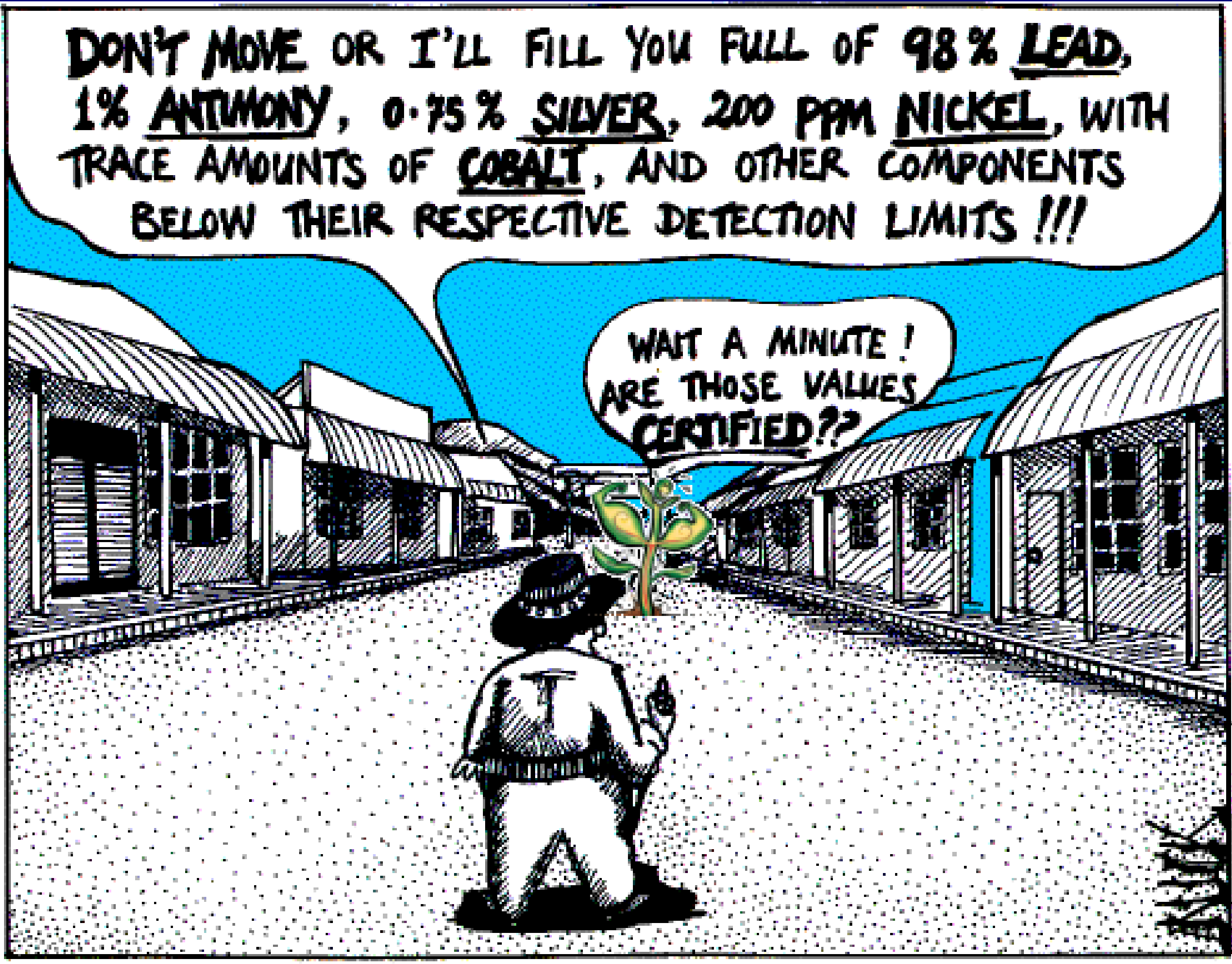
Advanced Course on Bioinorganic Chemistry & Biophysics of Plants – Introduction

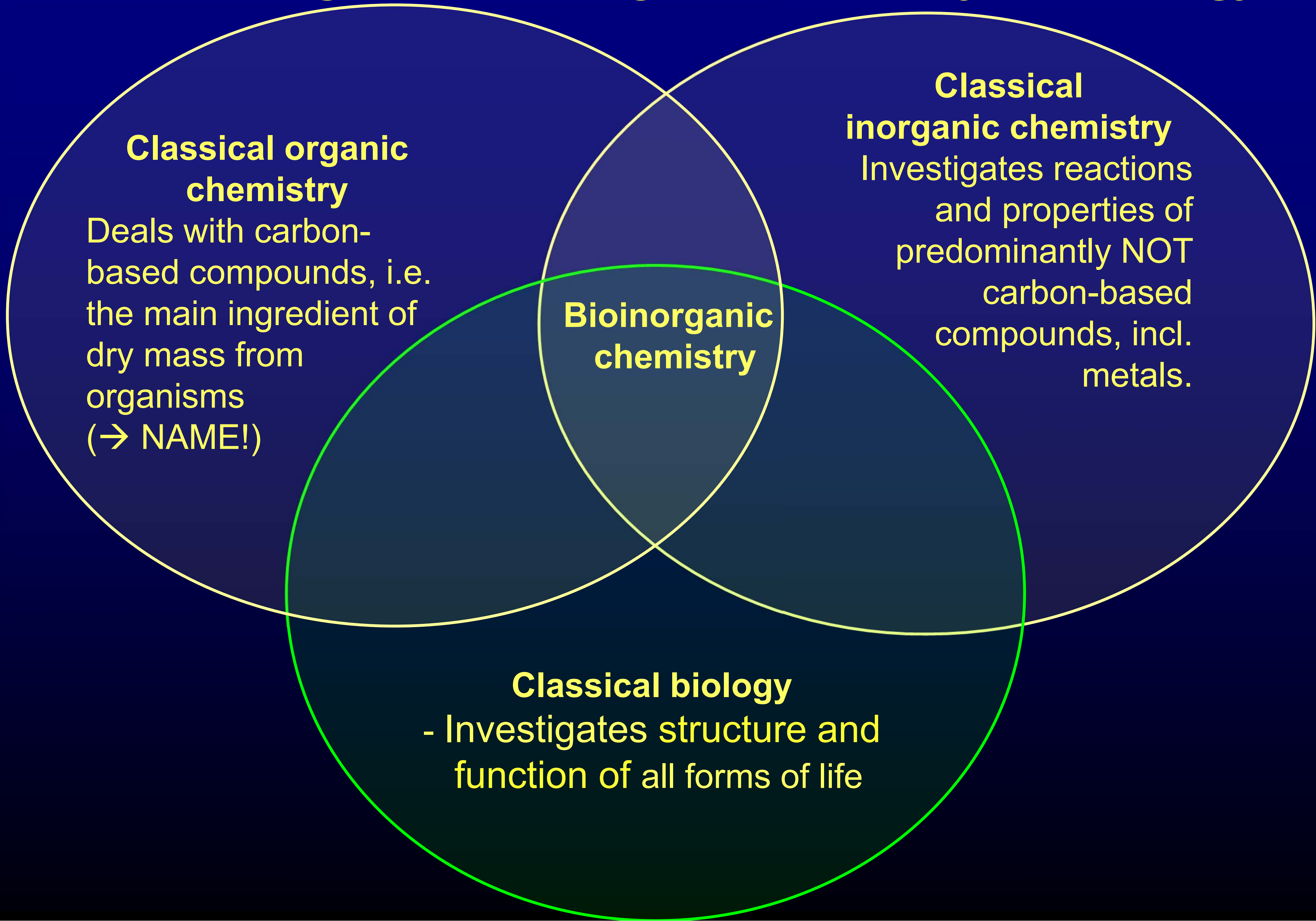


Schwermetall-Hyperakkumulation im Wilden Westen

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

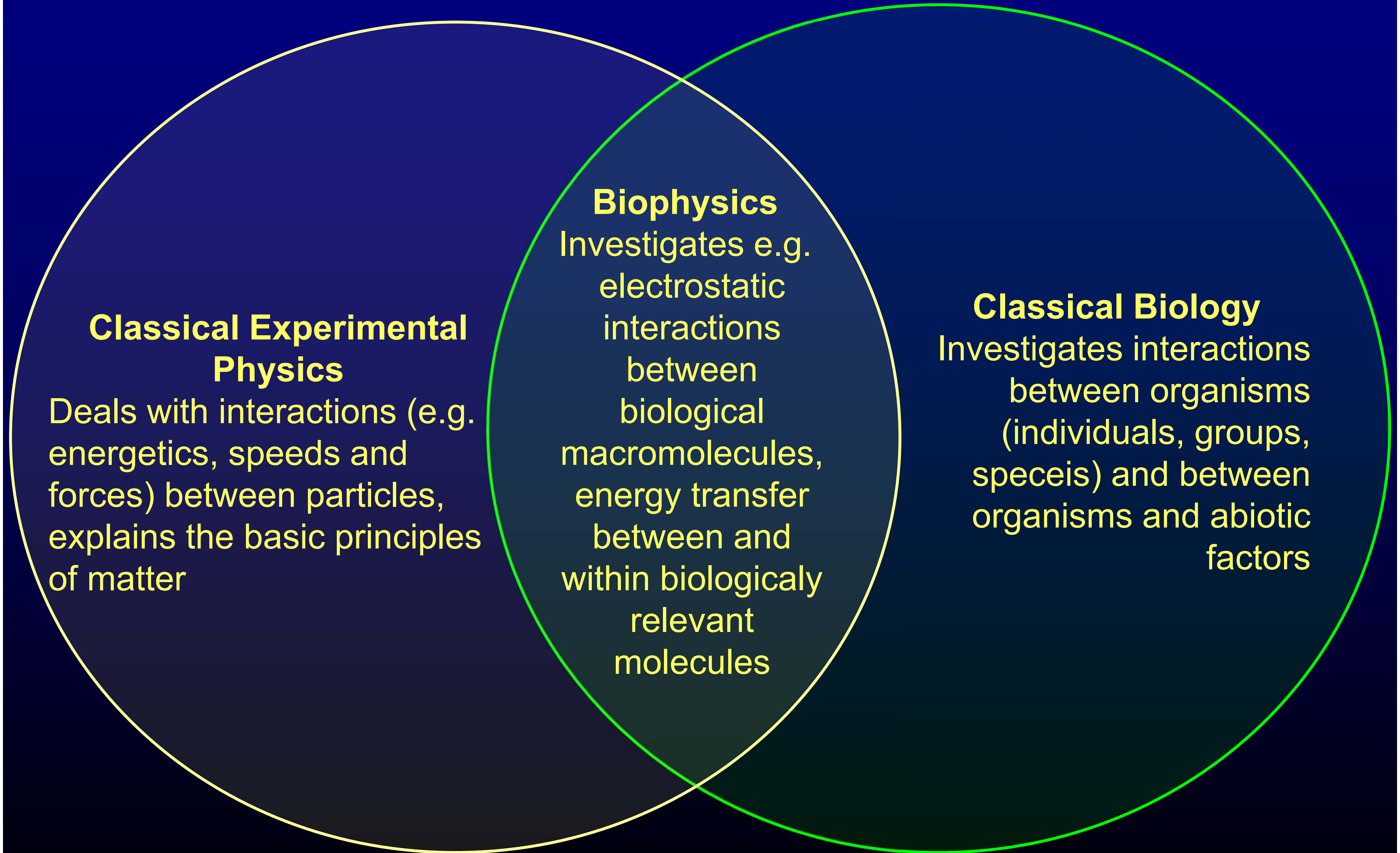
Metal coordination in biological ligands

→ 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

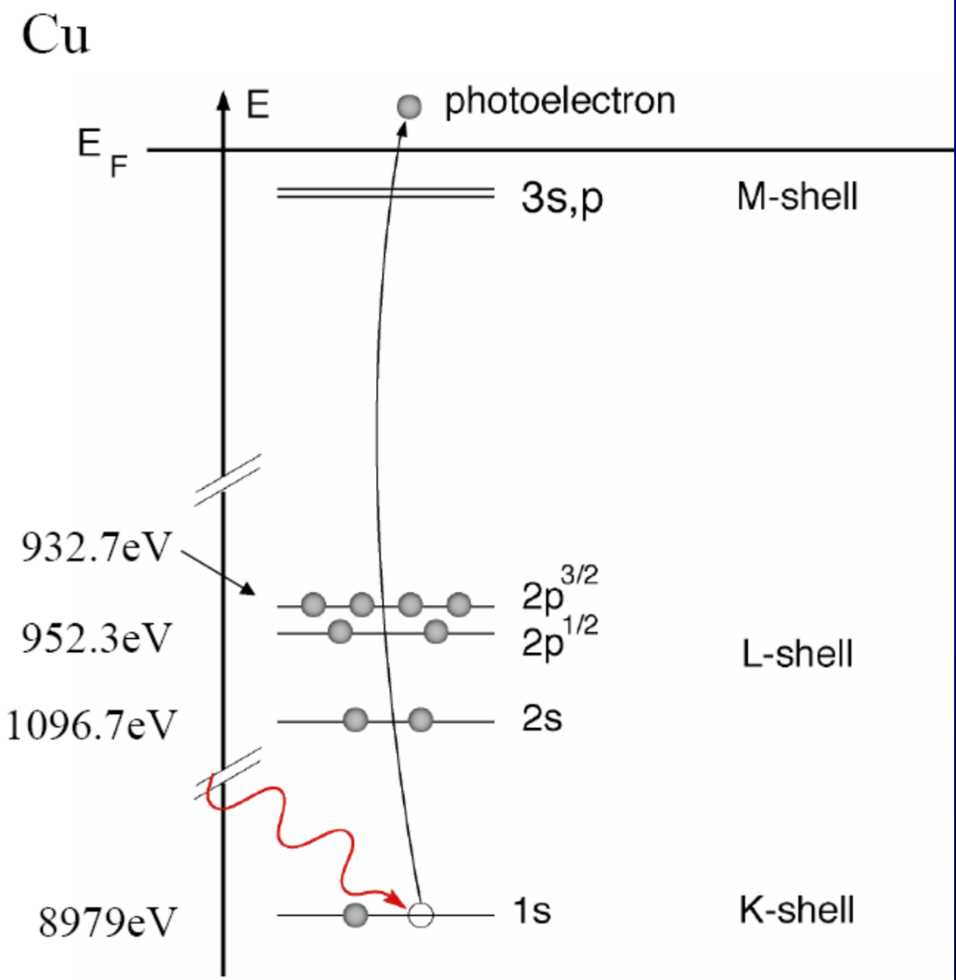
→ protein folding

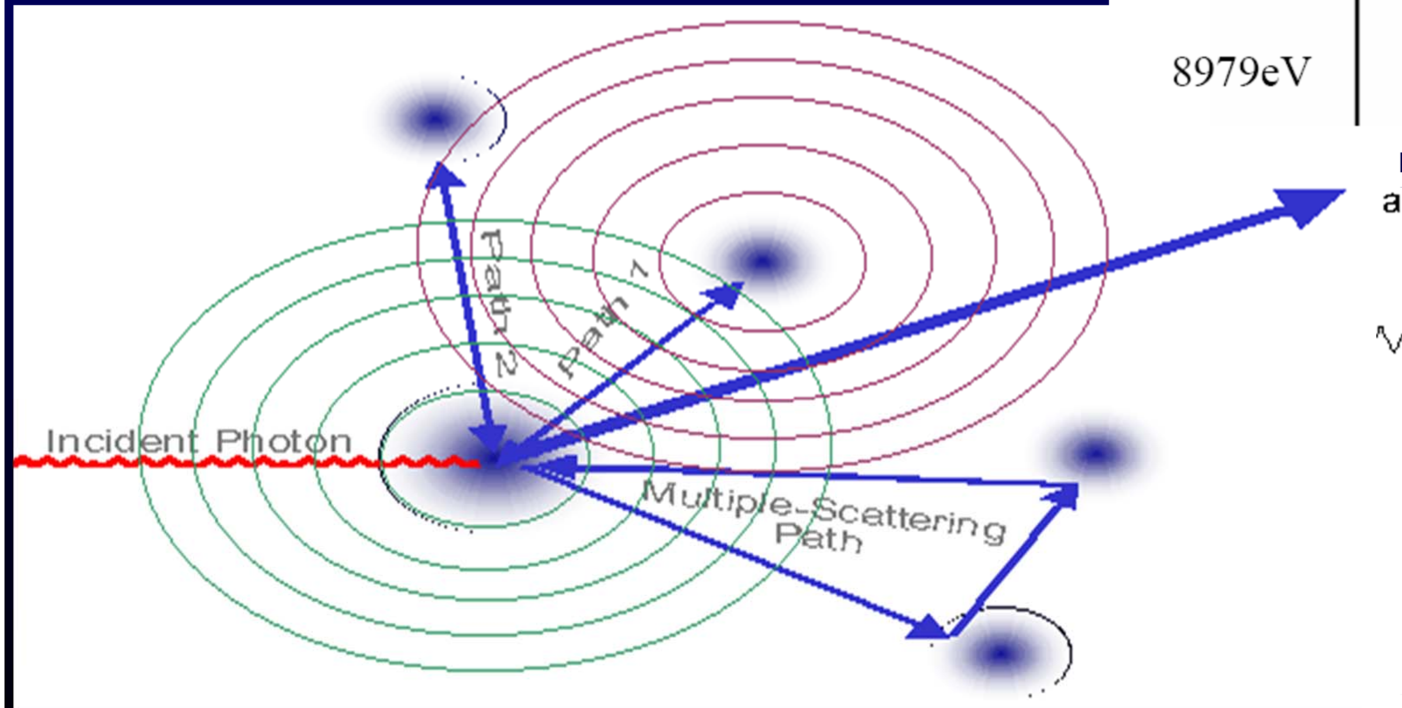
Methods used for investigating these questions include for example (in solutions, in models systems, but also in living cells)

