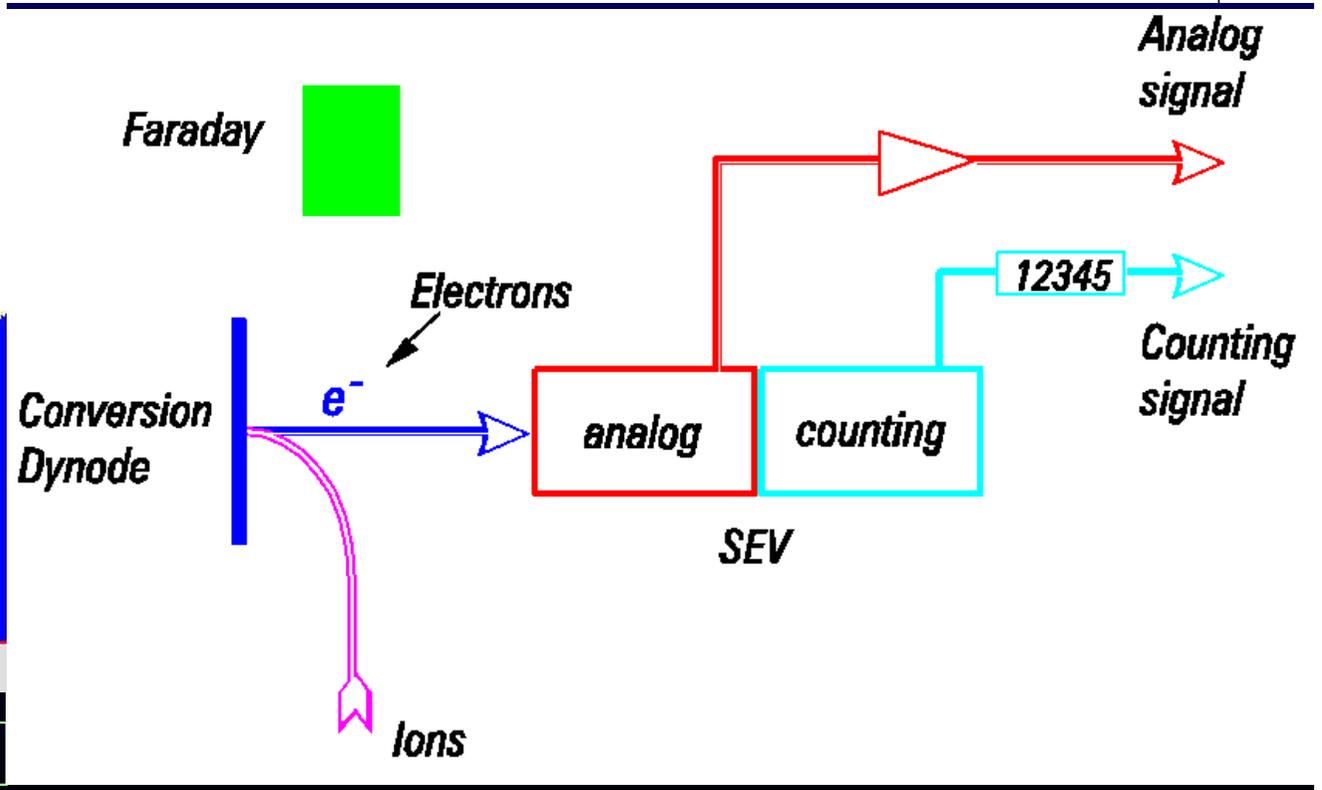
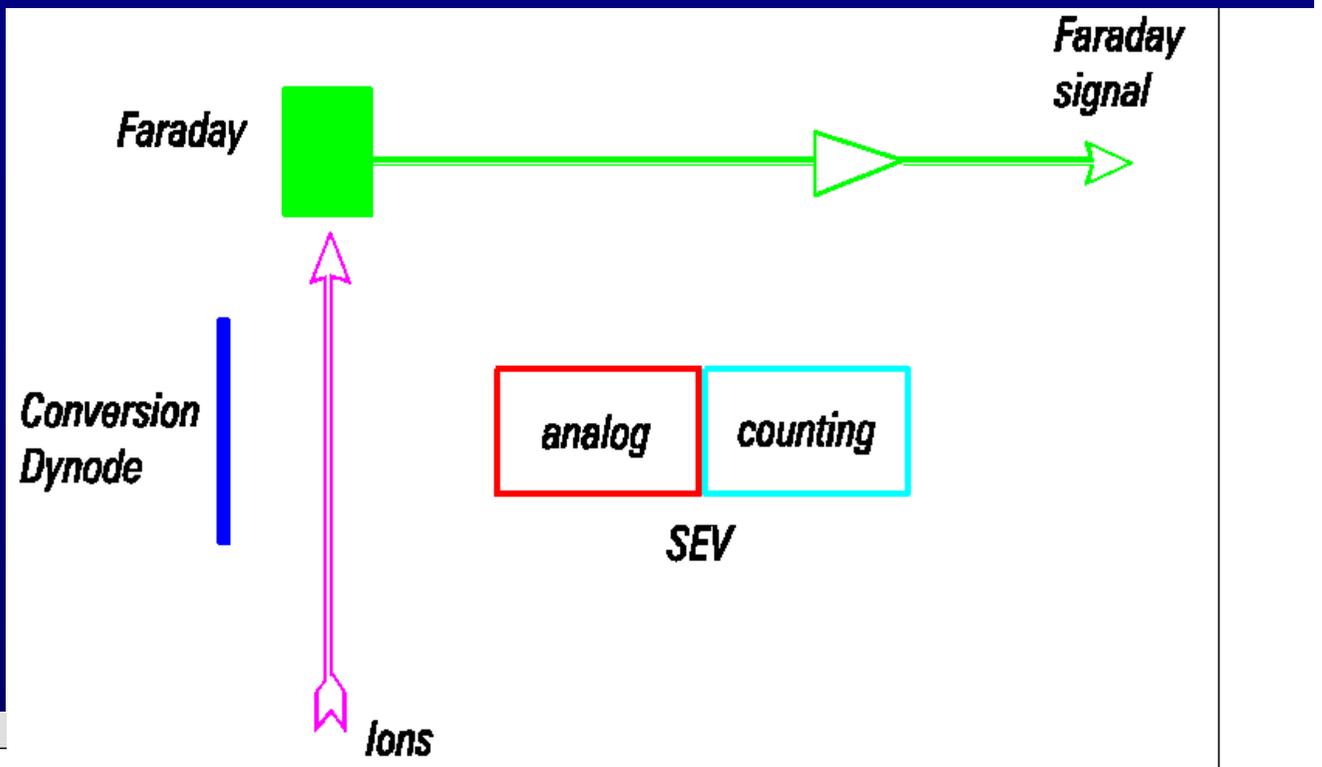
