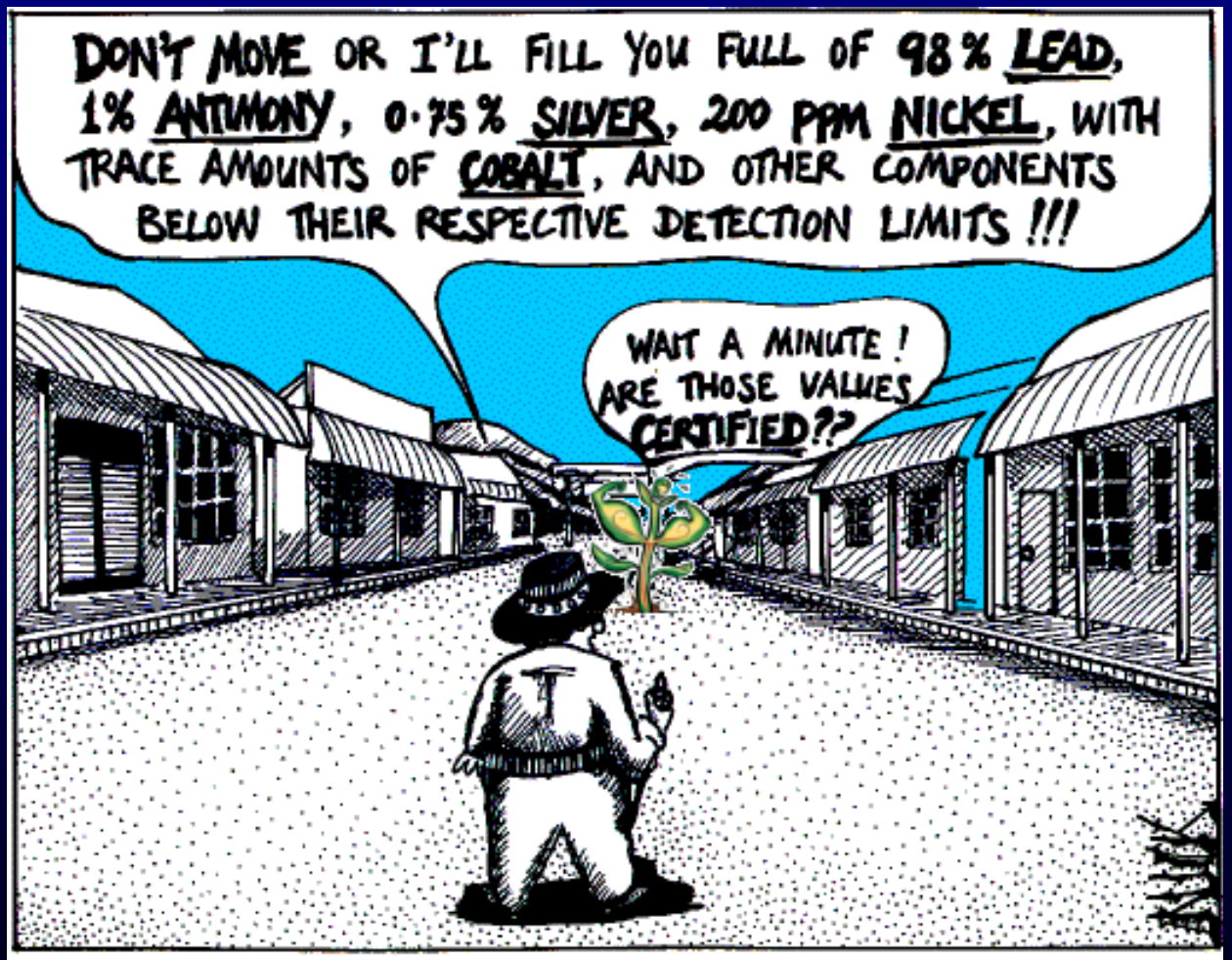
# Advanced Course on Bioinorganic Chemistry & Biophysics of Plants – Introduction

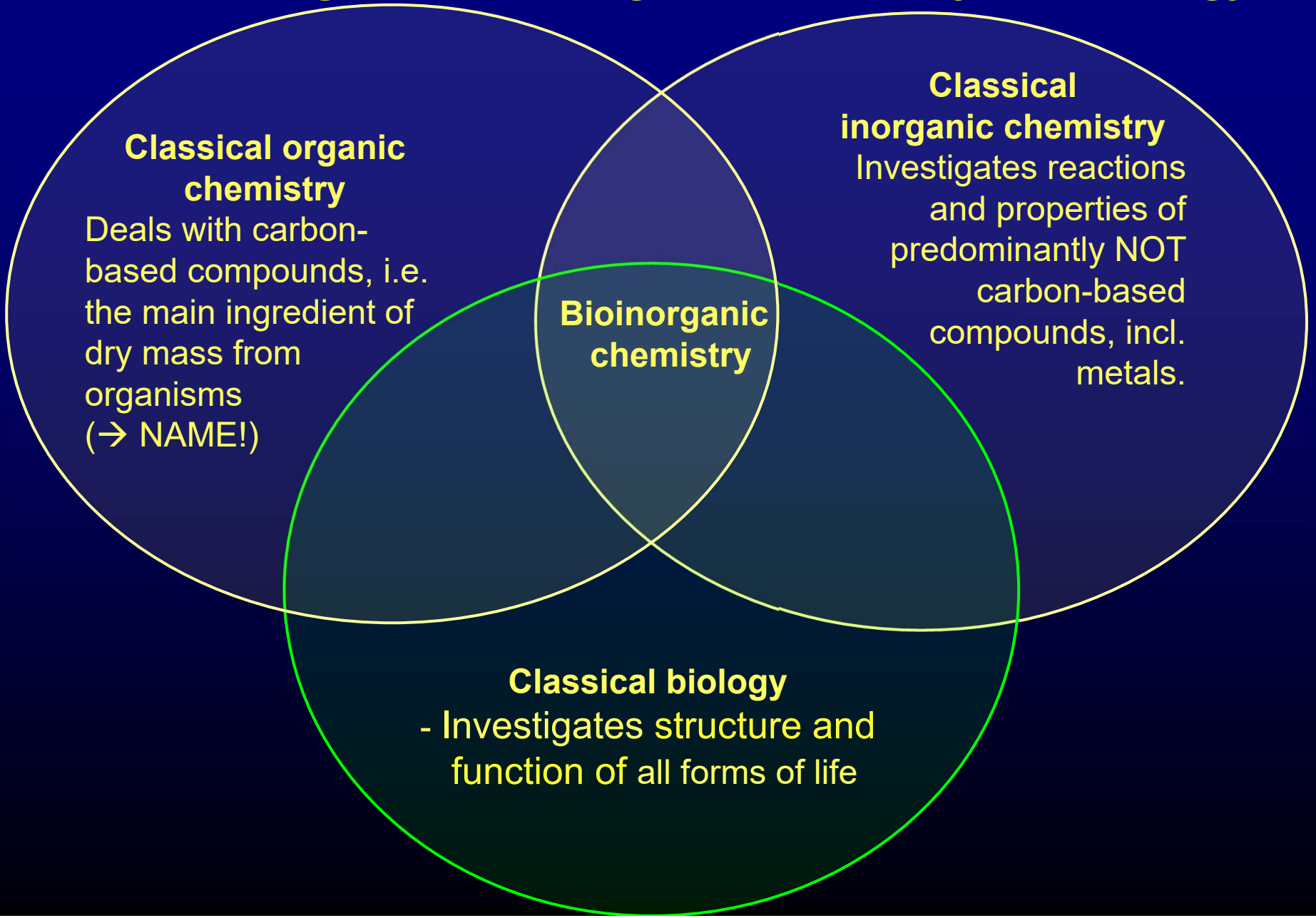



Schwermetall-Hyperakkumulation im Wilden Westen

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

# I.
# Basics of Bioinorganic Chemistry and Biophysis

# Bioinorganic Chemistry versus Classical organic and inorganic Chemistry and Biology

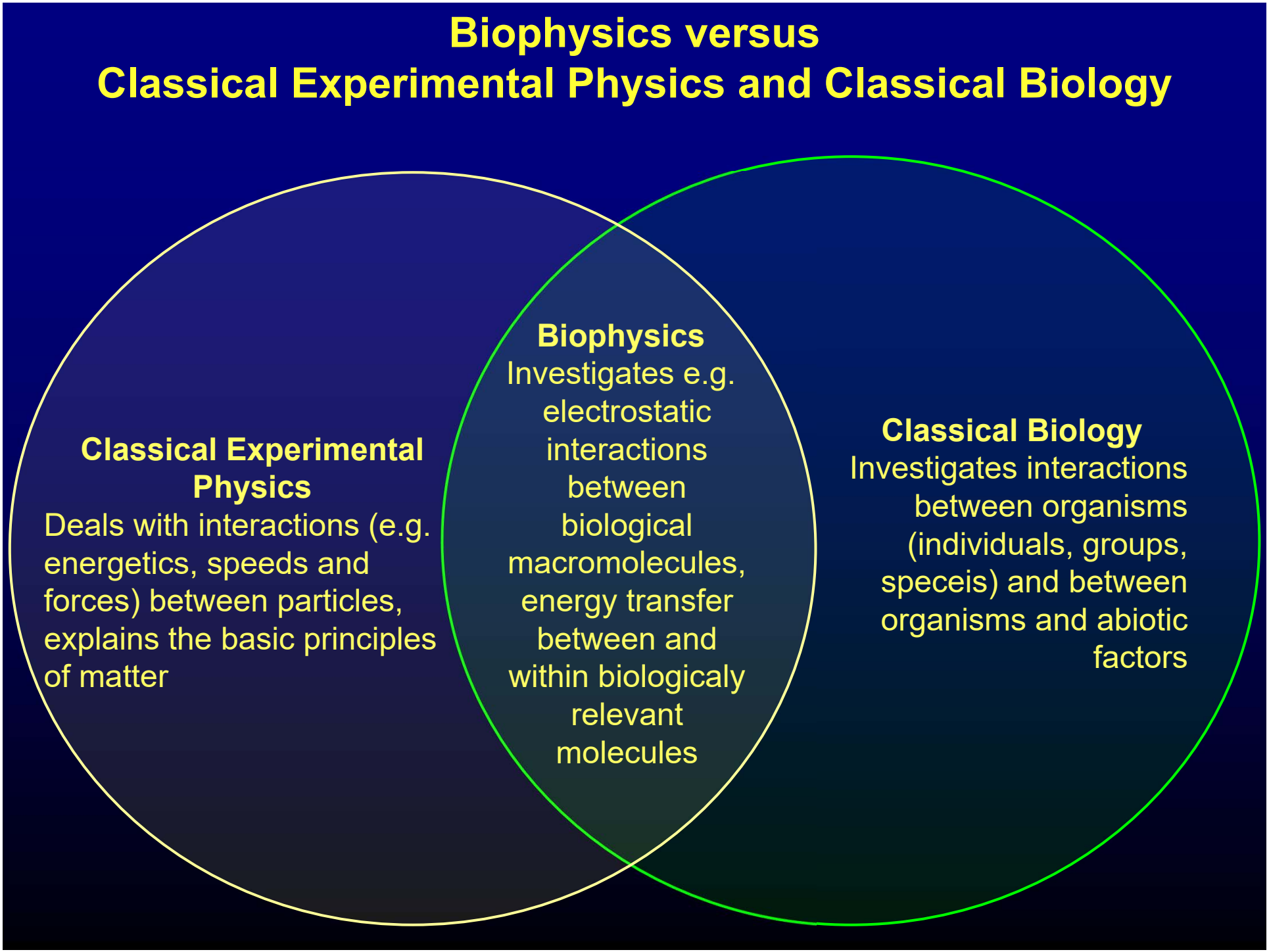


# Themes of bioinorganic chemistry research

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 models 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 characterisitics 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



