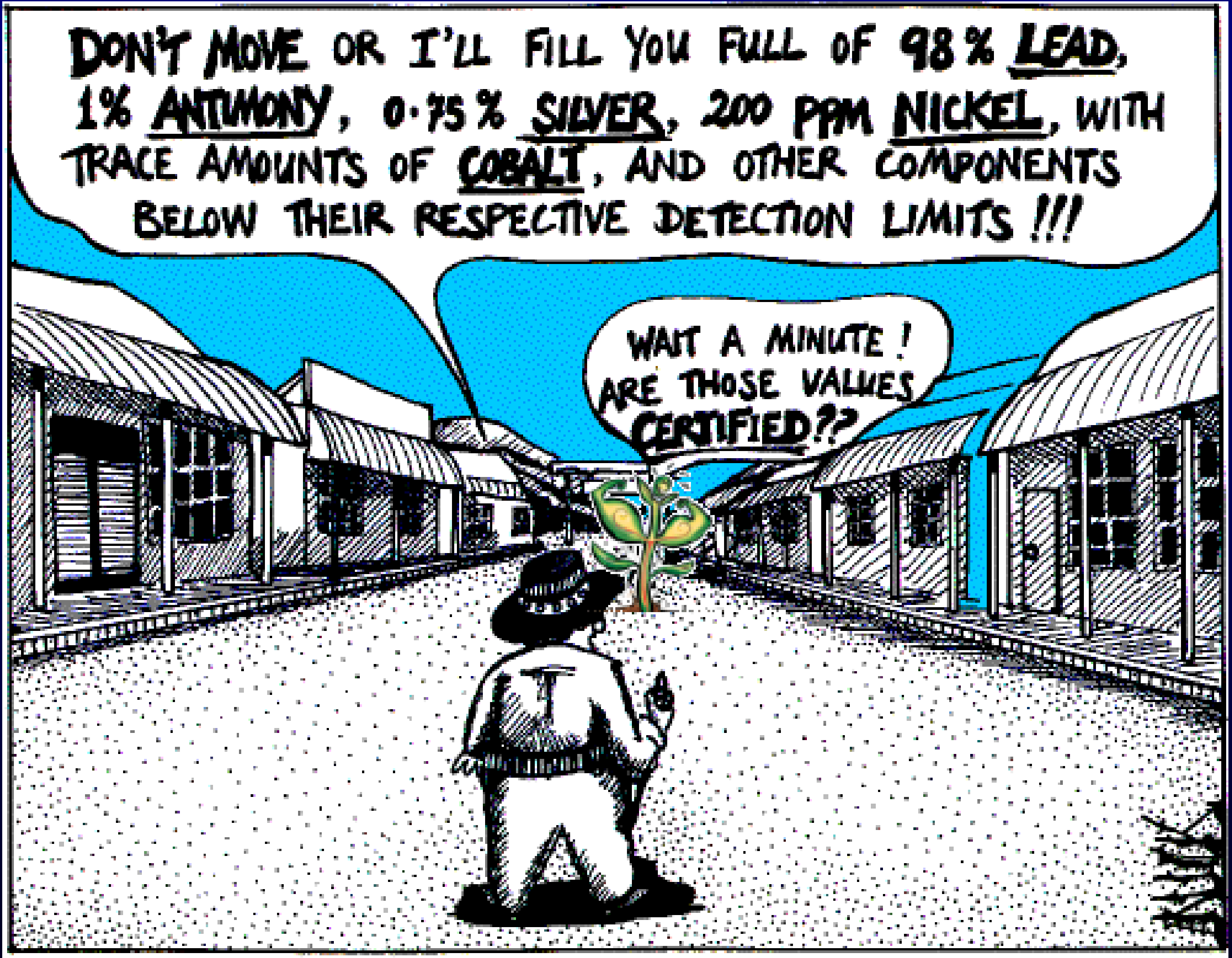
Heavy Metals and Plants – a complicated relationship
→ Heavy metal resistance



Heavy metal-hyperaccumulation in the Wild West

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

Variability of metal contents from deficiency to toxicity (I): A decisive factor for biodiversity

Plant communities in low metal habitats


Mount hood (Oregon, USA), From: commons.wikimedia.org

↑ Non-metalliferous alpine meadow


Alentejo, Portugal, From: commons.wikimedia.org

↑ Non-polluted site in the same region

Plant communities in high metal habitats

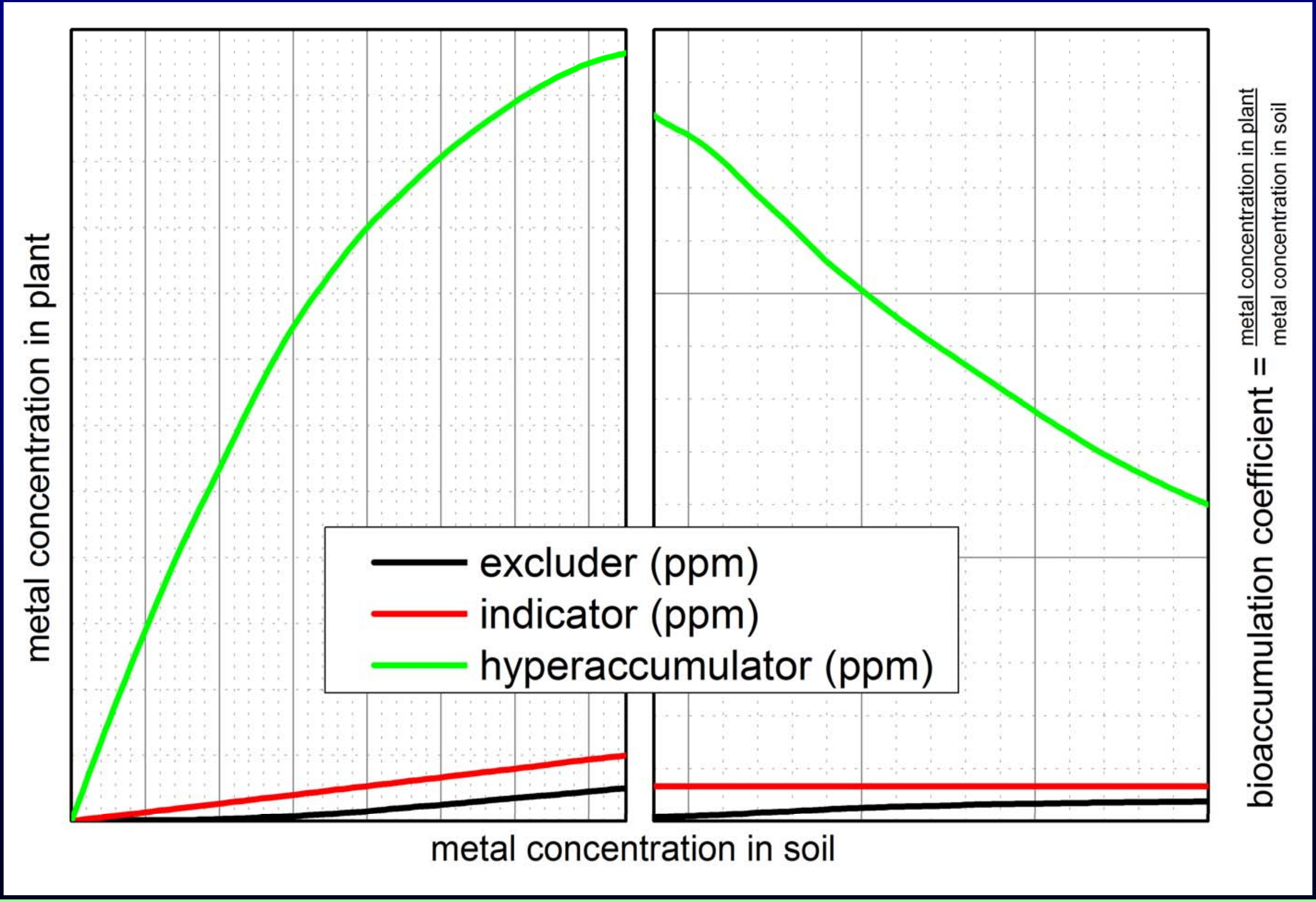

Slate Mountain serpentine barren (North Carolina, USA),
From: US forest service

↑ Natural serpentine barren


Sao Domingos mine (Alentejo, Portugal),
From: commons.wikimedia.org

↑ Antropogenic (mining) polluted site

Heavy metal uptake characteristics of plants

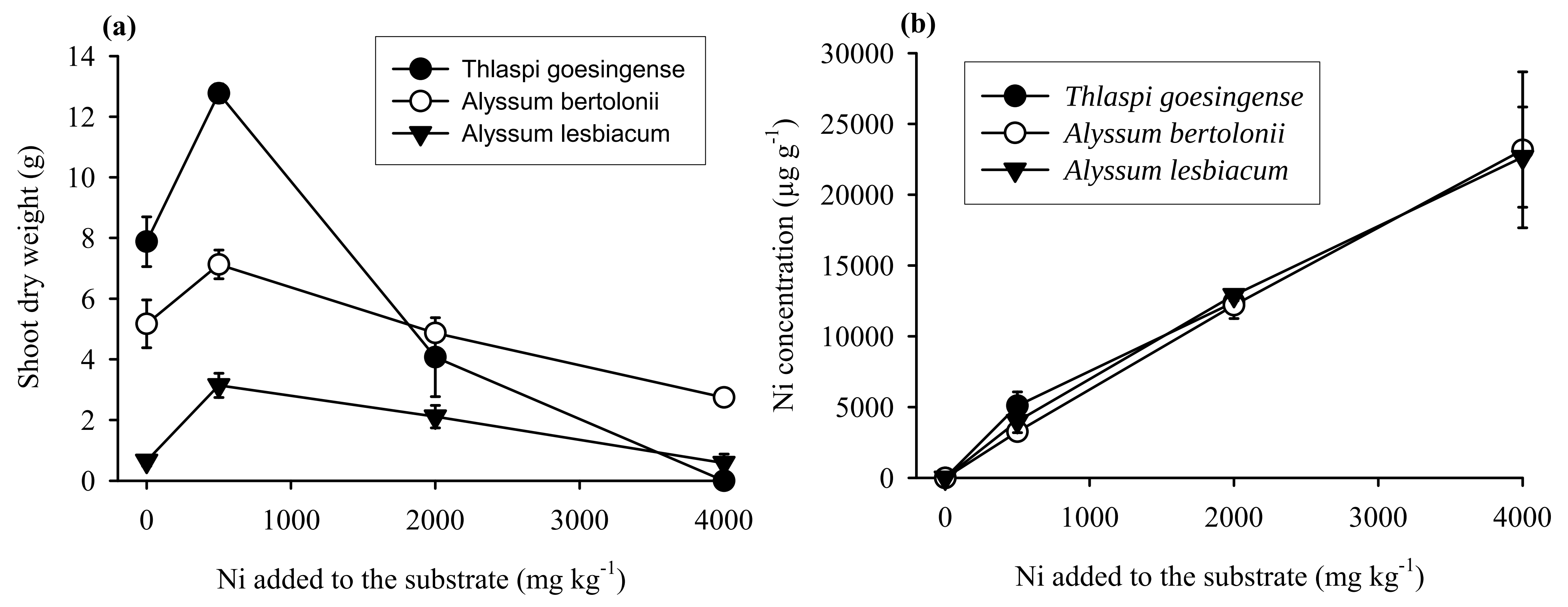


Reviews: Küpper H, Kroneck PMH (2005) Metal ions Life Sci 2, 31-62

Leitenmaier B, Küpper H (2013) Frontiers in Plant Science, https://doi.org/10.3389/fpls.2013.00374

Küpper H, Leitenmaier B (2013) Chapter 12, Volume 11 of series "Metal Ions in Life Sciences". (Eds: Sigel A, Sigel H, Sigel RKO). Springer Science + Business Media B.V., Dordrecht; pp. 373-394. https://doi.org/10.1007/978-94-007-5179-8_12

Plants with an unusual appetite: Heavy metal hyperaccumulation



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

Cadmium deficiency in the Cd/Zn hyperaccumulator *Noccaea* (formerly *Thlaspi*) *caerulescens*

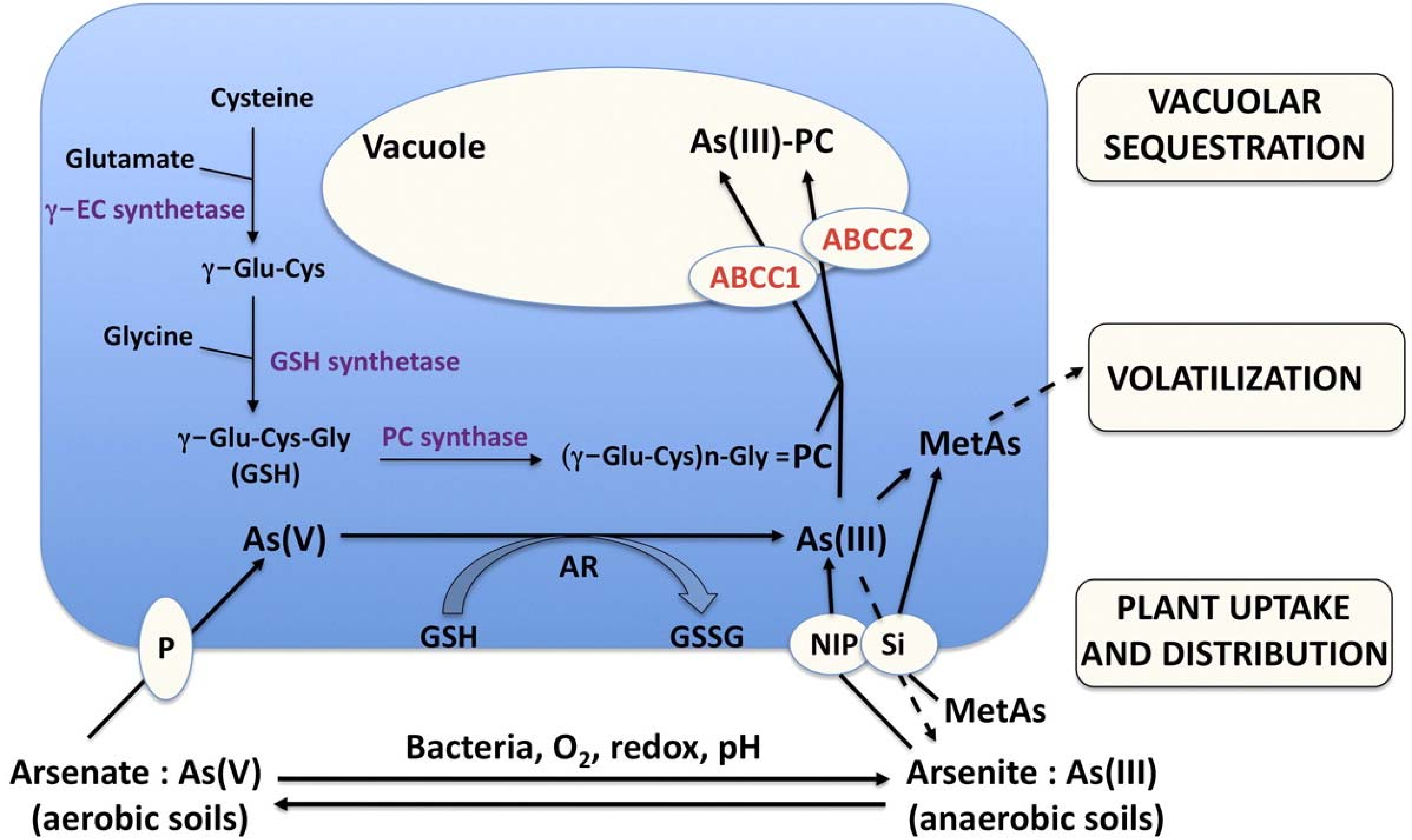
With 10 µM cadmium in the nutrient solution
--> healthy plants

Without cadmium in the nutrient solution
--> damage due to attack of insects

→ Various studies by many labs have shown that hyperaccumulation serves as defence against pathogens and herbivores

Küpper H, Kroneck PMH (2005) MIBS 44 (Sigel et al., eds), chapter 5

Metal(loid) detoxification: overview of mechanisms proposed for arsenic

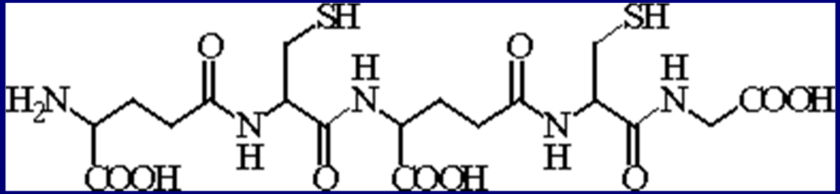


Briat J PNAS 2010;107:20853-20854

Metal detoxification by complexation

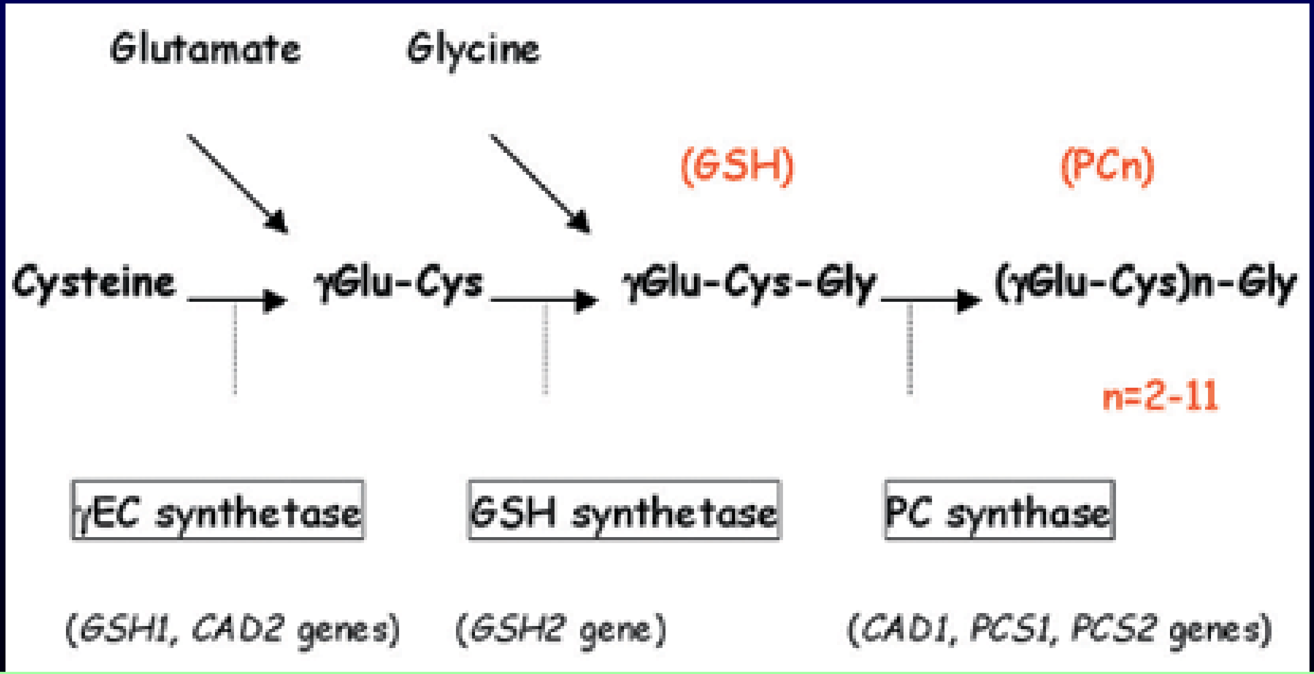
General Resistance-Mechanisms

Heavy metal detoxification with strong ligands



Phytochelatins (PCs)

- Bind Cd^{2+} with very high affinity, also As(III) and As(V), but many other heavy metal ions with low affinity
- Synthesized by phytochelatin-synthase
- PC synthase activated by blocked thiols of glutathion and similar peptides