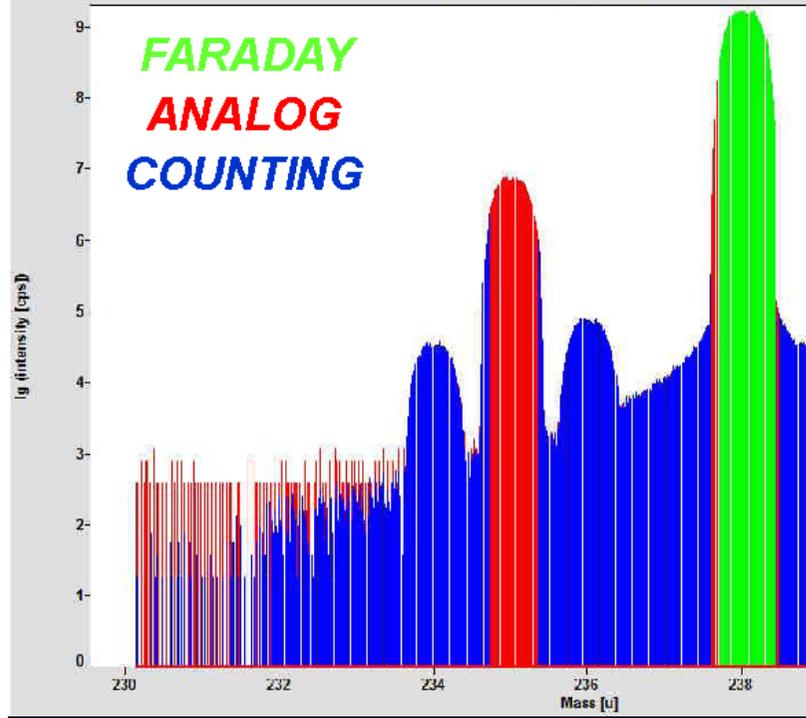
Advantages of element XR vs. quadrupole ICP-MS