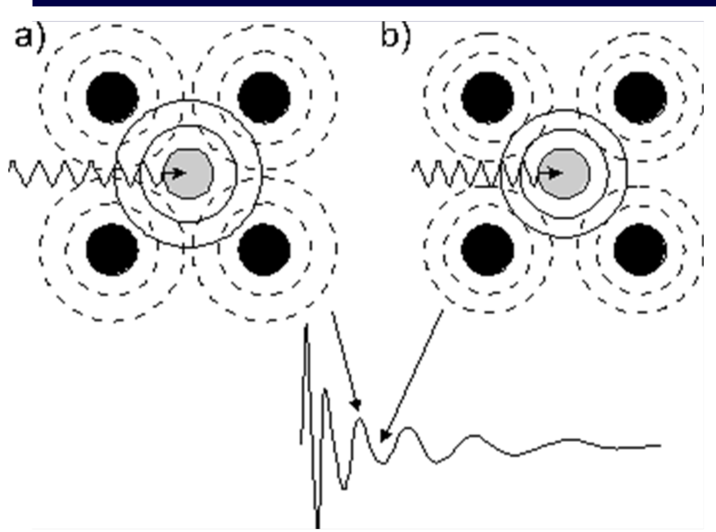
- UV/VIS absorption and fluorescence spectroscopy (→ electronic transitions to/from excited states → e.g. analysis of chromophore coupling)
- X-ray absorption spectroscopy (→ ionisation energies = X-ray absorption edges and emission bands, their element-specific characteristics and their modification 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?

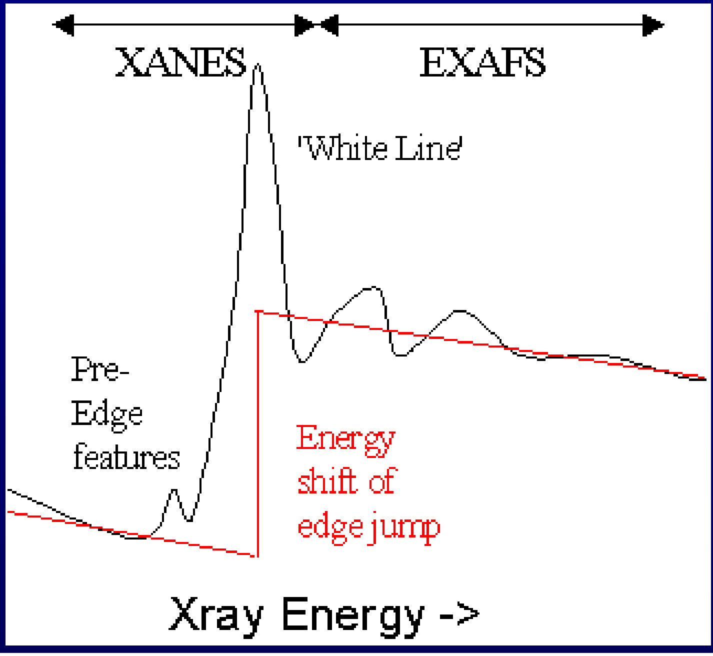
Theoretical Background of important biochemical and biophysical methods…

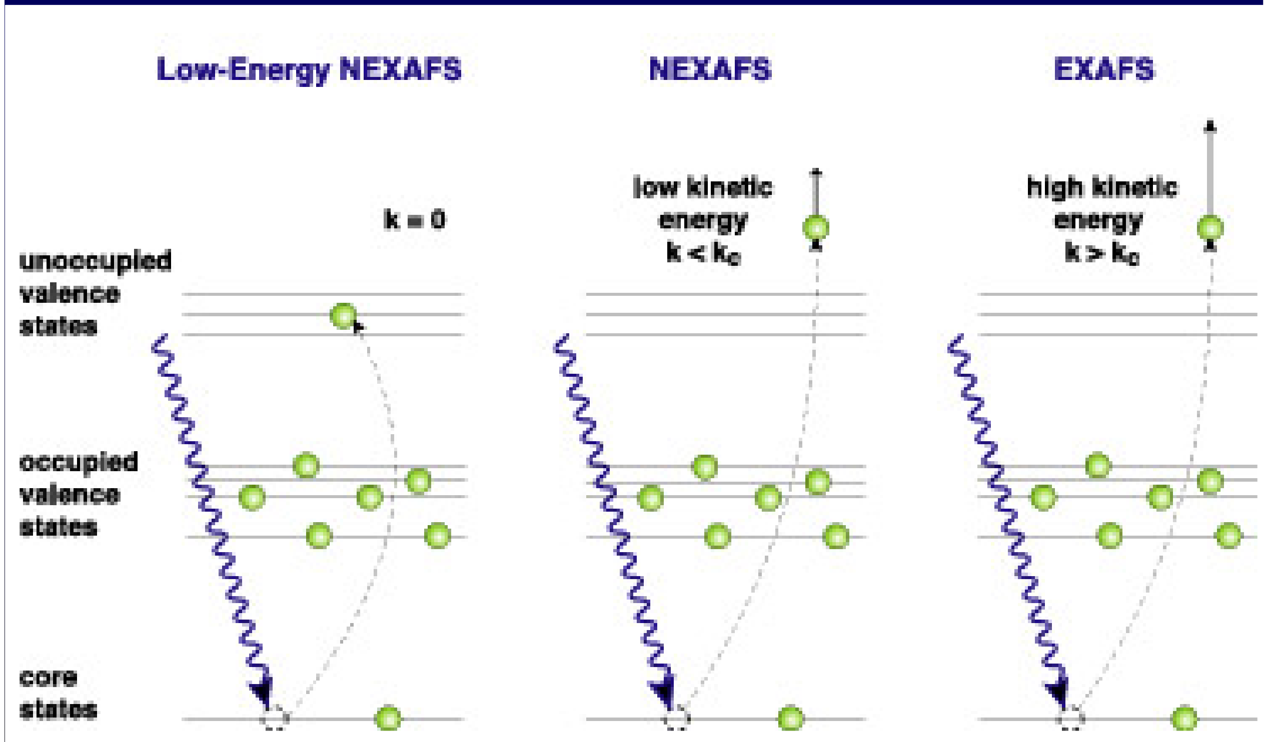


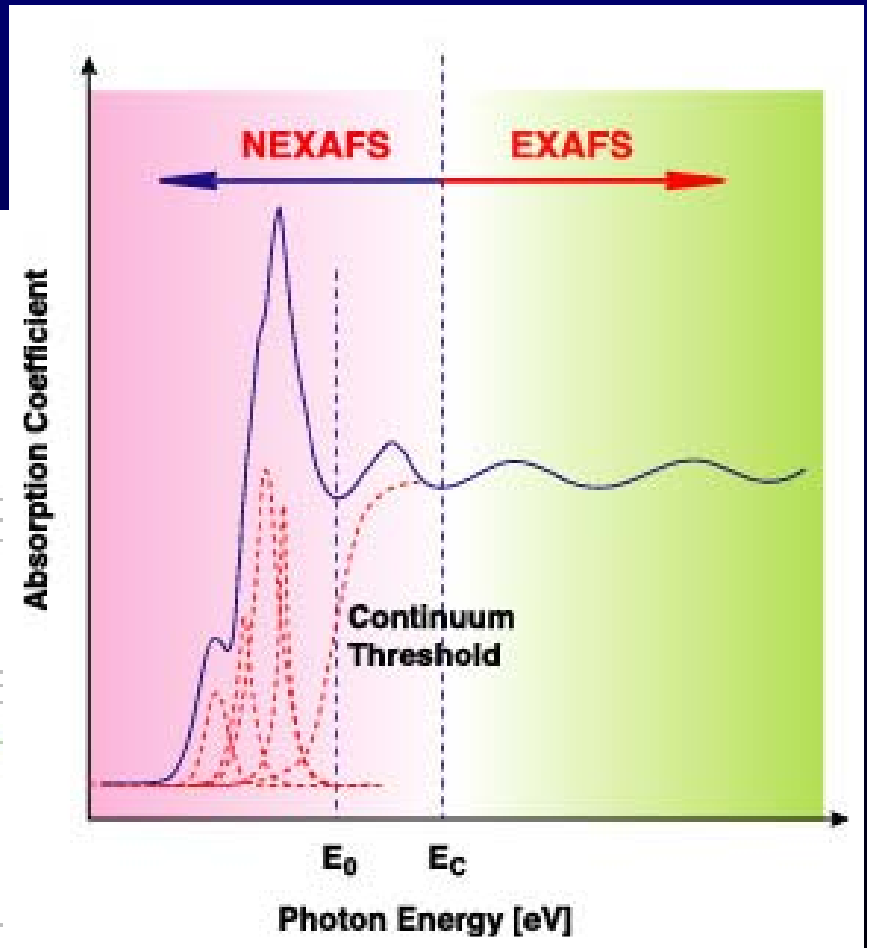




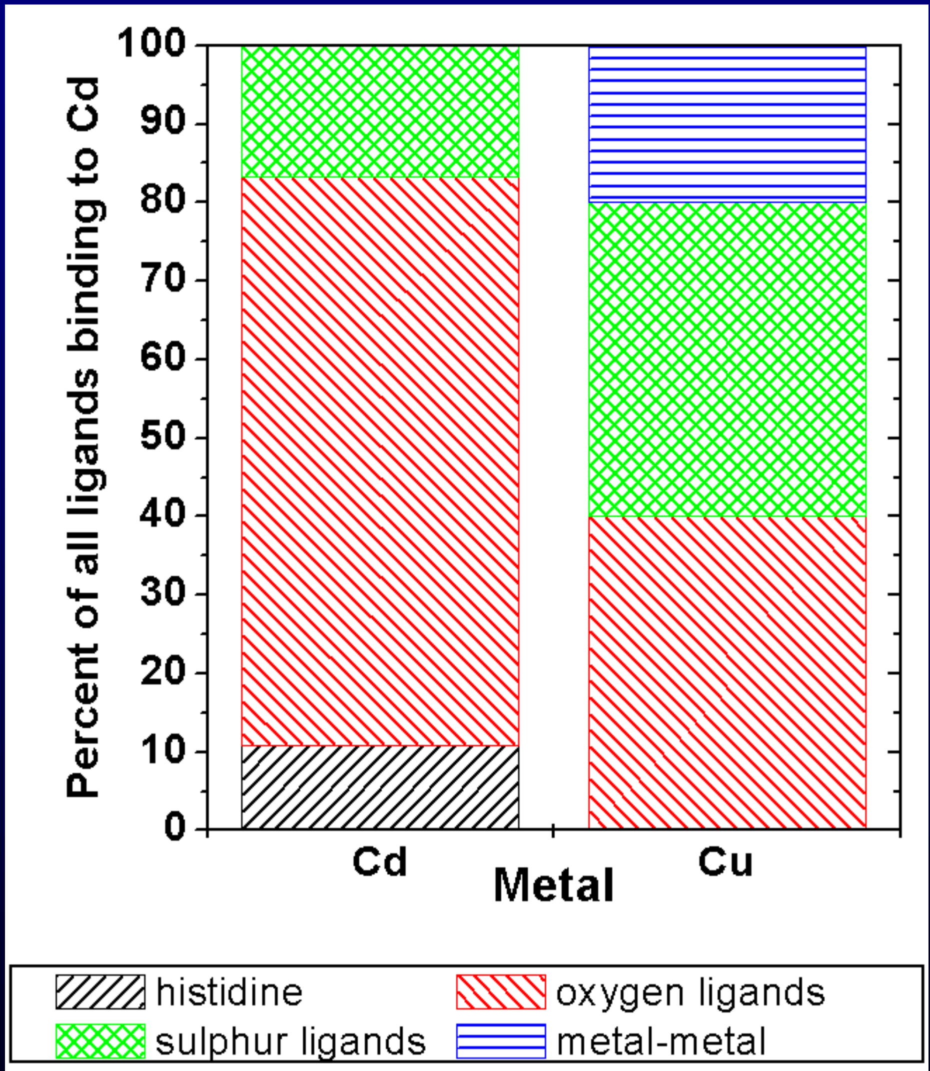
… differences in the basics and applications between related methods…

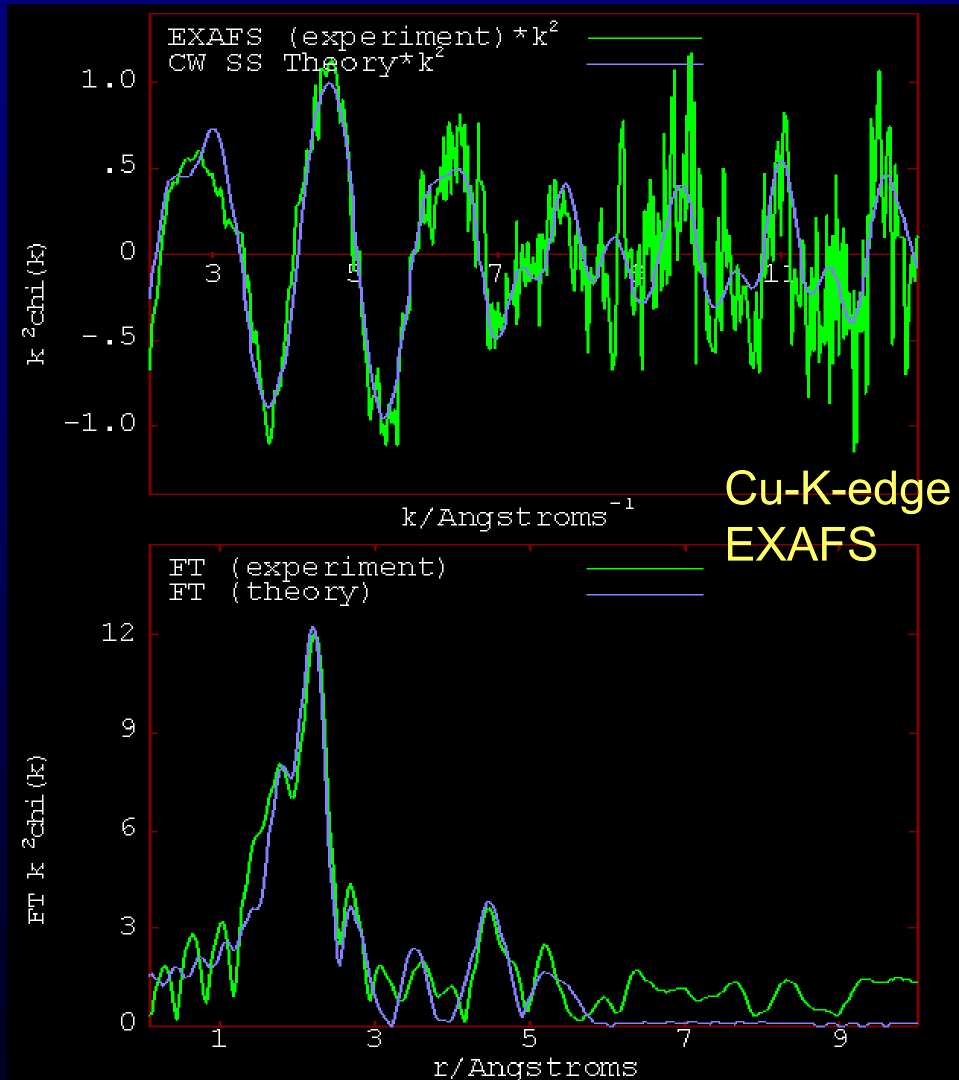






…and the use of these methods for answering questions in bioinorganic chemistry and biophysics.