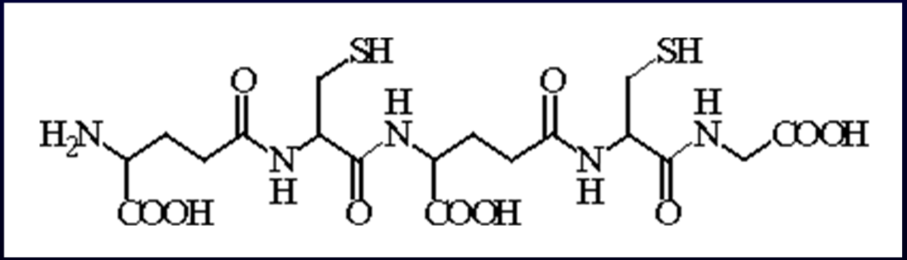
From: Inouhe M (2011) Braz J Plant Physiol 17, 65-78

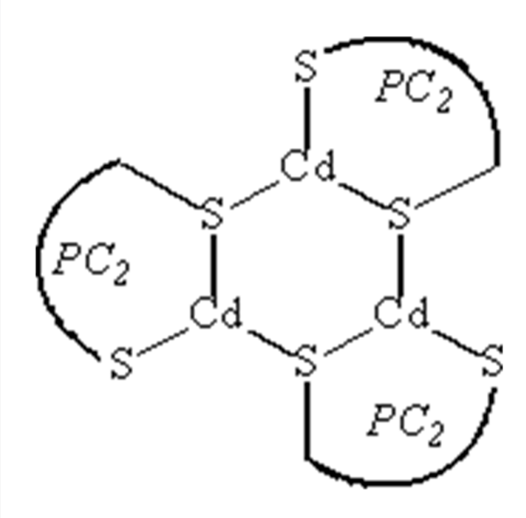
General Resistance-Mechanisms

Heavy metal detoxification with strong ligands

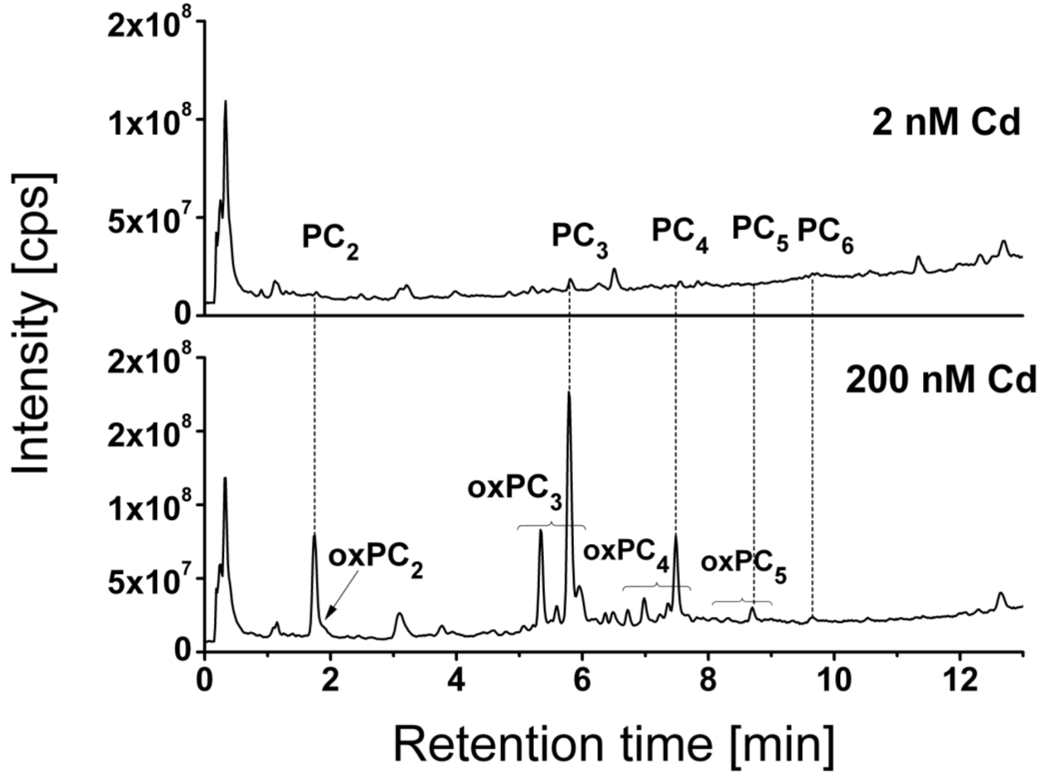
Phytochelatins (PCs)

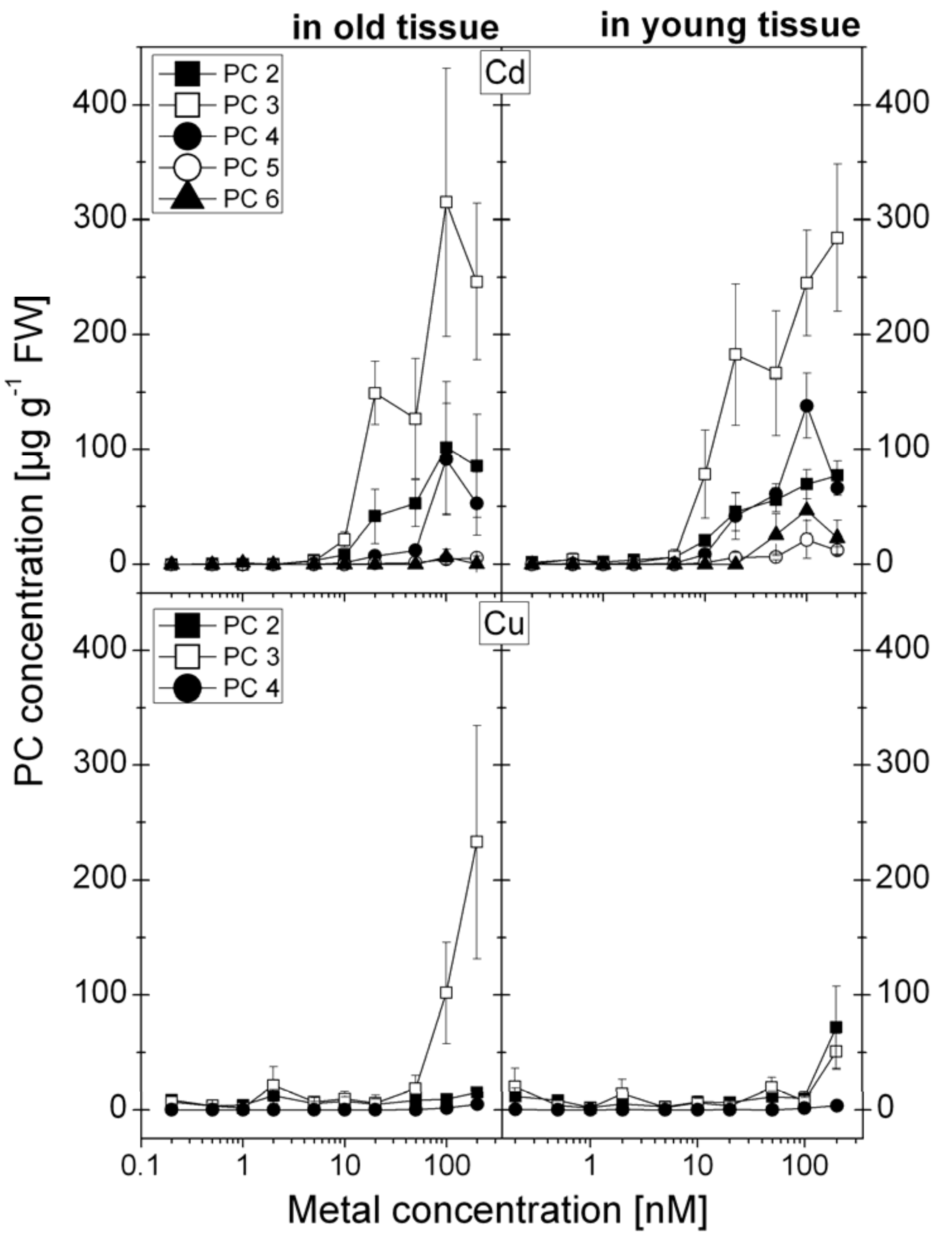
- They are the main Cd-resistance and As-resistance mechanism in most plants (**except** hyperaccumulators) and many animals
- PCs bind Cd^{2+} in the cytoplasm, then the complex is sequestered into the vacuole.
- Phytochelatin-Cd-aggregates are formed in vacuoles





Detoxification in non-accumulator plants:
Induction of phytochelatins in *Ceratophyllum demersum*





-Higher Cd concentration → more different PCs
+ much higher amount of PC 2-4
-Threshold concentration specific for each PC
-Most prominent: PC3 at 20nM
- **PCs induced → no role in homeostasis**,
reported presence at „0“ metal(loid) stress most
likely due to trace contaminations of chemicals

Andresen et al., 2013, Metallomics, 5, 1377-1386

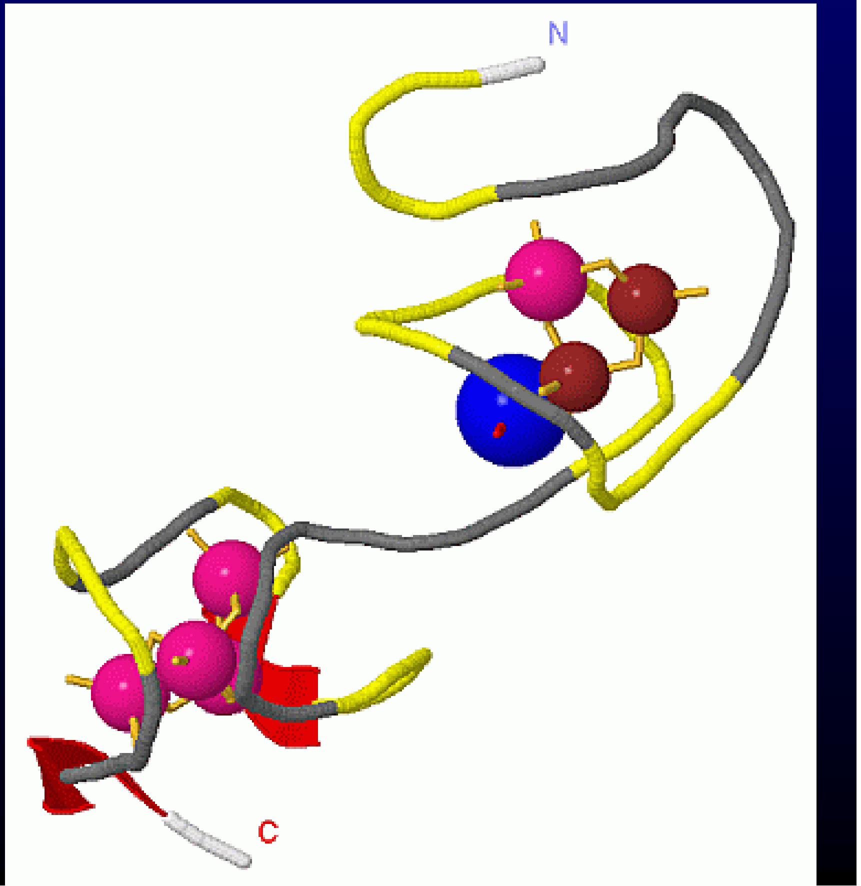
General Resistance-Mechanisms

Heavy metal detoxification with strong ligands

Glutathion

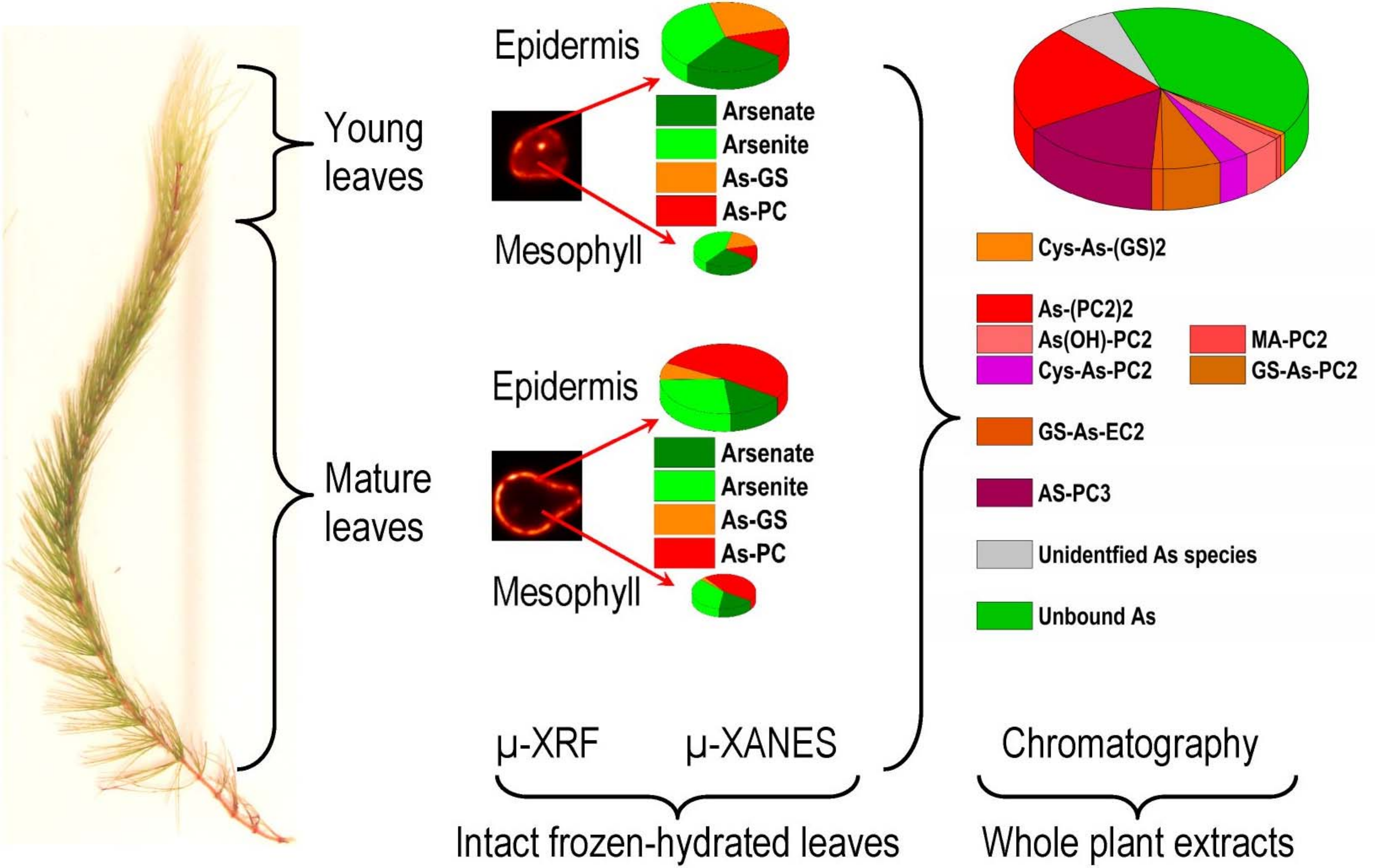
- Also glutathione itself, the building block of phytochelatins, can bind and thus detoxify Cd and As - the *in vivo* relevance is questionable, but occurring under heavy stress

Metallothionins



- MTs of type I und II bind Cu^+ with high affinity and seem to be involved in its detoxification.
- Maybe Cd binding by MTs is relevant for detoxification as well
- BUT: Main role of MTs in plants seems to be metal distribution during the normal (non-stressed) metabolism

Speciation of arsenic in a non-accumulator plant



Young leaves

Epidermis

Mesophyll

- 🟩 Arsenate
- 🟩 Arsenite
- 🟧 As-GS
- 🟥 As-PC

Mature leaves

Epidermis

Mesophyll

- 🟩 Arsenate
- 🟩 Arsenite
- 🟧 As-GS
- 🟥 As-PC

- 🟧 Cys-As-(GS)2
- 🟥 As-(PC2)2
- 🟥 As(OH)-PC2
- 🟥 MA-PC2
- 🟪 Cys-As-PC2
- 🟧 GS-As-PC2
- 🟧 GS-As-EC2
- 🟪 AS-PC3
- ⬜ Unidentfied As species
- 🟩 Unbound As

µ-XRF µ-XANES

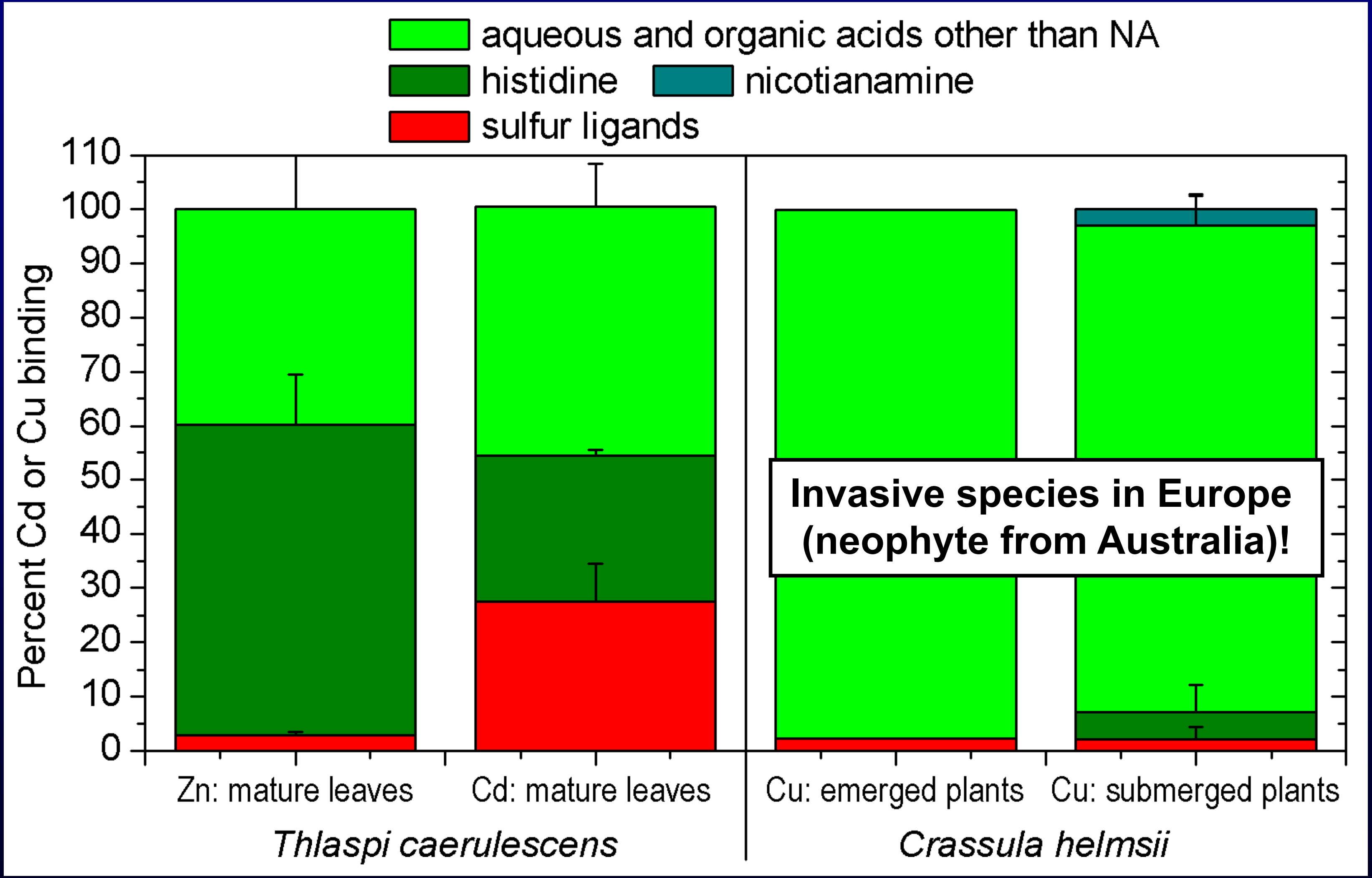
Intact frozen-hydrated leaves

Chromatography

Whole plant extracts

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

Speciation of hyperaccumulated metals revealed by EXAFS: Cd in the Cd/Zn-hyperaccumulator *N. caerulescens* and Cu in the Cu-hyperaccumulator *C. helmsii*



