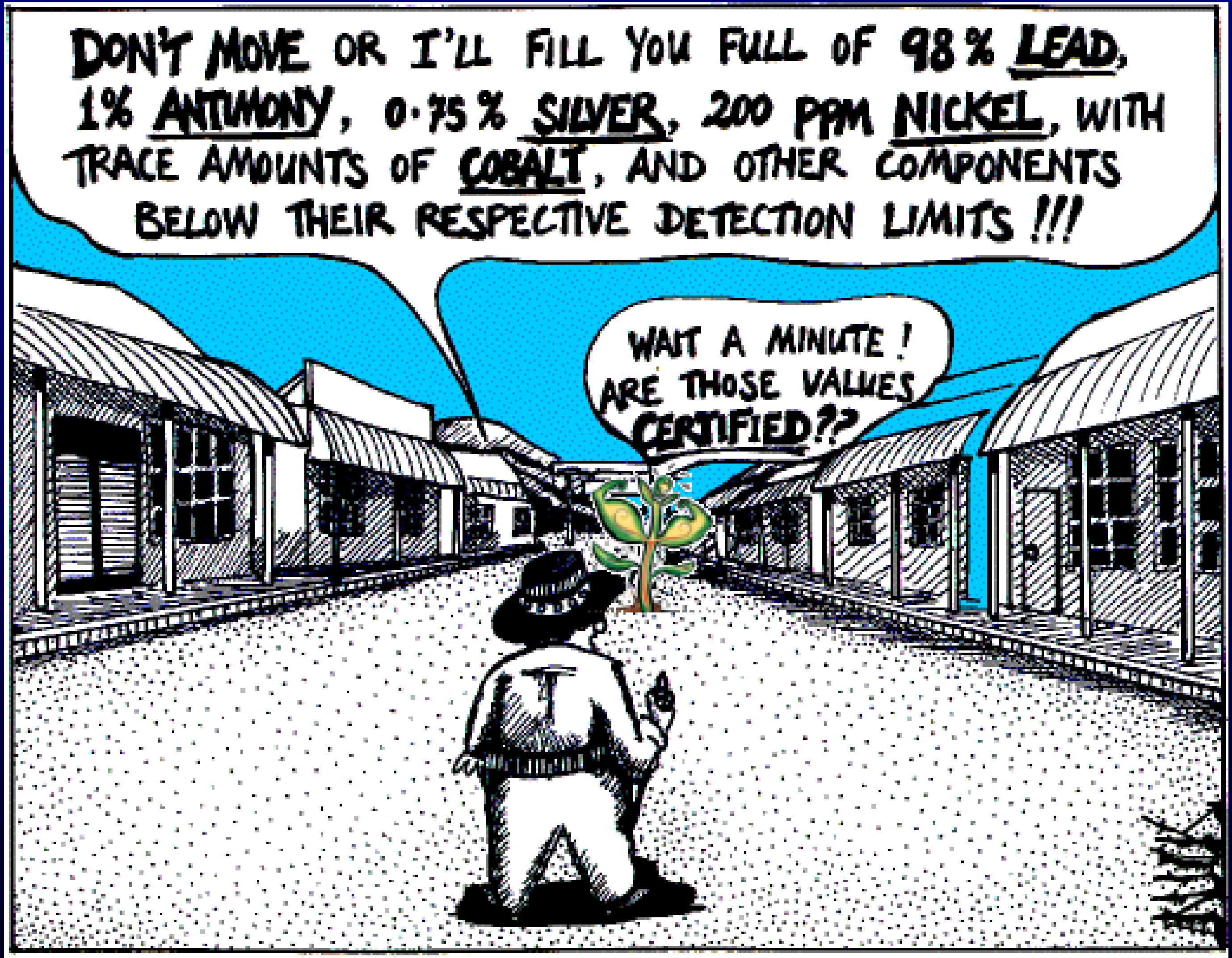