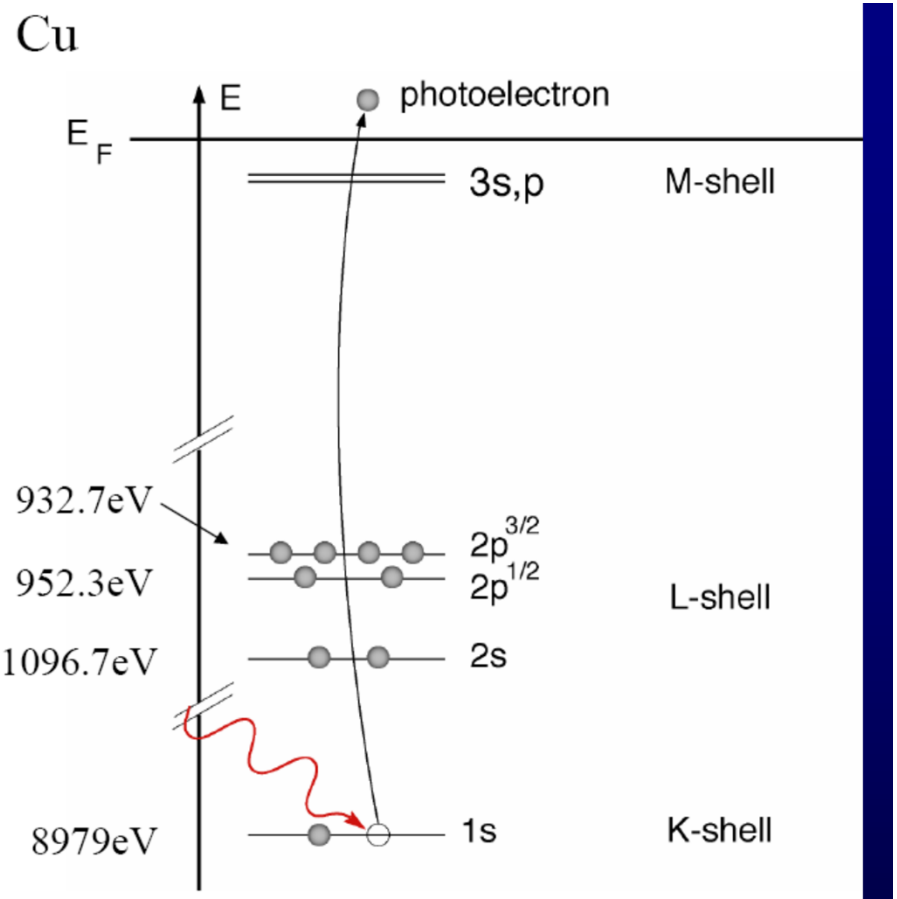
# Themes of biophysical research

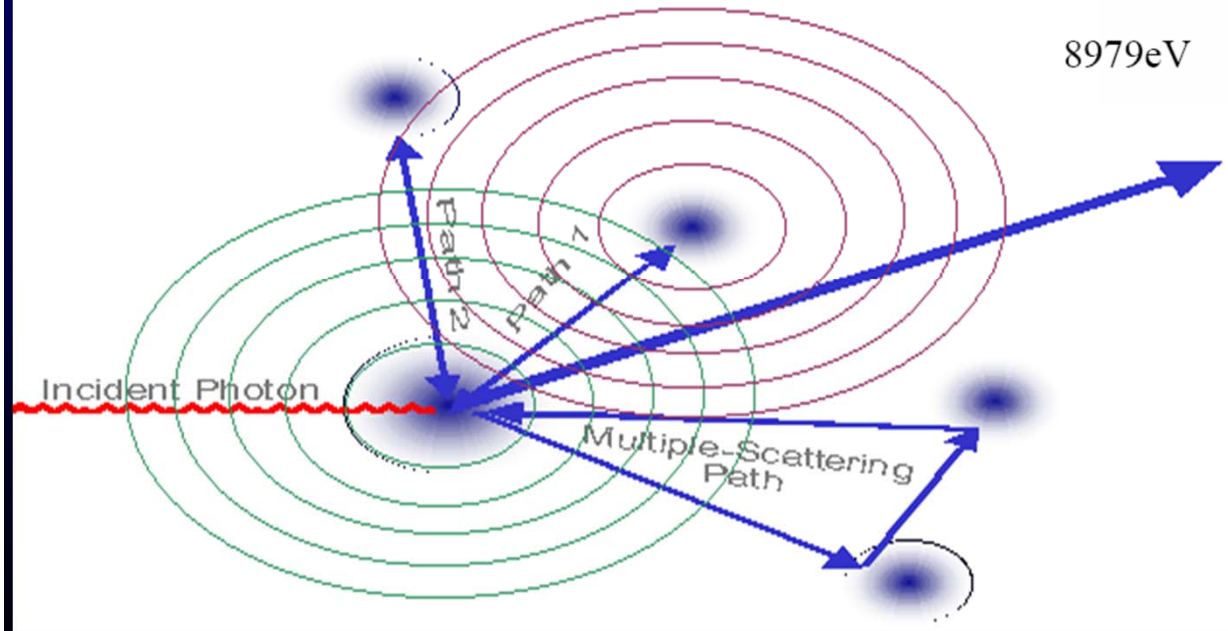
Energetics and kinetics of biological processes
- → transport (e.g. of metals)
- → catalysis in biology, usually via metal-based active sites in enzymes
- → reversible coupling of biologically relevant molecules without bond formation/breakage
- → protein folding

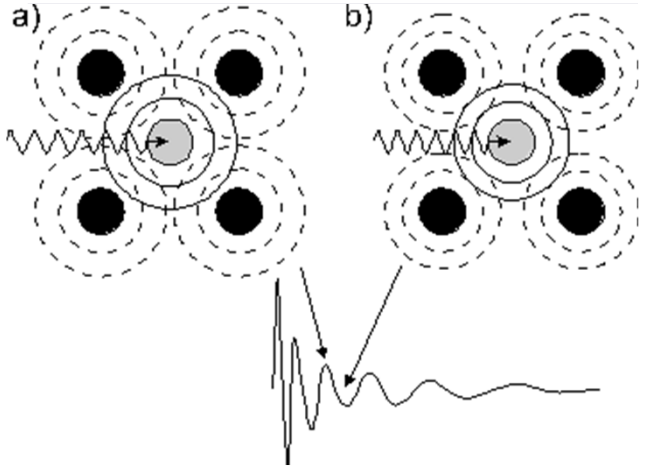
Methods used for investigating these questions include for example (in solutions, in models systems, but also in living cells)
- UV/VIS absorption and fluorescence spectroscopy (→ electronic transitions to/from excited states → e.g. analysis of chromophore coupling)
- X-ray absorption spectroscopy (→ 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?

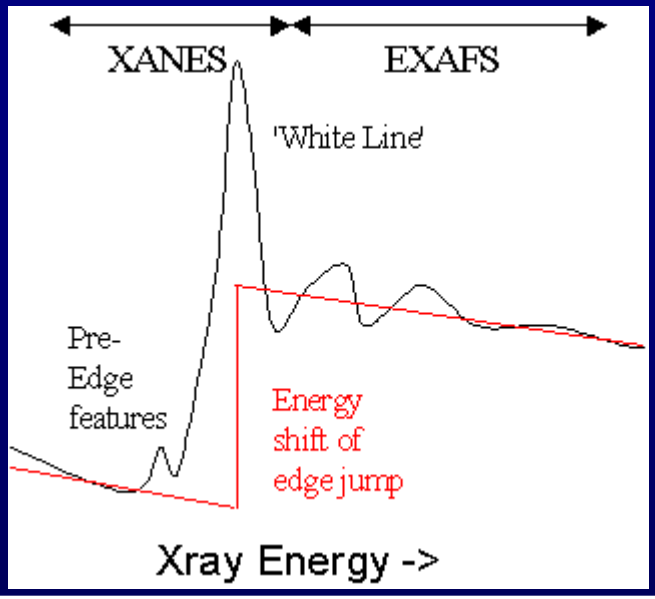
# Theoretical Background of important biochemical and biophysical methods…

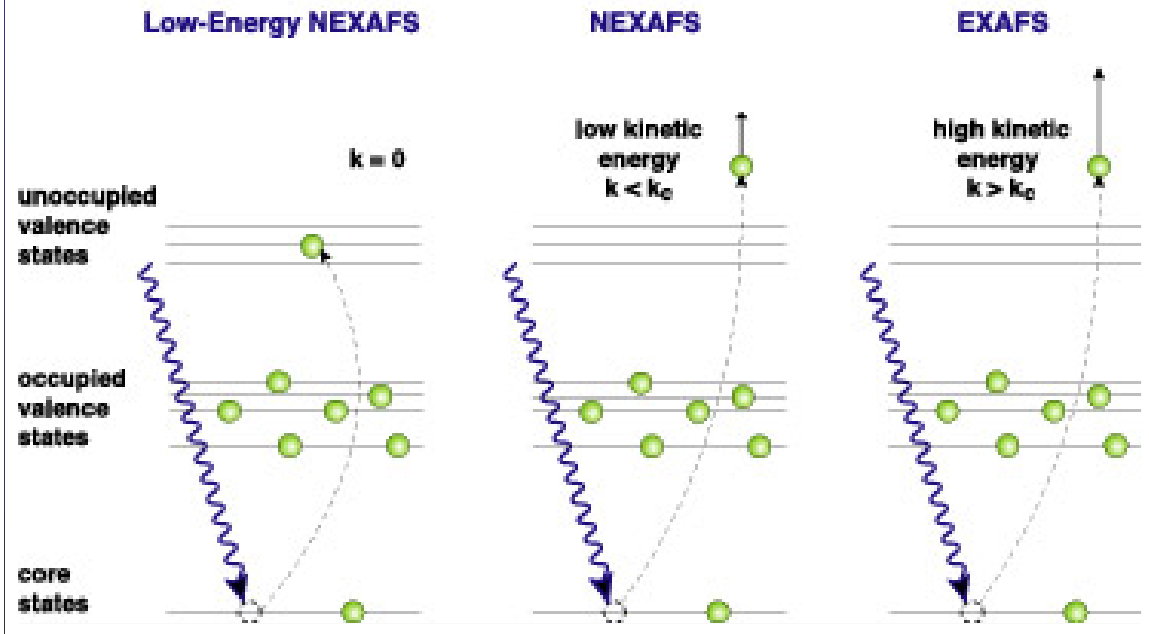


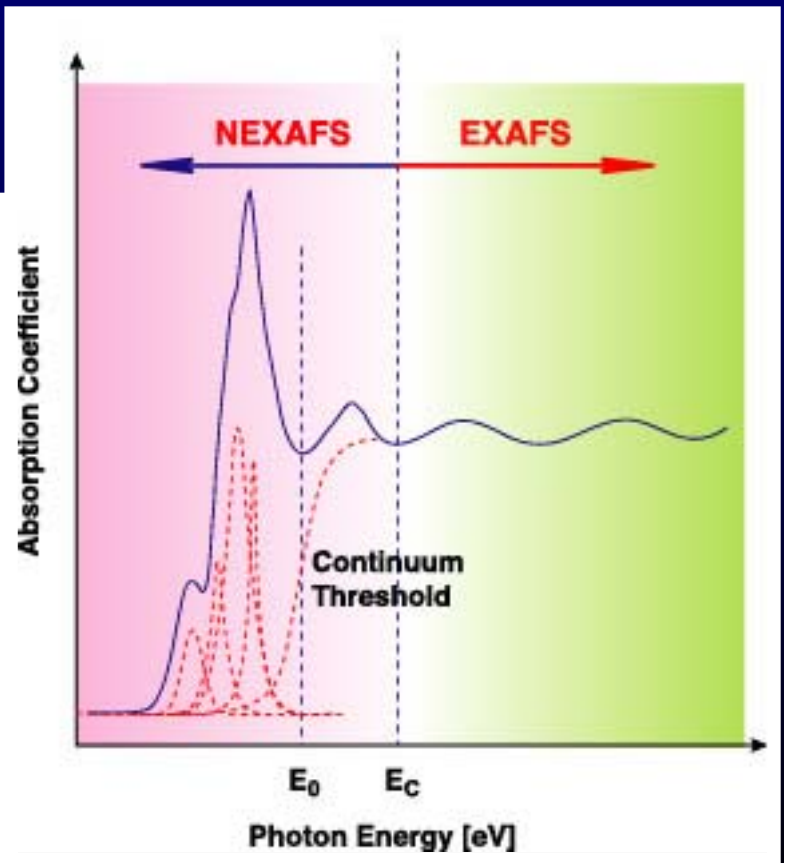



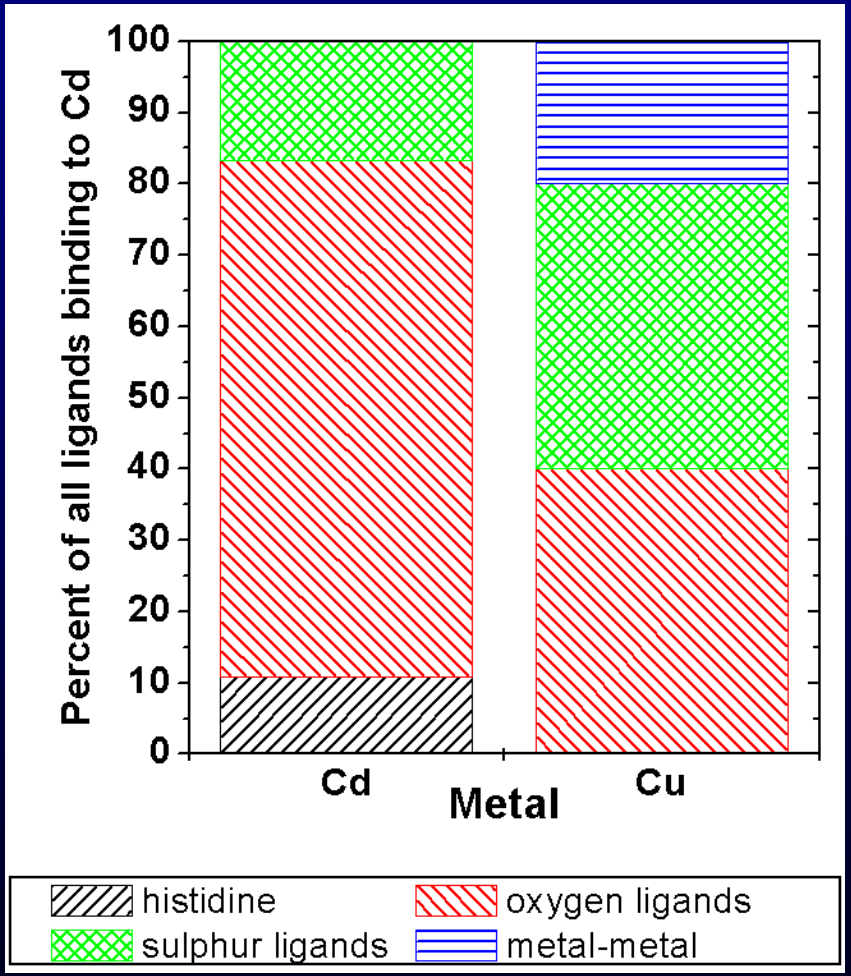
# … differences in the basics and applications between related methods…