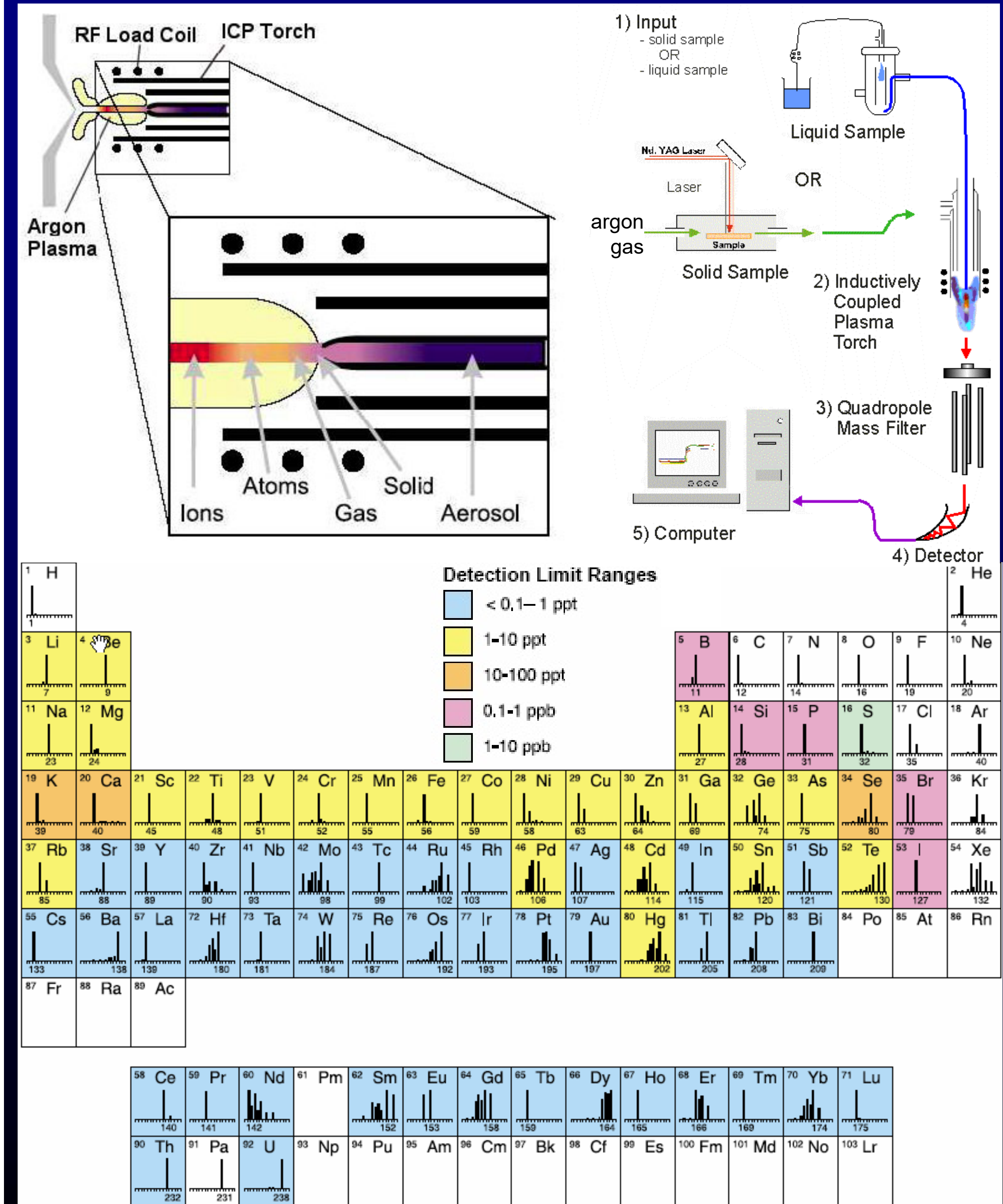




Cd: Küpper H, Mijovilovich A, Meyer-Klaucke W, Kroneck PMH (2004) Plant Physiology 134 (2), 748-757

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:

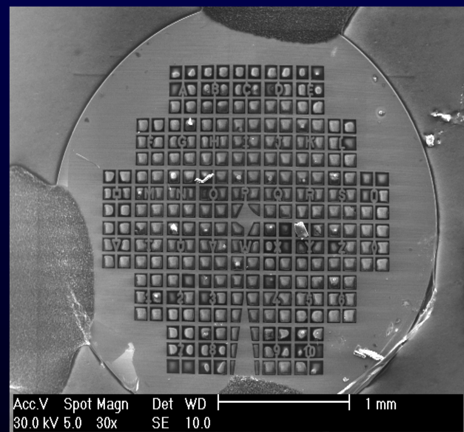
- more complicated technique than AAS
- much more expensive than AAS
- elements that prefer to form negative ions, such as Cl, I, F, etc. are very difficult to determine via ICP-MS because ions formed by the ICP discharge are typically positive ions.

Principles of sample preparation for specific methods…

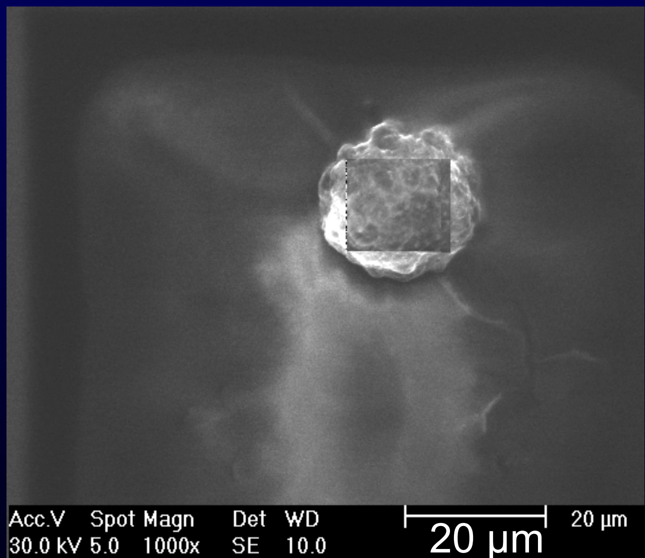
micropipette filled with silicon oil, connected to air-filled syringe for controlling pressure difference

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 grid

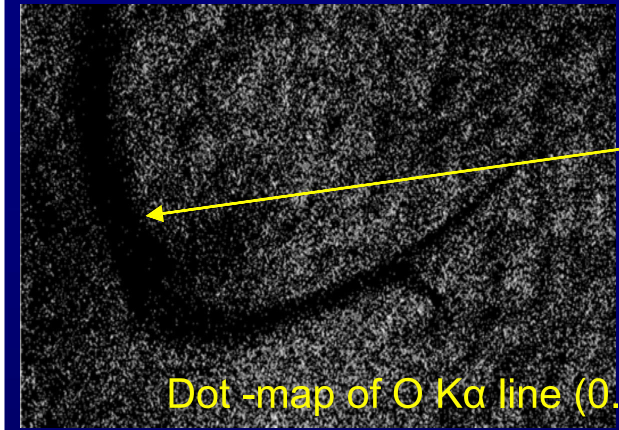


typical dried sample

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

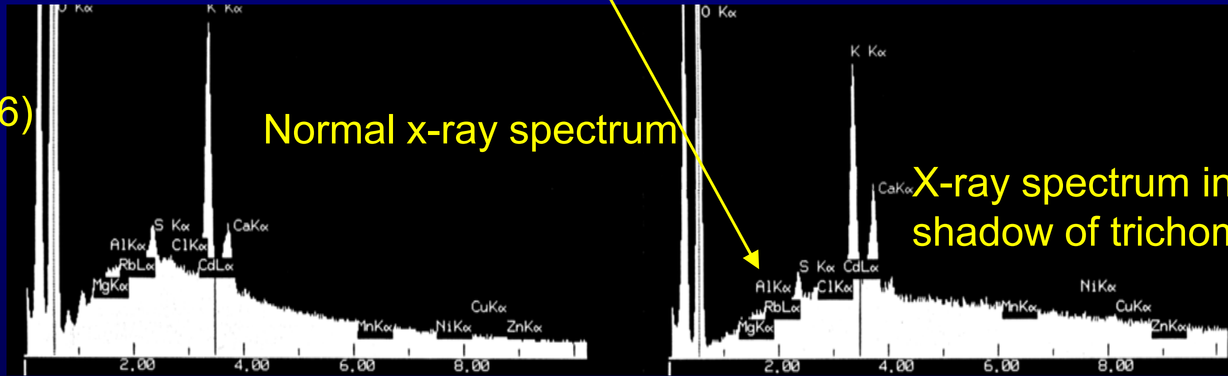
Küpper H, Zhao F, McGrath SP (1999) Plant Physiol 119, 305-11

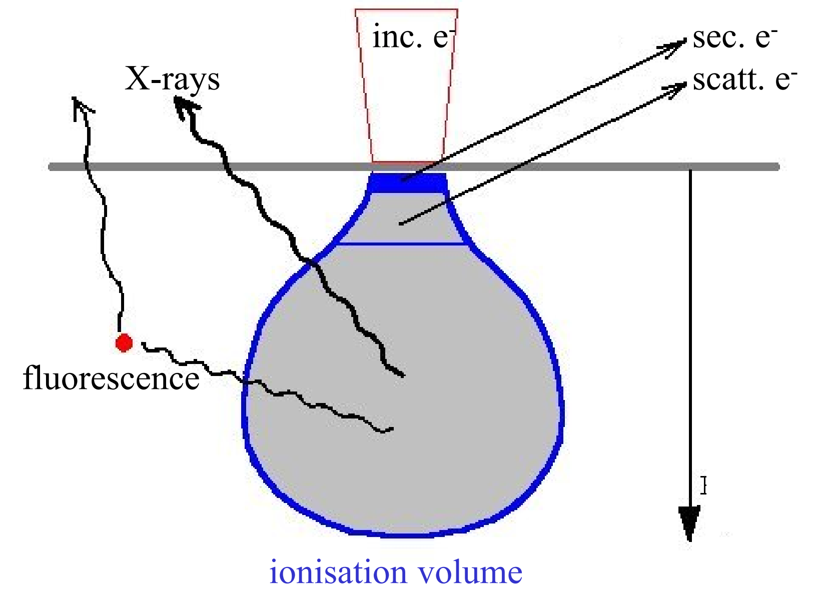
…and problems associated with these samples.



Effect of shading

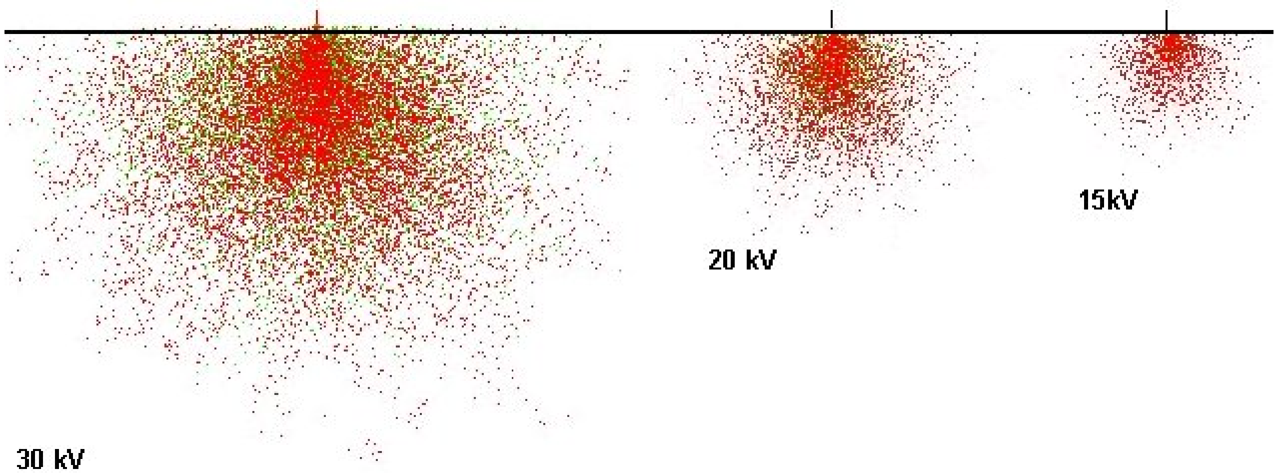
shading inside a sample leads to absorption of low-energy x-rays