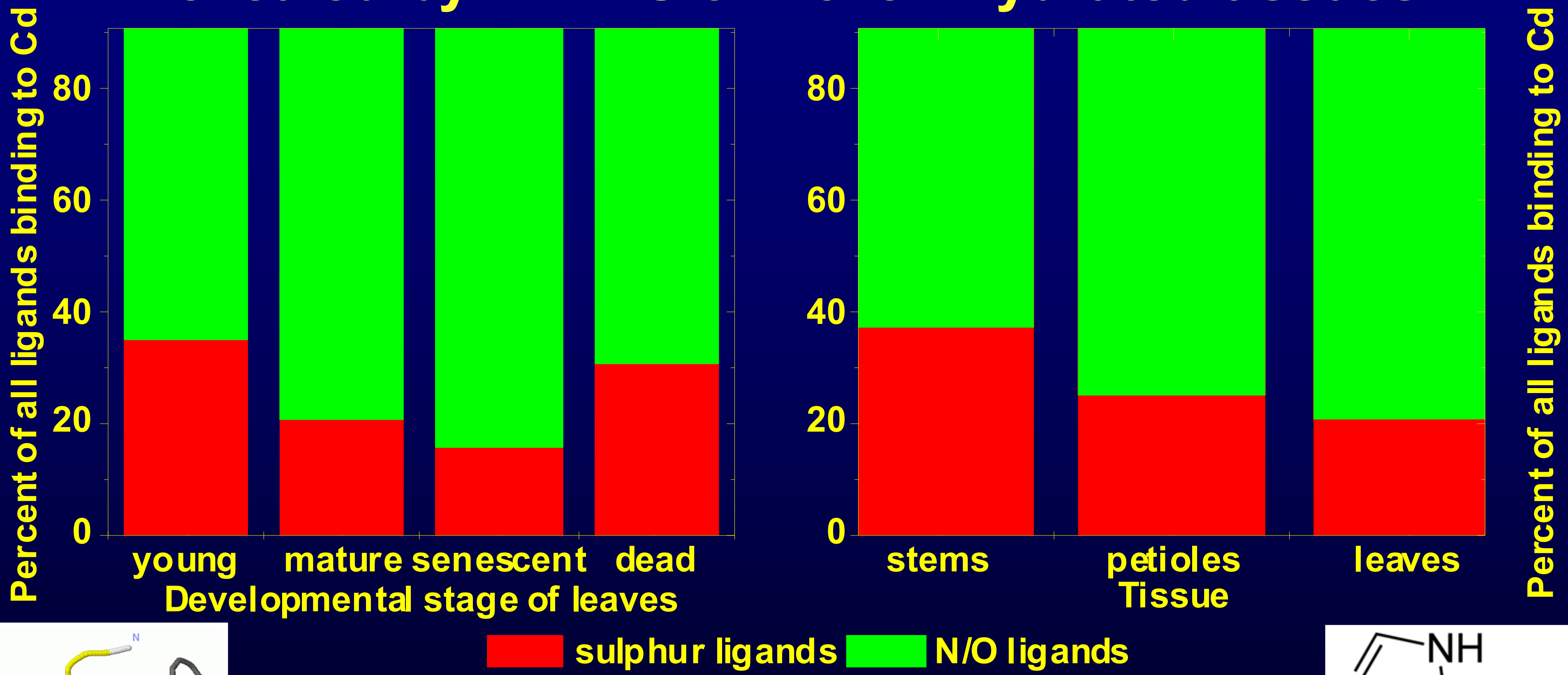
Invasive species in Europe (neophyte from Australia)!

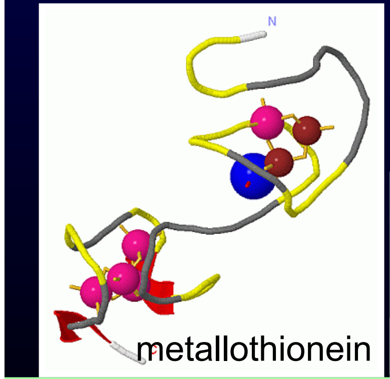
Hyperaccumulated metals are stored in weakly bound form, i.e. ideal for **defence**

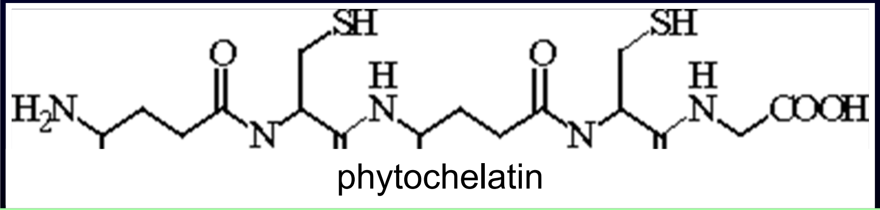
Cd: Küpper H, Mijovilovich A, Meyer-Klaucke W, Kroneck PMH (2004) Plant Physiology 134 (2), 748-757

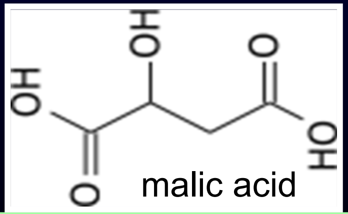
Cu: Küpper H, Mijovilovich A, Götz B, Küpper FC, Wolfram Meyer-Klaucke W (2009) Plant Physiol. 151, 702-14

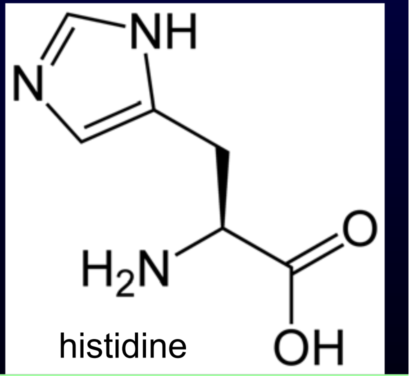
Speciation of cadmium and zinc hyperaccumulated by *Noccaea caerulescens* (Ganges ecotype) revealed by EXAFS of frozen-hydrated tissues




metallothionein

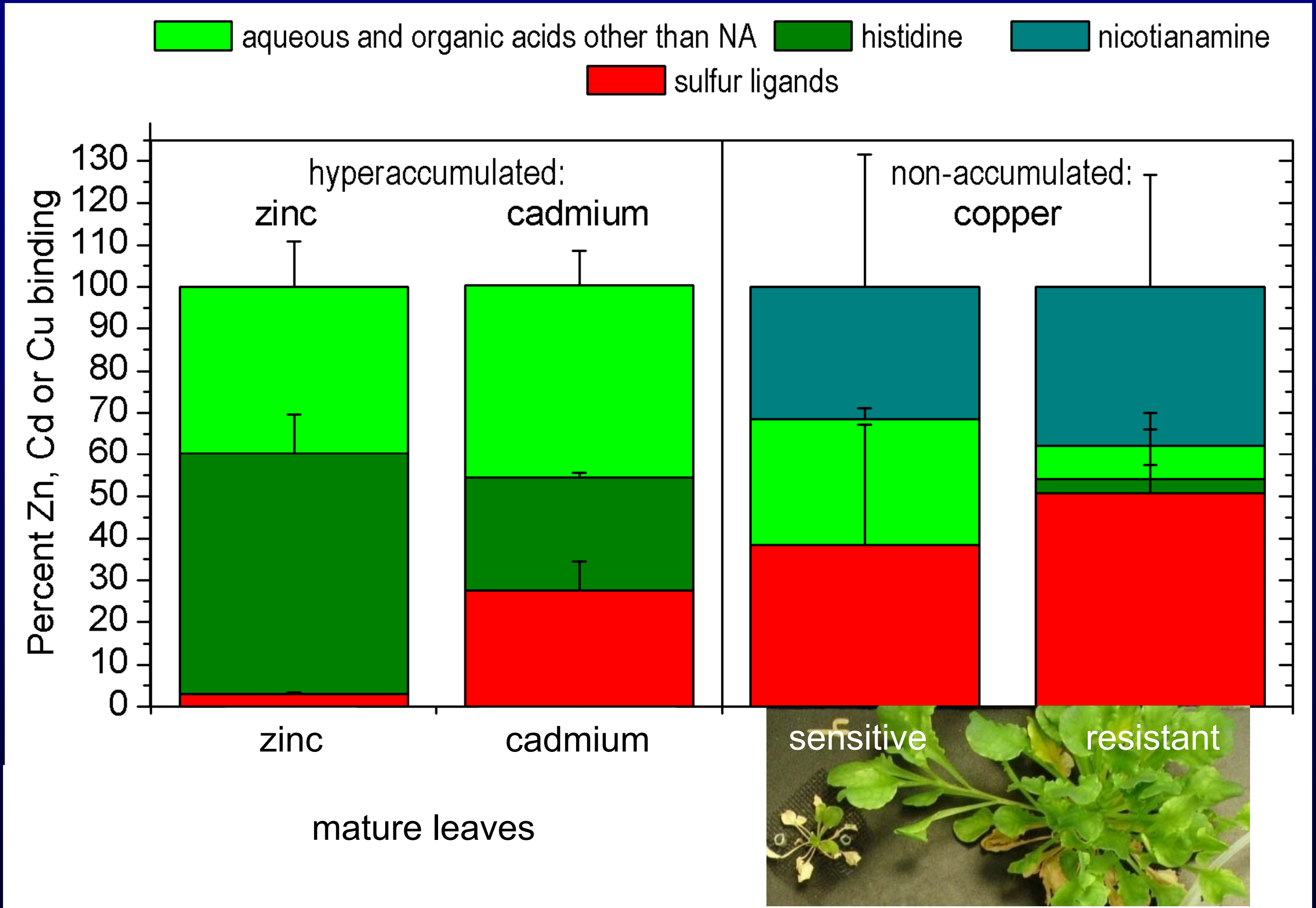

phytochelatin


malic acid


histidine

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

Differences in ligands between hyperaccumulated and non-hyperaccumulated metals: zinc, cadmium and copper in the Cu-sensitive Cd/Zn-hyperaccumulator *N. caerulescens*

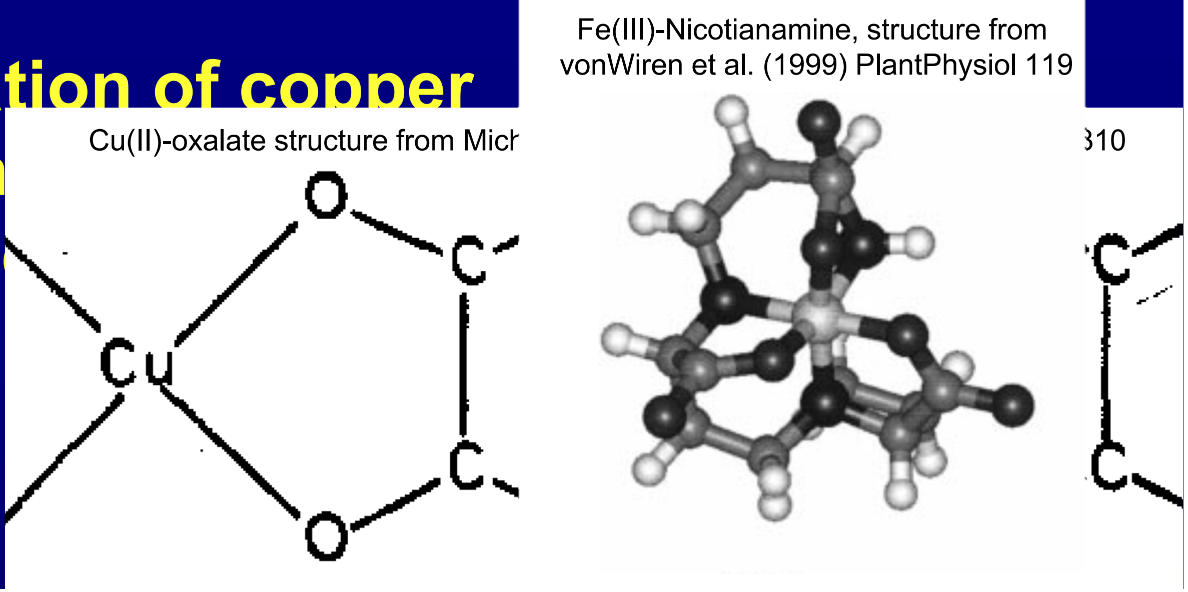


- Hyperaccumulated metals are stored in weakly bound form, i.e. ideal for **defence**
- Non-hyperaccumulated metals in hyperaccumulator plants are stored in strongly bound form

Cd, Zn: 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, 715-731
Küpper H, Mijovilovich A, Götz B, Küpper FC, Wolfram Meyer-Klaucke W (2009) Plant Physiol. 151, 702-14

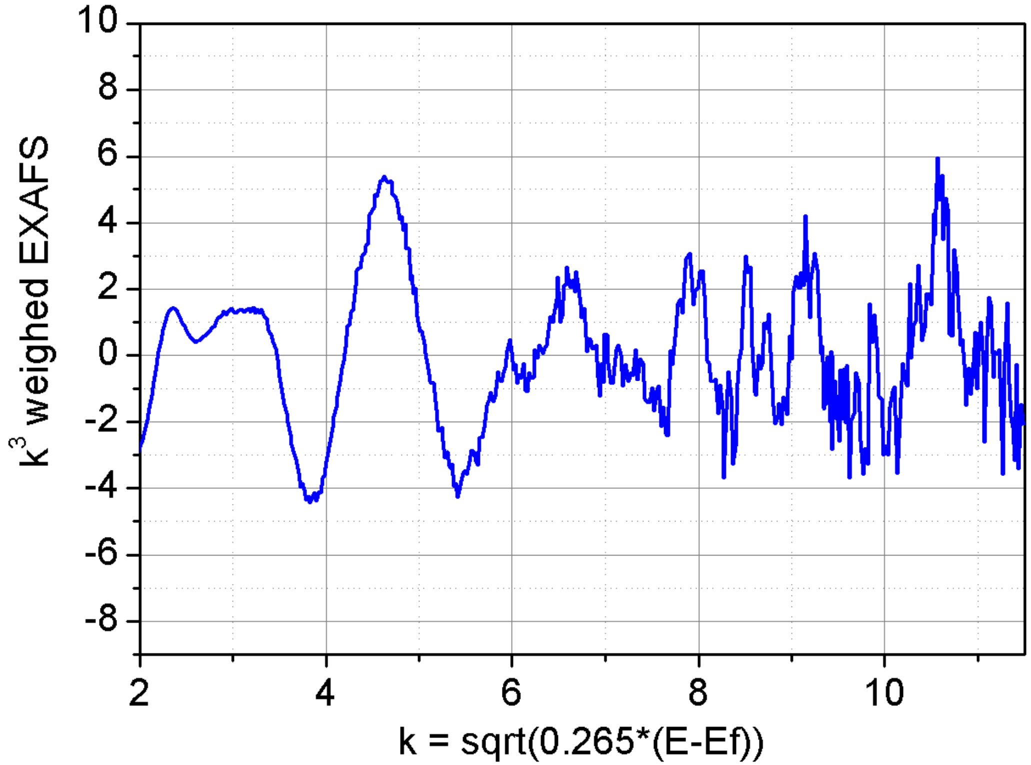
Speciation of copper in the Cu-sensitive CdZn-h

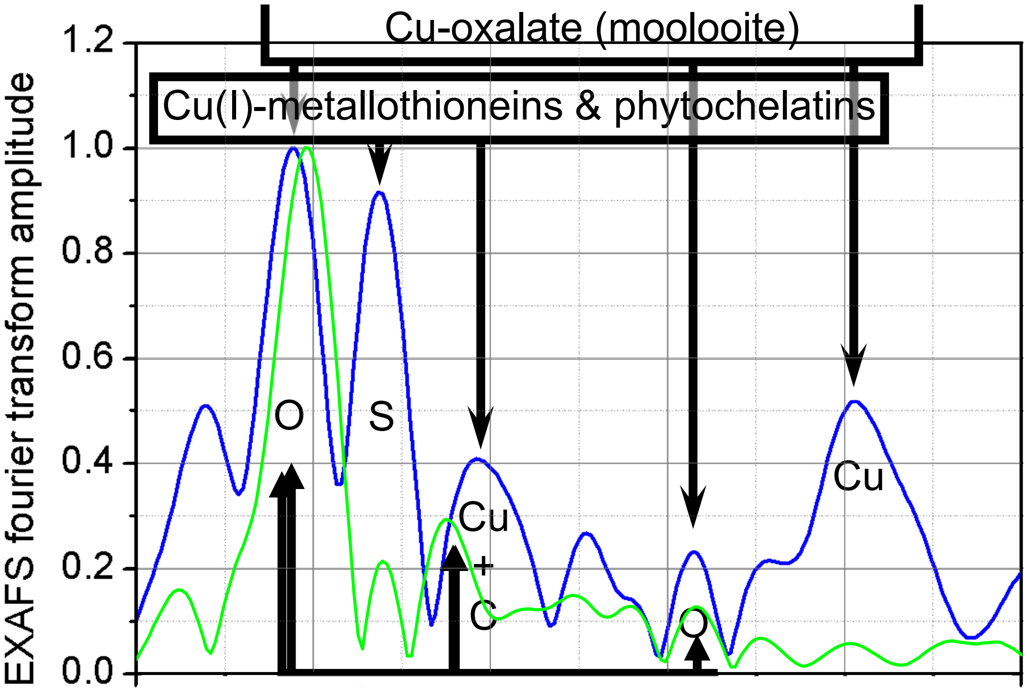
Analysed by XAS

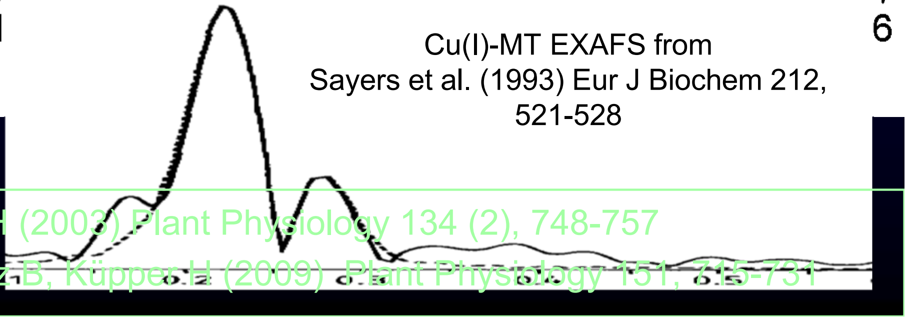


Fe(III)-Nicotianamine, structure from vonWiren et al. (1999) PlantPhysiol 119

Cu(II)-oxalate structure from Mich







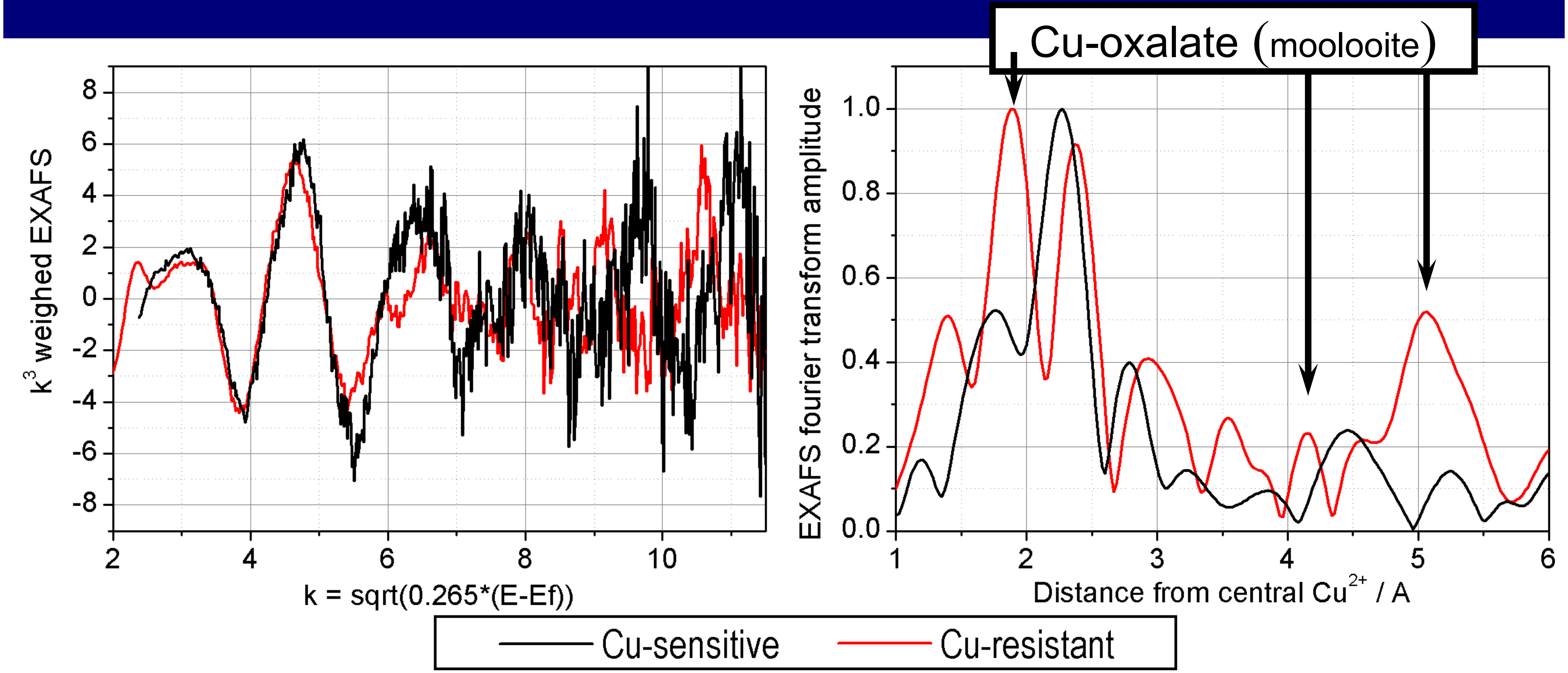
Cu(I)-MT EXAFS from Sayers et al. (1993) Eur J Biochem 212, 521-528