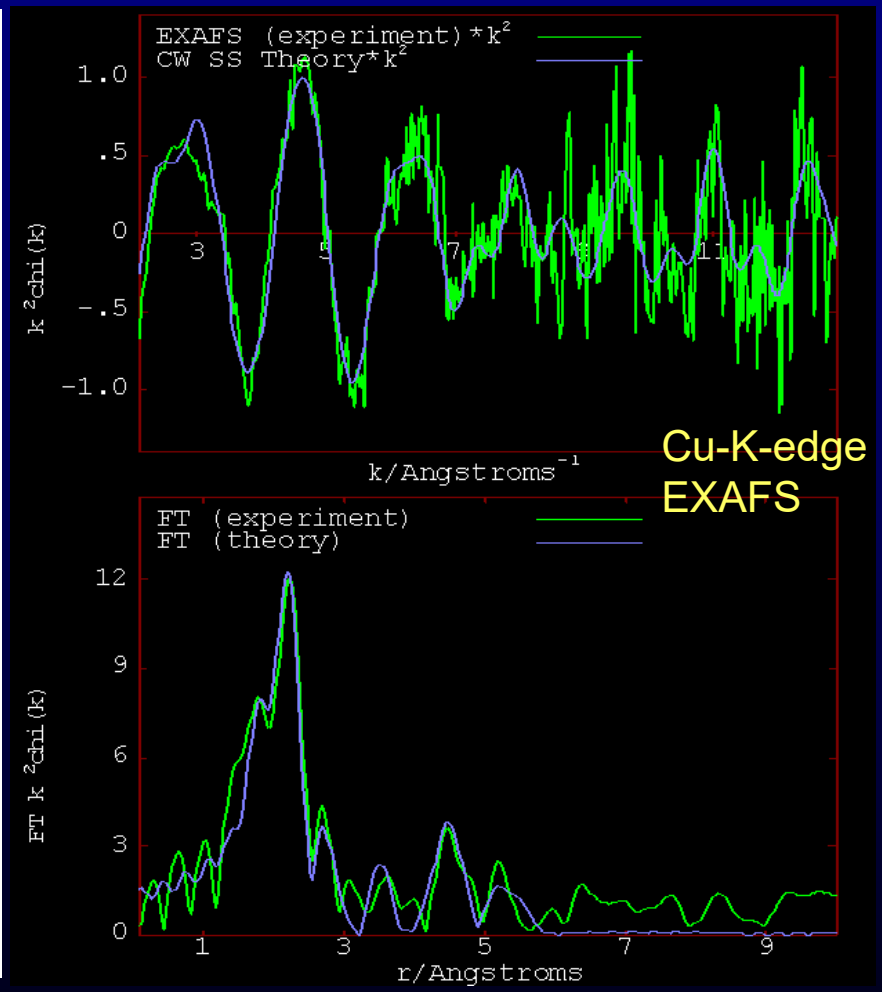


# …and the use of these methods for answering questions in bioinorganic chemistry and biophysics.



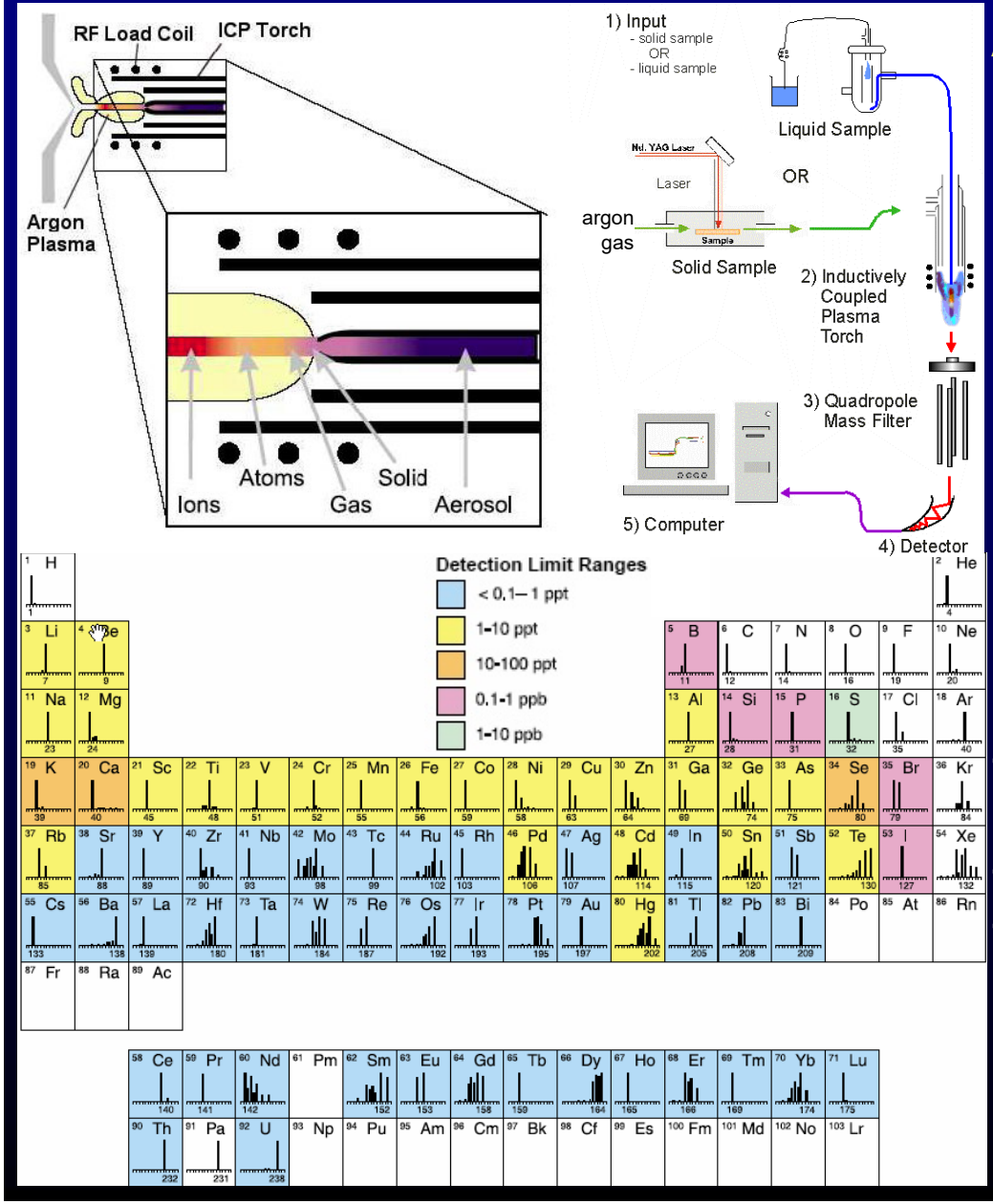





Cd: Küpper H, Mijovilovich A, Meyer-Klaucke W, Kroneck PMH (2004) Plant Physiology 134 (2), 748-757

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

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




Advantages:

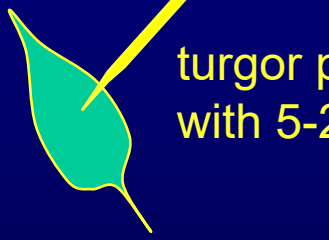
- Detection limits for most elements equal to or better than those obtained by Graphite Furnace –AAS (GFAAS)
- Higher throughput than GFAAS
- minimum of matrix interferences due to the high-temperature of the ICP source
- Superior detection capability to ICP-AES with the same sample throughput
- Ability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

# Principles of sample preparation for specific methods…

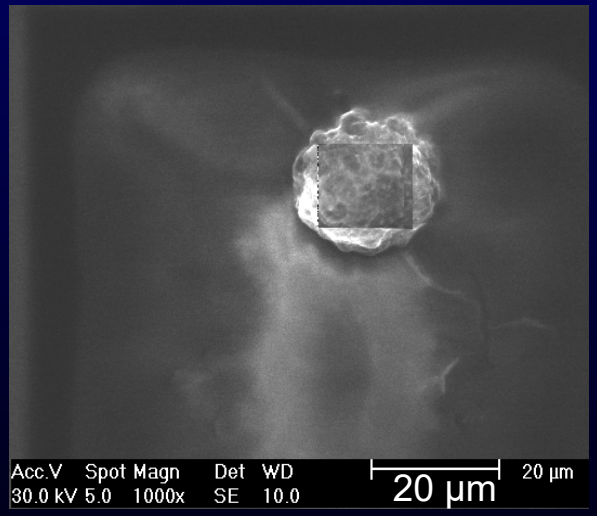
micropipette filled with silicon oil, connected to air-filled syringe for controlling pressure difference



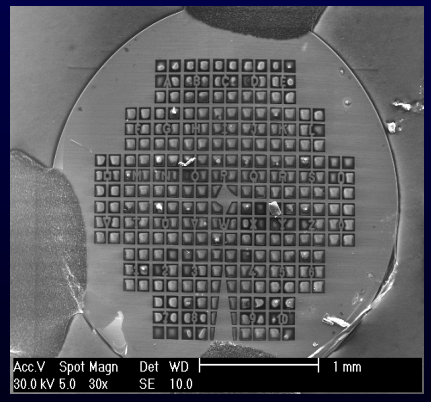
turgor pressure of punctured cell fills pipette with 5-20 picolitres ($10^{-12}$ l) of cell sap

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



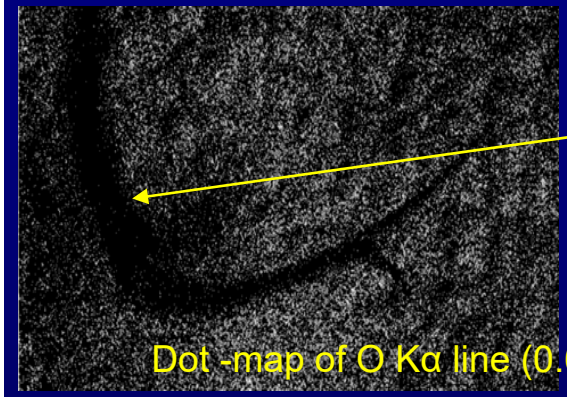

typical dried sample




analysis grid

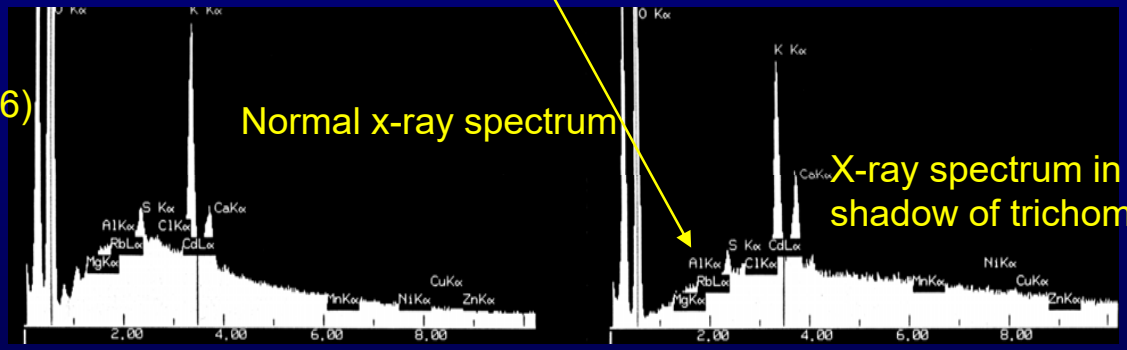
Küpper H, Zhao F, McGrath SP (1999) Plant Physiol 119, 305-11

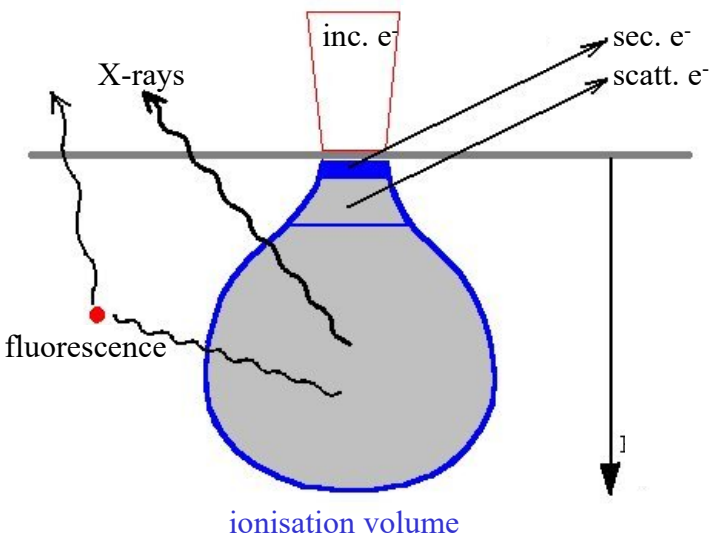
# …and problems associated with these samples.




## Effect of shading

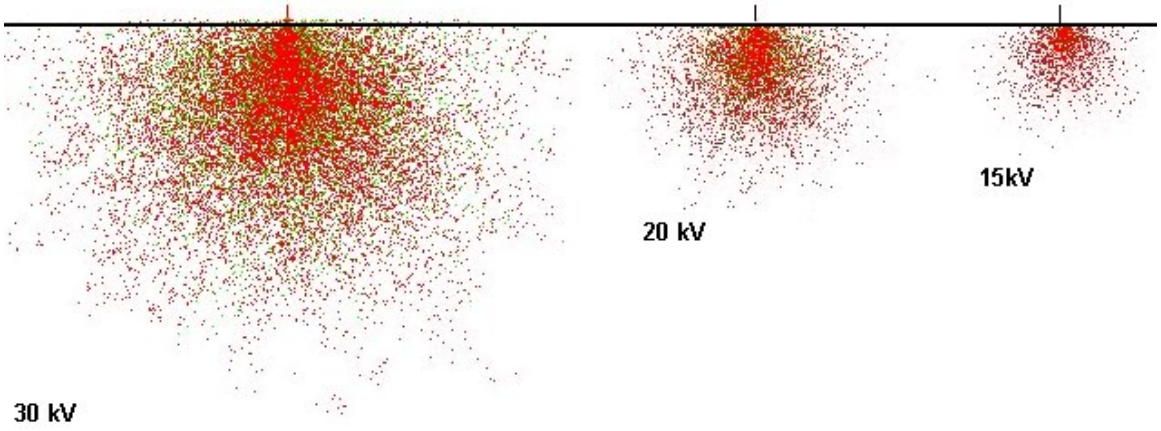
shading inside a sample leads to absorption of low-energy x-rays