- High mass resolution
- Still much higher sensitivity than regular ICP-MS (breaking ppq barrier)
- At the same time measurement of abundant elements via triple detector system



Inductively Coupled Plasma Mass Spectrometry (ICP-MS): automatic detector switching in Element XR

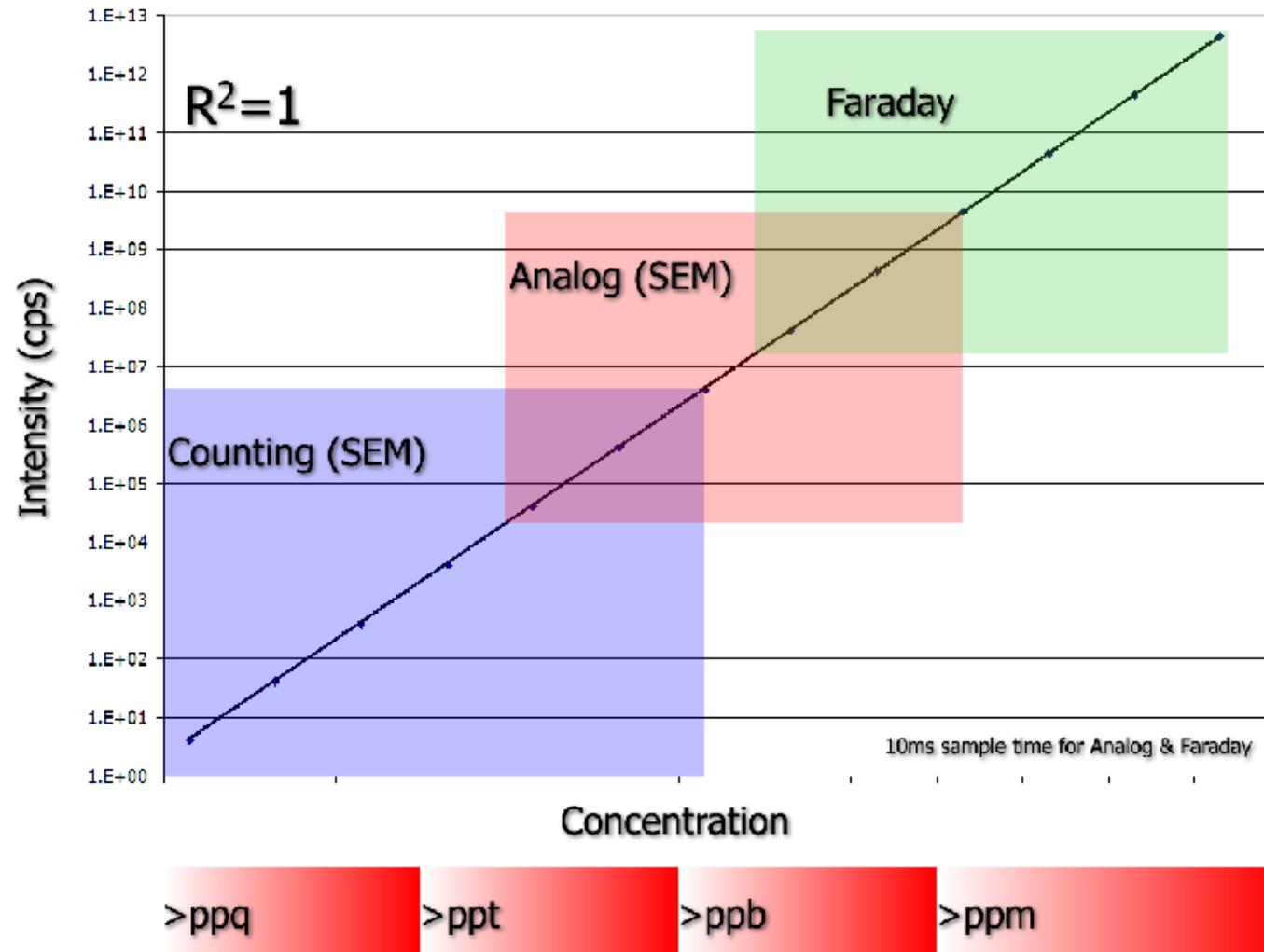
Nr. 1 Res. Low Date: Nov 25, 2005 15:49:05