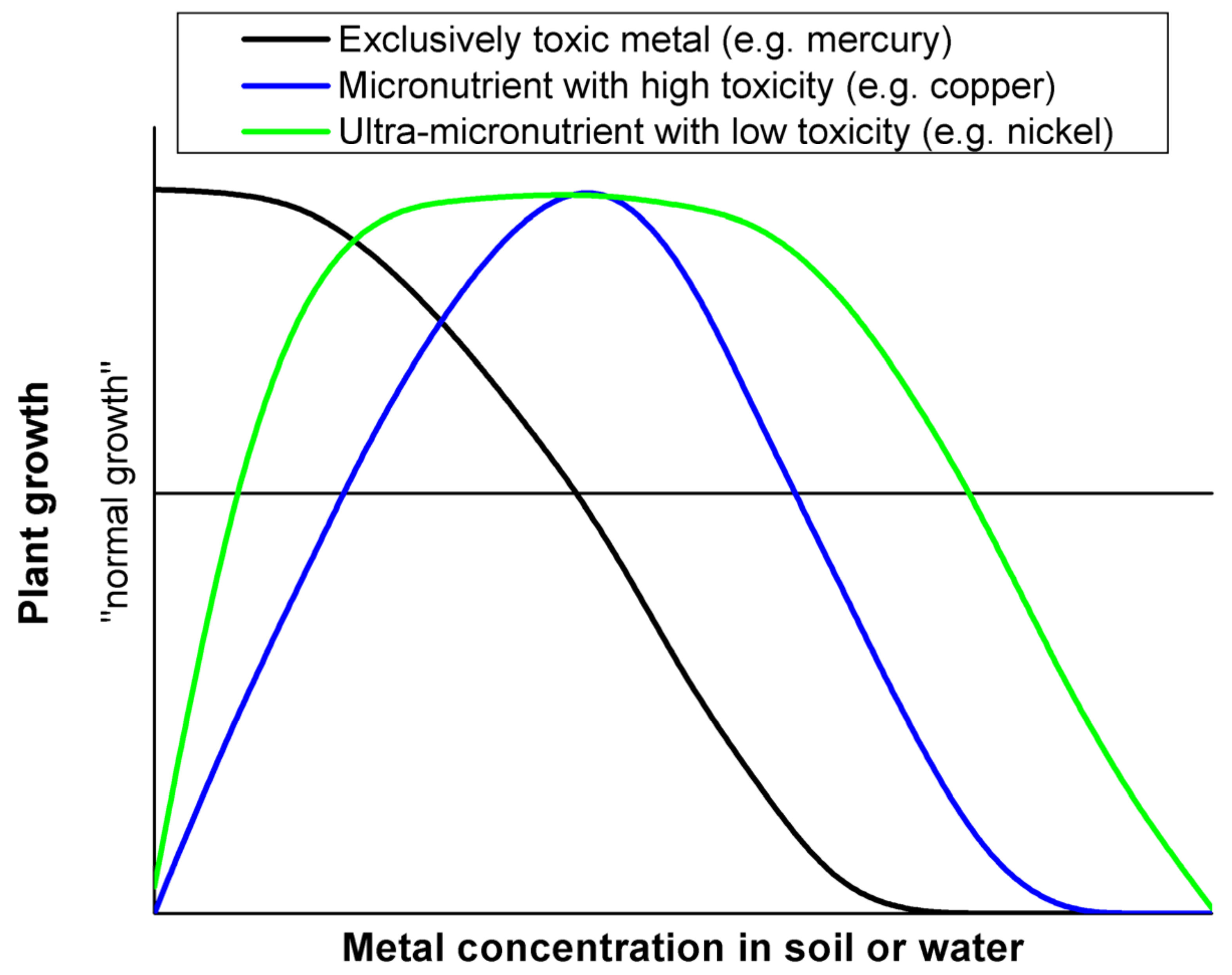
Trace metal deficiency & toxicity