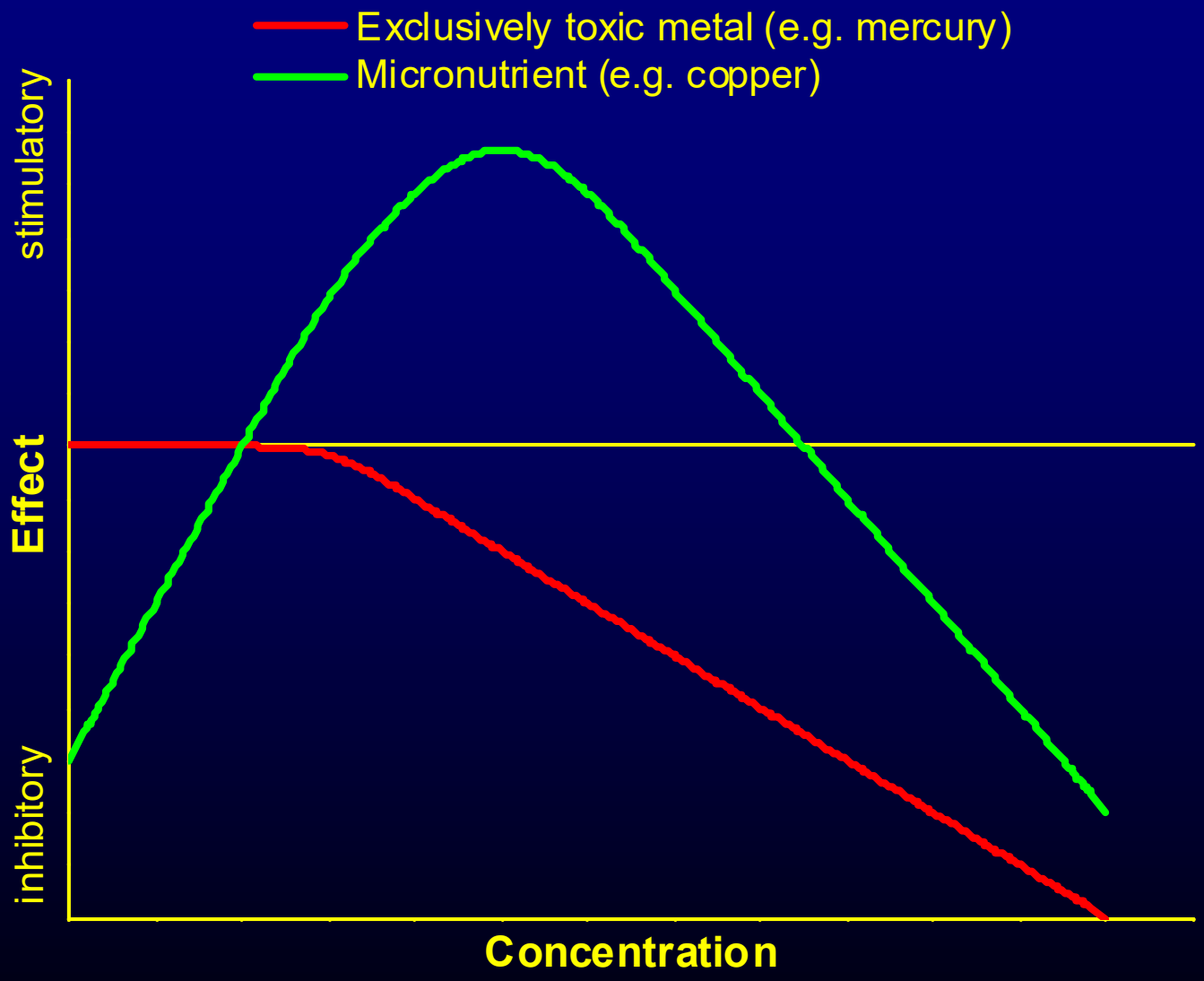



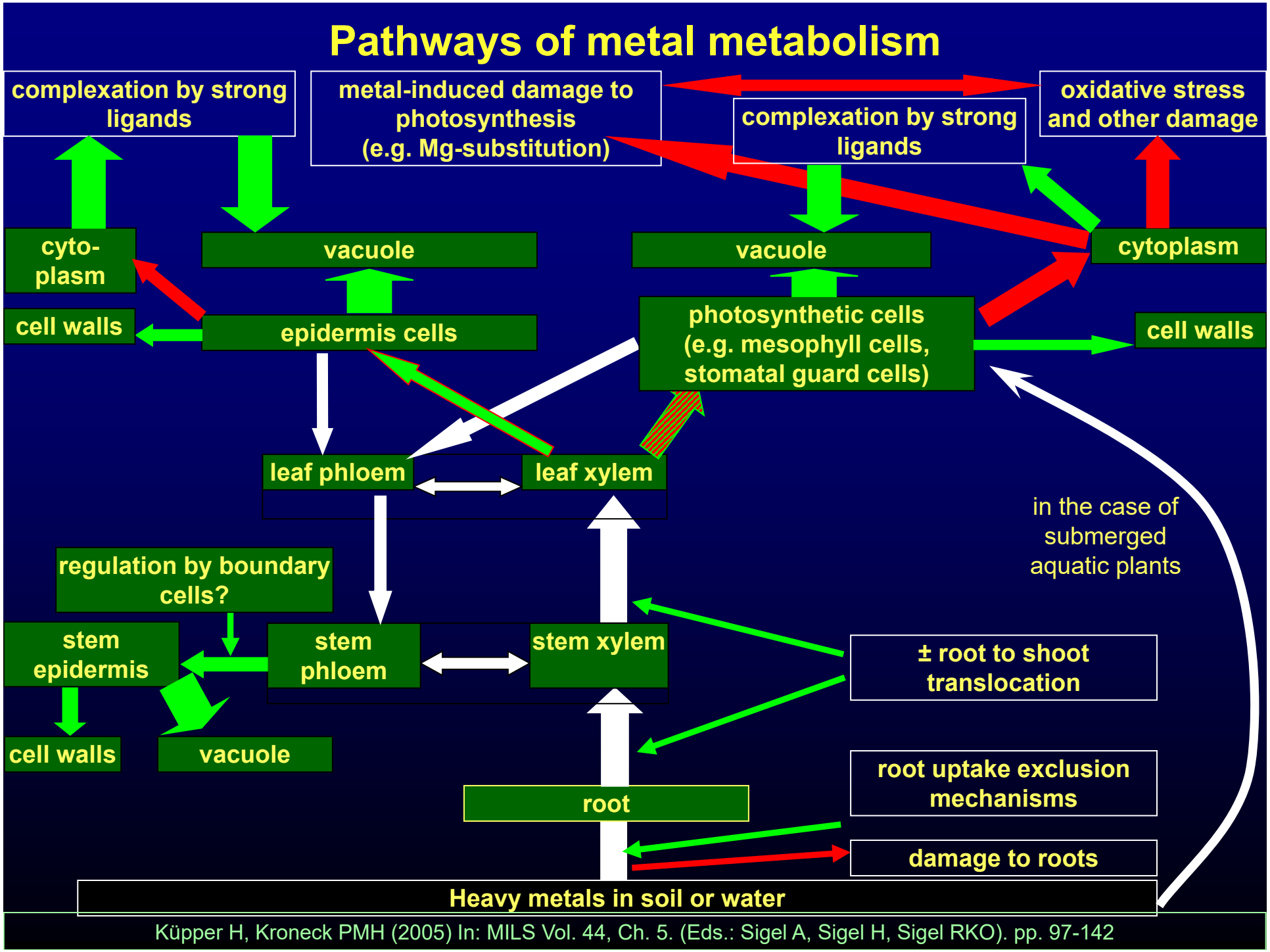

## Effect of acceleration voltage

high acceleration voltage leads to deeper penetration into the sample!



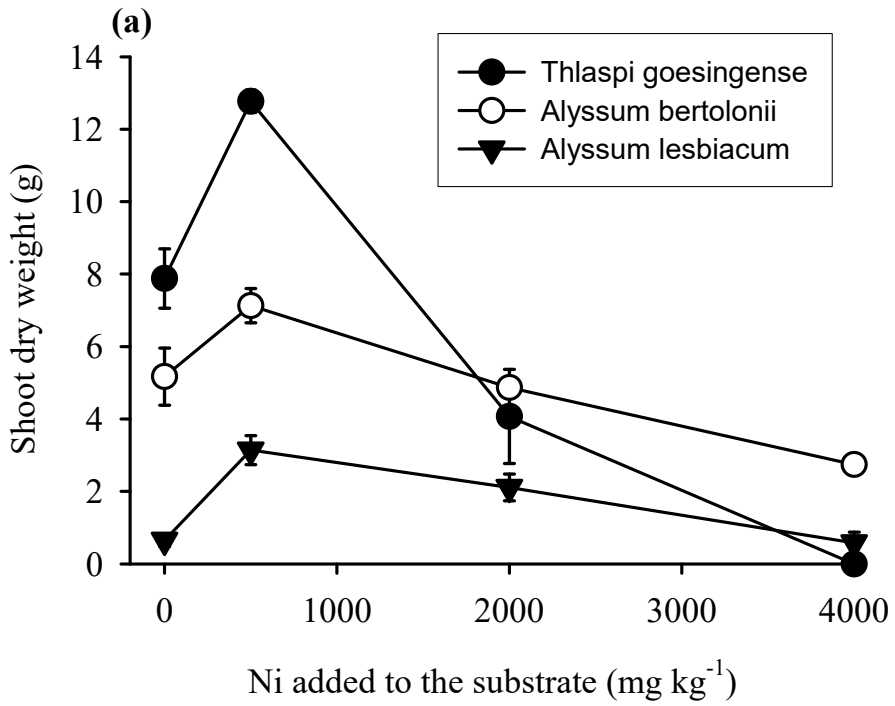
# Principles

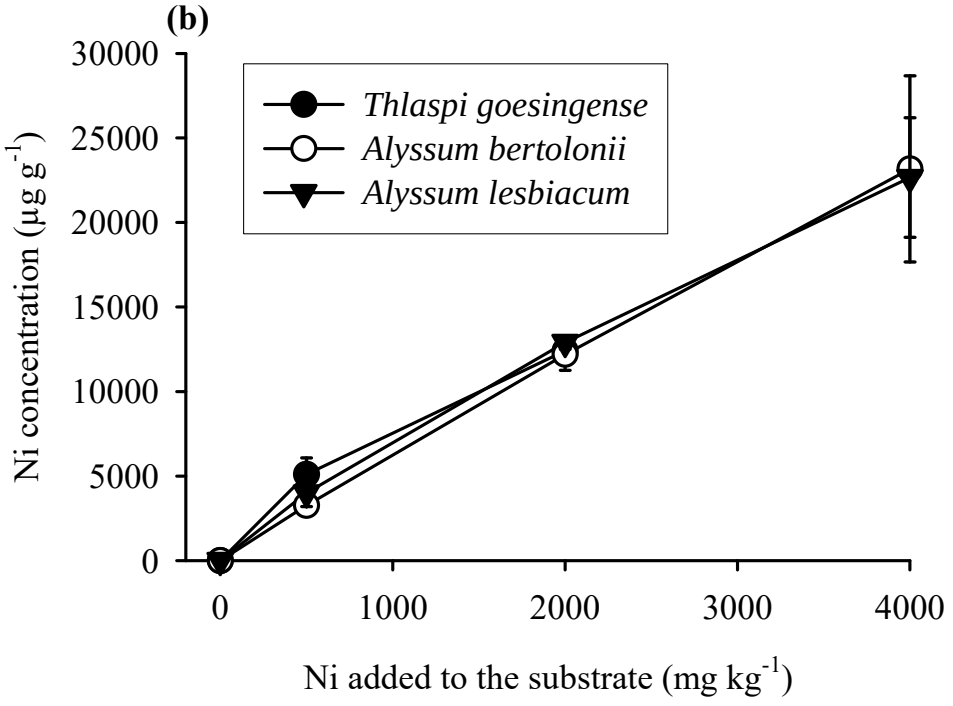

Exclusively toxic metal (e.g. mercury)
Micronutrient (e.g. copper)
stimulatory
Effect
inhibitory
Concentration




Küpper H, Kroneck PMH (2005) In: MILS Vol. 44, Ch. 5. (Eds.: Sigel A, Sigel H, Sigel RKO). pp. 97-142

# We show you plants that strongly like potentially toxic metals…

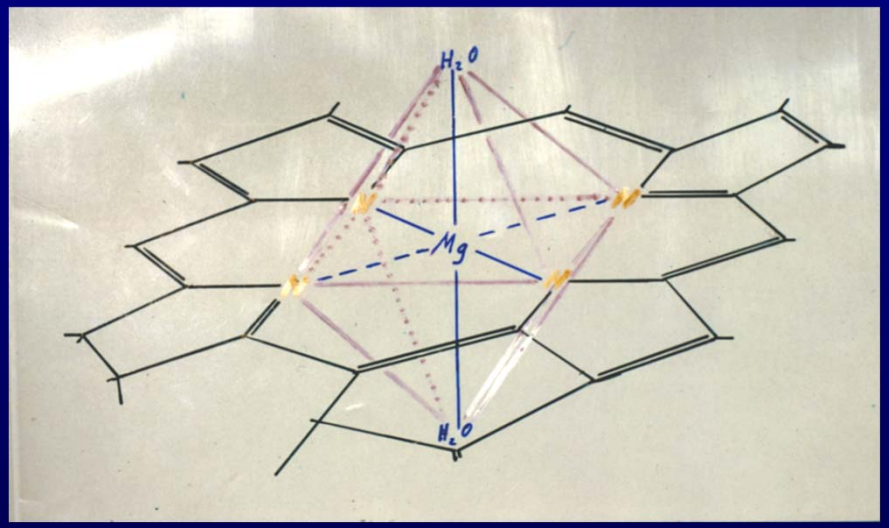


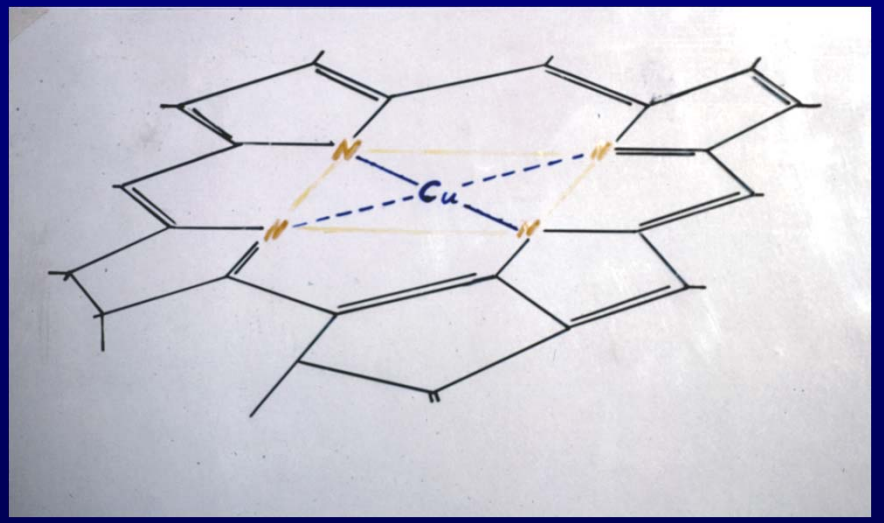




Effects of $Ni^{2+}$ addition on hyperaccumulator plant growth and $Ni^{2+}$ concentration in shoots

Küpper H, Lombi E, Zhao FJ, Wieshammer G, McGrath SP (2001) J Exp Bot 52 (365), 2291-2300

# …why trace metals can become toxic for plants…



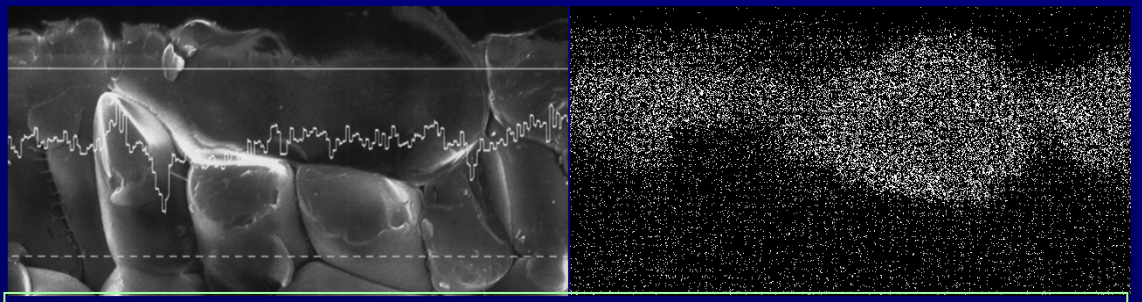




- shift of absorbance/fluorescence bands --> less energy transfer
- different structure --> proteins denature
- do not readily perform charge separation when in reaction centre
- unstable singlet excited state --> “black holes“ for excitons