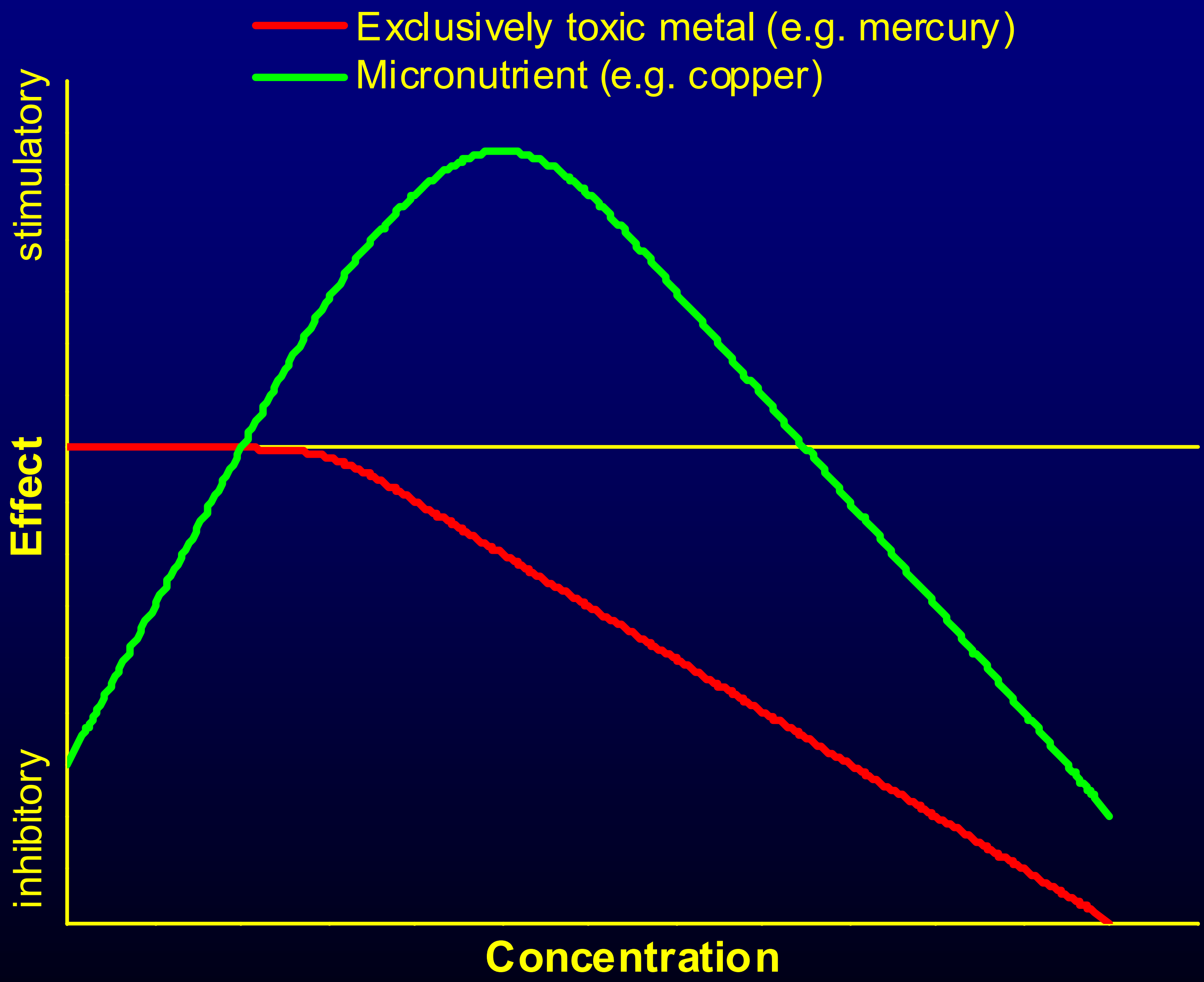


Effect of acceleration voltage

high acceleration voltage leads to deeper penetration into the sample!

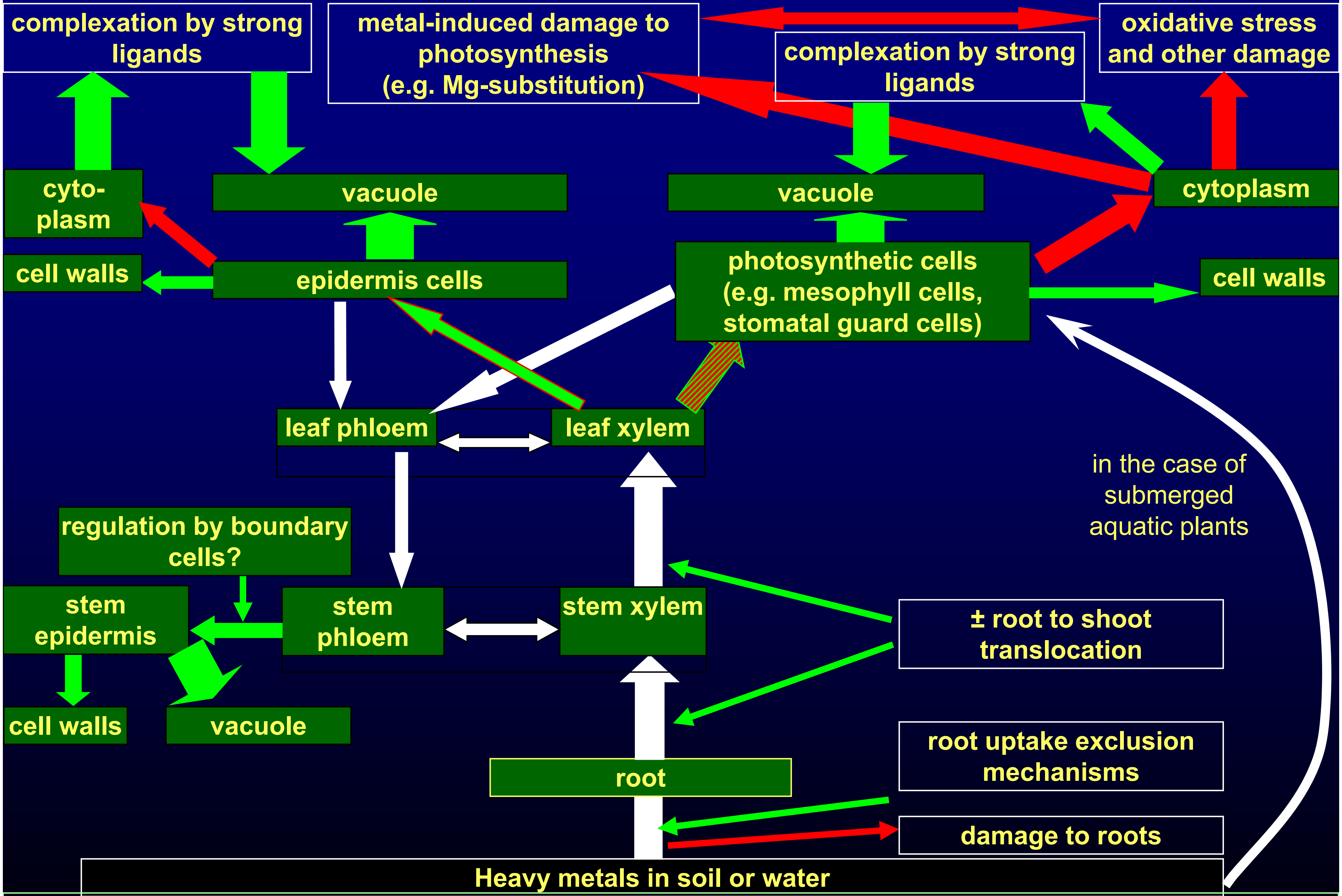


Principles



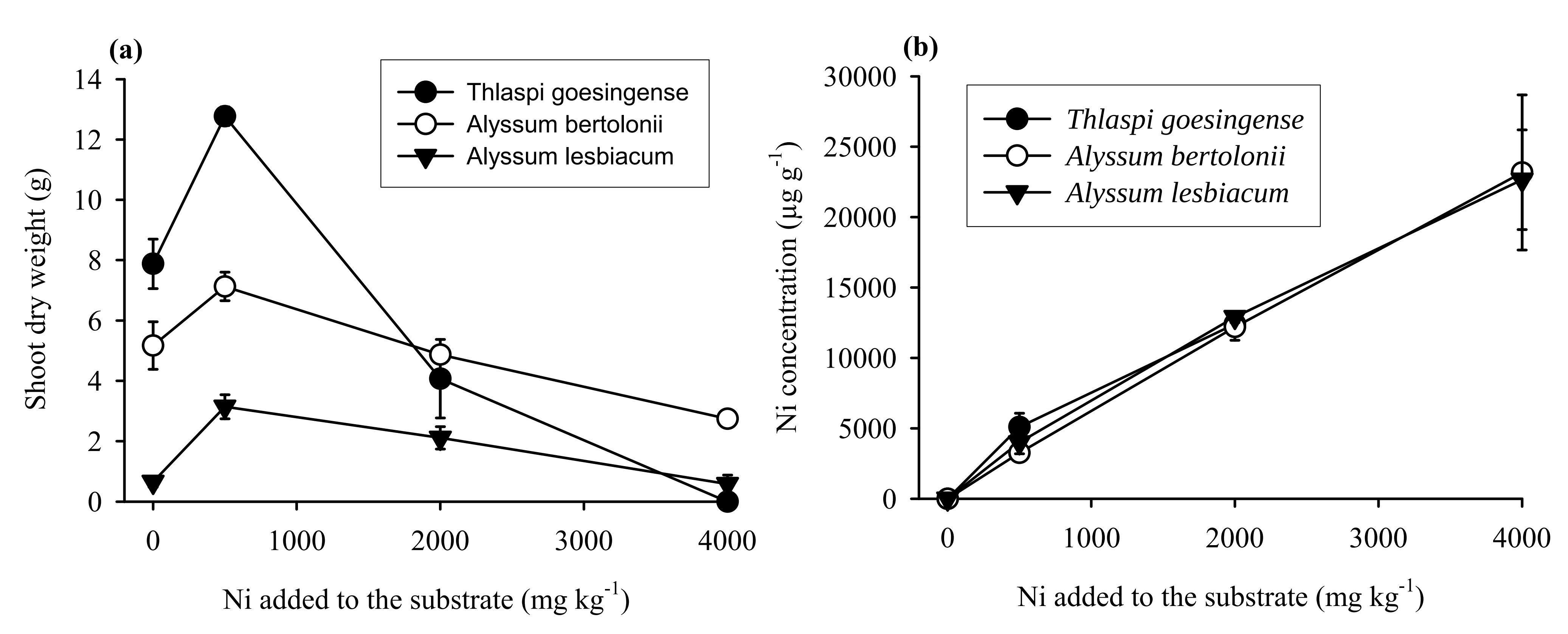
Exclusively toxic metal (e.g. mercury)
Micronutrient (e.g. copper)
stimulatory
Effect
inhibitory
Concentration

Pathways of metal metabolism



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

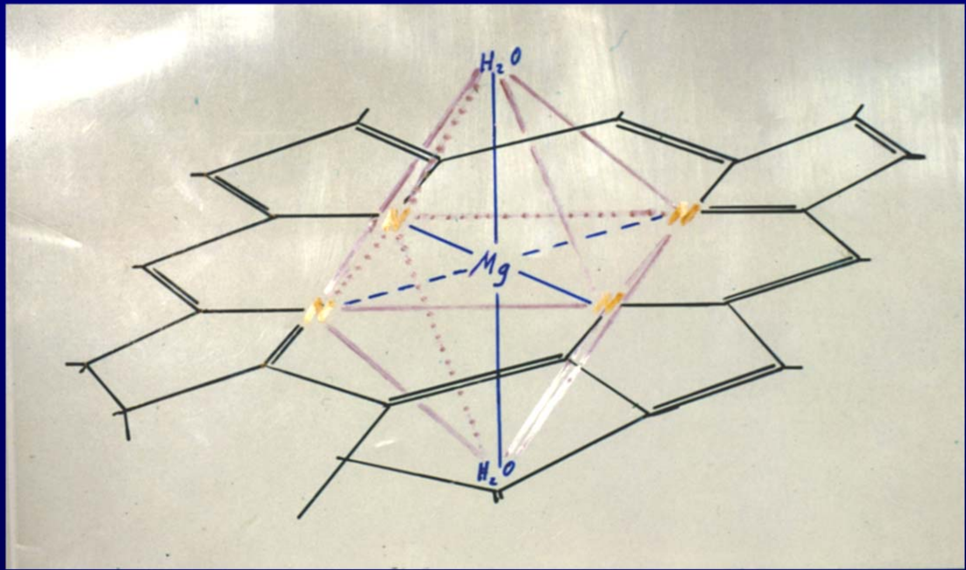
We show you plants that strongly like potentially toxic metals…

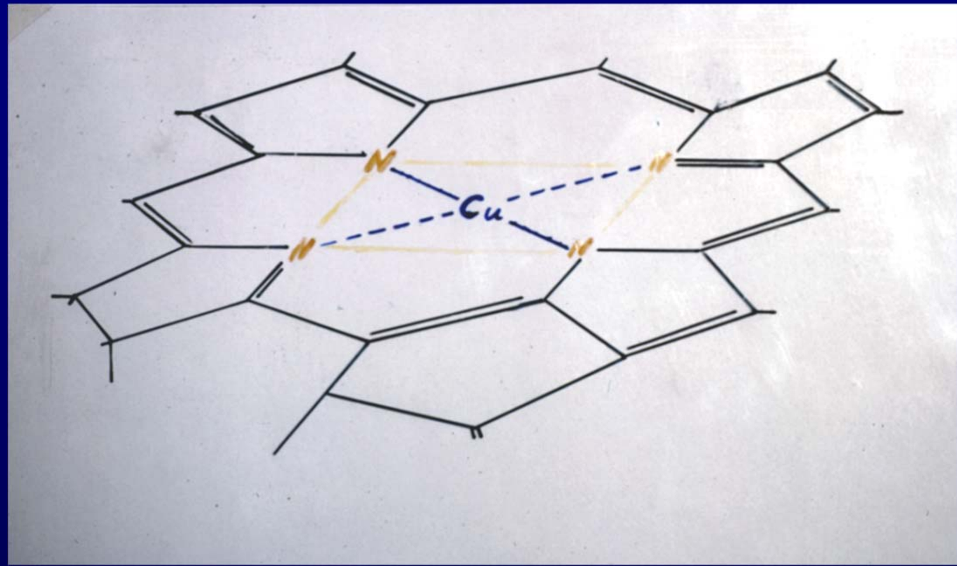


Effects of Ni^{2+} addition on hyperaccumulator plant growth and Ni^{2+} concentration in shoots

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…



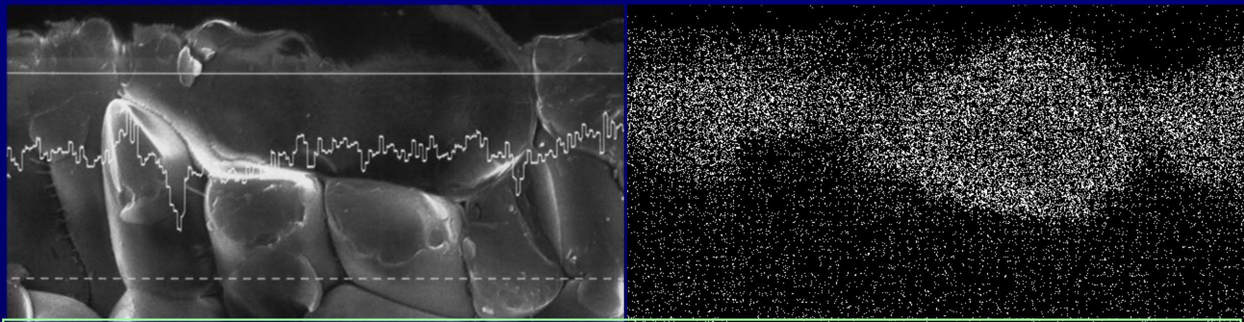


- shift of absorbance/fluorescence bands --> less energy transfer
- different structure --> proteins denature
- do not readily perform charge separation when in reaction centre
- unstable singlet excited state --> “black holes“ for excitons

…and how plants defend themselves against that toxicity.