Cd: Küpper H, Mijovilovich A, Meyer-Klaucke W, Kroneck PMH (2003) Plant Physiology 134 (2), 748-757

Cu: Mijovilovich A, Leitenmaier B, Meyer-Klaucke W, Kroneck PMH, Götz B, Küpper H (2009) Plant Physiology 151, 715-731

Speciation of copper in the Cu-sensitive CdZn-hyperaccumulator *N. caerulescens*

comparison of sensitive vs. resistant individuals



→ *Copper bio-mineralisation as hardly soluble Cu-oxalate serves as additional detoxification in Cu-resistance*

Mijovilovich A, Leitenmaier B, Meyer-Klaucke W, Kroneck PMH, Götz B, Küpper H (2009) Plant Physiology 151, 715-731

General Resistance-Mechanisms

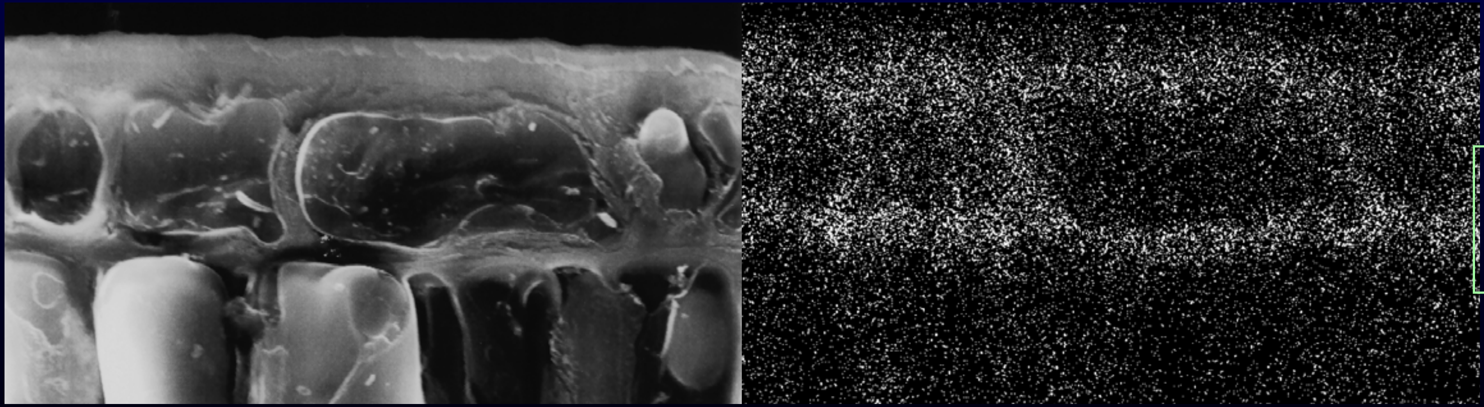
Heavy metal detoxification with strong ligands

Other Ligands

- Non-proteogenic amino acid nicotianamine (also involved in normal transport)
- Anthocyanins: seem to be involved in Brassicacae in molybdemum binding (detoxification or storage?)

Hale et al_2001, PlantPhysiol 126, 1391-1402

- Cell wall: main Al binding site in the Al-hyperaccumulator *Camellia sinensis* (tea)

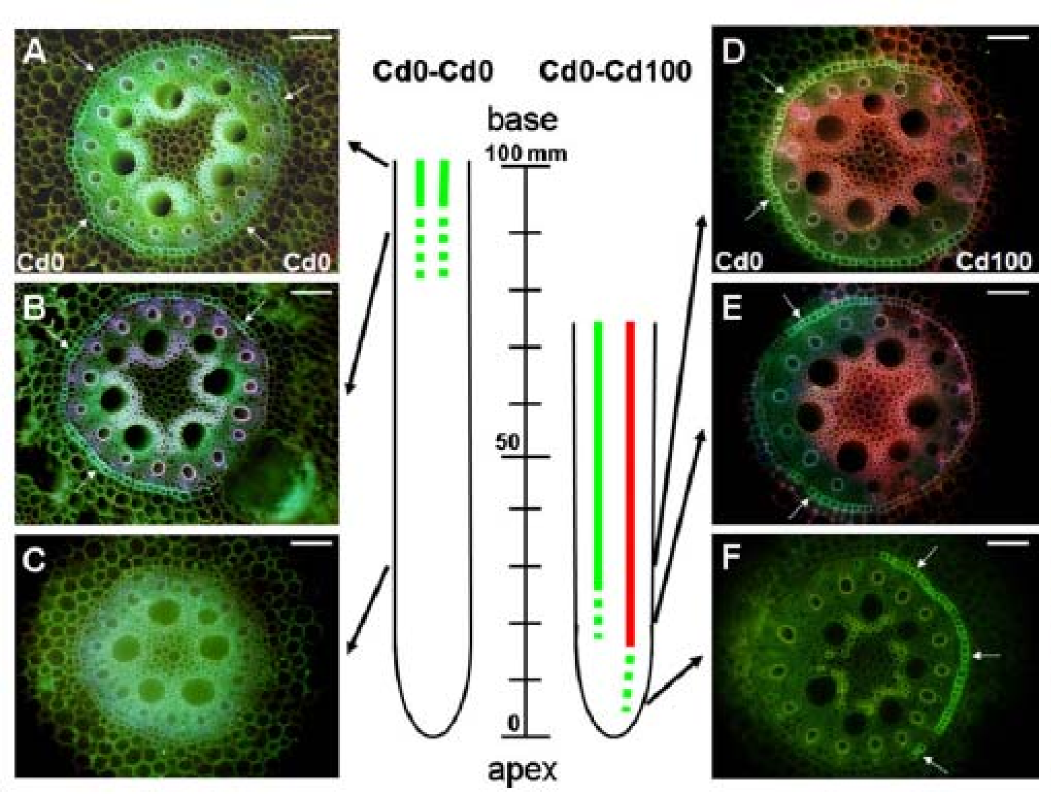


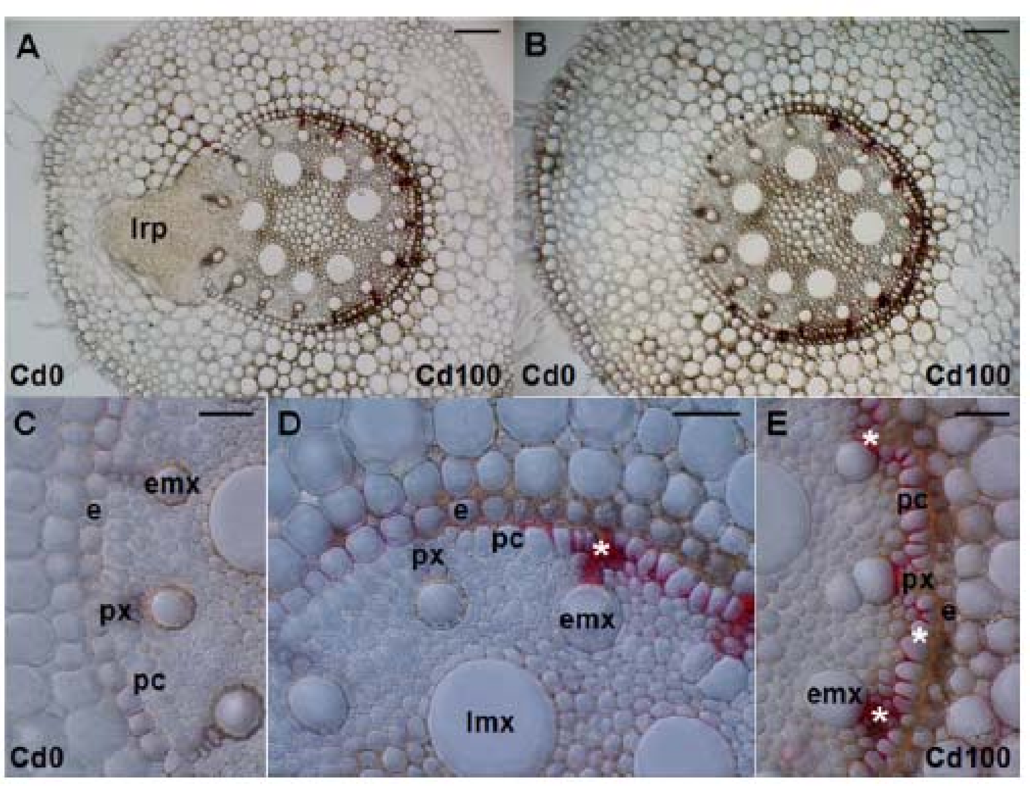
Carr HP, Lombi E, Küpper H, McGrath SP, Wong MH (2003) Agronomie 23, 705-10

- Some algae release unidentified thiol-ligands during Cu-stress

Metal detoxification by sequestration within the plant

Maize seedlings with proper roots placed between 2 agar blocks, one of which contained Cd (50 or 100 µM)





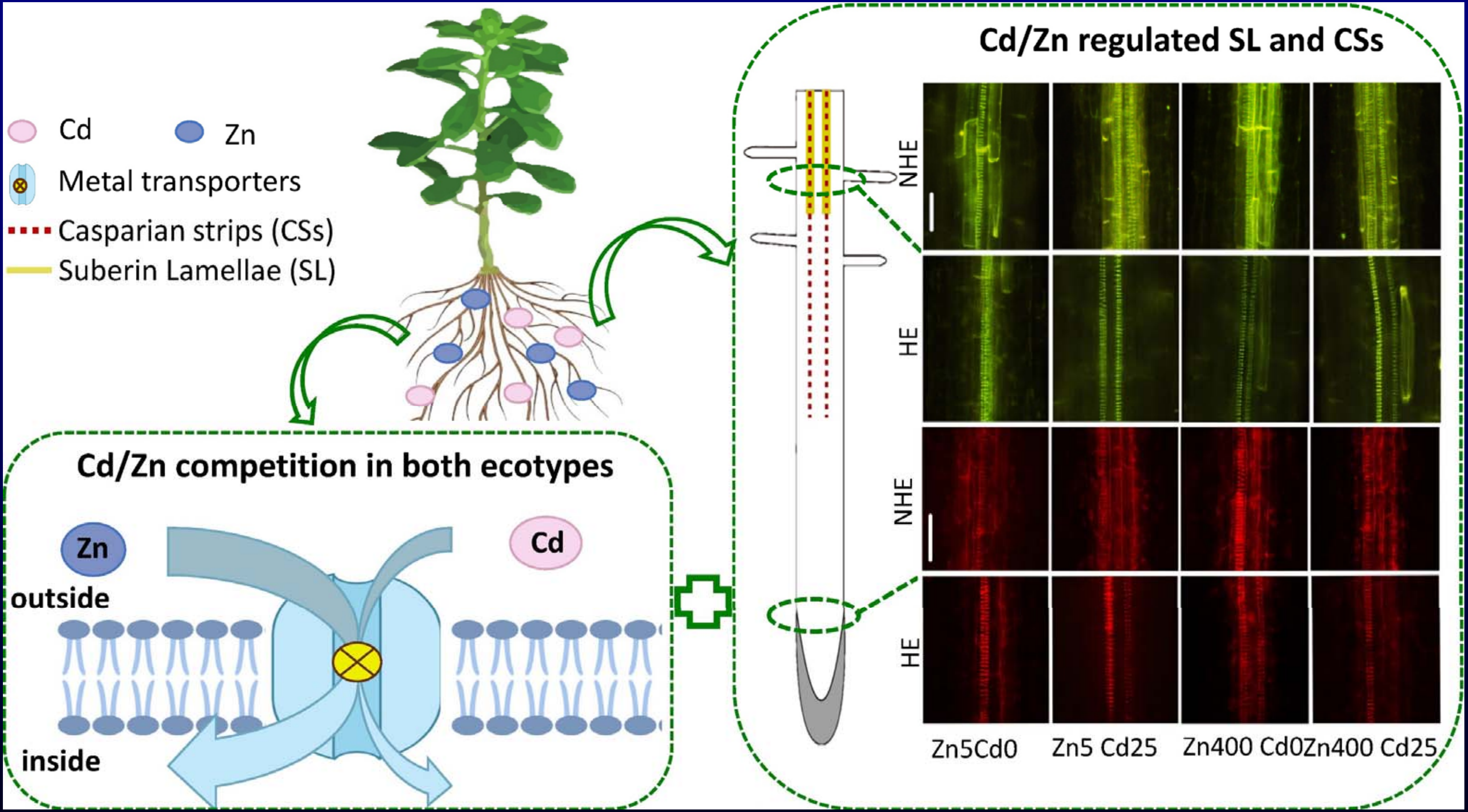
Gradual development of endodermal suberin lamellae in untreated roots

In Cd-exposed roots, suberin already 5mm from apex (F), but

Lignification at Cd-exposed side

→ Suberin formation + lignification to reduce unspecific permeability of root membranes

Cd toxicity defence in *Sedum alfredii*

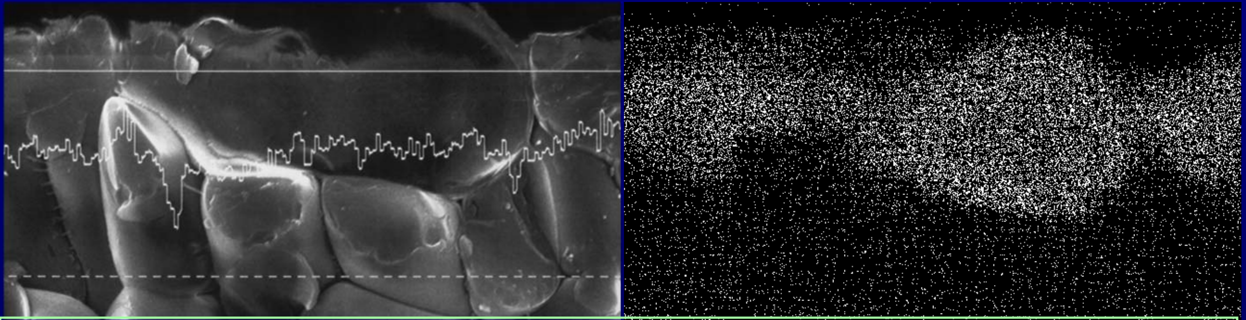


From: Cao K, Jaime-Pérez N, Mijovilovich A, Morina F, Bokhari SNH, Liu Y, Küpper H*, Tao Q (2024) Ecotoxicology and Environmental Safety, 275, 116272, https://doi.org/10.1016/j.ecoenv.2024.116272

Heavy metal detoxification by compartmentation

Mechanisms

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

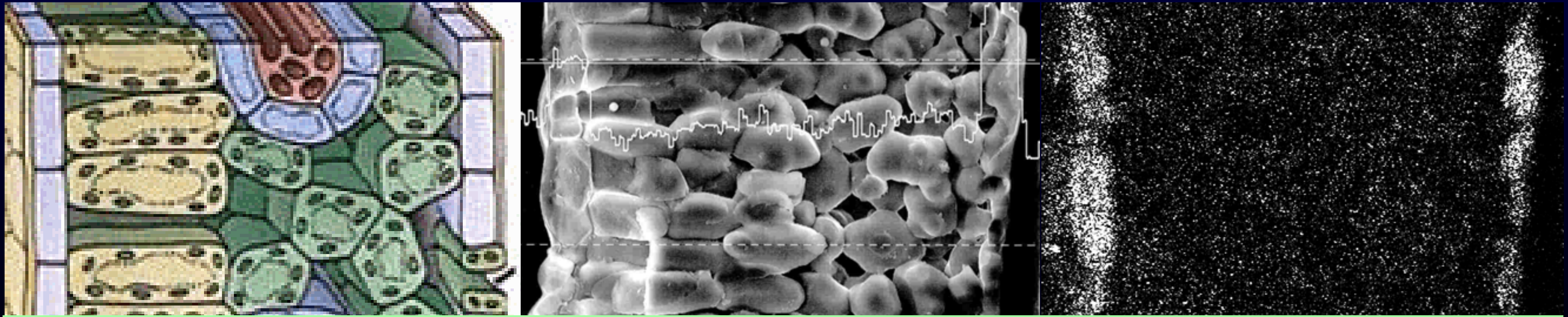


Küpper H et al., 2001, J Exp Bot 52 (365), 2291-2300

- 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

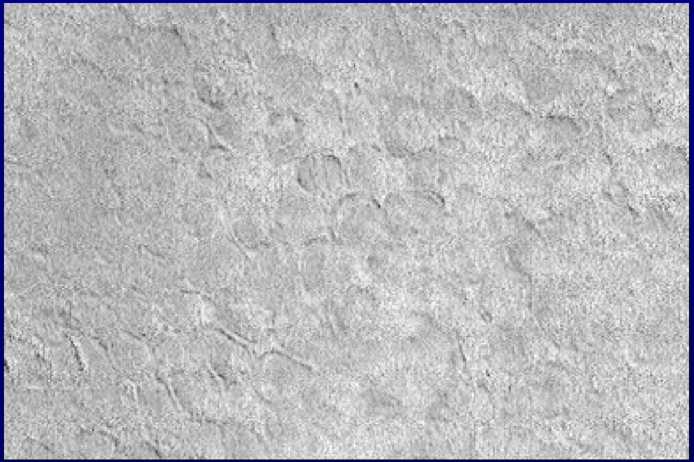
- Sequestration in least sensitive tissues, e.g. the epidermis instead of the photosynthetically active mesophyll



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

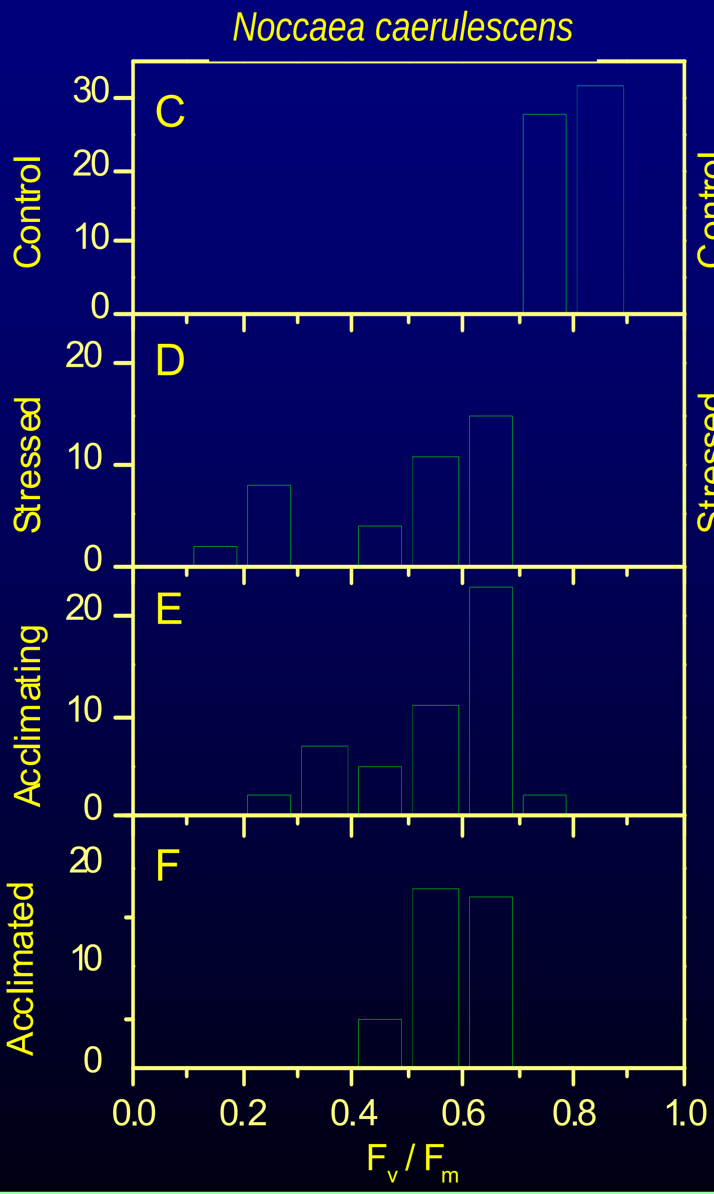
Differences between species and on a cellular level:

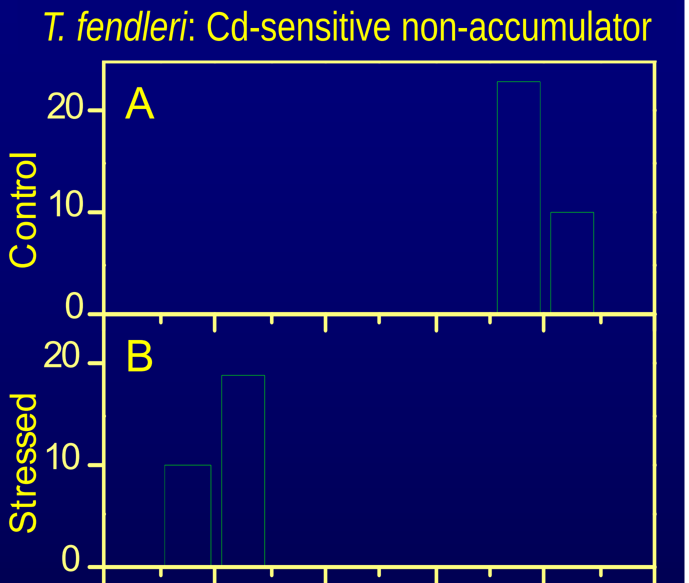
distribution of photosystem II activity parameters during Cd toxicity in the Zn/Cd-hyperaccumulator *N. caerulescens*



Cellular F_v/F_m distribution in a control plant

Distribution of F_v/F_m in a plant stressed with Cd^{2+}

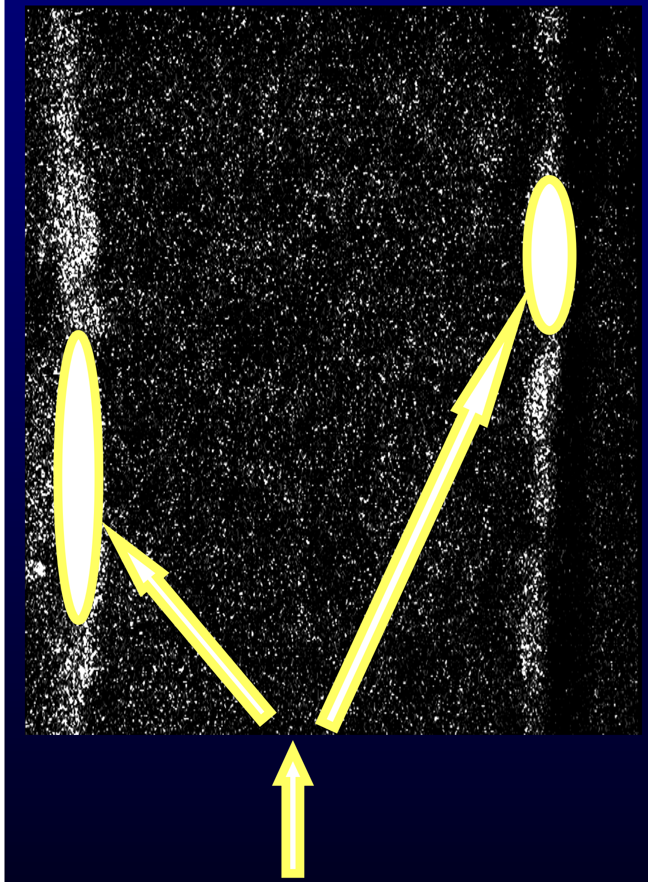


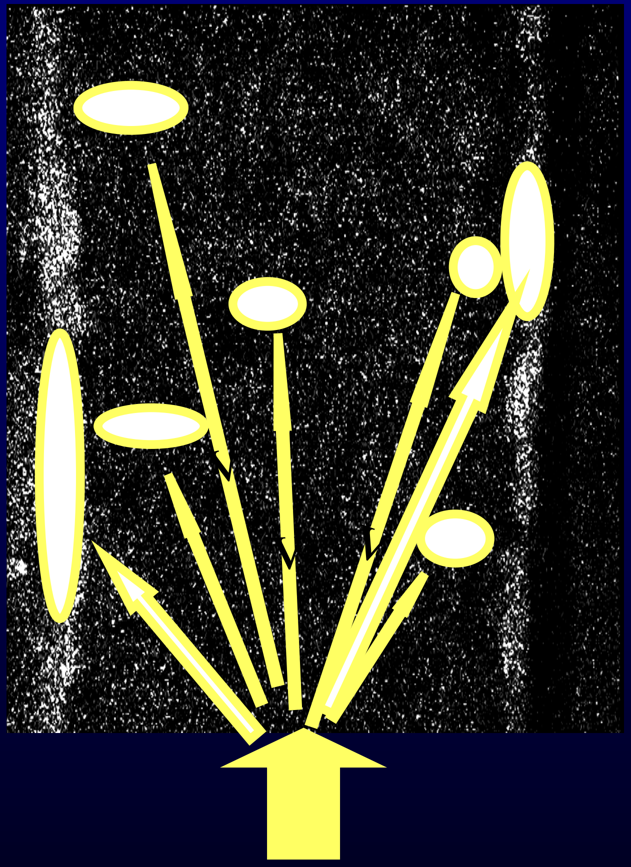


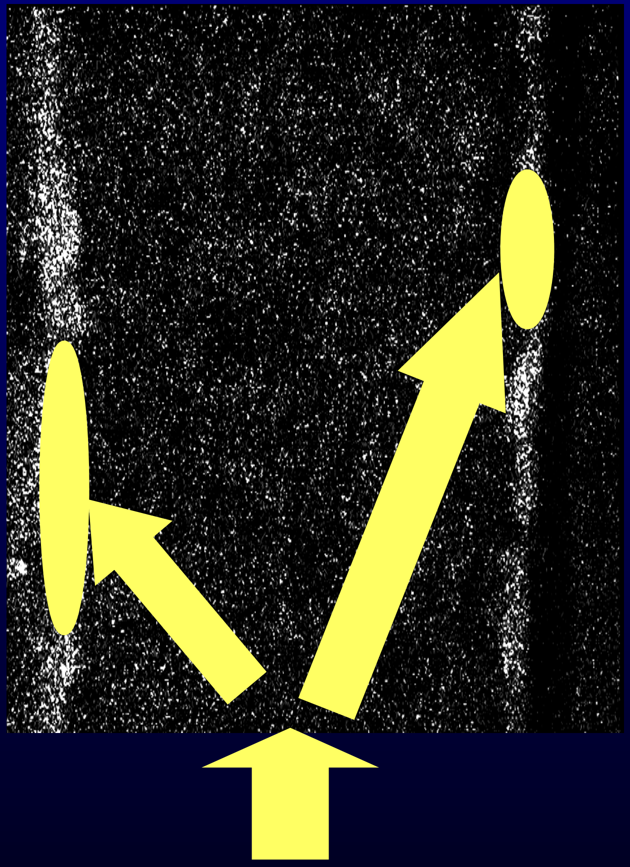
Stress was applied as 10µM Cd^{2+} in the nutrient solution that was continuously exchanged for 6 months

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

Proposed mechanism of emergency defence against heavy metal stress


Normal: Sequestration in epidermal storage cells

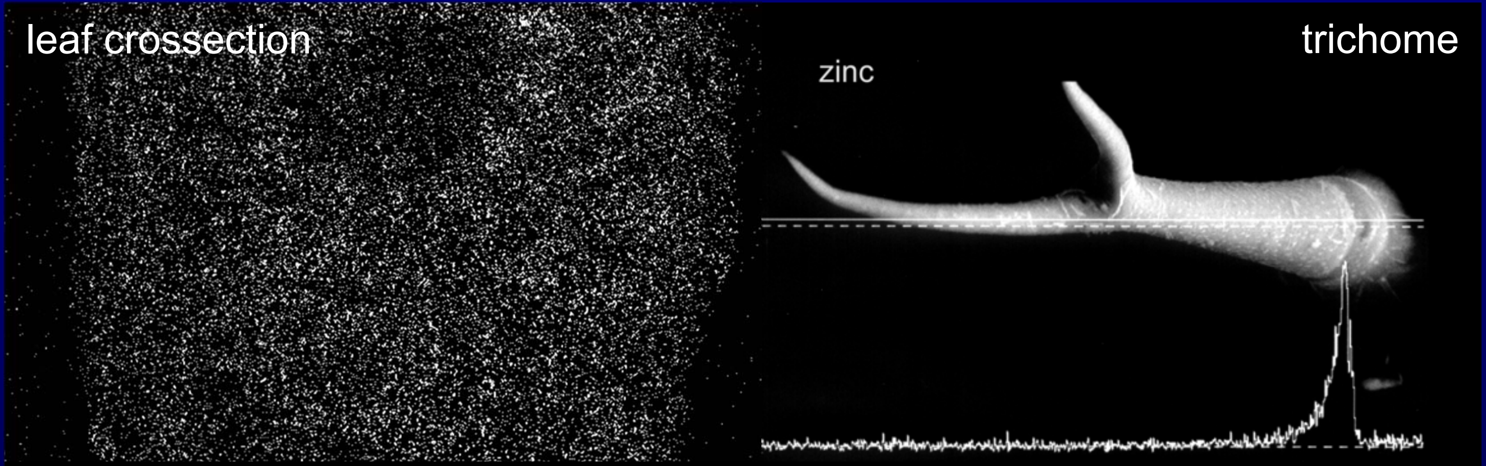

Stressed: additional sequestration in selected mesophyll cells


Acclimated: Enhanced sequestration in epidermal storage cells

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

Heavy metal detoxification by compartmentation: variations of the pattern as revealed by EDX

species-specific in *Arabidopsis halleri*

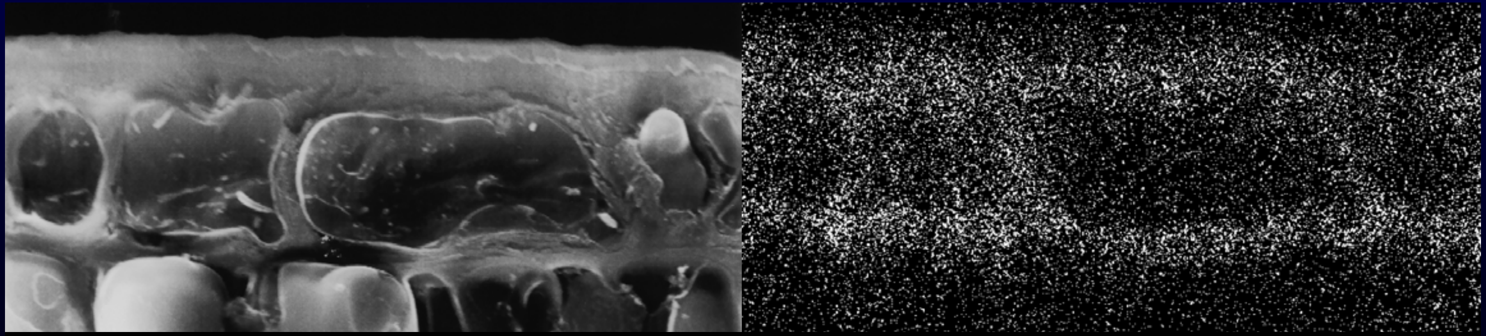


zinc distribution: 2D map (left), line scan (right)

Accumulation of Zn mainly in the mesophyll instead of the epidermis,
but highest concentrations (up to 1M) in epidermal trichomes (→ defence)

Küpper H, Lombi E, Zhao FJ, McGrath SP (2000) Planta 212, 75-84

metal-specific for Al in *Camellia sinensis* (tea)



epidermis of leaf crossection: electronoptic image (left), Al distribution (right)

Accumulation of Al in the cell walls instead of the vacuoles, but again in the epidermis (→ defence?)

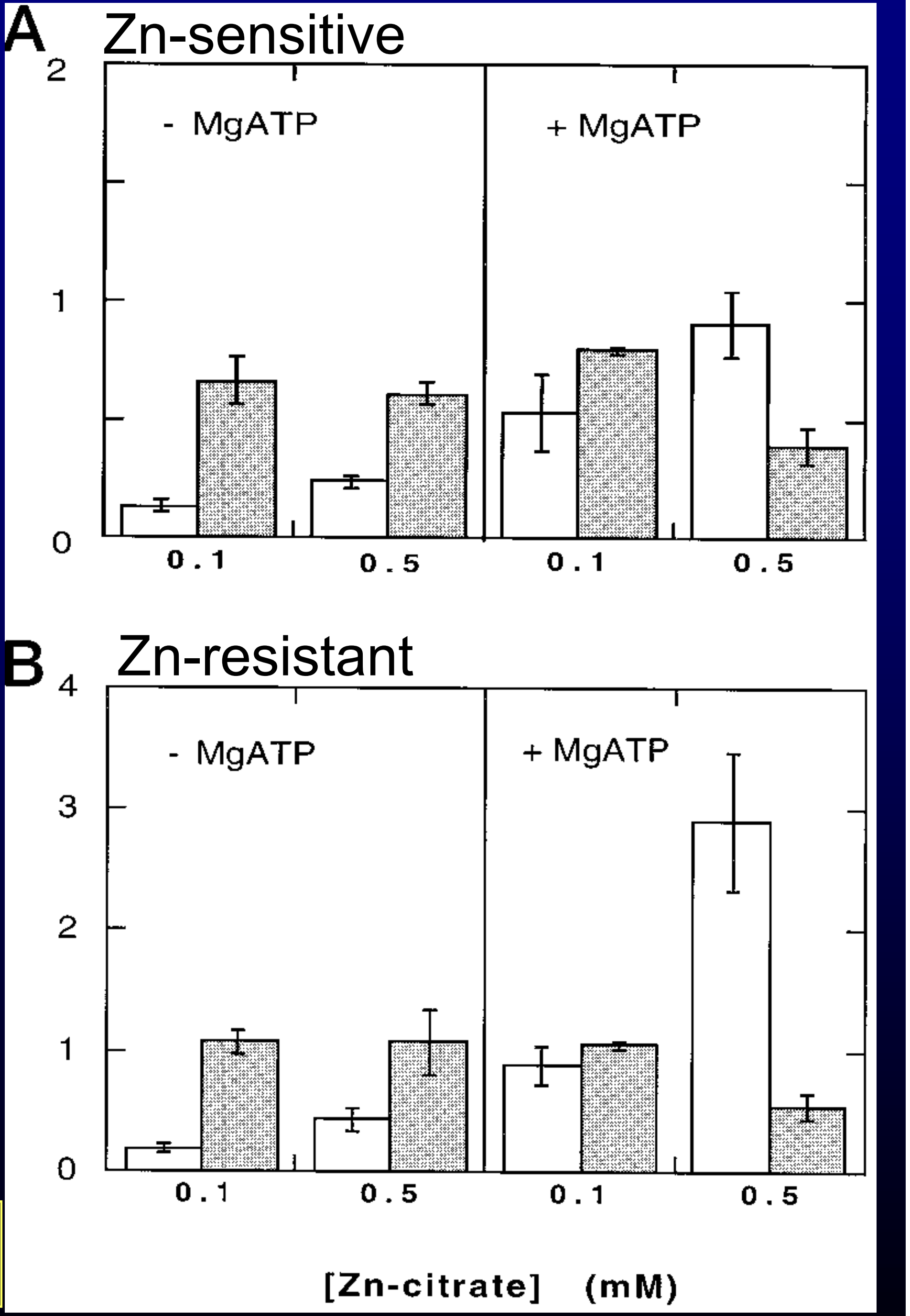
Carr HP, Lombi E, Küpper H, McGrath SP, Wong MH (2003) Agronomie 23, 705-10

Root-specific resistance mechanisms

Strategies

- Reduction of the unspecific permeability of the root for unwanted heavy metals: expression of peroxidases enhances lignification
- Active (ATP-dependent) discharge by efflux-pumps: was shown for Cu in *Silene vulgaris* (and for diverse metals in bacteria).

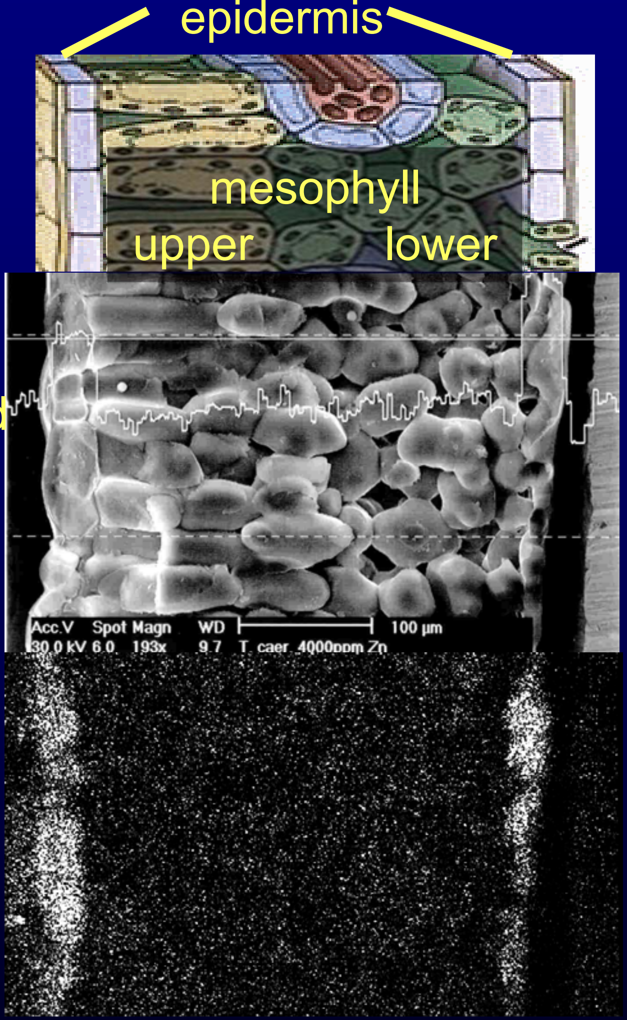
Chardonnens AN, Koevoets PLM, vanZanten A, Schat H, Verkleij JAC, 1999, PlantPhysiol120_779-785



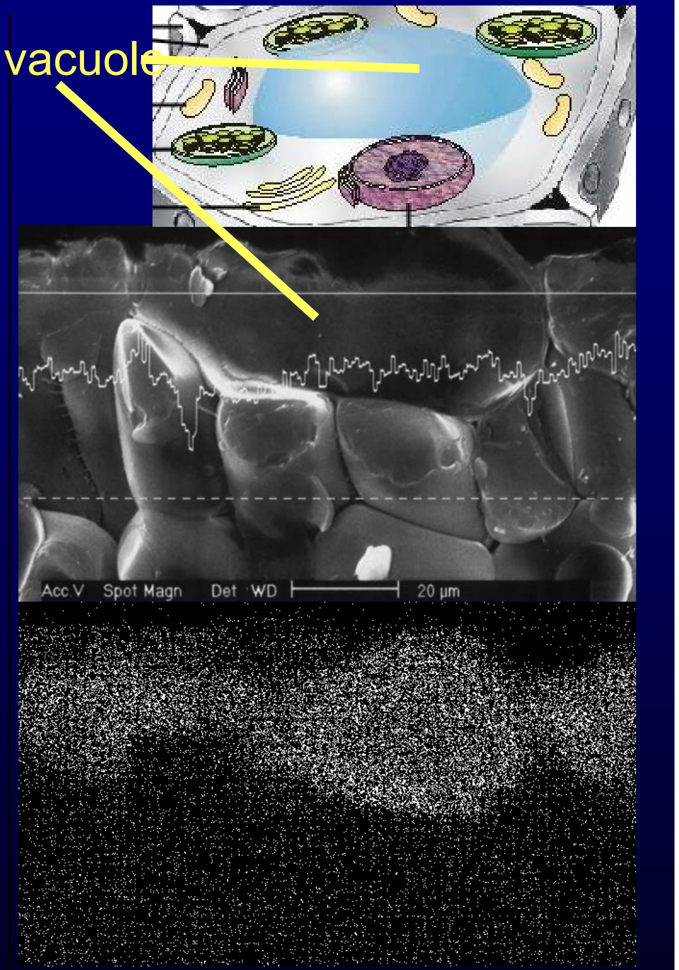
Most common pattern of heavy metal detoxification by compartmentation in hyperaccumulators: Enhanced sequestration in epidermal vacuoles

- Sequestration in the vacuole: plant-specific mechanism (animals+bacteria usually don‘t have storage vacuoles...)
- Sequestration in least sensitive tissues, e.g. the epidermis instead of the photosynthetically active mesophyll

Generally: active transport against the concentration gradient → transport proteins required!