Metal uptake in the Wild West

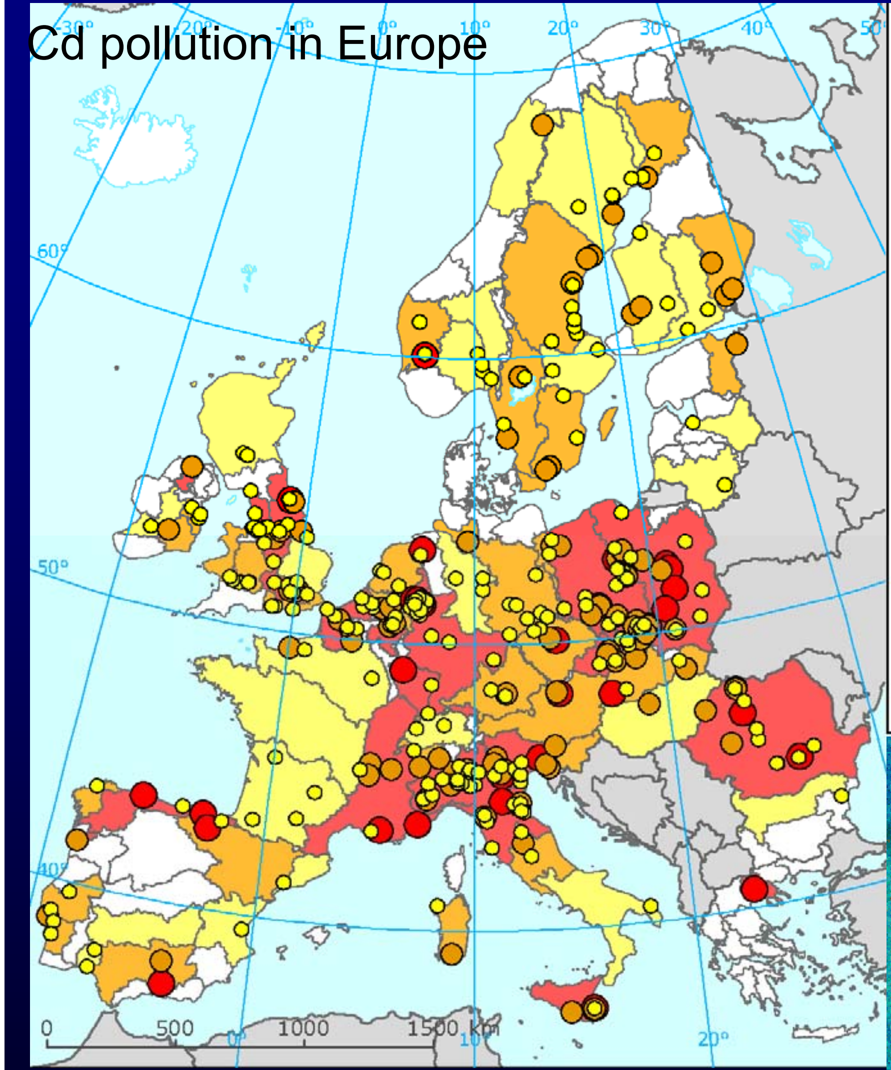
modified from: http://strangematter.sci.waikato.ac.nz/

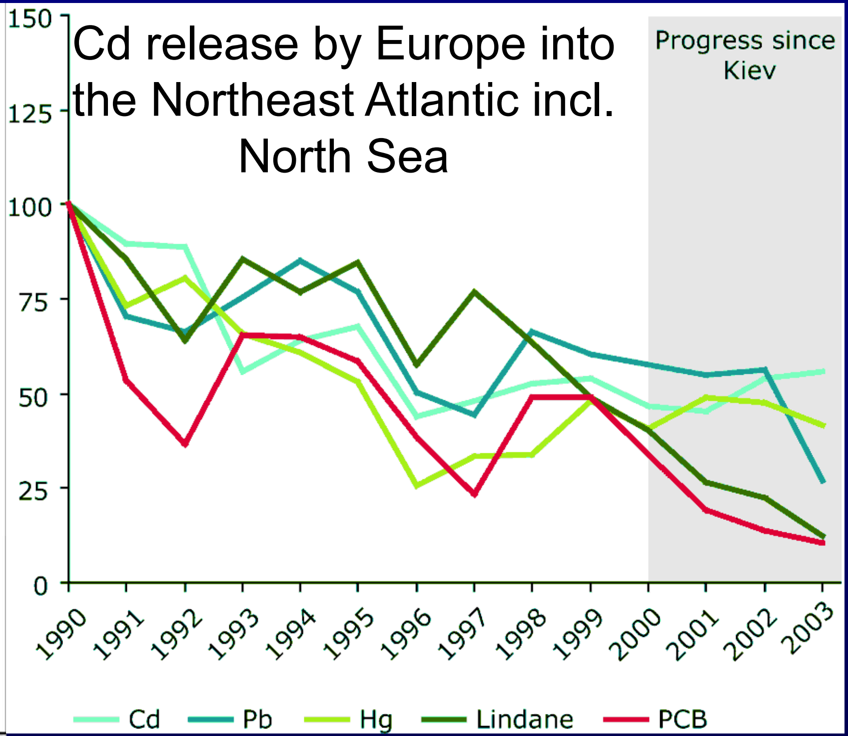
Dose-response principle for heavy metals