Mechanisms

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

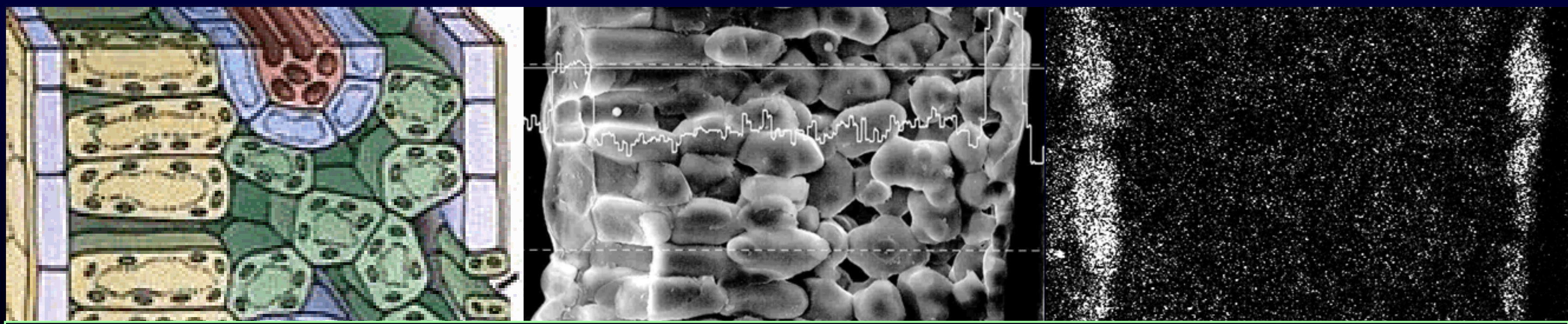


Küpper H et al., 2001, J Exp Bot 52 (365), 2291-2300

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

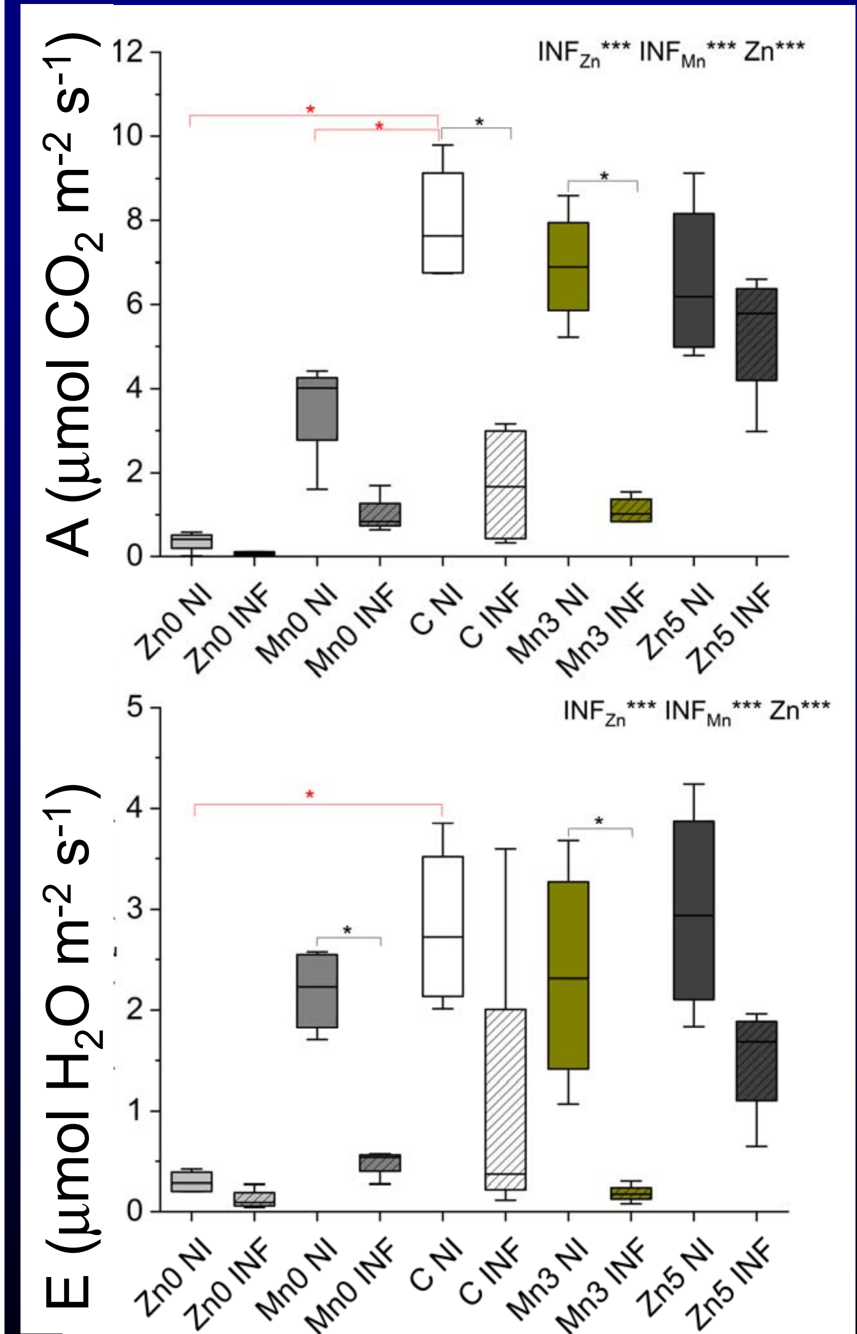
- Sequestration in the vacuole:
  - plant-specific mechanism (animals+bacteria usually don‘t have vacuoles...)
  - very efficient, because the vacuole does not contain sensitive enzymes
  - saves the investment into the synthesis of strong ligands like phytochelatins
  - main mechanism in hyperaccumulators

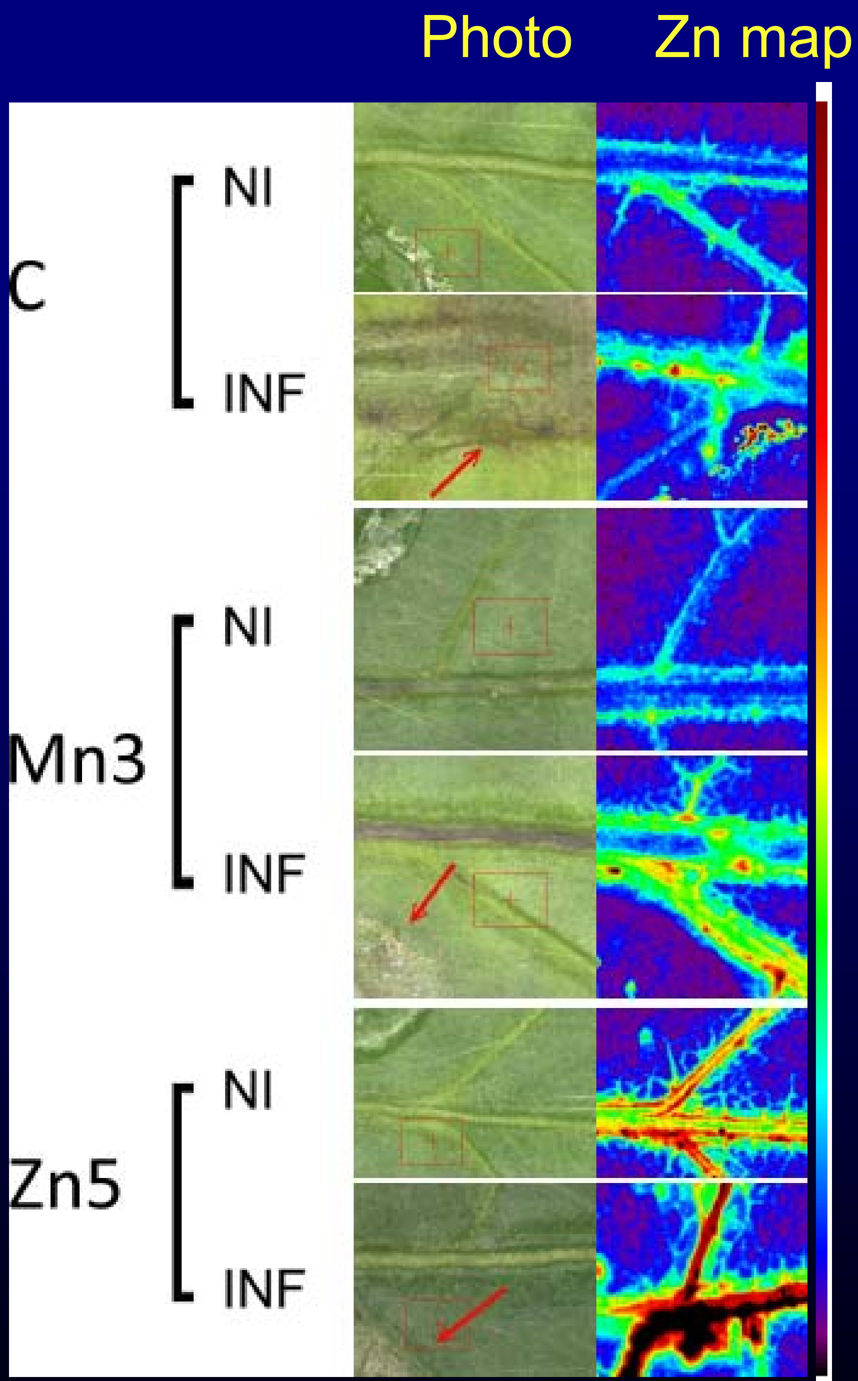
- Sequestration in least sensitive tissues, e.g. the epidermis instead of the photosynthetically active mesophyll



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

We will show you original data from recent research…

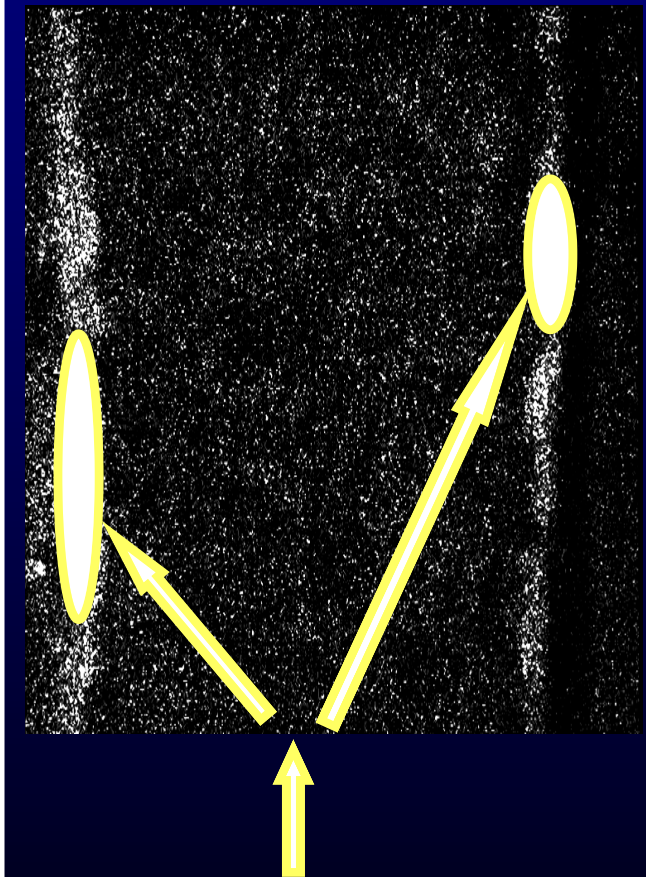




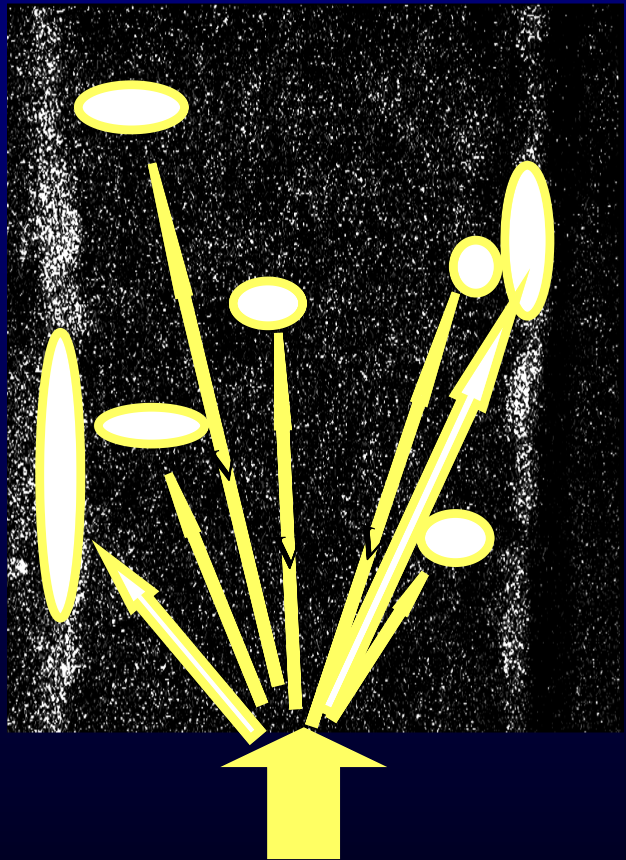
Kuvelja et al. Plant Sci. 2024, 112060.

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

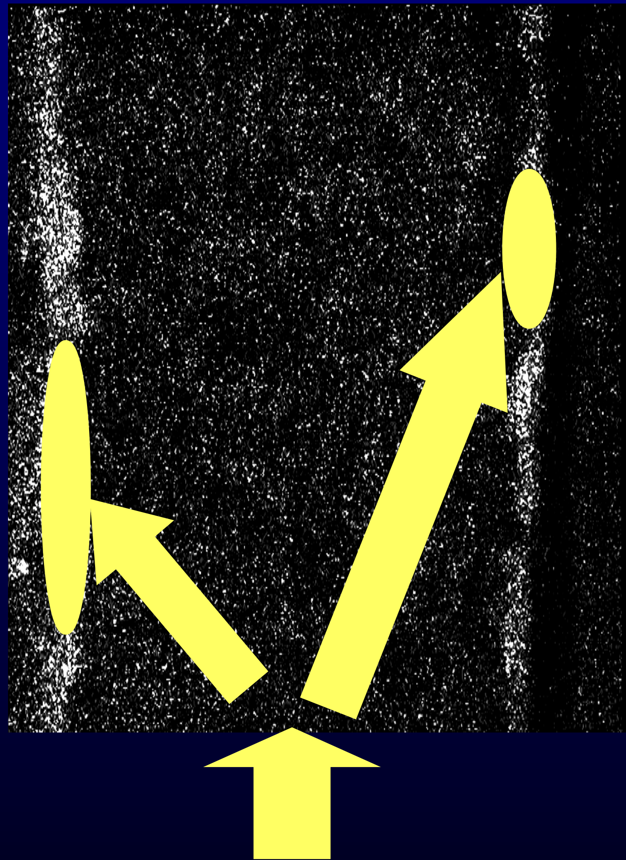
Normal: Sequestration in epidermal storage cells



Stressed: additional sequestration in selected mesophyll cells



Acclimated: Enhanced sequestration in epidermal storage cells



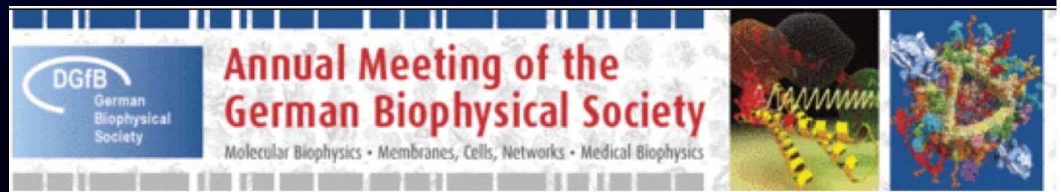
Küpper H, Aravind P, Leitenmaier B, Trtilek M, Šetlík I (2007) New Phytologist 175, 655-674