Zn K α line scan and dot map of a *N. caerulescens* leaf

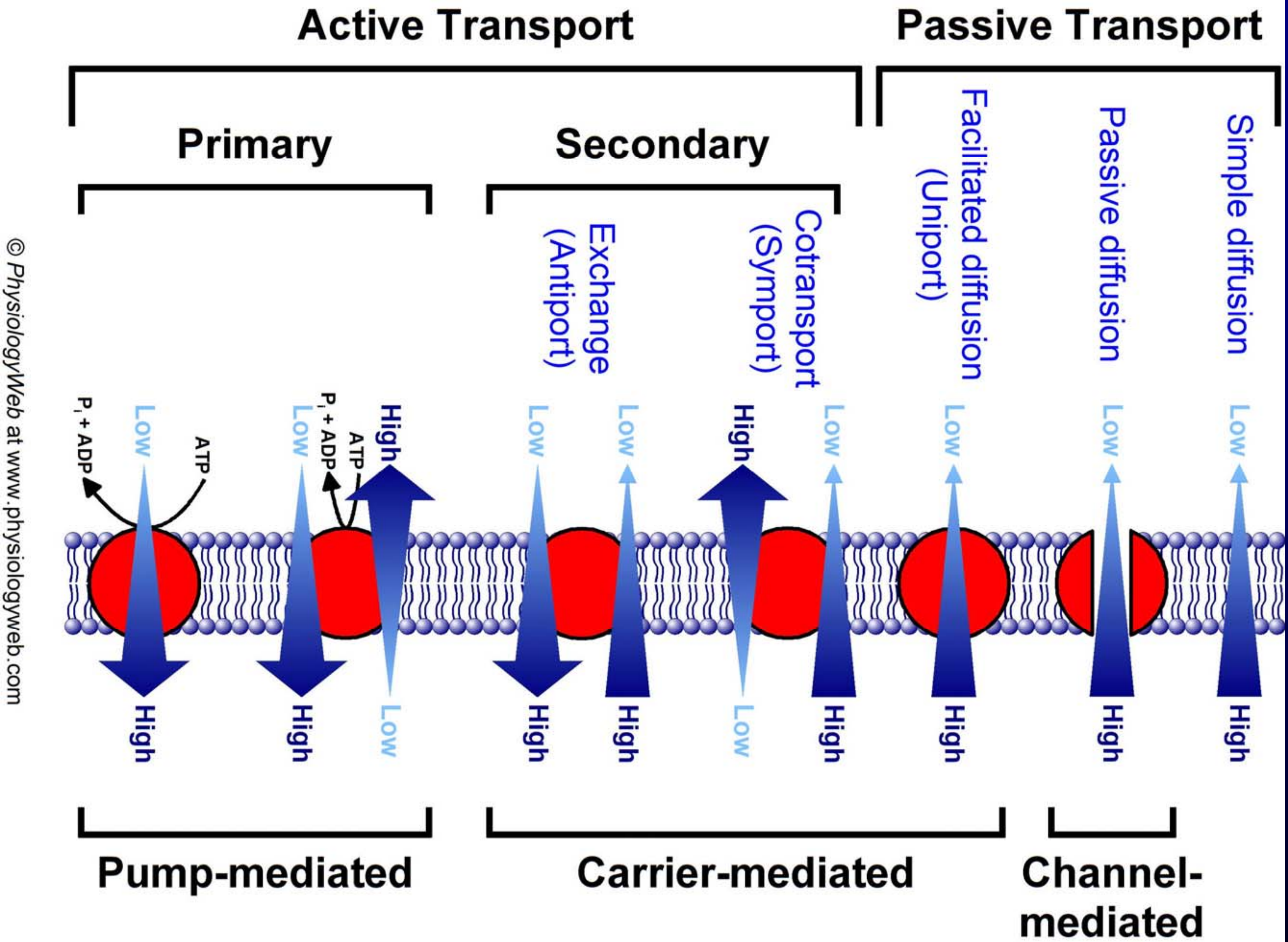


Ni K α line scan and dot map of a *A. bertolonii* leaf

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

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

Mechanisms of Metal transport proteins



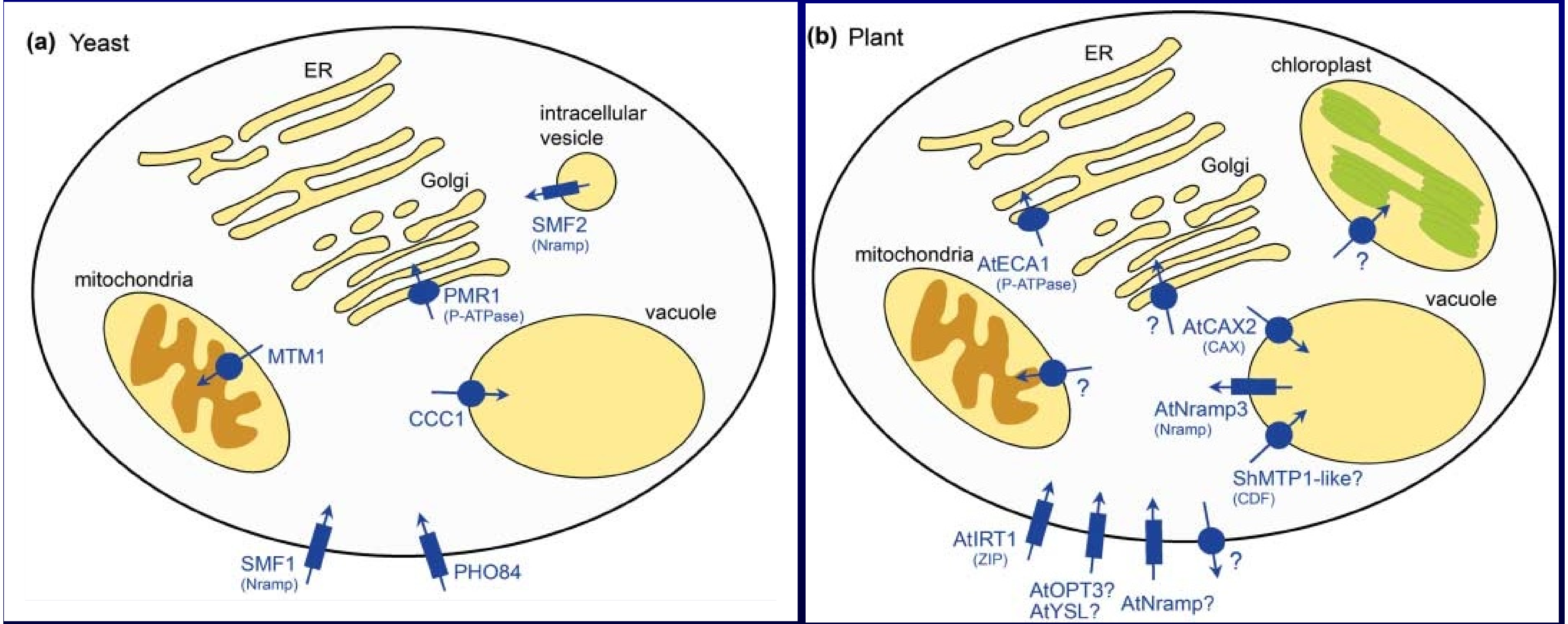
© *PhysiologyWeb* at www.physiologyweb.com

$$\Delta G = n_{Ionen} * R * T * \ln (c_{inside} / c_{outside}) + 3F (\varphi_{outside} - \varphi_{inside})$$

(R = gas constant, T = temperature, F = Faraday constant, φ = electrochemical potential)

Mechanisms of metal uptake in Eucaryotes: Main families of metal transport proteins

example: manganese transport in yeast and plants

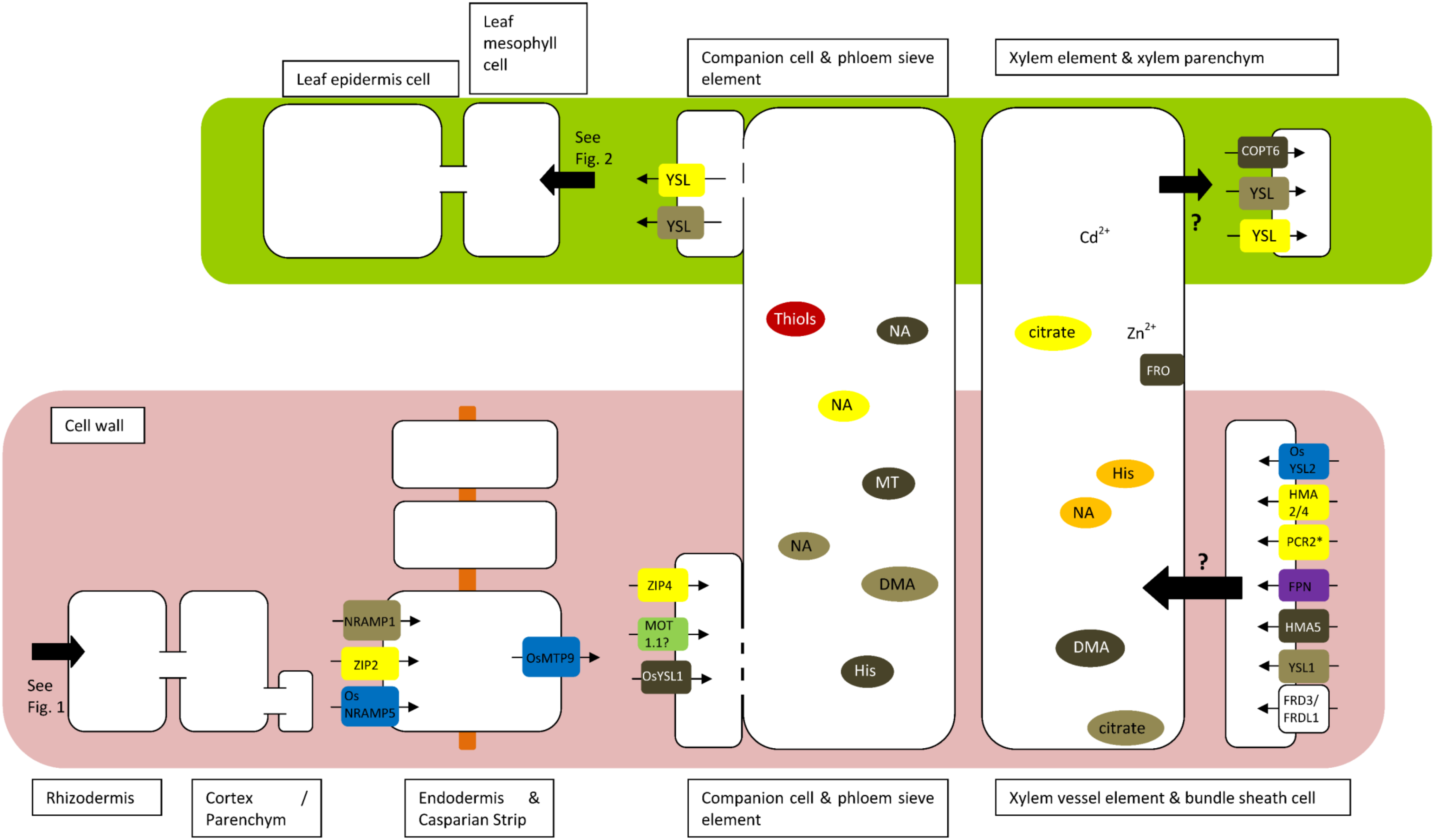


From: Pittman JK, 2005, NewPhytol167, 733-742

4 main families of transmembrane metal transport proteins

- P-type **ATPases**
- cation diffusion facilitators (**CDF-**transporters)
- ZRT-/IRT-like proteins (**ZIP**-transporters)
- Natural resistance associated Macrophage proteins (**Nramp-**transporters)

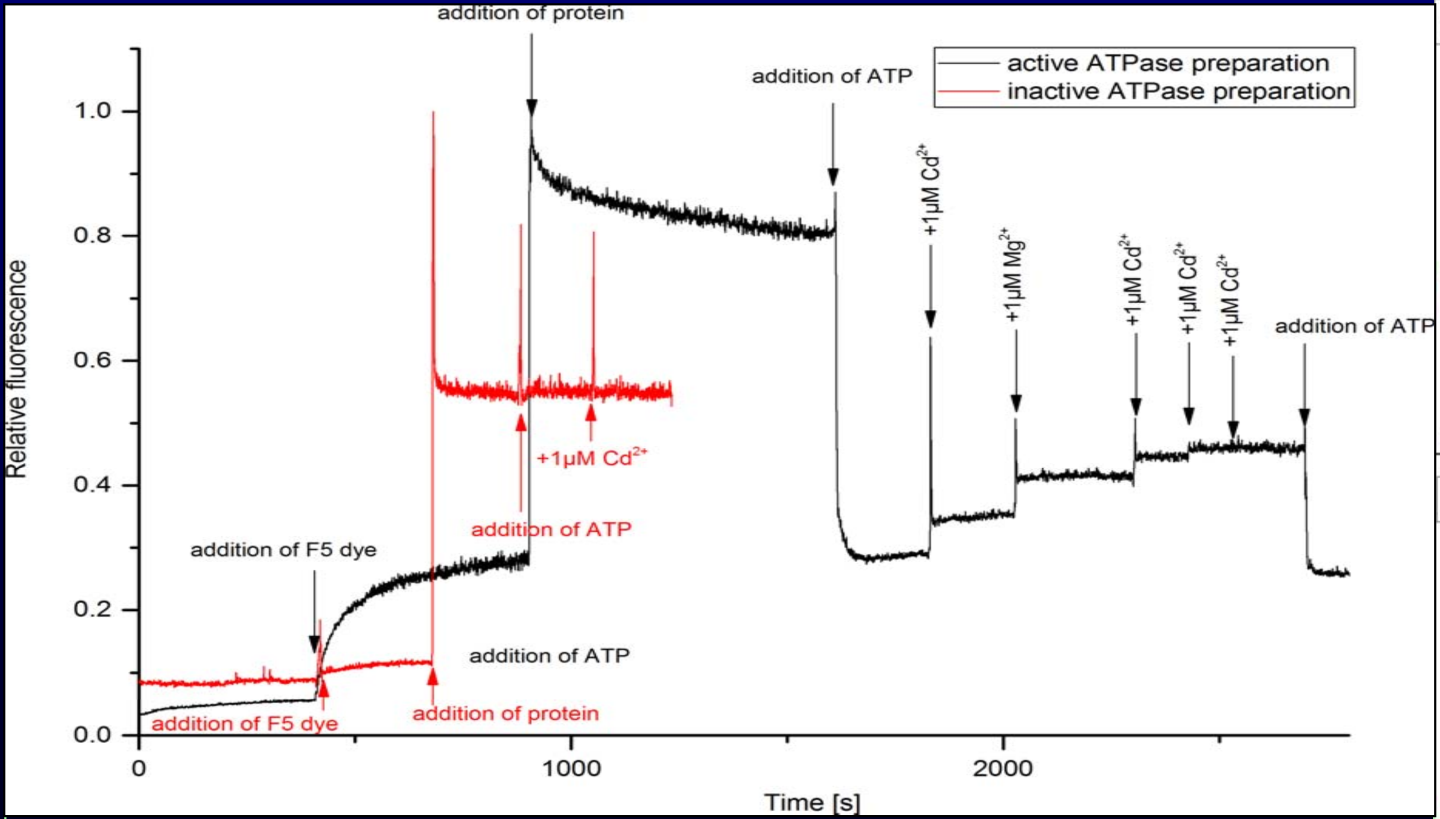
Mechanisms of metal uptake in plants: Different transport steps require different transporters Translocation. Root-to-shoot: Xylem, shoot-to-root: phloem

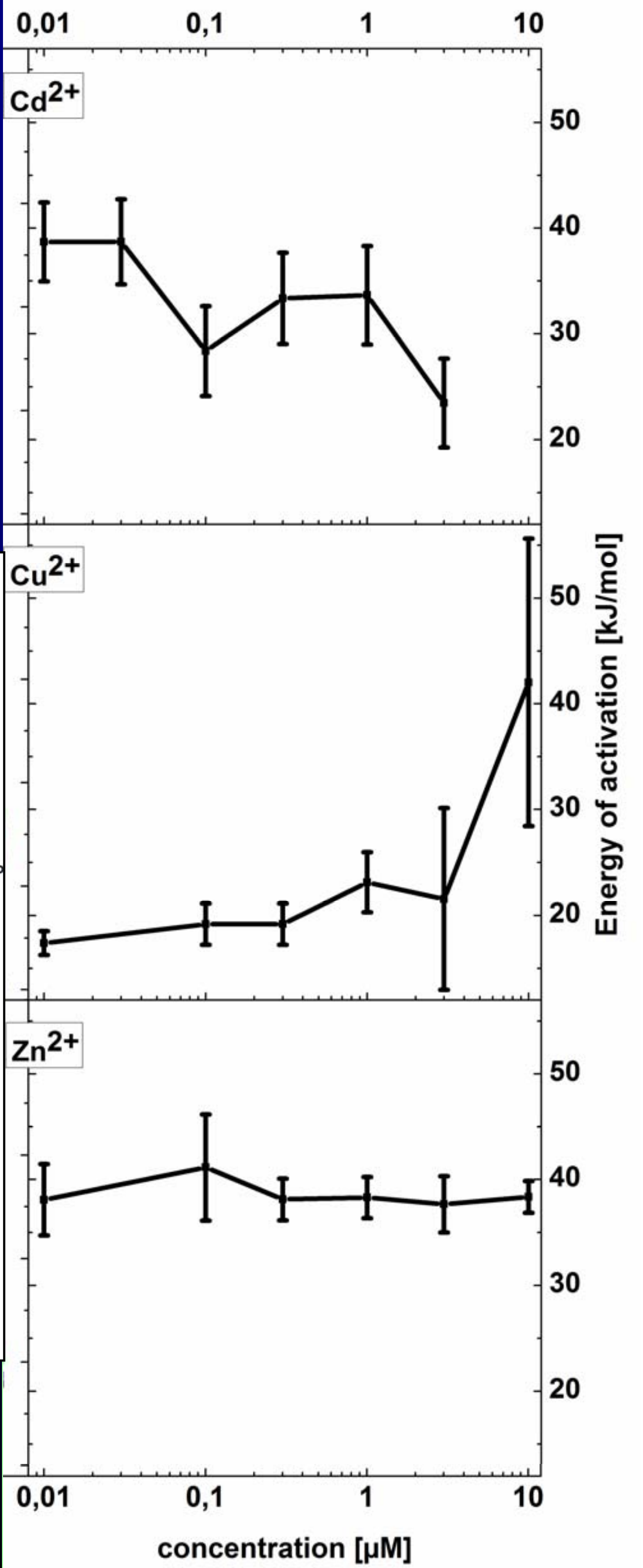


From: Andresen E, Peiter E, Küpper H (2018) Journal of Experimental Botany 69, 909-954