# …and how plants defend themselves against that toxicity.

## Mechanisms

**• Generelly: aktive transport processes against the concentration gradient**
**→ transport proteins involved.**

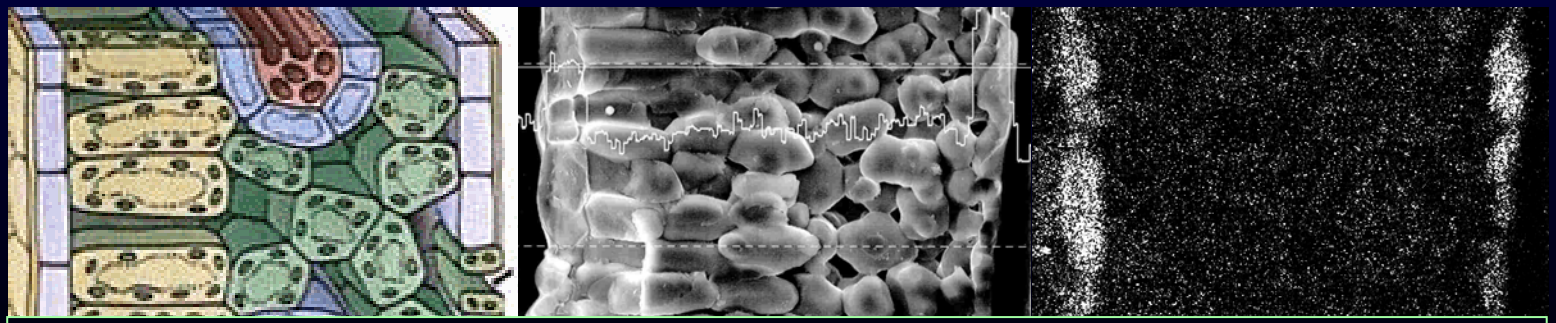


Küpper H et al., 2001, J Exp Bot 52 (365), 2291-2300

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

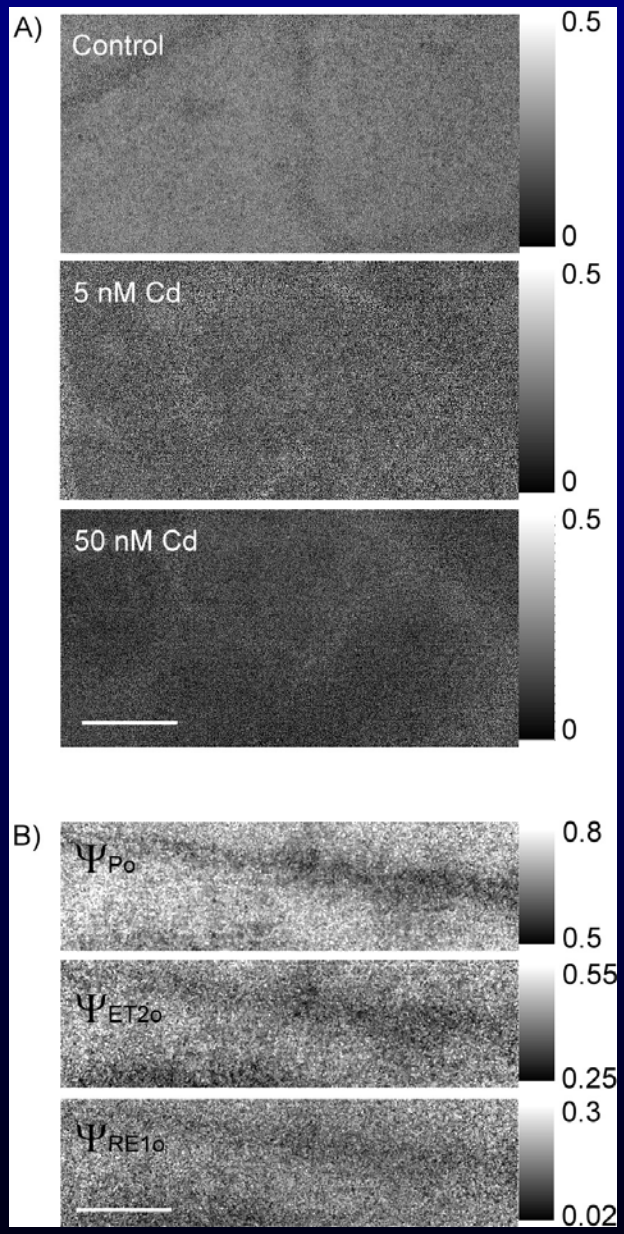
• Sequestration in the vacuole:
- plant-specific mechanism (animals+bacteria usually don‘t have vacuoles...)
- very efficient, because the vacuole does not contain sensitive enzymes
- saves the investment into the synthesis of strong ligands like phytochelatins
- main mechanism in hyperaccumulators

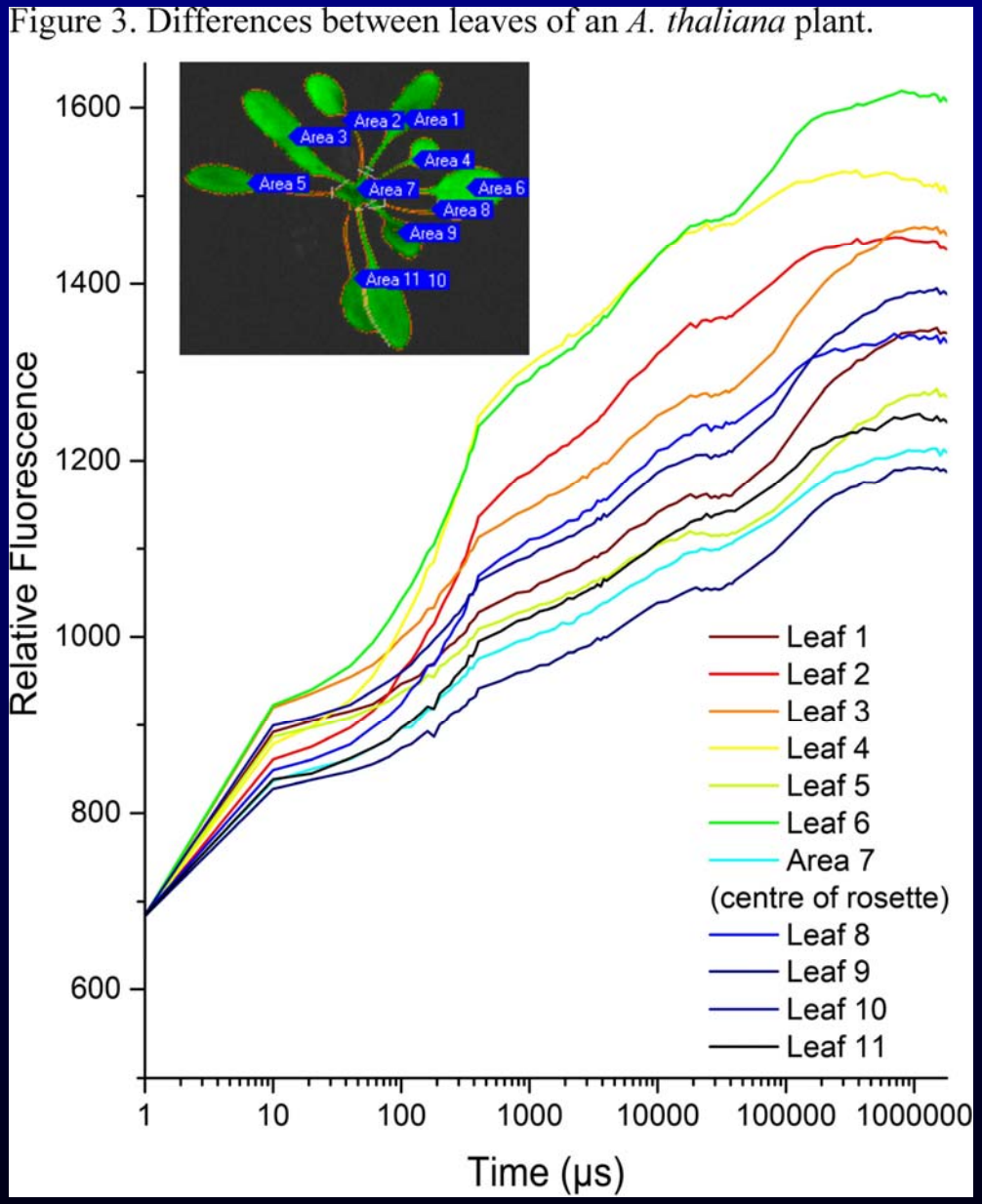
• Sequestration in least sensitive tissues, e.g. the epidermis instead of the photosynthetically active mesophyll



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

# We will show you original data from recent research…

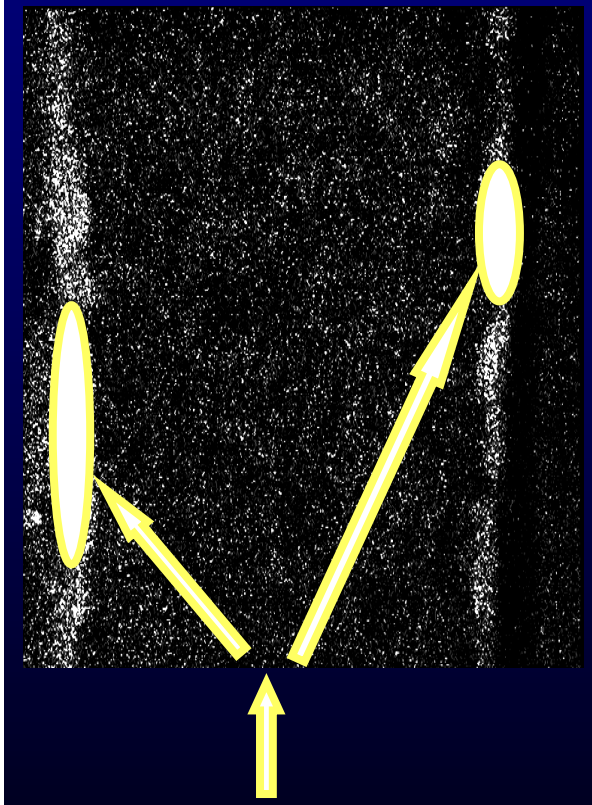


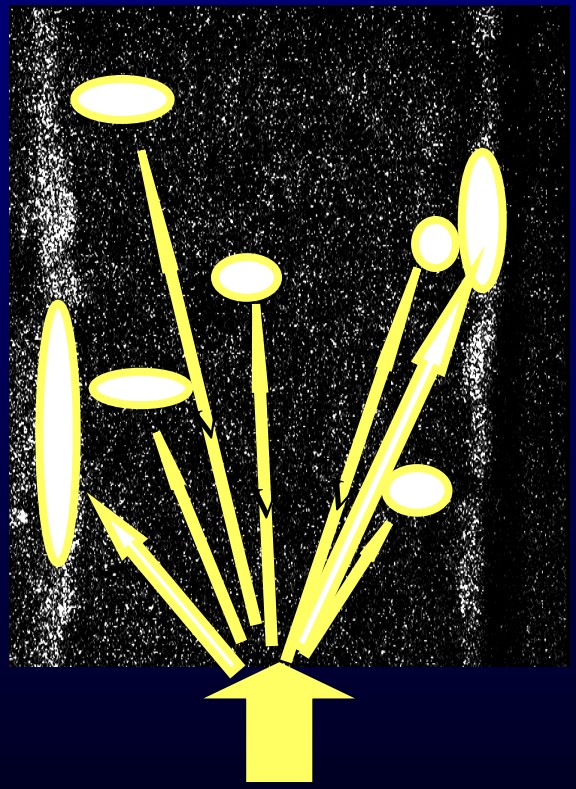

Küpper H*, Benedikty Z, Morina F, Andresen E, Mishra A, Trtílek M (2019) Plant Physiology 179, 369-381

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

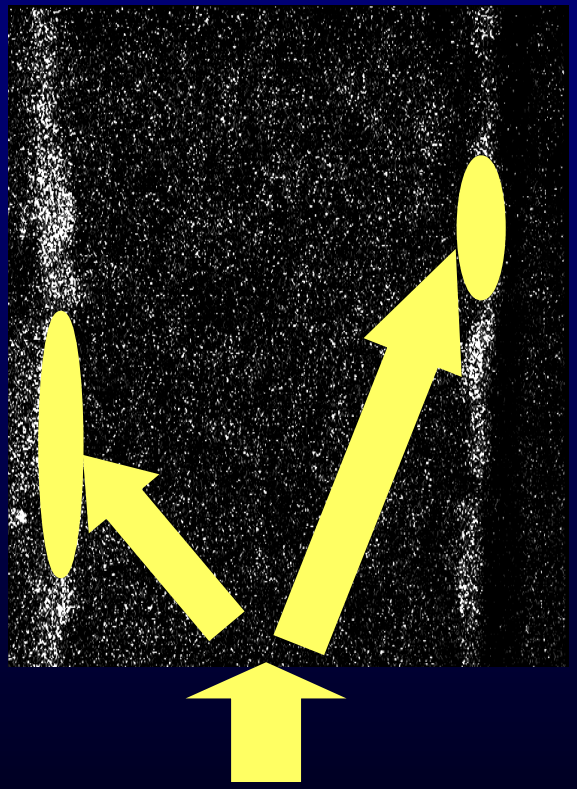
Normal: Sequestration in epidermal storage cells



Stressed: additional sequestration in selected mesophyll cells



Acclimated: Enhanced sequestration in epidermal storage cells



Küpper H, Aravind P, Leitenmaier B, Trtilek M, Šetlík I (2007) New Phytologist 175, 655-674