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

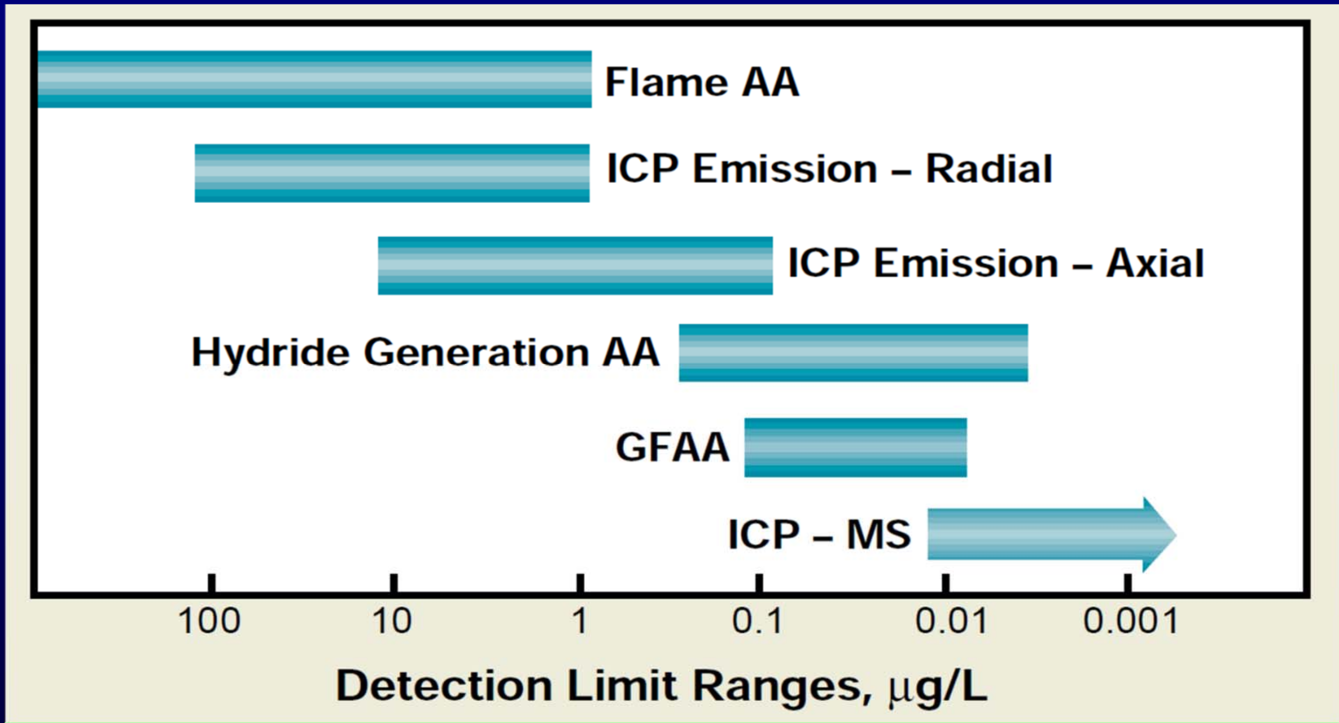
… and how to publish them

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



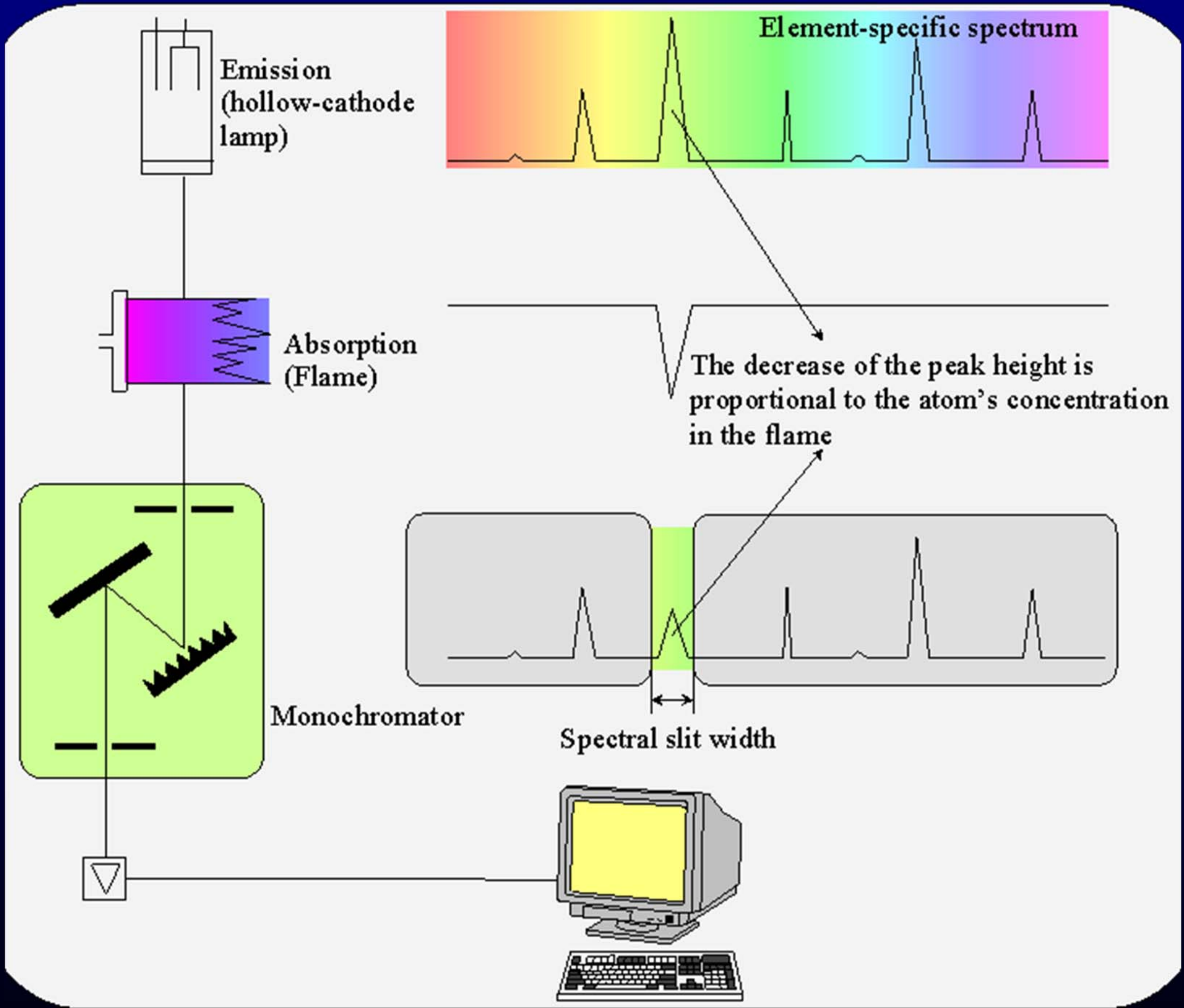
From: Thermo electron product information

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)

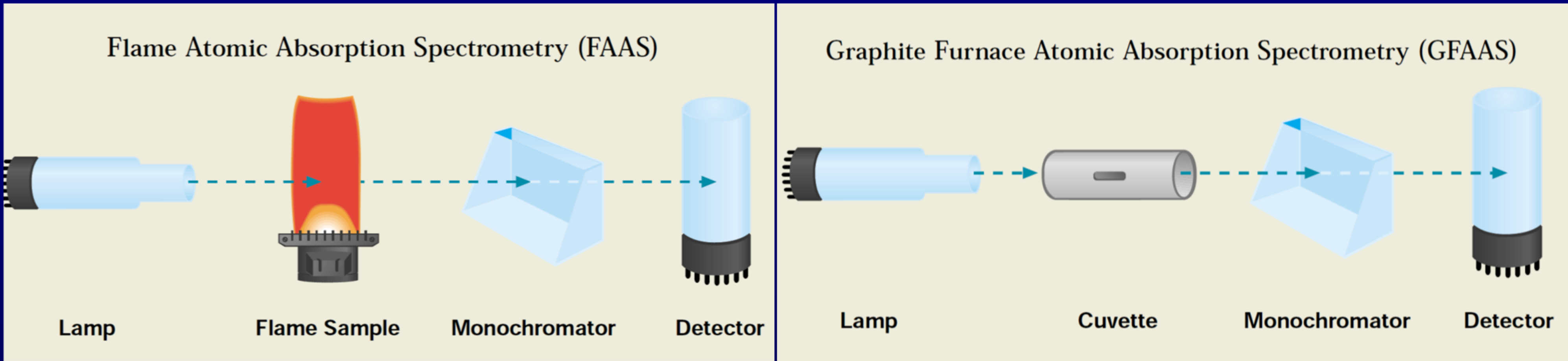


Advantages:
- easy to use,
- fast if only 1 element is needed
- affordable

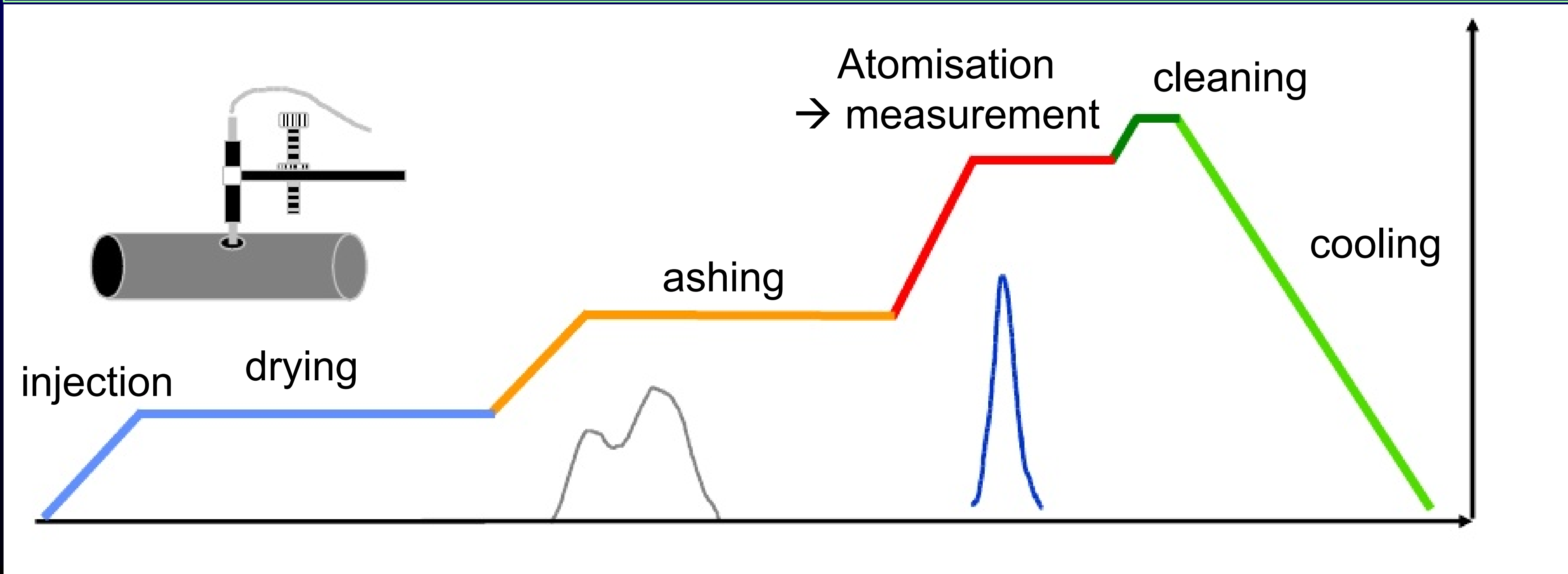
Disadvantages:
- insensitive for some elements (e.g. sulphur)
- slow if many elements are needed

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

Principle



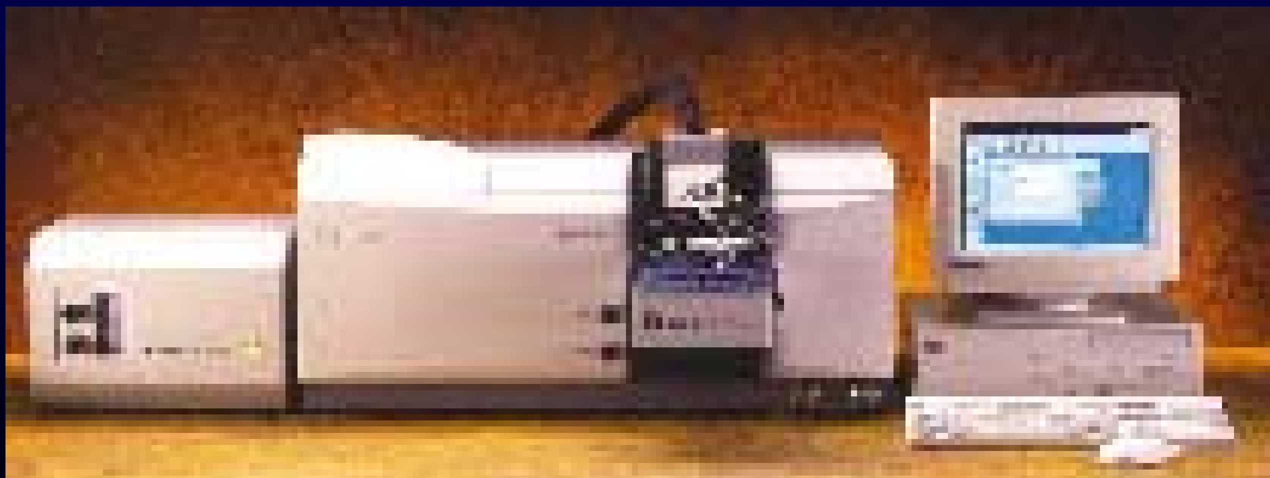
From: http://www.scribd.com/doc/15784148/Graphite-Furnace-Analysis (bottom); Thermo electron teaching file (top)



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

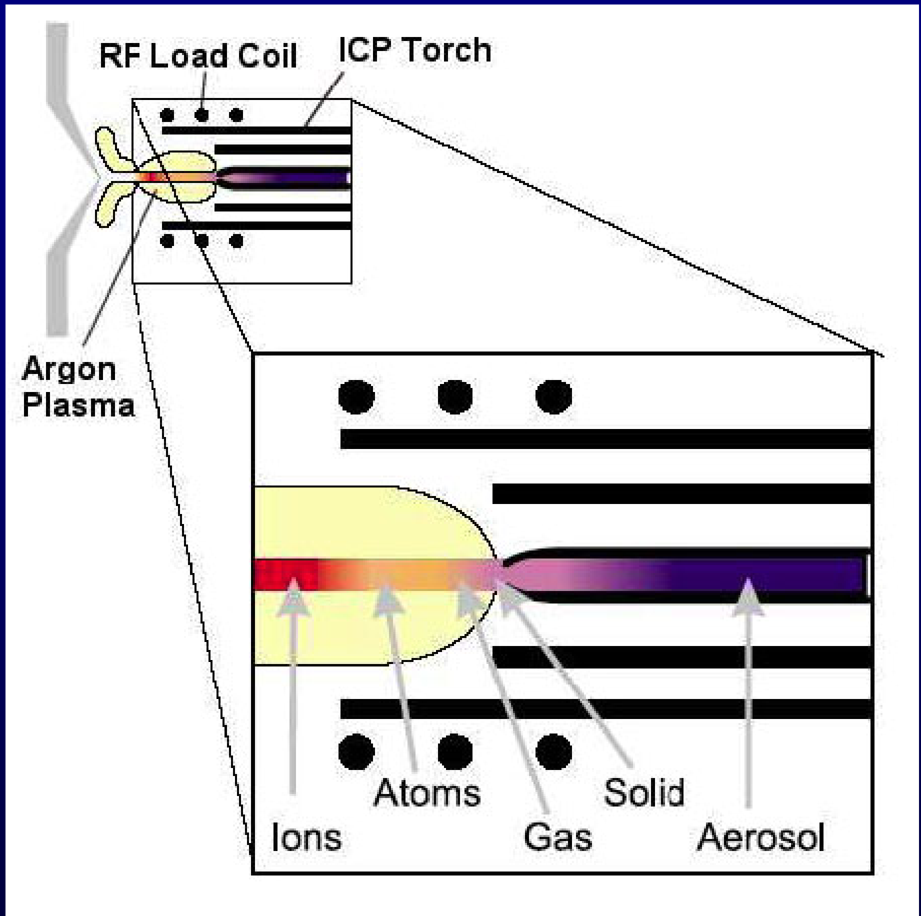
Criteria	Flame	Furnace
Elements	67	48
Sensitivity	ppm - %	ppt – ppb
Precision	Good	Fair
Interferences	Few	Many
Speed	Rapid	Slow
Simplicity	Easy	More complex
Flame Hazards	Yes	No
Automation	Yes	Yes (unattended)
Operating Cost	Low	Medium