Metal-dependent differences in energetics of NcHMA4

- Activation energy changes with the concentration and type of the metal to be pumped.
- Maximal activity after saturation of all high-affinity Cd binding sites





top: Mishra S, Mishra A, Küpper H (2017) Frontiers in Plant Scince, https://doi.org/10.3389/fpls.2017.00835

right: Leitenmaier B, Witt A, Witzke A, Stemke A, Meyer-Klaucke W, Kroneck PMH, Küpper H (2011) Biochimica et Biophysica Acta (Biomembranes) 1808, 2591-2599

Different expression patterns of closely related Cd/Zn-pumping ATPases as revealed by Quantitative mRNA In Situ Hybridisation

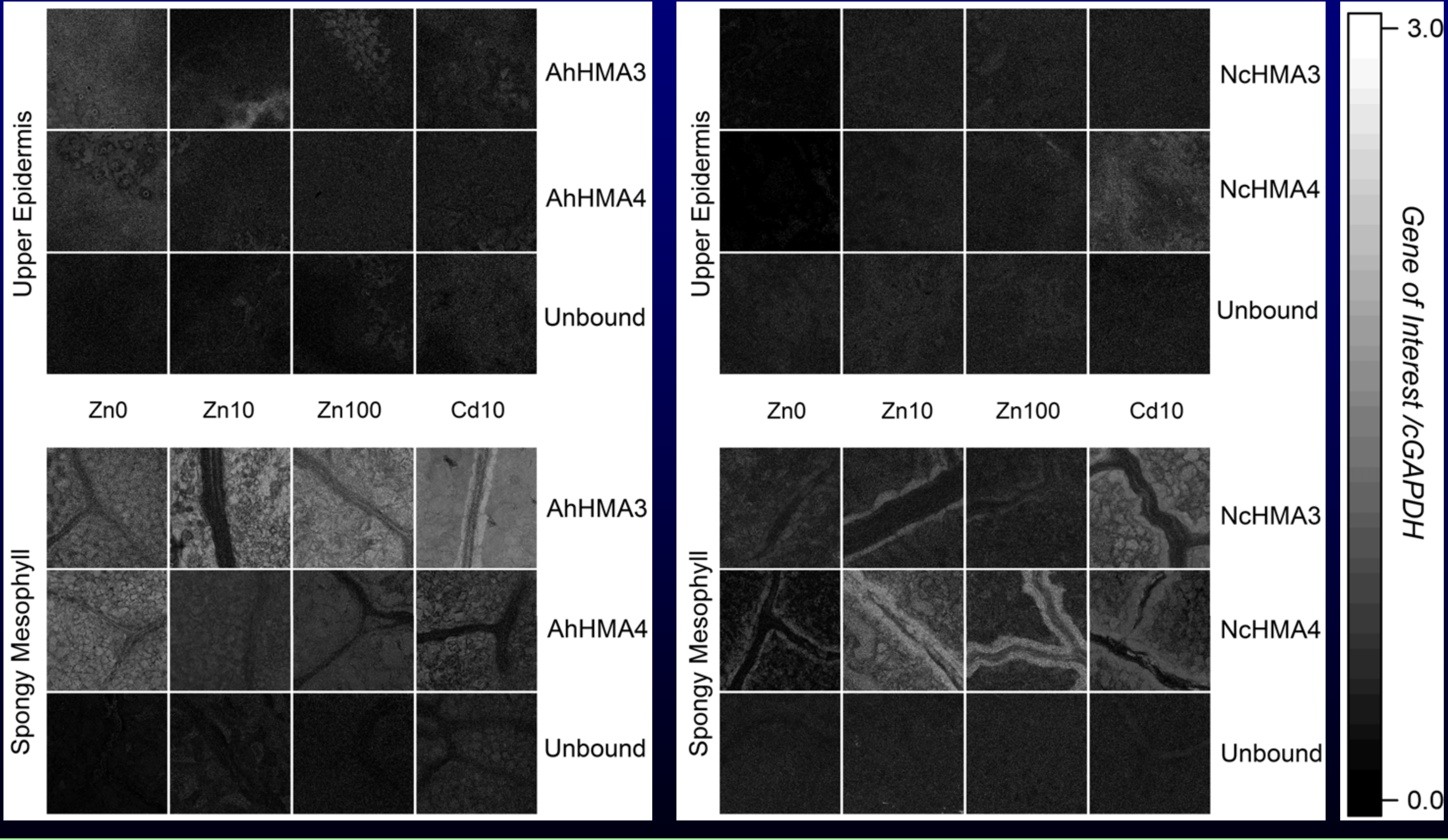
Arabidopsis halleri

→ mostly in mesophyll (final storage)

→ HMA4 up-regulated in Zn-deficiency

Noccaea caerulescens

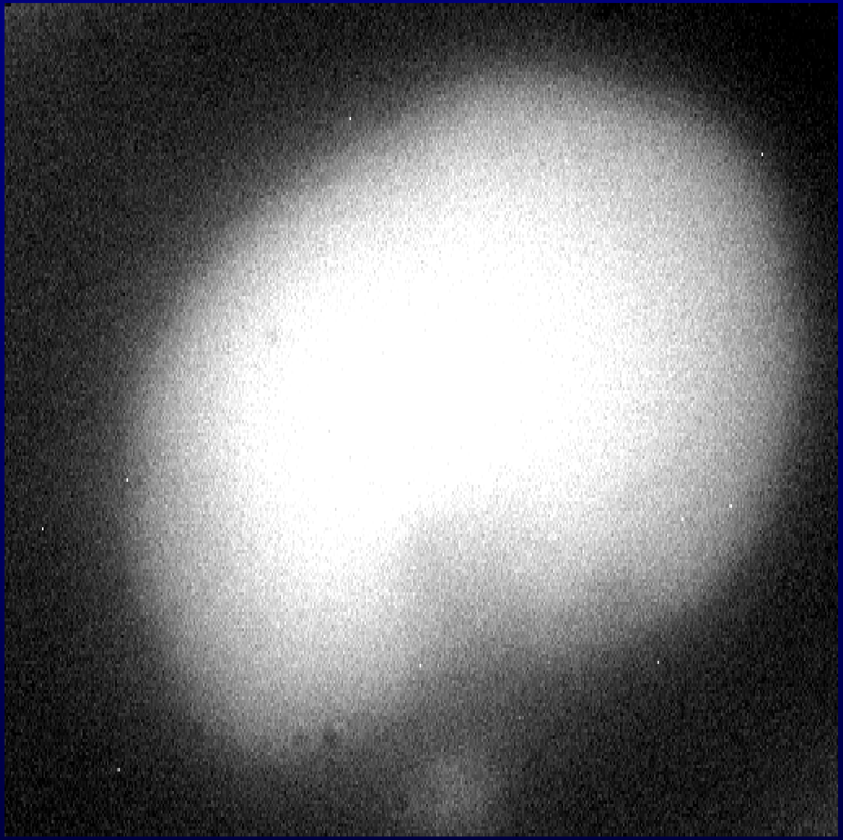
→ HMA4 much stronger in bundle sheath, likely because final storage is in epidermis



Mishra S, Mishra A, Küpper H (2016) Frontiers in Plant Scince, https://doi.org/10.3389/fpls.2017.00835.

Cd-transport into protoplasts isolated from the hyperaccumulator plant *Noccaea caerulescens*... (II)

In almost all measured cells, a bright cytoplasmatic ring appeared first after start adding Cd to the medium.

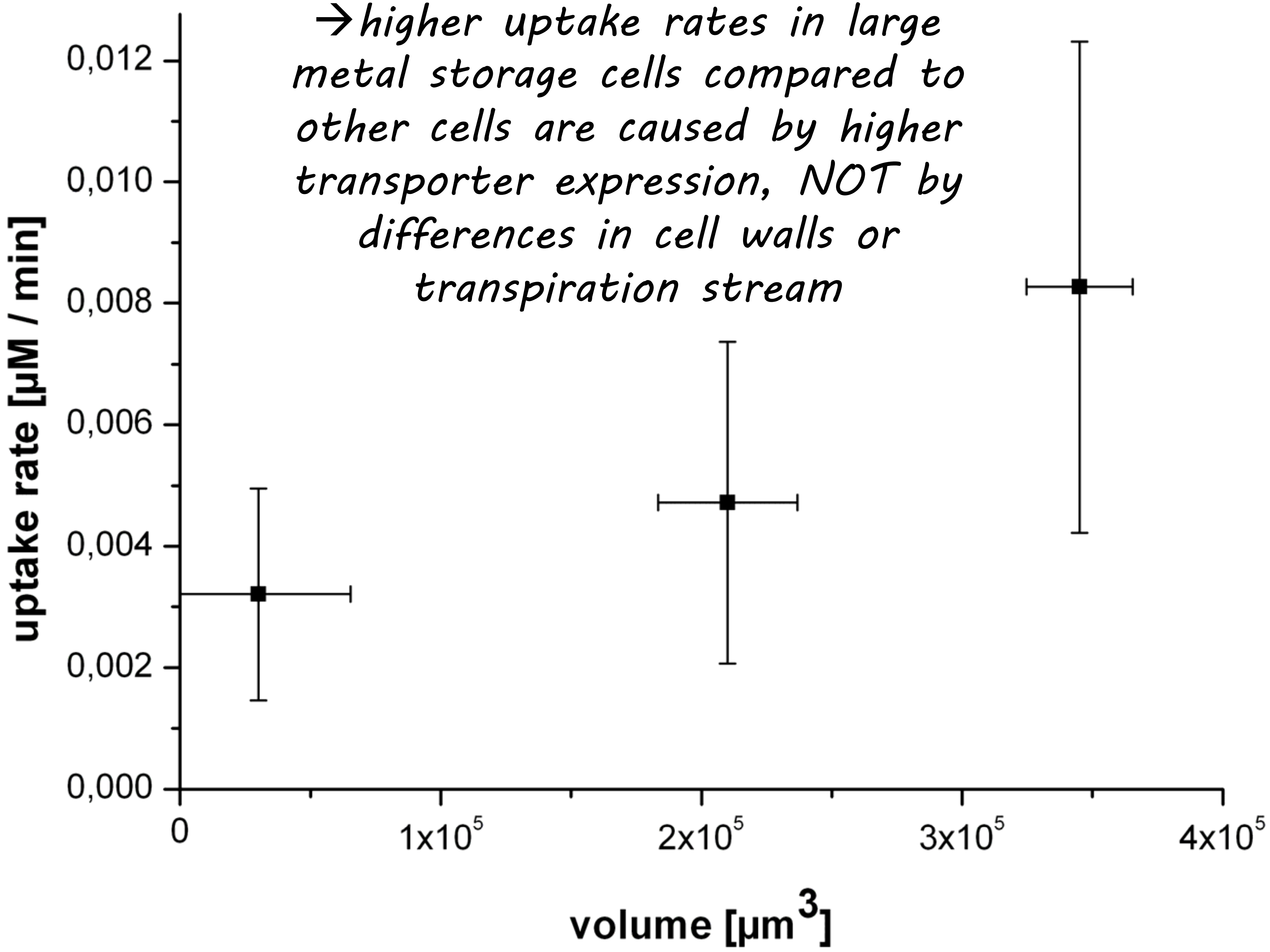


A cell that was incubated with Cd over night is completely filled with Cd, which means that the transport into the vacuole took place

The transport into the vacuole is the time-limiting step in metal uptake!

Leitenmaier B, Küpper H (2011) Plant Cell & Environment 34, 208-219

Cd-transport into protoplasts isolated from the hyperaccumulator plant *Noccaea caerulescens*...(III)

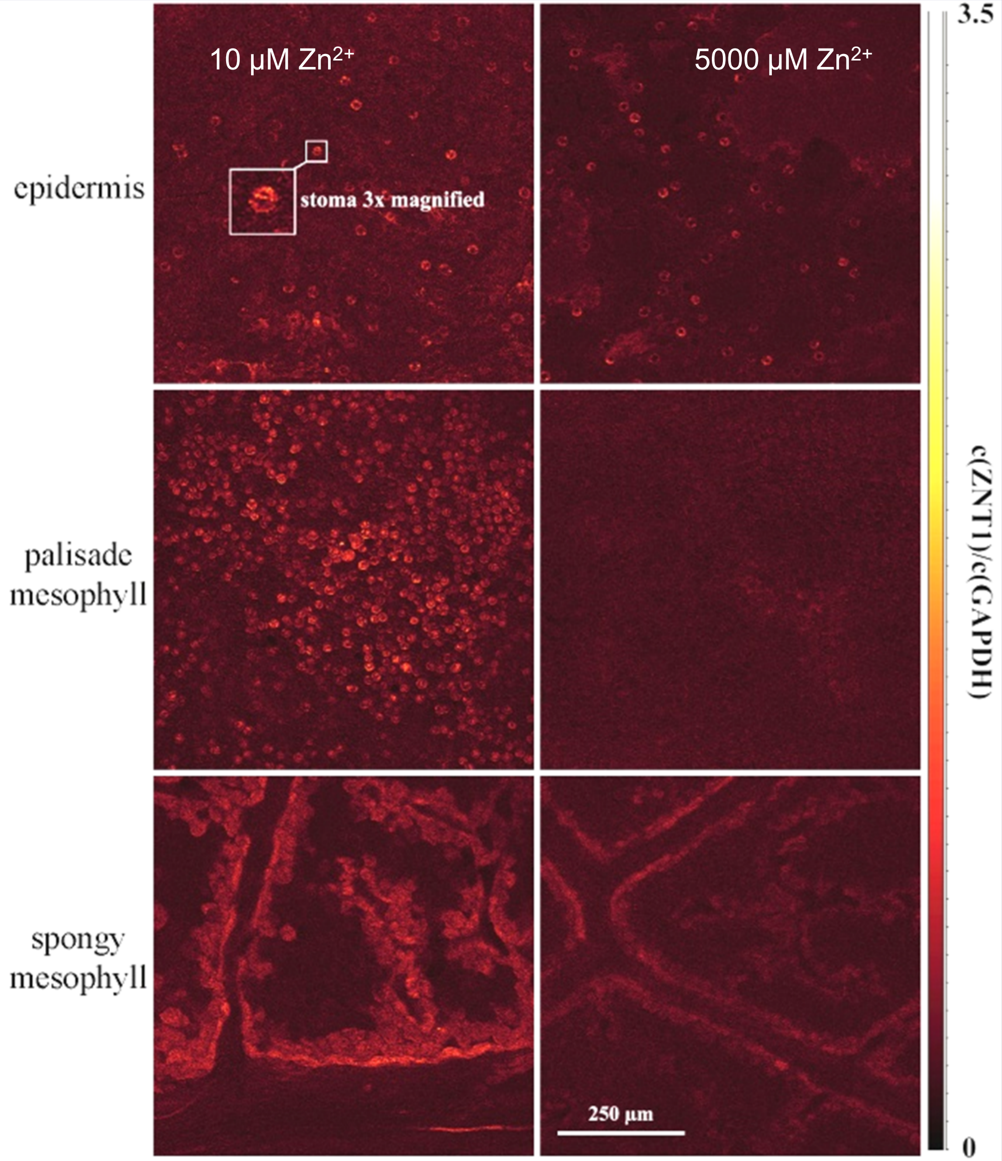


→ higher uptake rates in large metal storage cells compared to other cells are caused by higher transporter expression, NOT by differences in cell walls or transpiration stream

Leitenmaier B, Küpper H (2011) Plant Cell & Environment 34, 208-219

Different expression patterns of closely related Zn-specific ZIP transporters as revealed by QISH

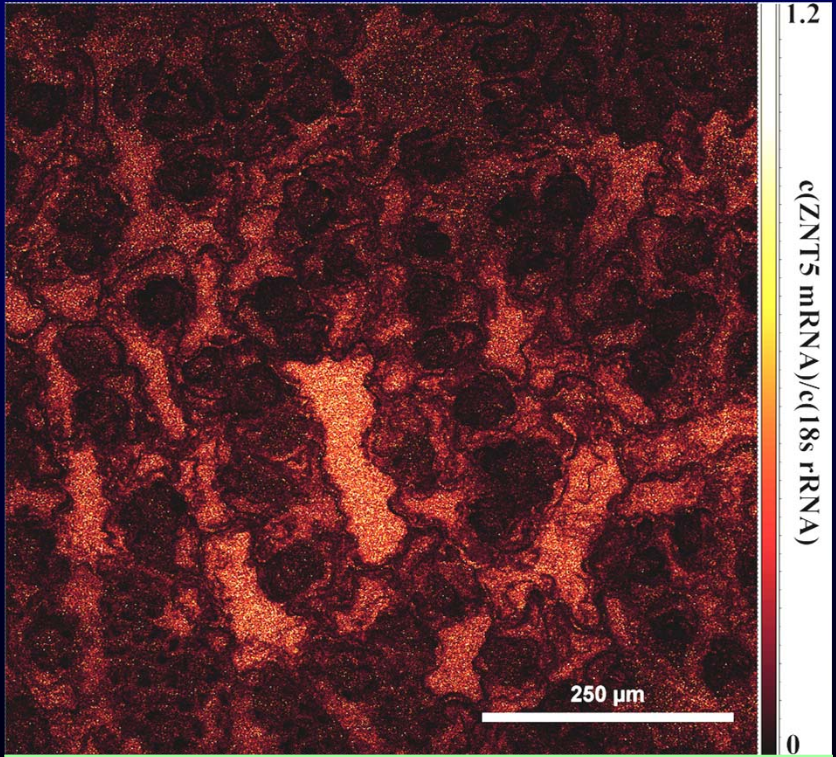
Expression of ZNT1 mainly in metabolically active cells, not metal storage cells



Küpper H, Seib LO, Sivaguru M, Hoekenga OA, Kochian LV, 2007 The Plant Journal 50(1), 159-187

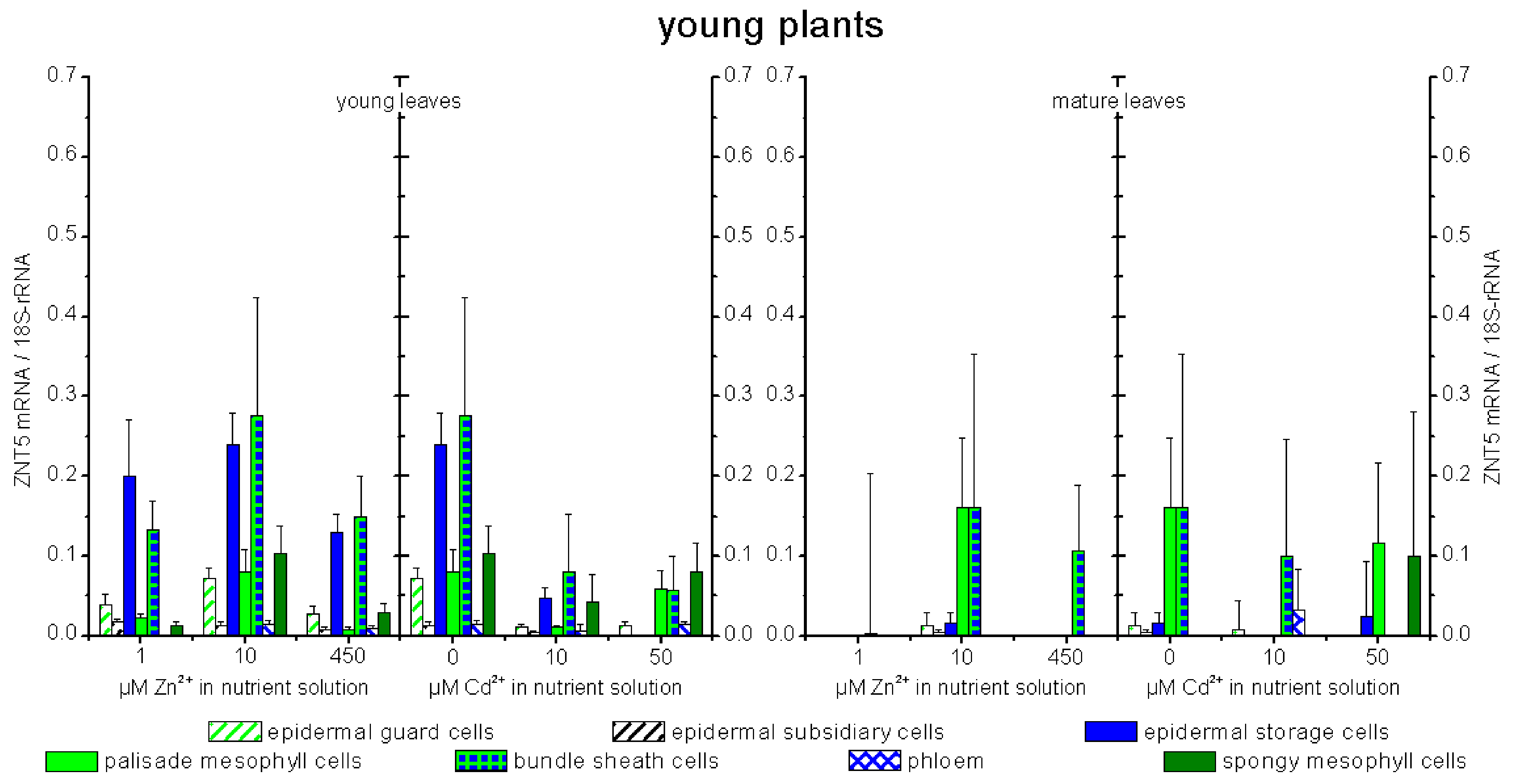
Expression of ZNT5 mainly in metal storage cells