# 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

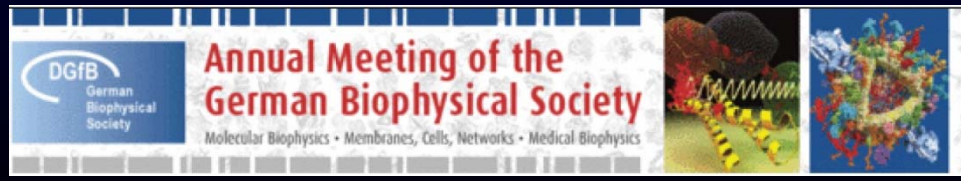
## … and how to publish them


New Phytologist



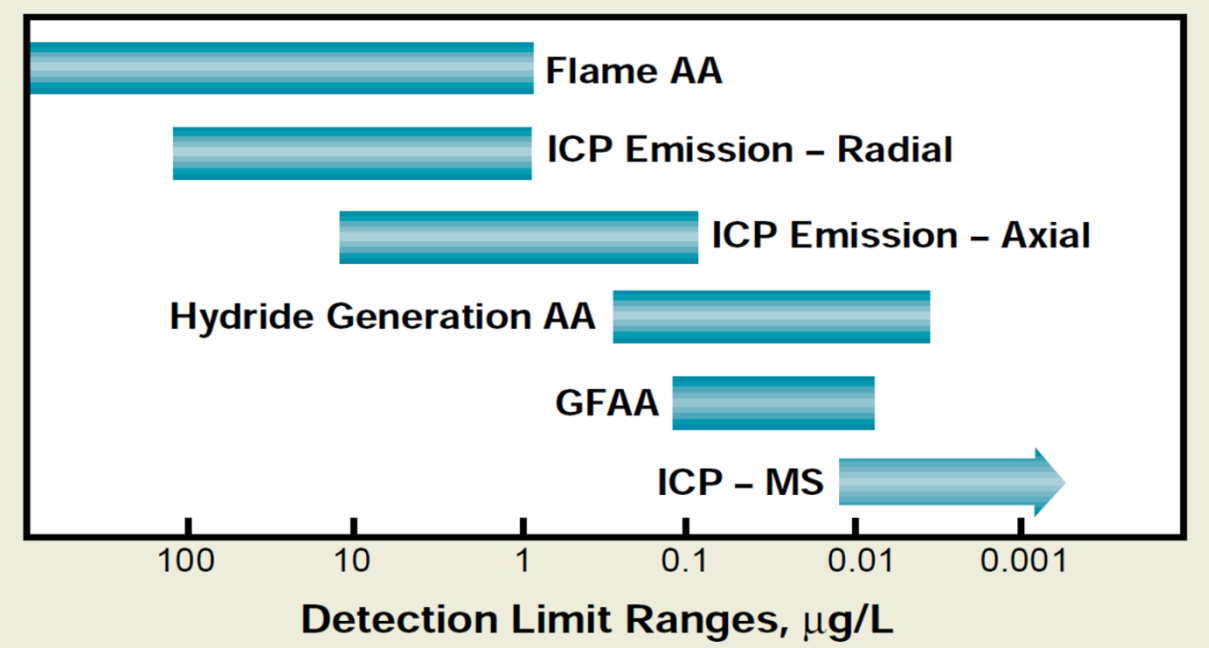
Plant, Cell & Environment

*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



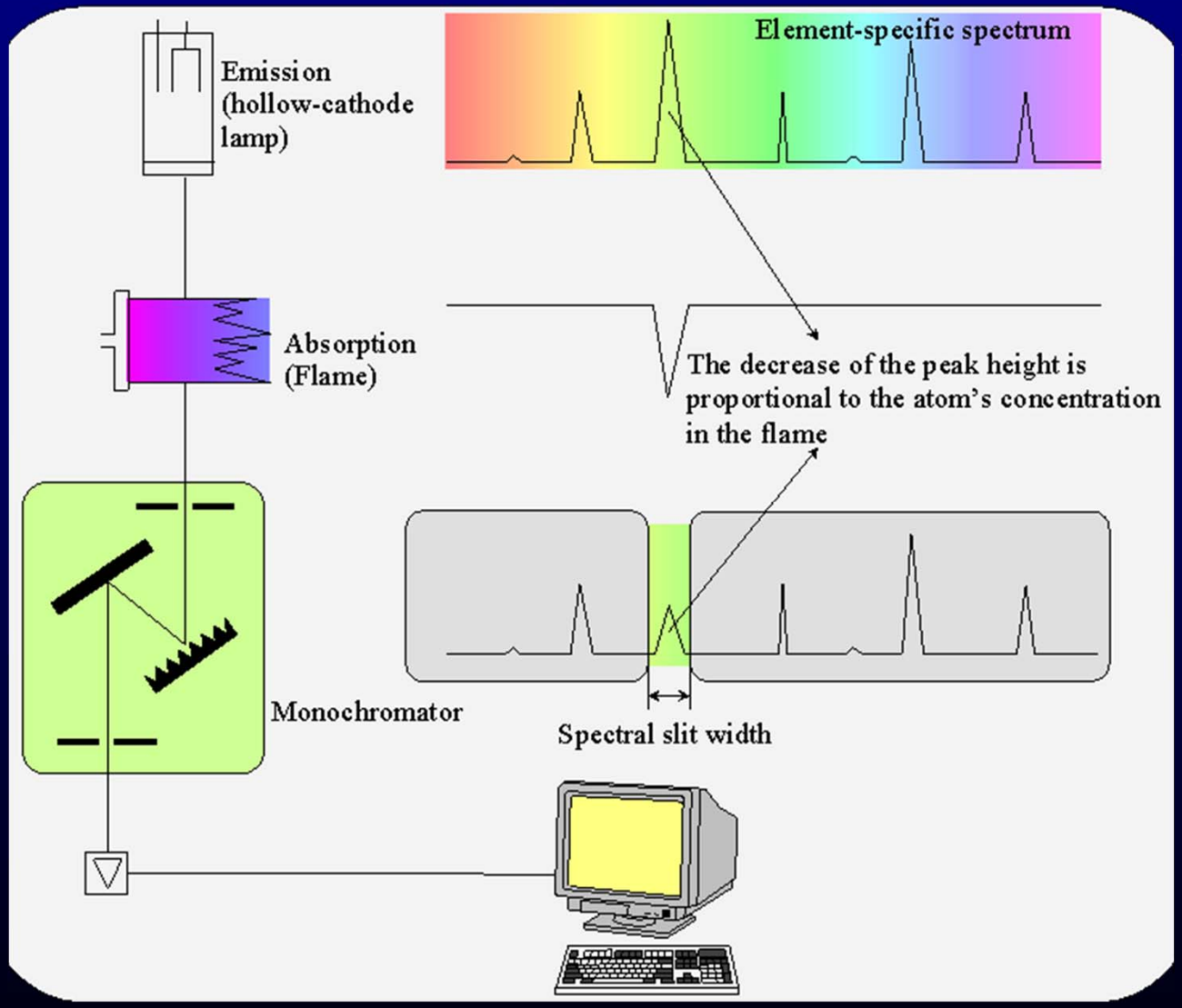

From: Thermo electron product information

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 µg/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)

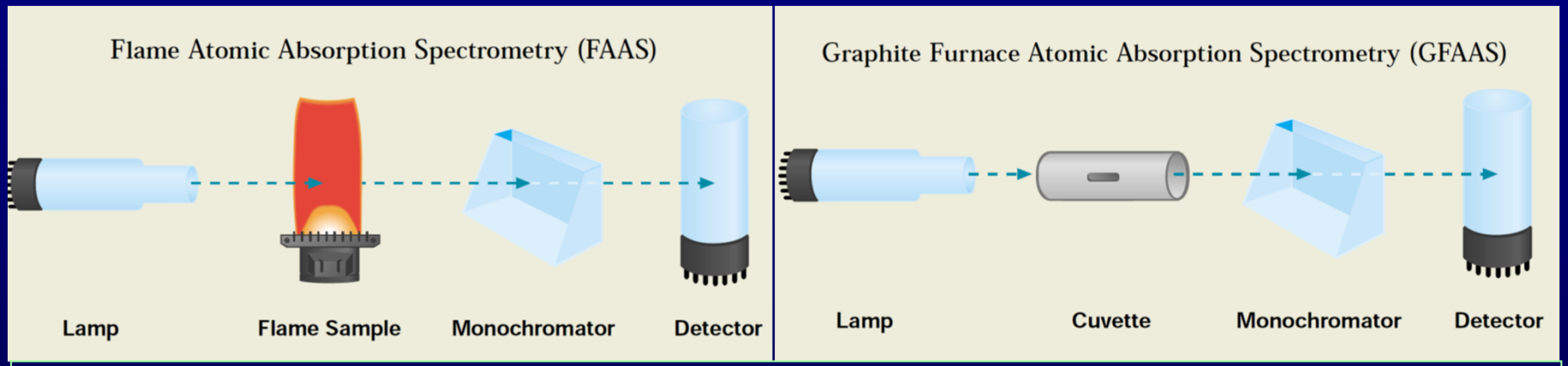



Advantages:
- easy to use,
- fast if only 1 element is needed
- affordable

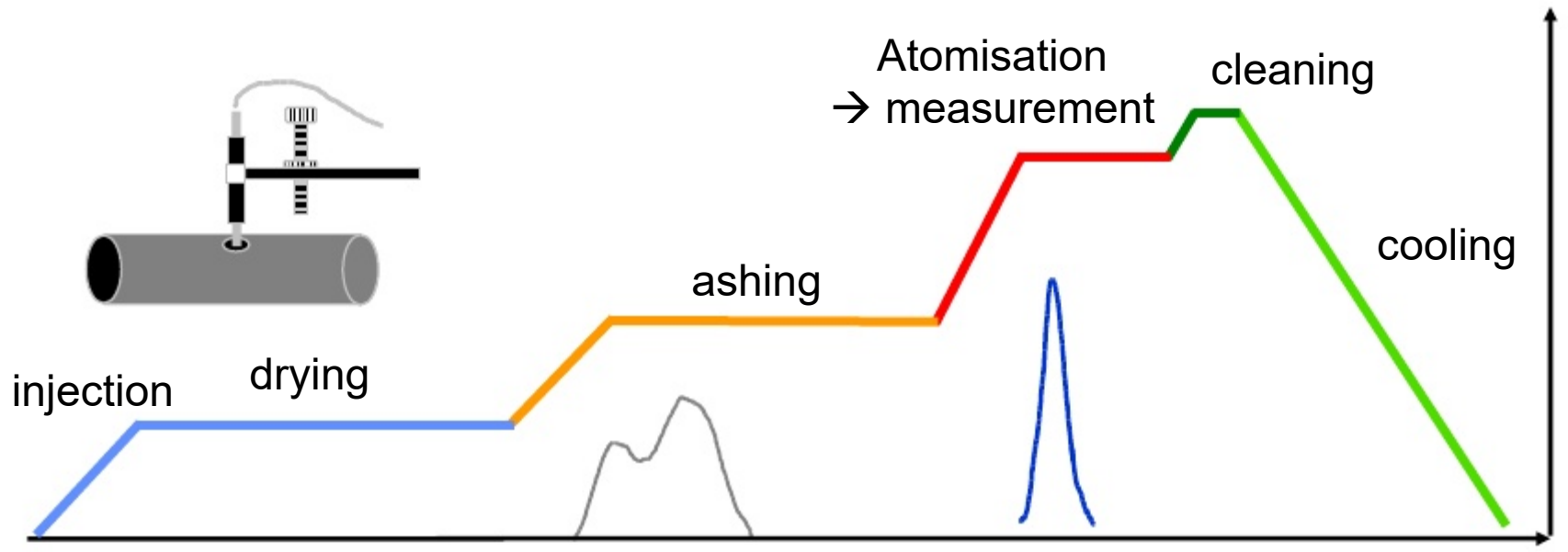
Disadvantages:
- insensitive for some elements (e.g. sulphur)
- slow if many elements are needed

# Metal content – methods of Measurement (II)
# Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS)

## Principle



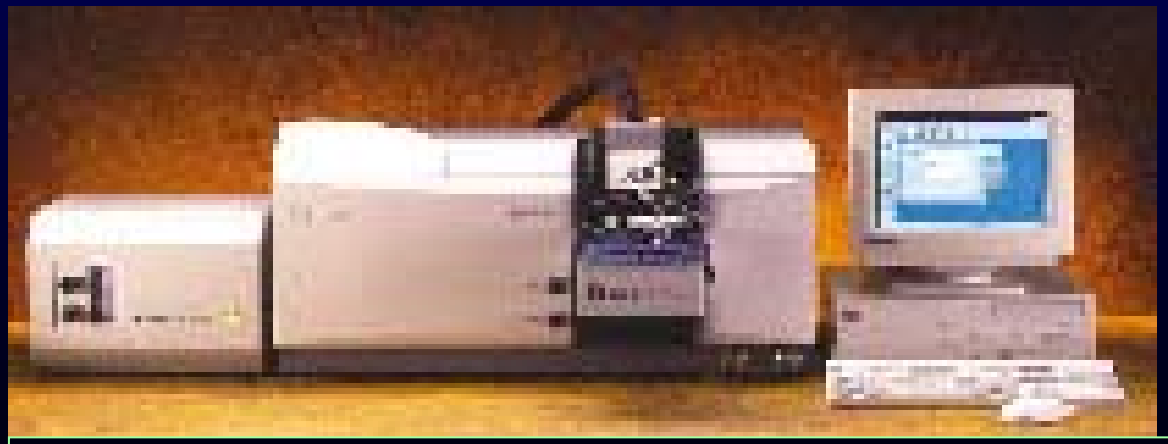

From: http://www.scribd.com/doc/15784148/Graphite-Furnace-Analysis (bottom); Thermo electron teaching file (top)



# Metal content – methods of Measurement (II)
# Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS)

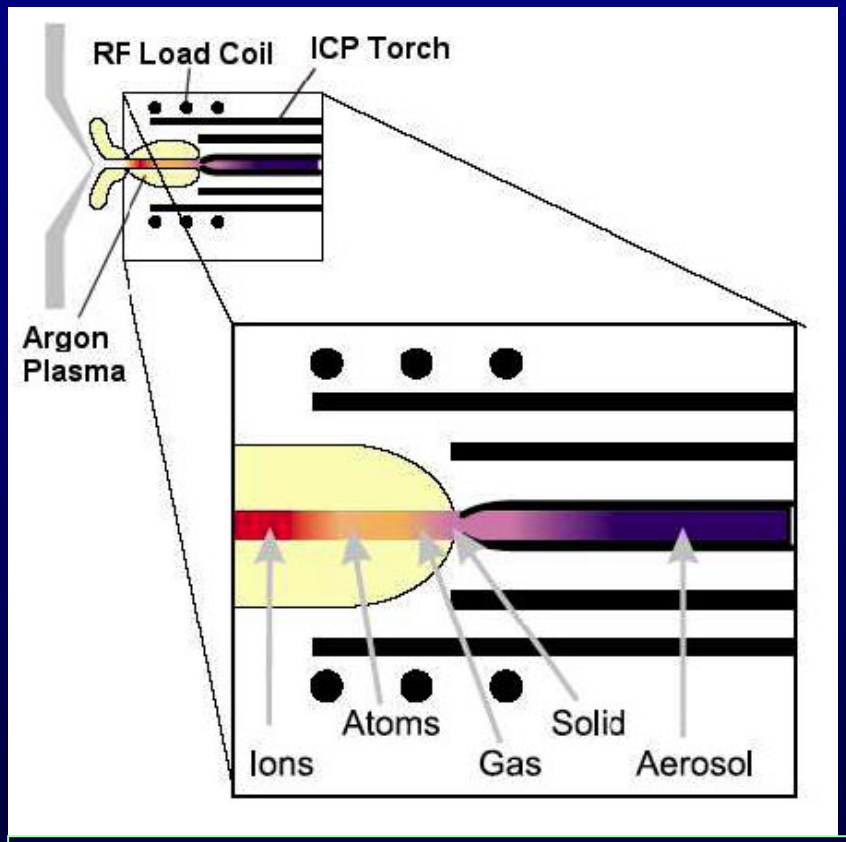
| Criteria | Flame | Furnace |
|---|---|---|
| Elements | 67 | 48 |
| Sensitivity | ppm - % | ppt – ppb |
| Precision | Good | Fair |
| Interferences | Few | Many |
| Speed | Rapid | Slow |
| Simplicity | Easy | More complex |
| Flame Hazards | Yes | No |
| Automation | Yes | Yes (unattended) |
| Operating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