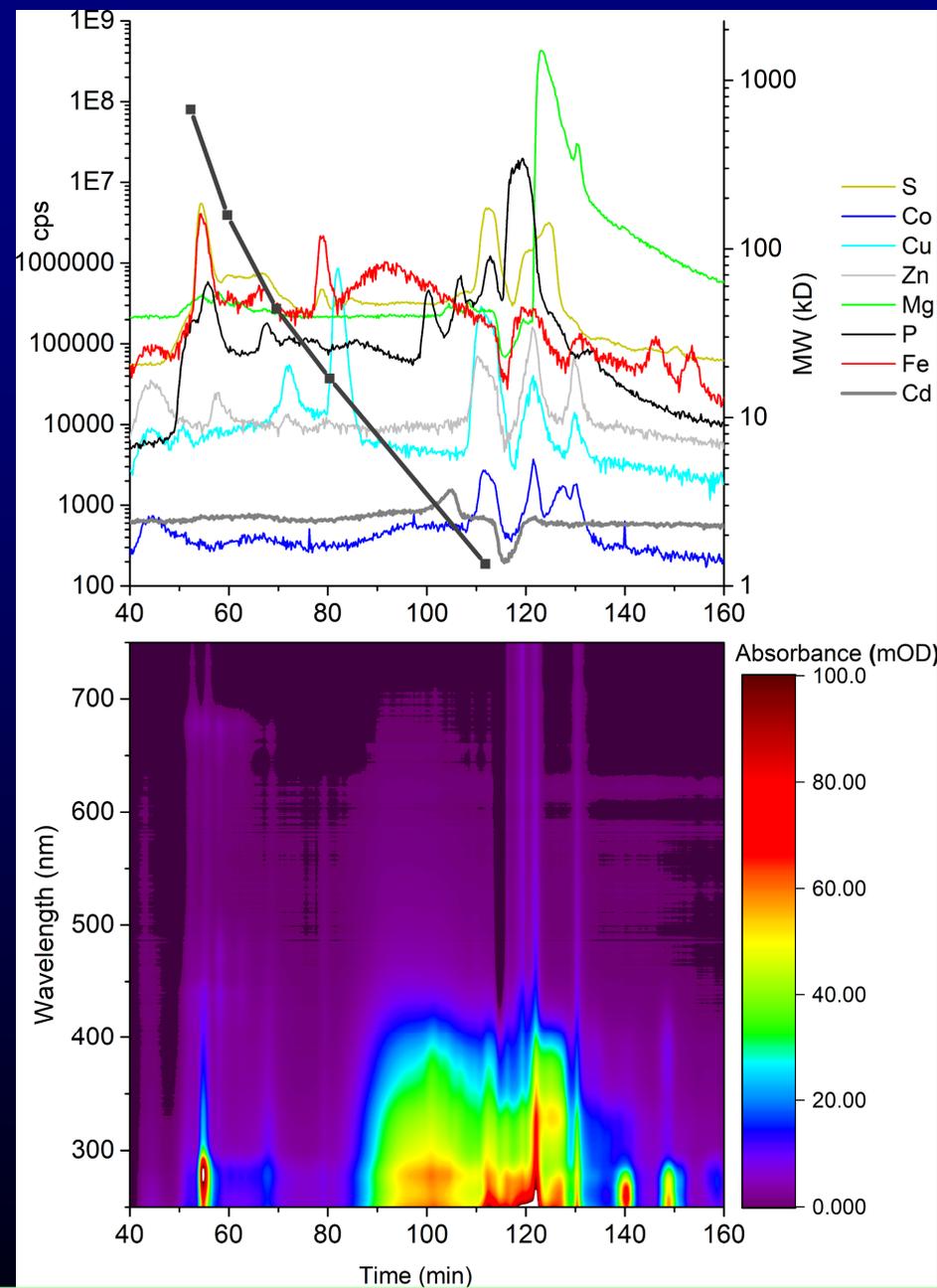
Metal content – methods of Measurement

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Extended Dynamic Range in the Finnigan ELEMENT XR



Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Coupling to HPLC



Left: Photo from our lab; right: Küpper H, Hussain Bokhari SN, Jaime Perez N, Lyubenova L, Ashraf N, Andresen E (2019)
Analytical Chemistry 91, 1710961-10969

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