→ judged by its expression pattern in the epidermis that matches known accumulation patterns for Zn and Ni, ZNT5 may be a key player in hyperaccumulation of Zn



Küpper H, Kochian LV (2010) New Phytologist 185, 114-129

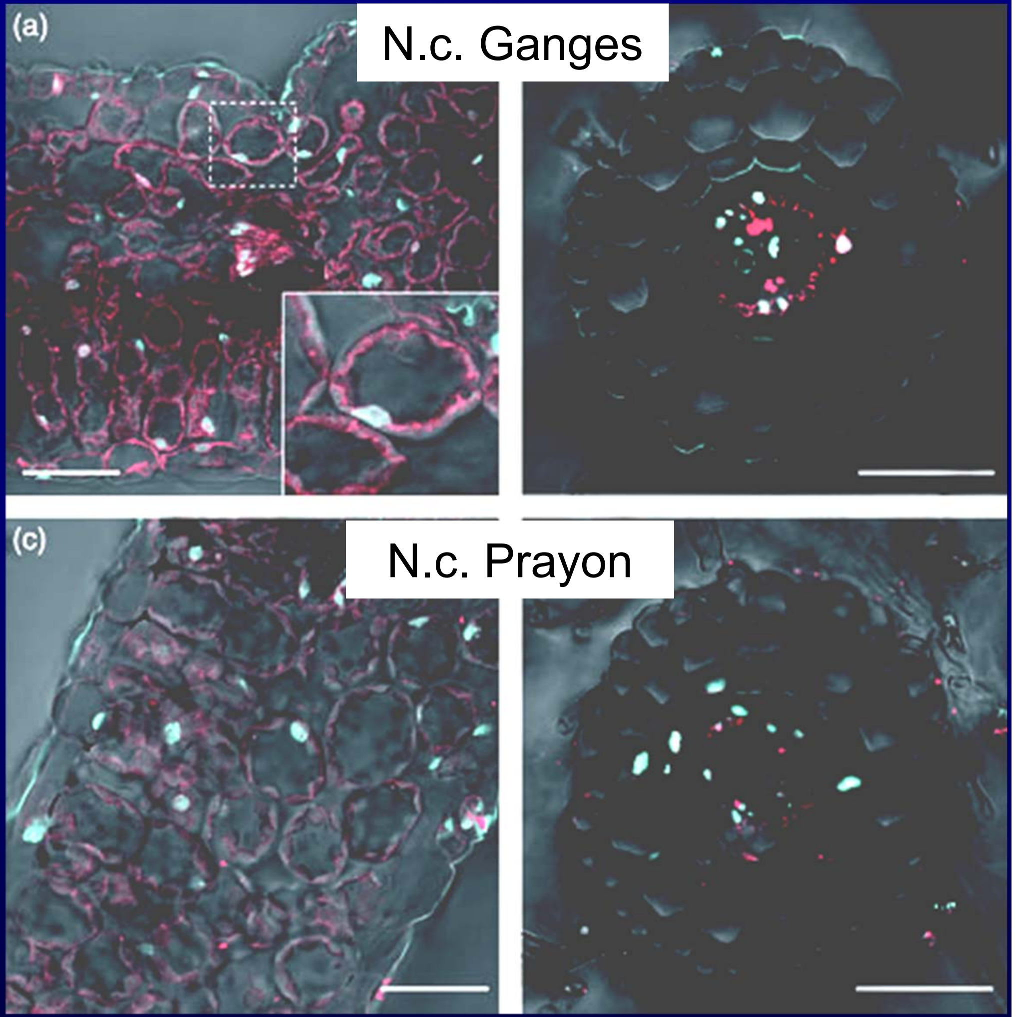
Regulation of ZNT5 transcription in young leaves of *Noccaea caerulescens (Ganges ecotype)* analysed by QISH



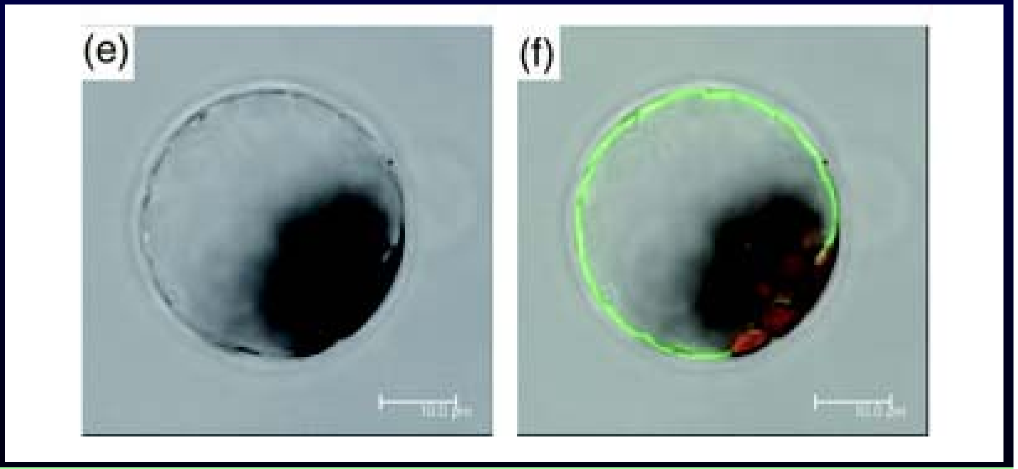
→ ZNT5 seems to be involved both in unloading Zn from the veins and in sequestering it into epidermal storage cells

Küpper H, Kochian LV (2010) New Phytologist 185, 114-29

HMA3 as a likely candidate for the vacuolar Cd sequestration in *N. caerulescens* and elevated Cd-accumulation in the Ganges vs. Prayon ecotype



HMA3 is much stronger expressed in T.c. Ganges



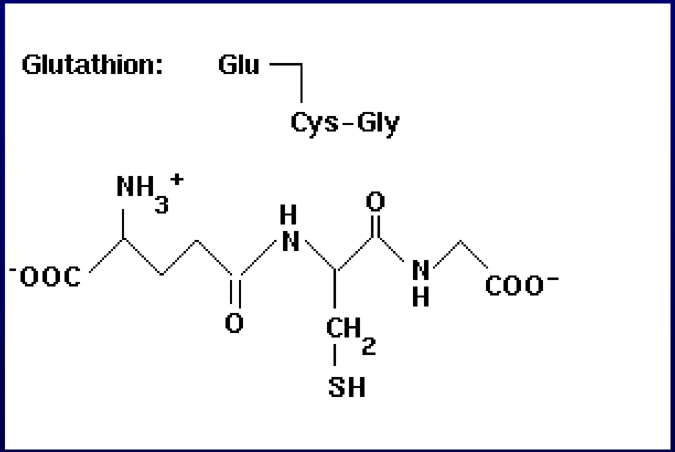
HMA3 is localised in the vacuolar membrane

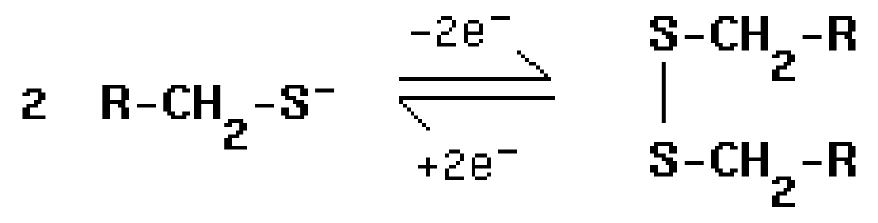
Ueno D et al. et Ma JF, 2011, PlantJ66_852–62

Other mechanisms of metal resistance

Resistance mechanisms against oxidative stress

- Enhanced expression of enzymes that detoxify reactive oxygen species (superoxide dismutase+catalase. Problem: inhibition of Zn-uptake (→SOD) during Cd-Stress.
- Synthesis of non-enzyme-antioxidants, e.g. ascorbate and glutathione





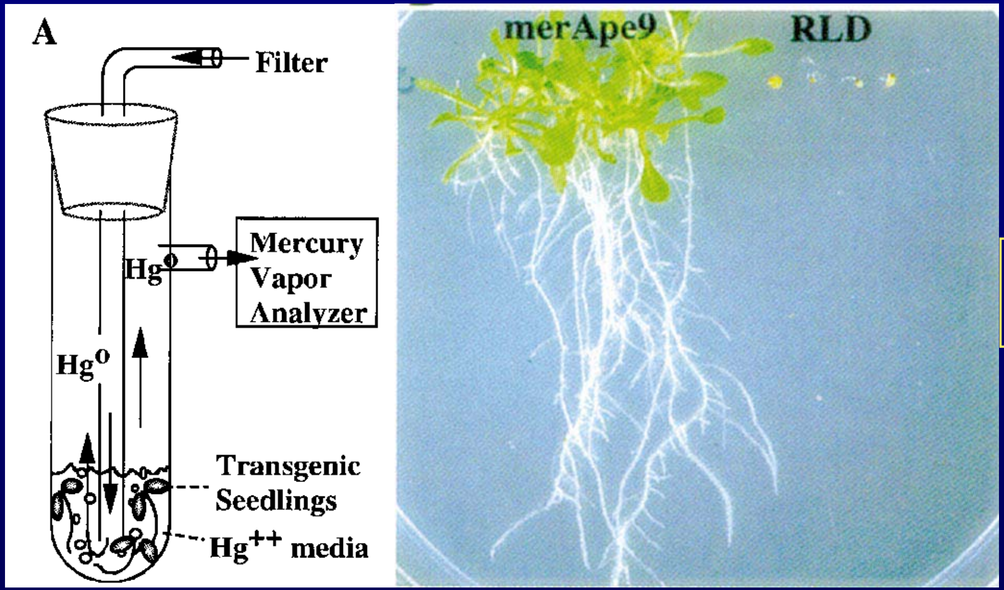
$$2\ R-CH_2-S^- \underset{+2e^-}{\overset{-2e^-}{\rightleftharpoons}} \begin{array}{l} S-CH_2-R \\ | \\ S-CH_2-R \end{array}$$

- Changes in the cell membranes to make them more resistant against the attack of reactive oxygen species:

- Lipids with less unsaturated bonds
- Exchange of phosphatidyl-choline against phosphytidyl-ethanolamine as lipid-“head“
- Diminished proportion of lipids and enhanced proportion of stabilising proteins in the membrane

Other detoxification mechanisms

- Reduction by reductases, e.g. Hg^{2+} --> Hg_0, Cu^{2+} --> Cu^+

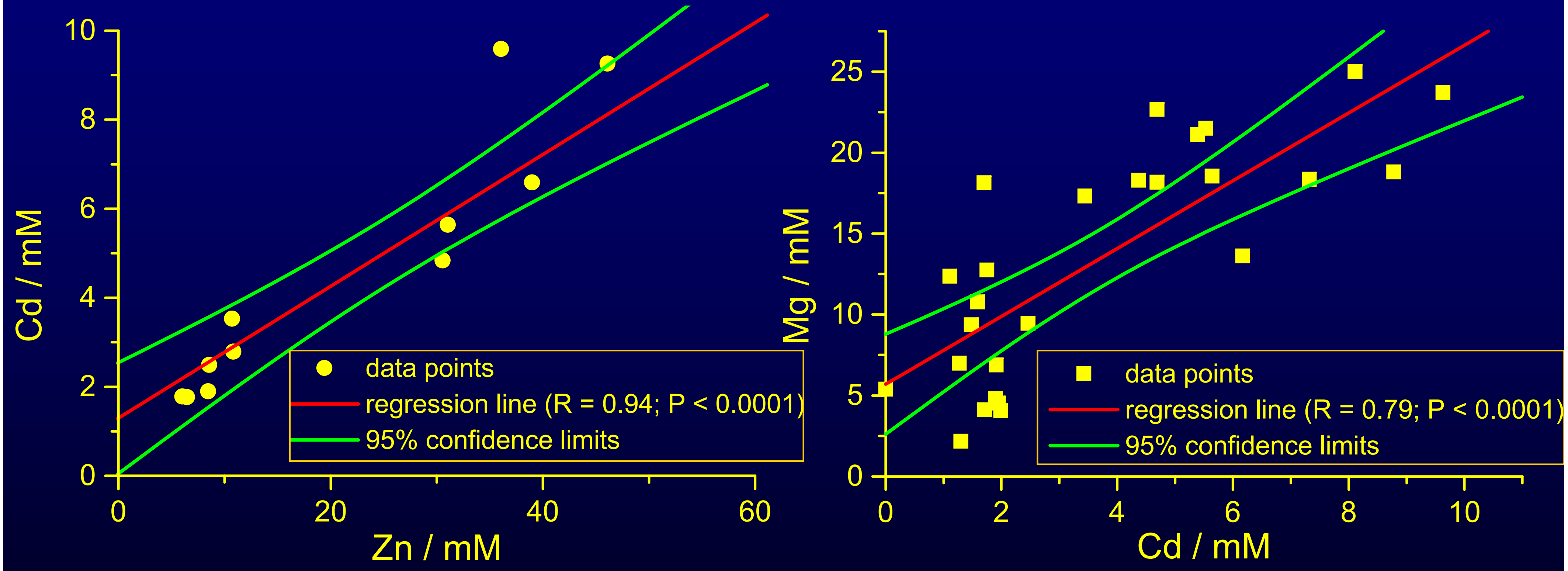


Rugh CL, et al, 1996, PNAS 93, 3182-3187

- Methylation of arsenic → historically regarded as „detoxification“, but more recent evidence suggests an increase in toxicity in terms of mutagenic/cancerogenic effects upon methylation
- Precipitation of insoluble sulfides outside the cell (on the cell wall)

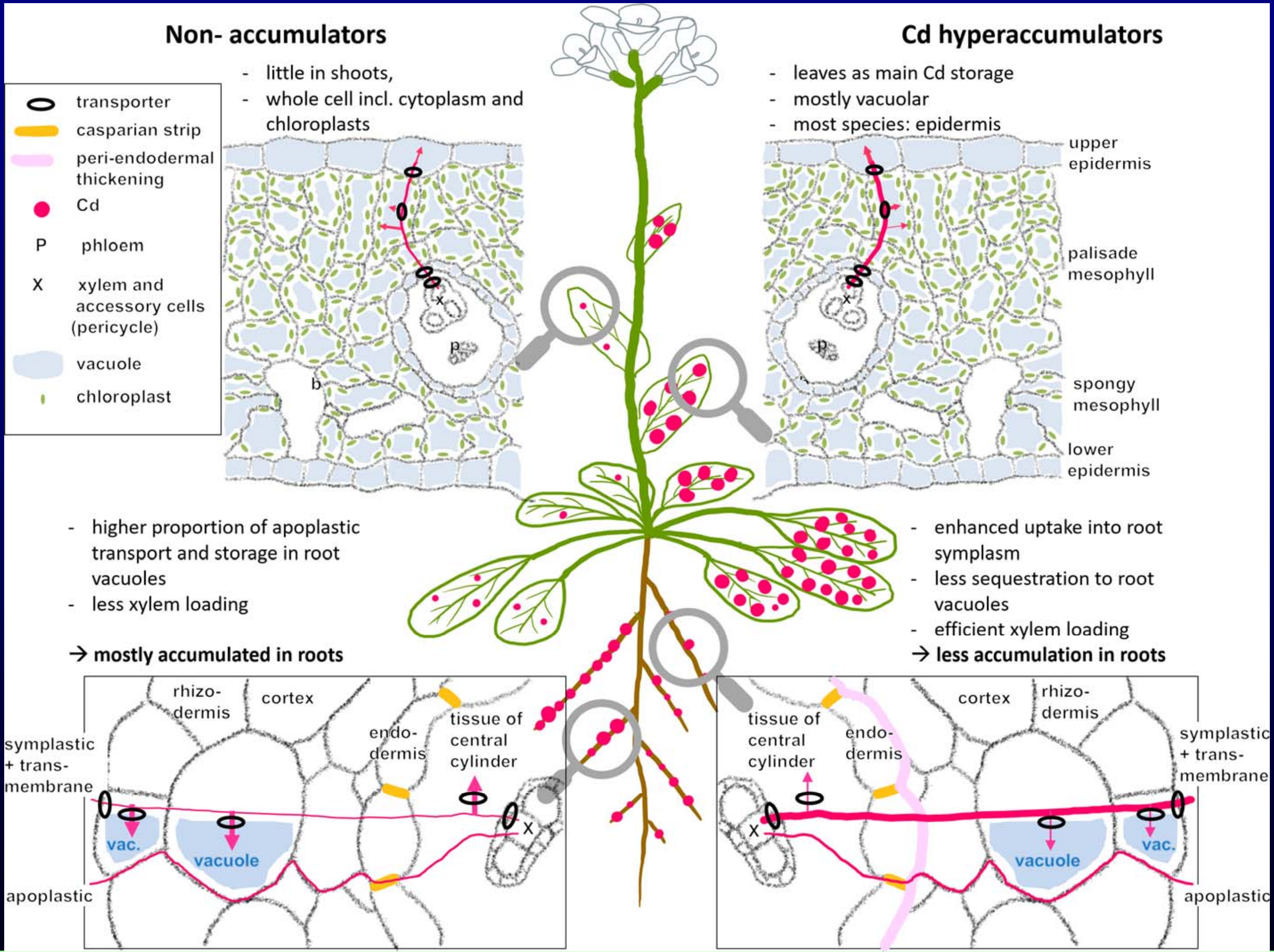
Compartmentation of metals in leaves

Up-regulation of Mg uptake in response to Cd toxicity in the mesophyll of *Arabidospis halleri*



Küpper H, Lombi E, Zhao FJ, McGrath SP (2000) Planta 212, 75-84

Summary 1



Non- accumulators

- little in shoots,
- whole cell incl. cytoplasm and chloroplasts

- higher proportion of apoplastic transport and storage in root vacuoles
- less xylem loading

→ **mostly accumulated in roots**

Cd hyperaccumulators

- leaves as main Cd storage
- mostly vacuolar
- most species: epidermis

- enhanced uptake into root symplasm
- less sequestration to root vacuoles
- efficient xylem loading

→ **less accumulation in roots**

Review: Küpper H, Tao Q, Morina F (2025) Cadmium hyperaccumulation. In: Cadmium contamination in soils and plants: Mechanisms and mitigation
Editors: Fang-Jie Zhao, Peng Wang, Steve P. McGrath

Summary 2

	Low trace metal content in soil			High trace metal content in soil		
	excluder	**indicator**	**hyperacc.**	**excluder**	**indicator**	**hyperacc.**
uptake	low	medium	medium-high	medium	high	very high
requirement	medium		high	medium		high
effect	deficiency	no stress	deficiency & pathogen attack	no stress	toxicity	no stress
costs	high	low	high	high		
growth	low	very high	very low	medium	very low	medium

Non-metalliferous alpine meadow

Mount hood (Oregon, USA). From: commons.wikimedia.org

Natural serpentine barren

Slate Mountain serpentine barren (North Carolina, USA), From: US forest service

Reviews:

Küpper H, Kroneck PMH (2005) Metal Ions Biol Syst 44, ch5, 97-142; Küpper H, Kroneck PMH (2007) Metal Ions Life Sci 2, 31-62;
Küpper H, Leitenmaier B (2013) Metal Ions Life Sci 11, ch12, 373-394; Andresen E, Küpper H (2013) Metal Ions Life Sci 11, ch13, 395-414

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