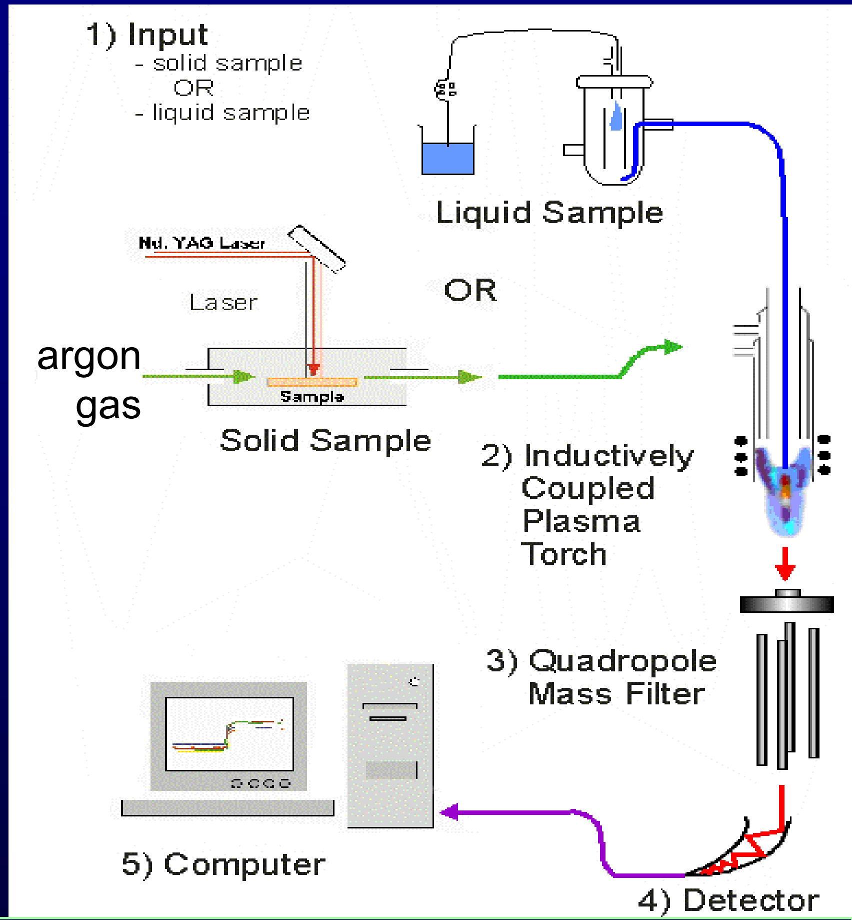
From: http://www.scribd.com/doc/15784148/Graphite-Furnace-Analysis



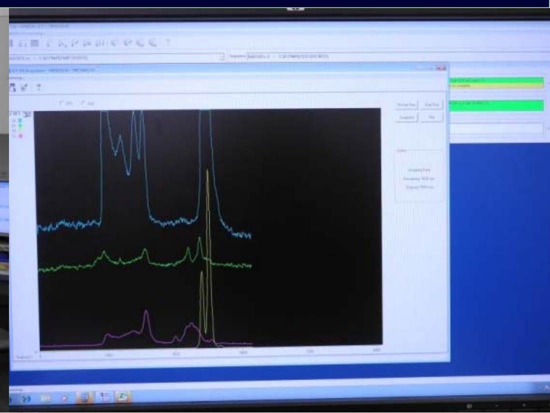
From: GBC product information

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

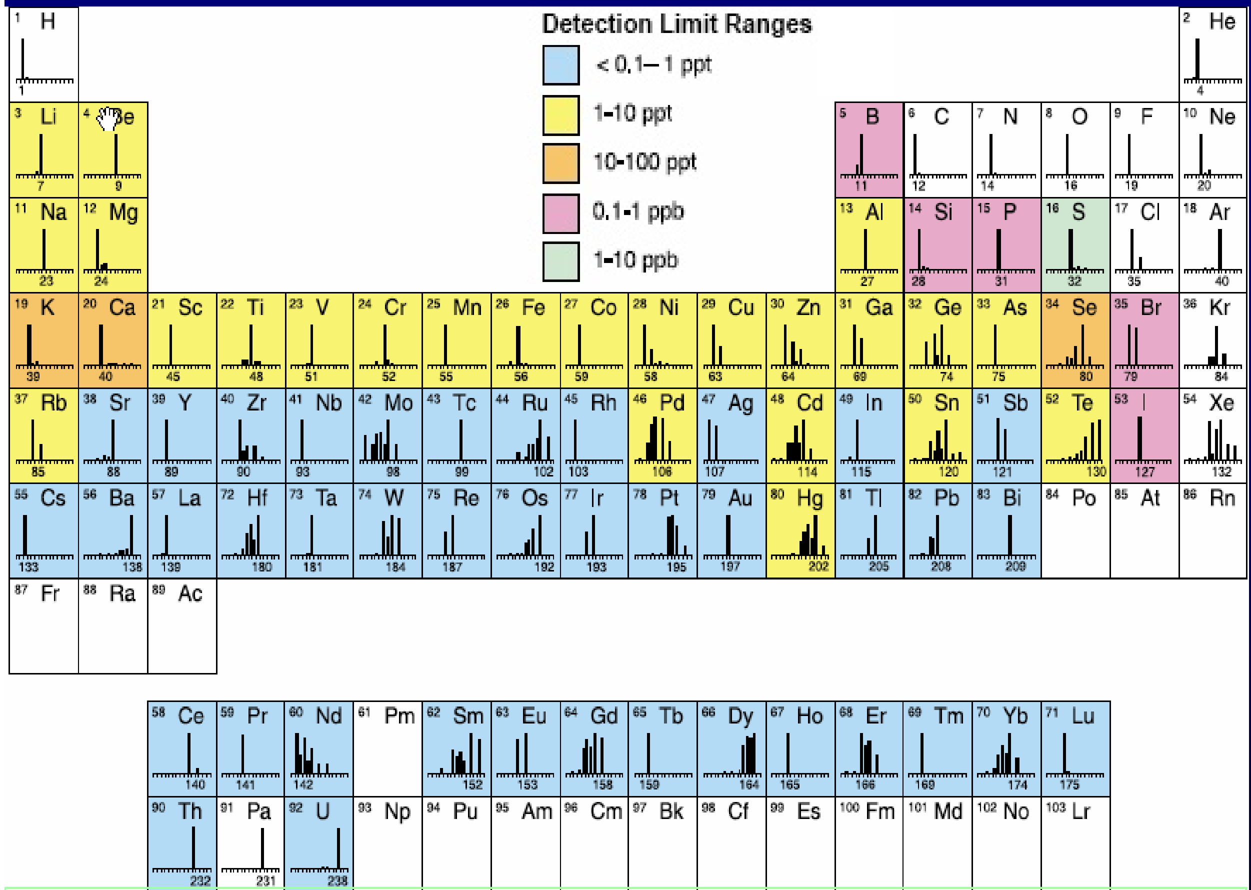




From: Perkin Elmer teaching file (top); LC-ICP-MS experiment at UFZ Leipzig (bottom)



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

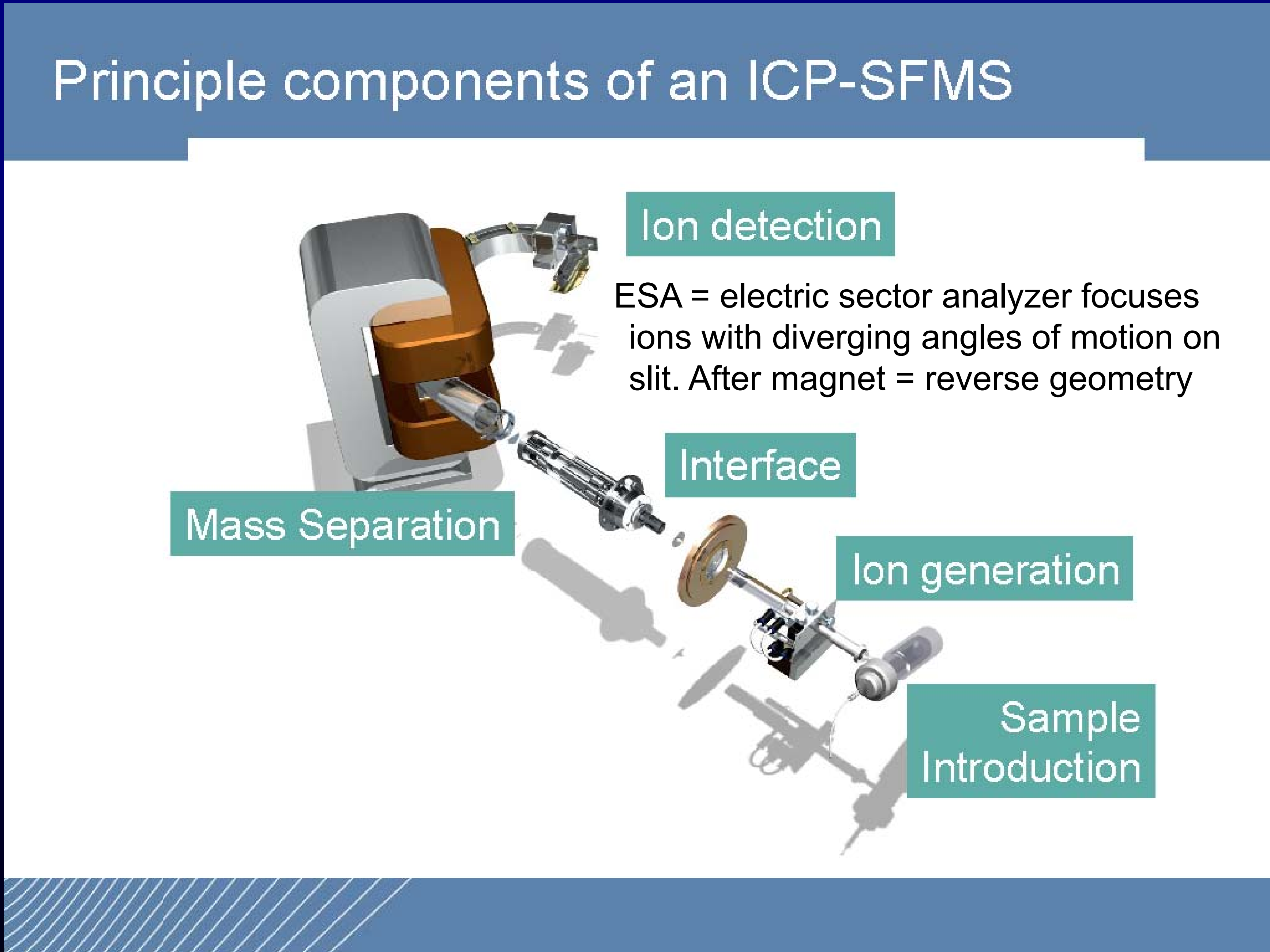


From: Perkin Elmer product information

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.

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



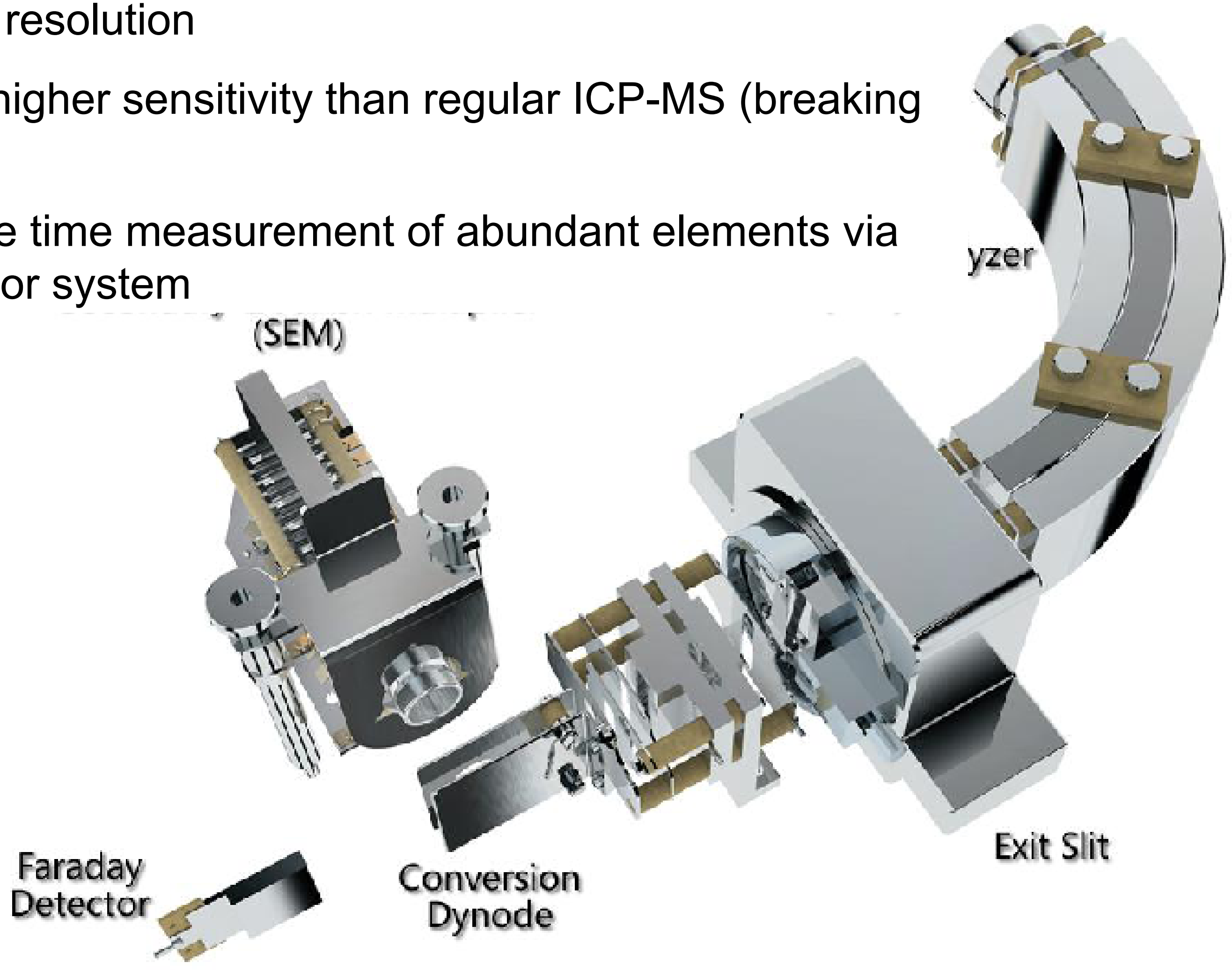
From: Thermo Scientific product information