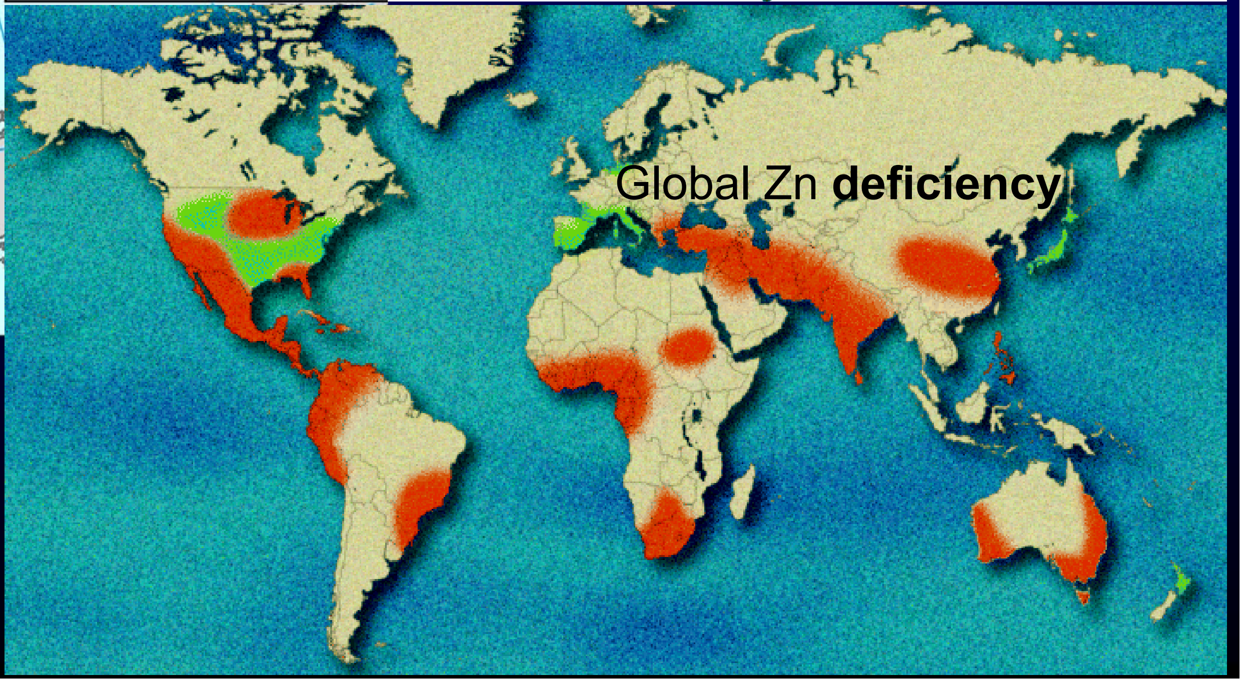


Küpper H, Kroneck PMH, 2005, Metal ions Life Sci 2, 31-62

Variability of Metal contents from deficiency to toxicity – a global problem for agriculture and human health