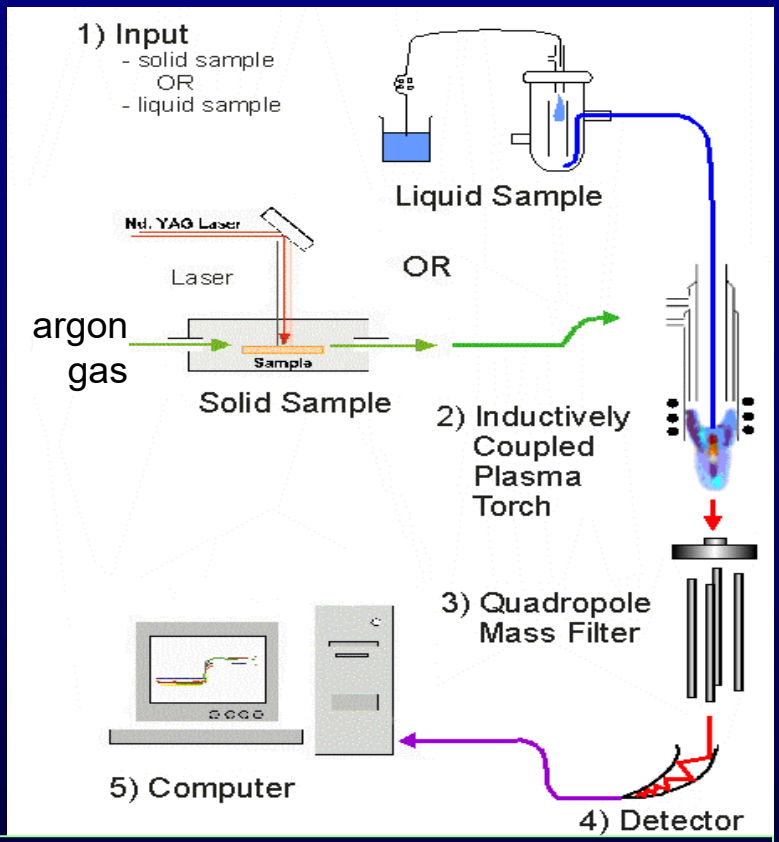
From: http://www.scribd.com/doc/15784148/Graphite-Furnace-Analysis



From: GBC product information

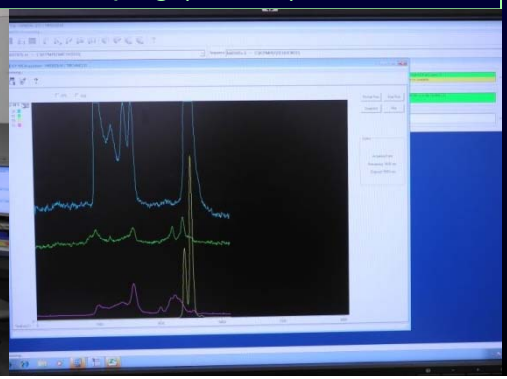
# Metal content – methods of Measurement (III)
# Inductively Coupled Plasma Mass Spectrometry (ICP-MS)



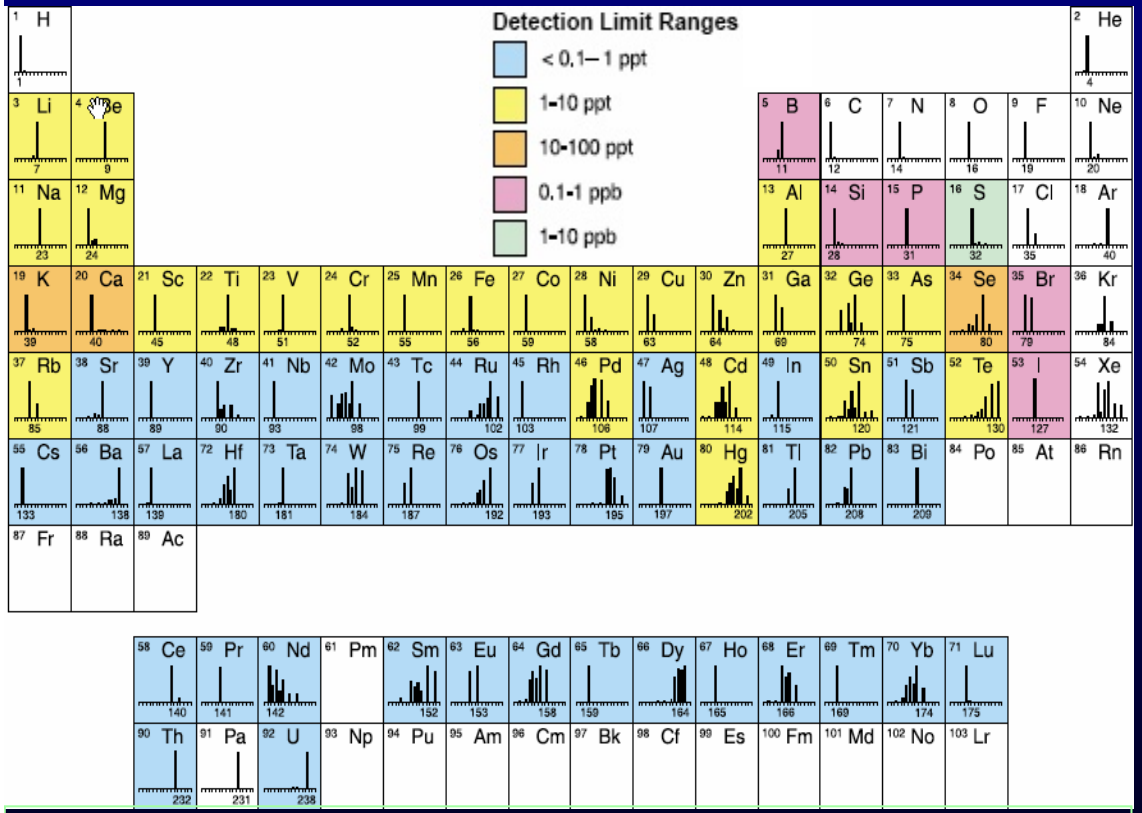




From: Perkin Elmer teaching file (top); LC-ICP-MS experiment at UFZ Leipzig (bottom)





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




From: Perkin Elmer product information

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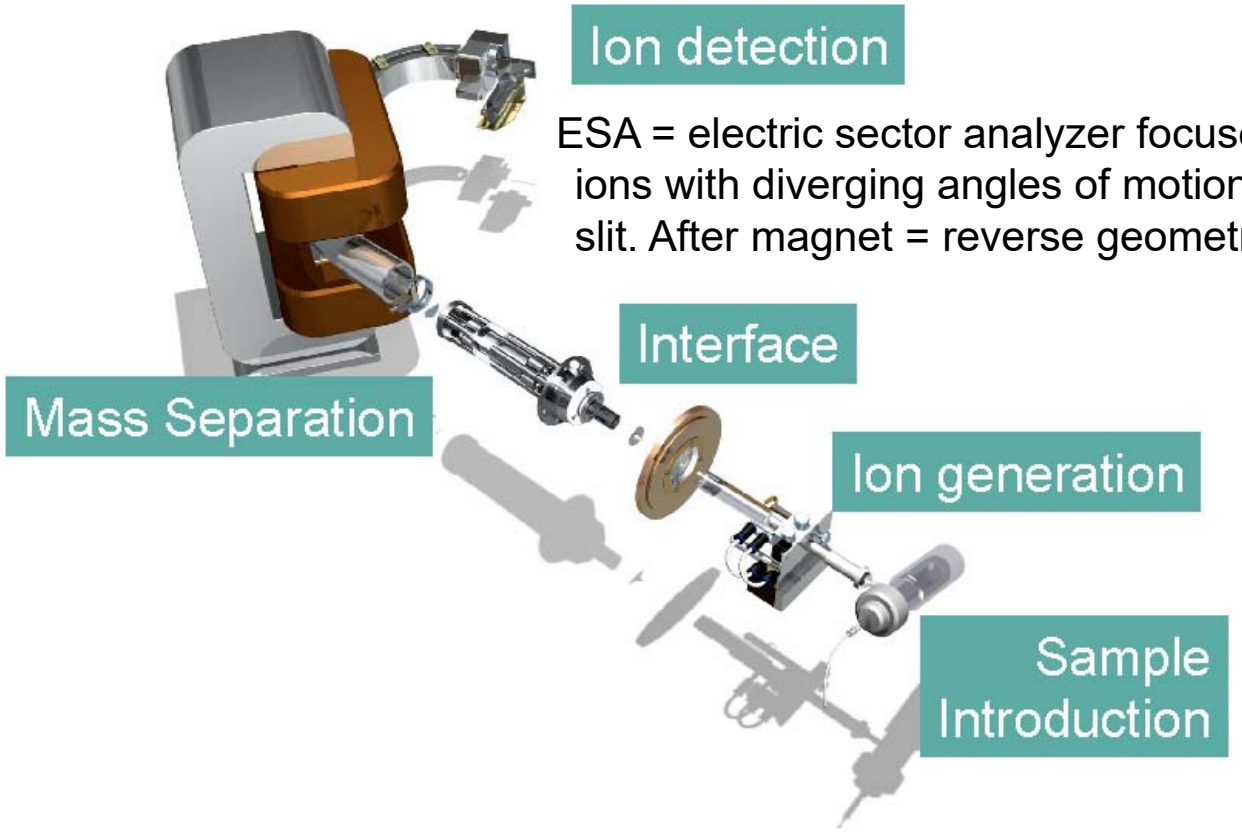
- Detection limits for most elements equal to or better than those obtained by Graphite Furnace –AAS (GFAAS)
- Higher throughput than GFAAS
- minimum of matrix interferences due to the high-temperature of the ICP source
- Superior detection capability to ICP-AES with the same sample throughput
- Ability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

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

## Principle components of an ICP-SFMS

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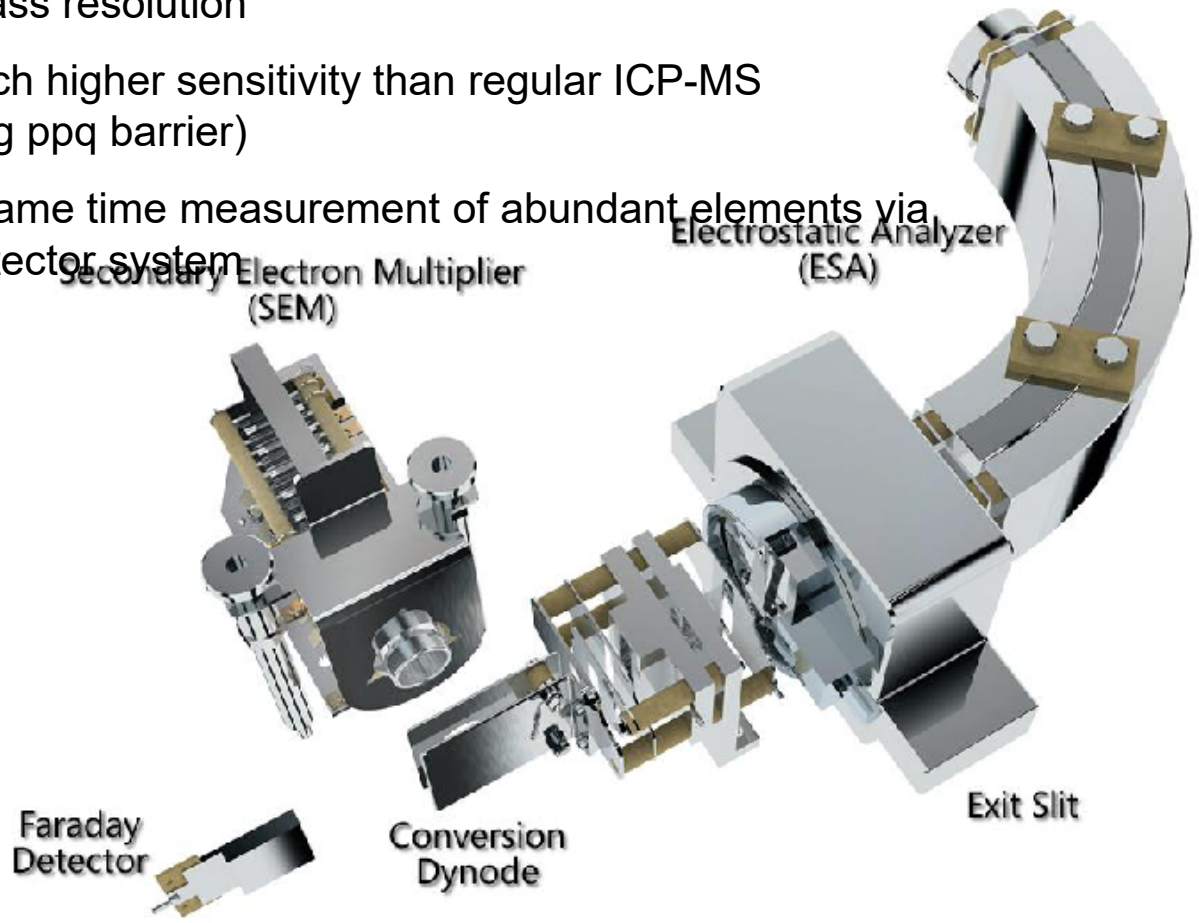
# Metal content – methods of Measurement
# Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