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

ELEMENT XR Ion Detection System

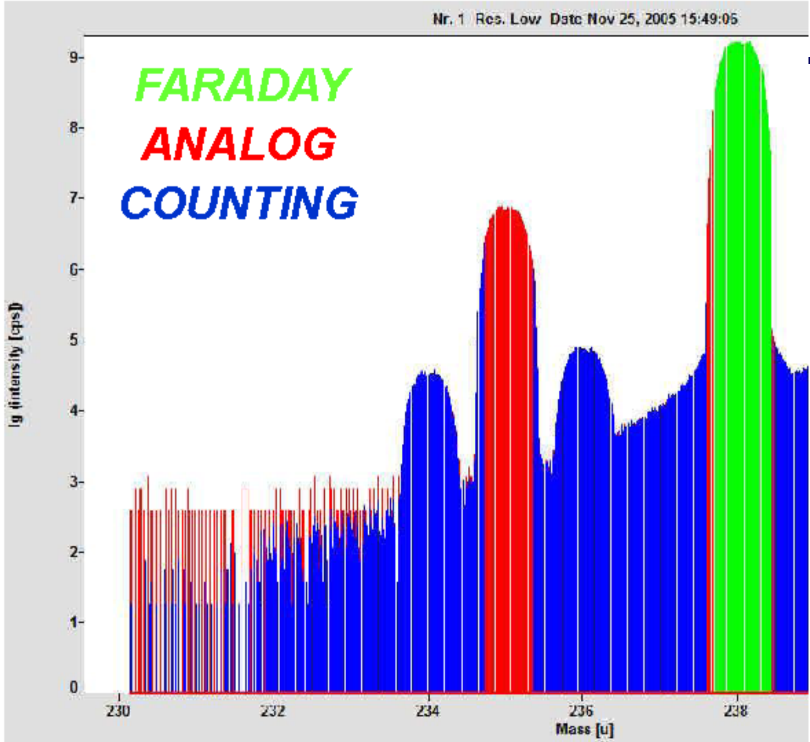
Advantages of element XR vs. quadrupole ICP-MS

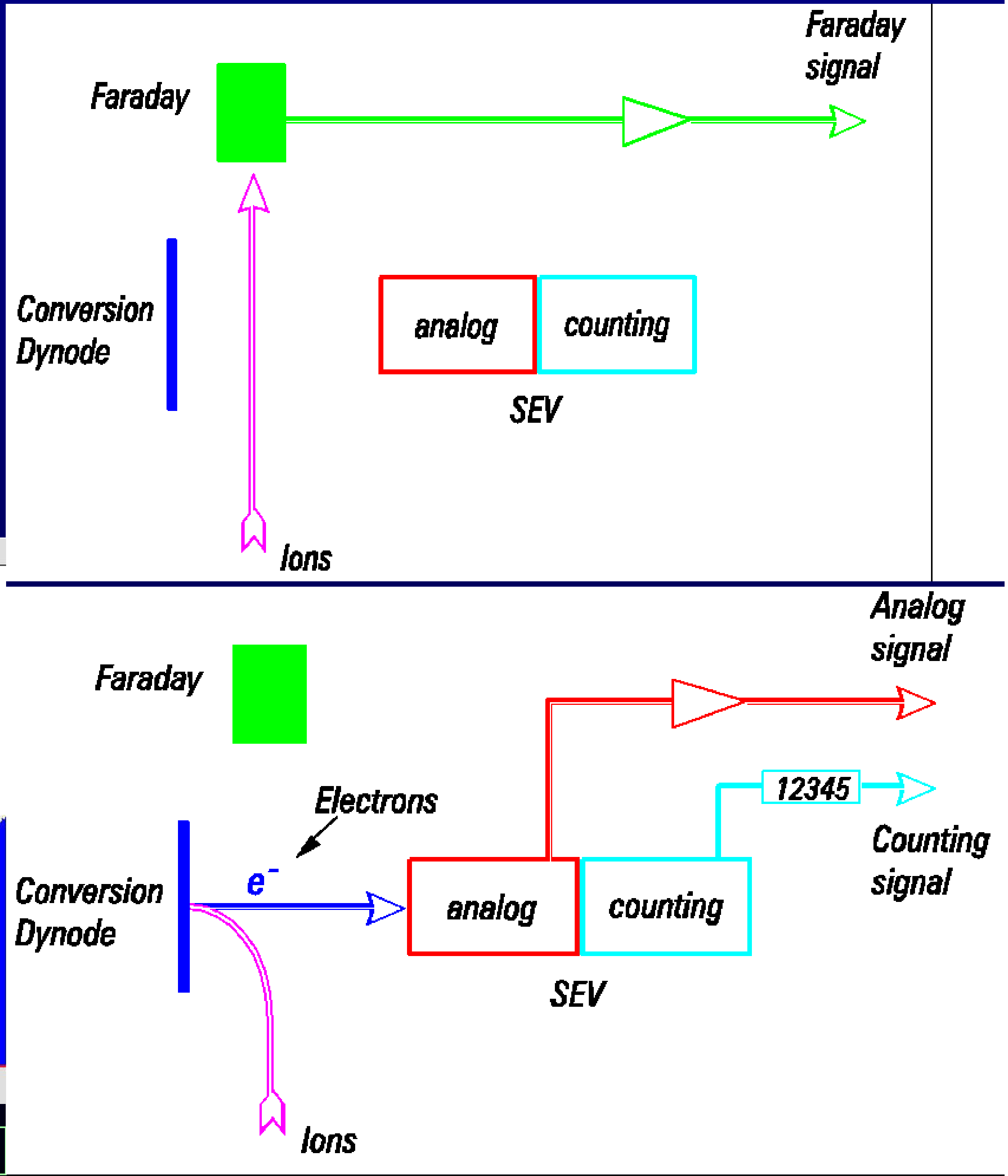
- High mass resolution
- Still much higher sensitivity than regular ICP-MS (breaking ppq barrier)
- At the same time measurement of abundant elements via triple detector system



From: Thermo Scientific product information

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

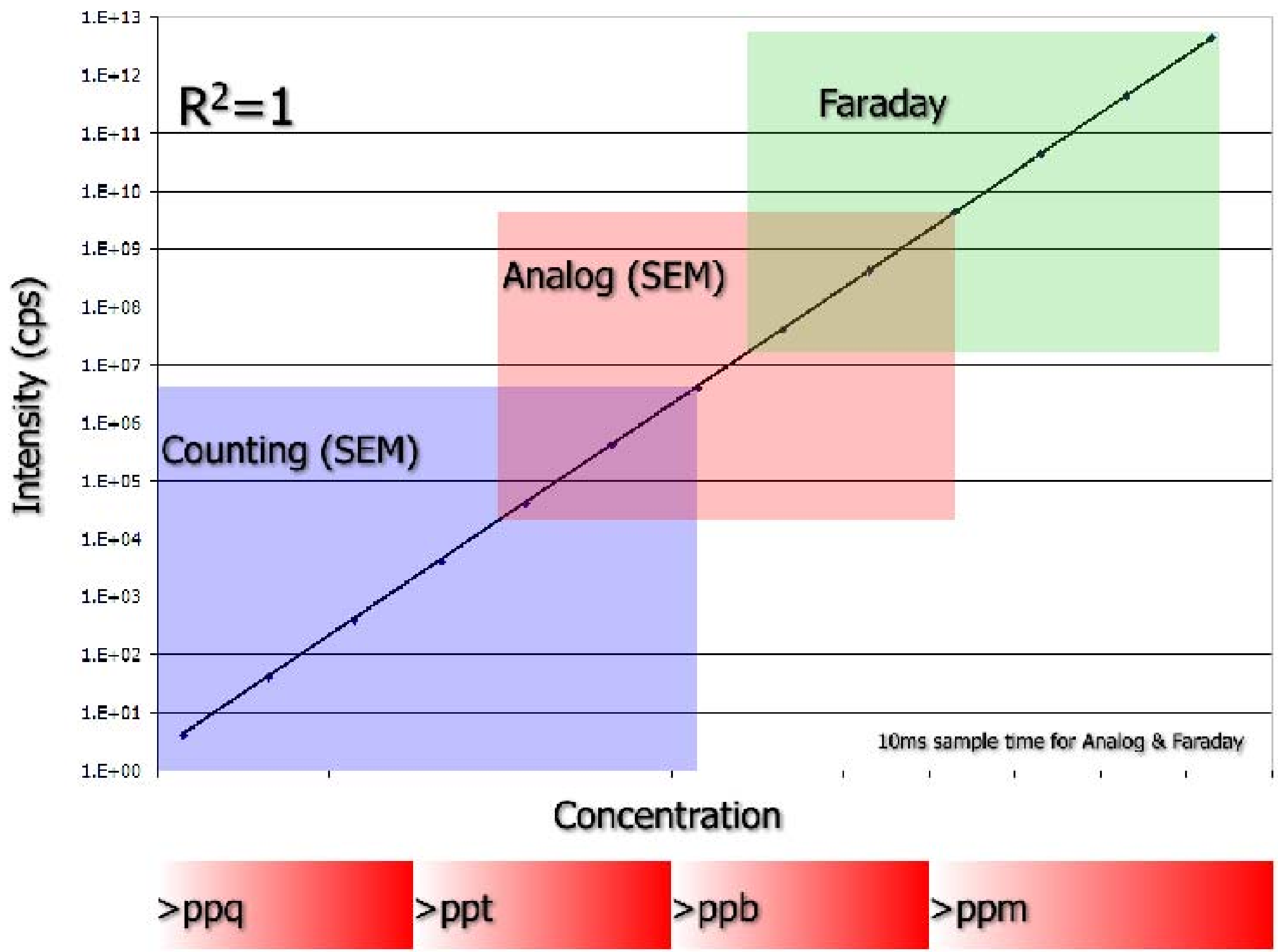




From: Thermo Scientific product information

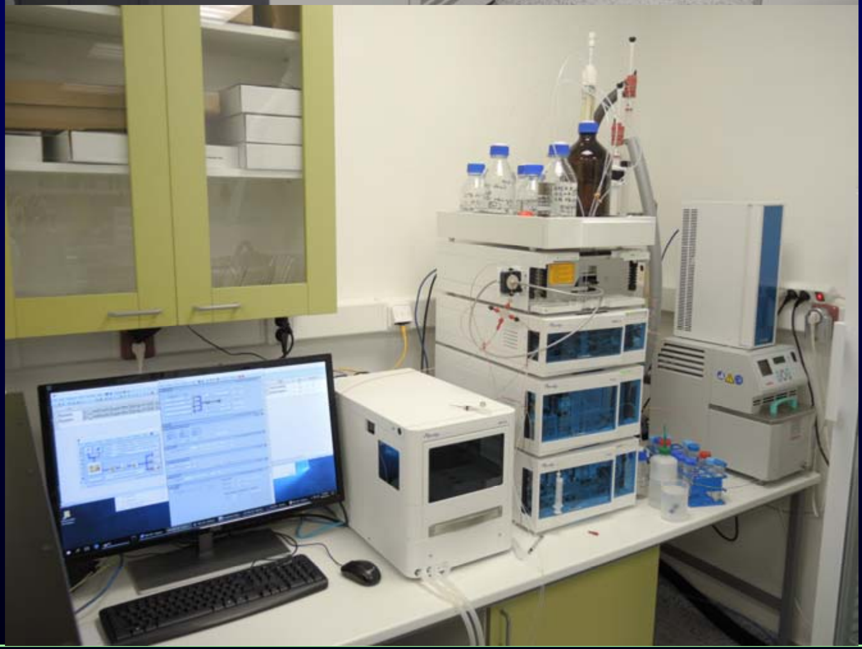
Metal content – methods of Measurement
Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

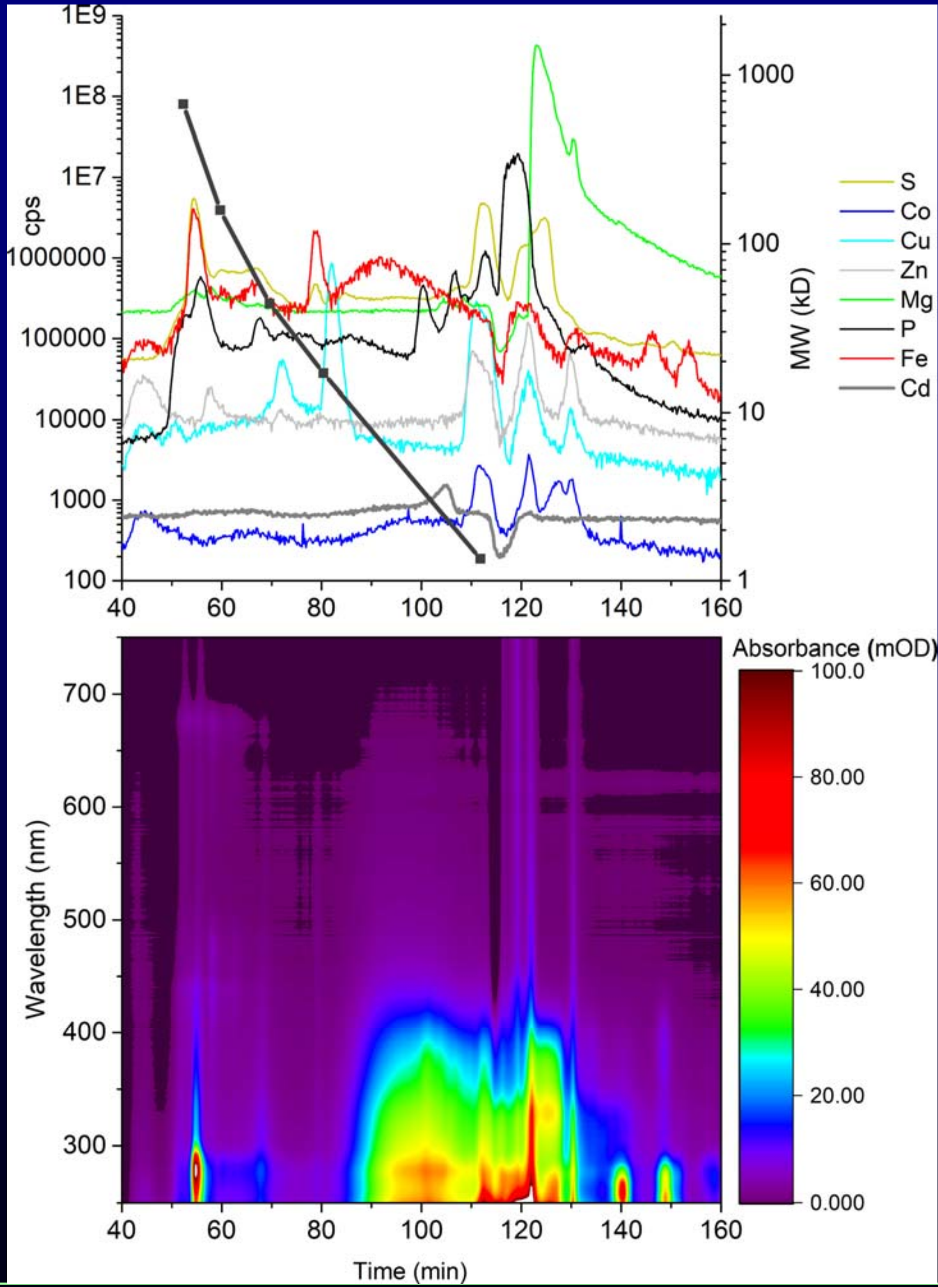
Extended Dynamic Range in the Finnigan ELEMENT XR



From: Thermo Scientific product information

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