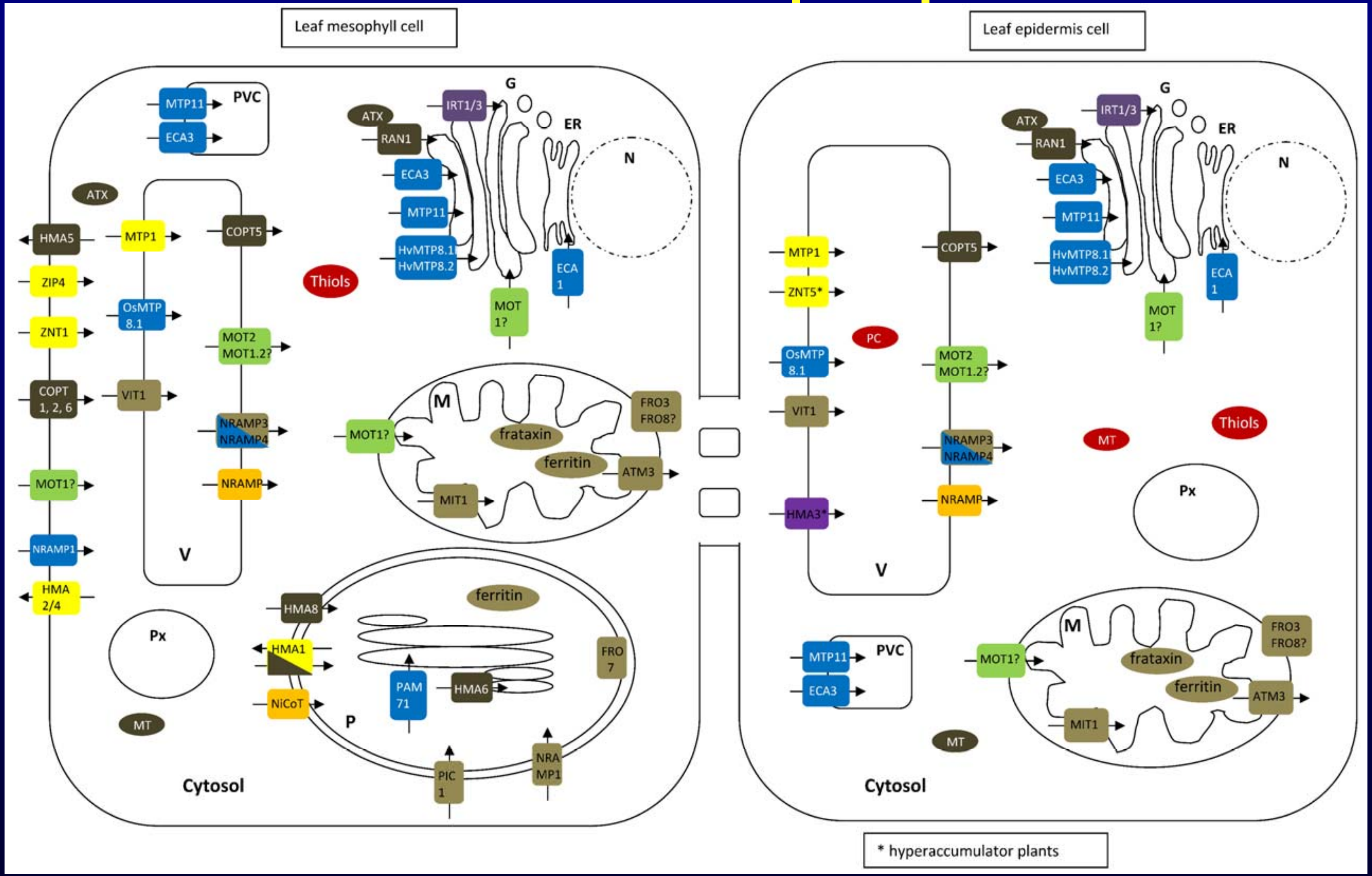


Cd map and trend from http://www.eea.europa.eu (European Environment Agency)

Zn map From: Alloway BJ. 2001. Zinc the vital micronutrient for healthy, high-value crops. Brussels, Belgium: International Zinc Association.