## 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

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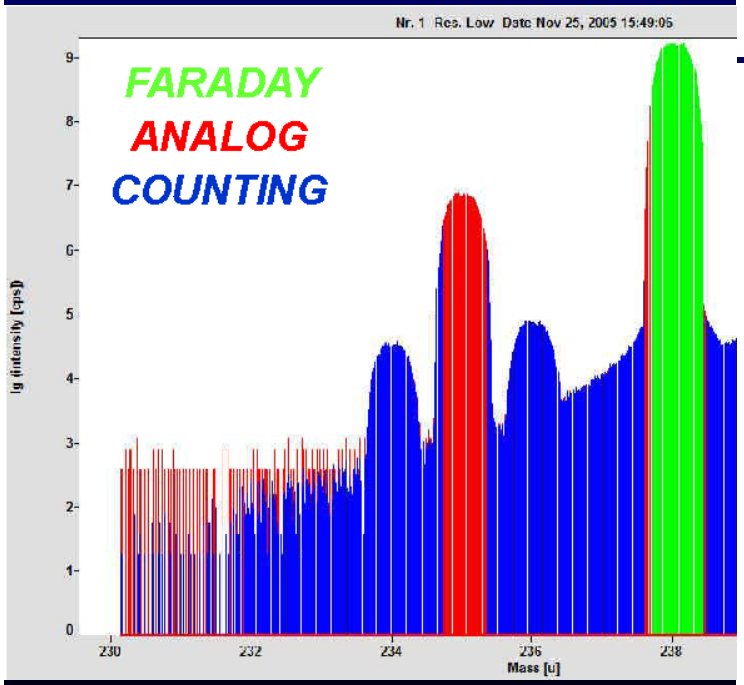
# Inductively Coupled Plasma Mass Spectrometry (ICP-MS):

automatic detector switching in Element XR

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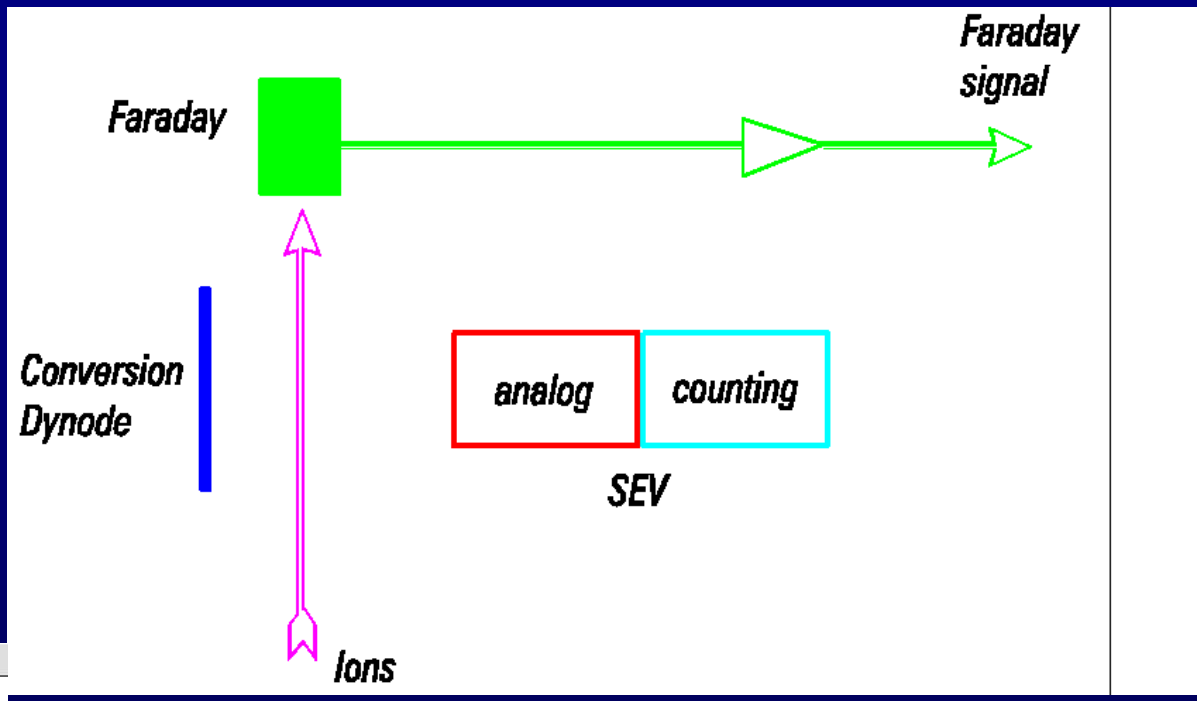


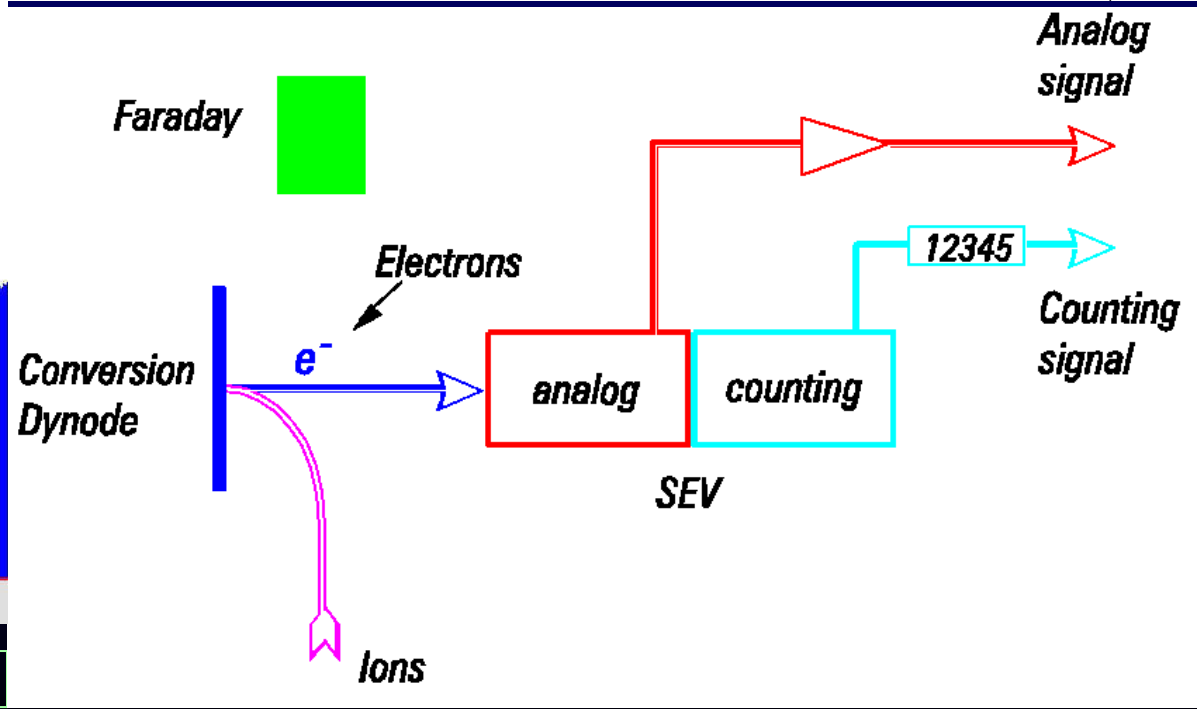

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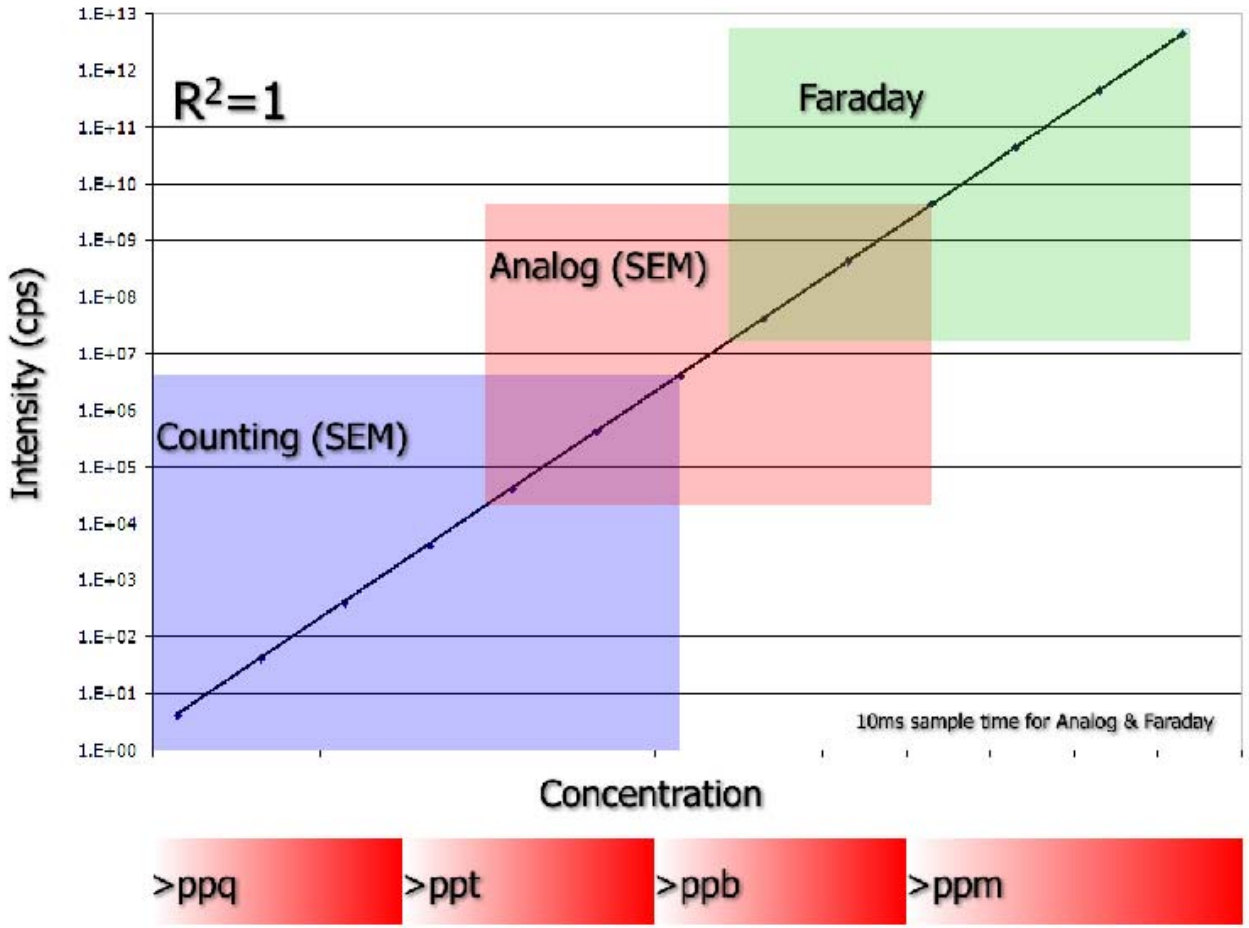
## Extended Dynamic Range in the Finnigan ELEMENT XR

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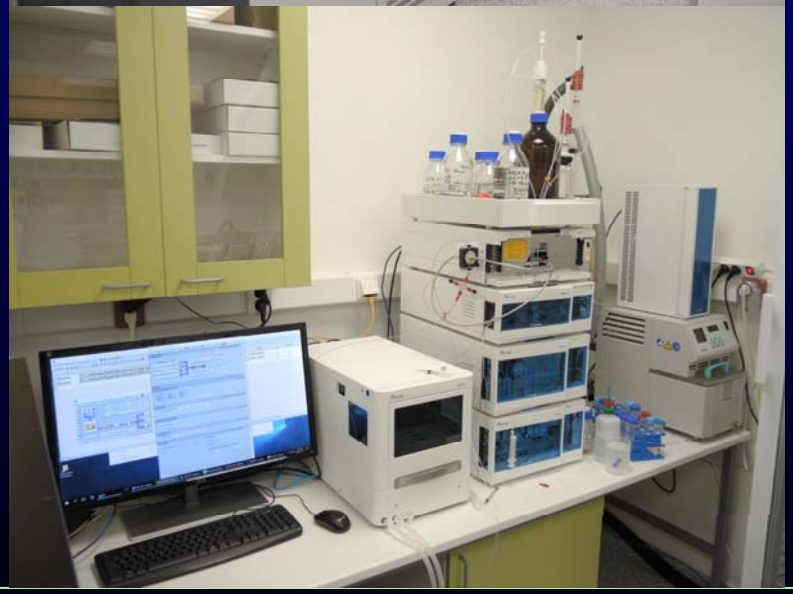
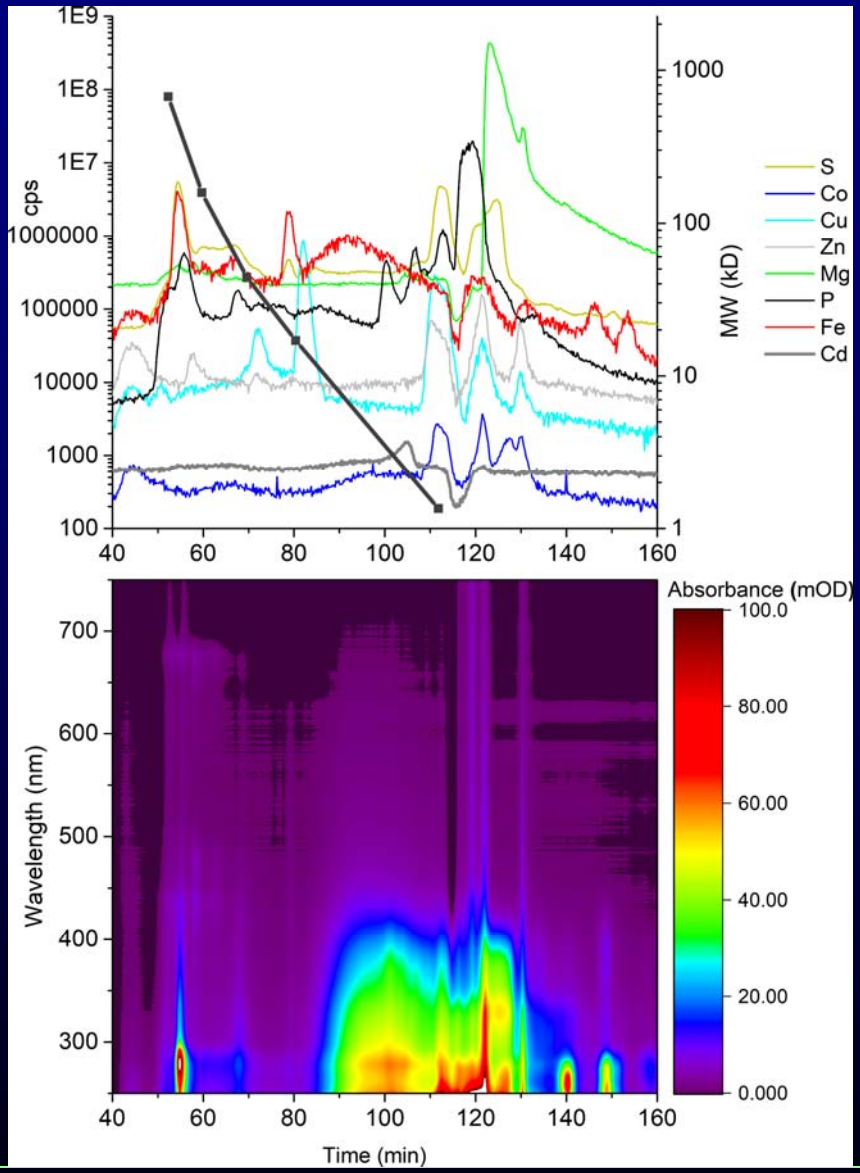
# Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Coupling to HPLC






*Left:* Photo from our lab;
*right:* Küpper H, Bokhari SNH, Jaime-Perez N, Lyubenova L, Ashraf N, Andresen E (2019) submitted to Analytical Chemistry

**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**