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

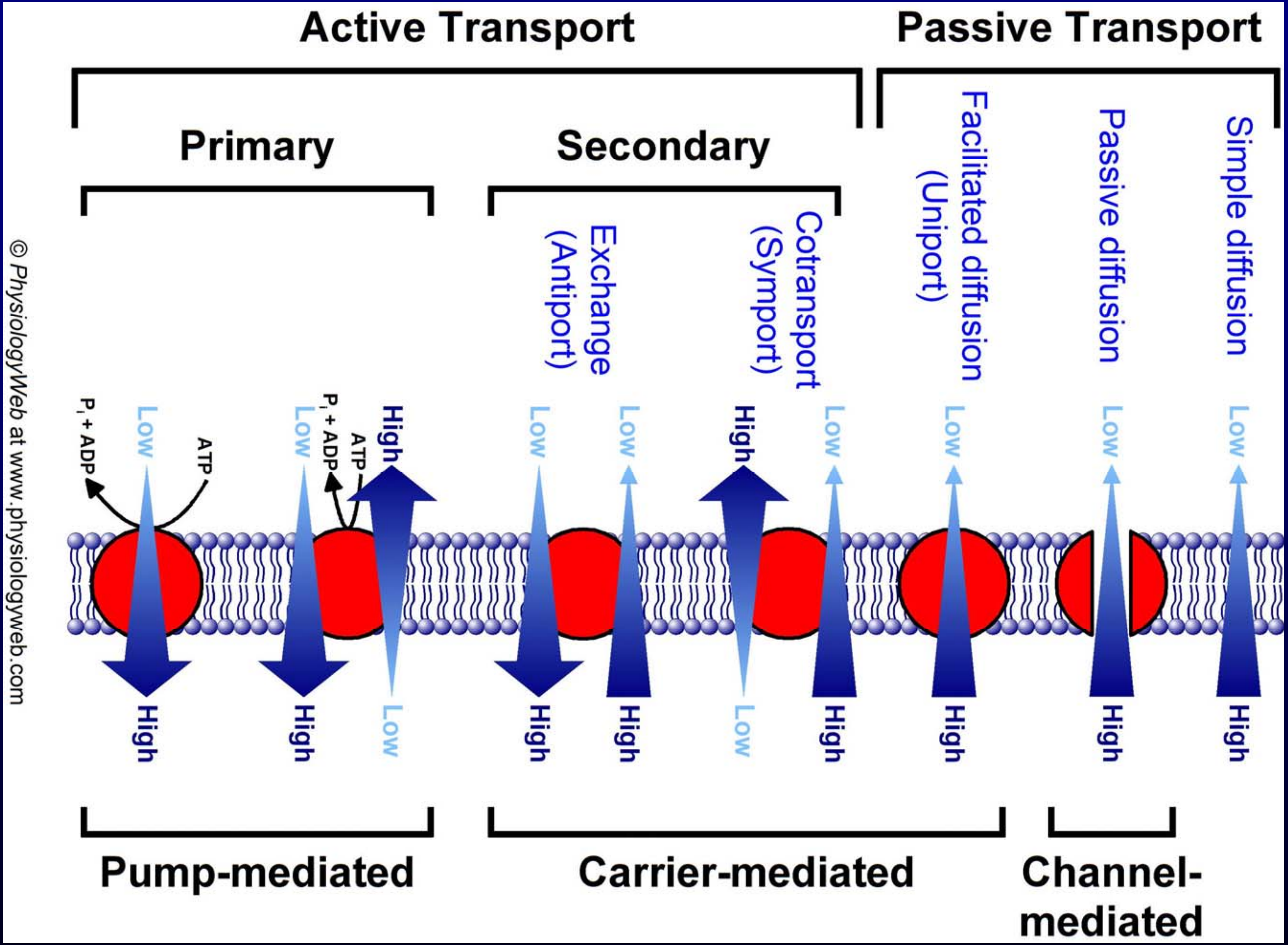
From: Andresen E, Peiter E, Küpper H (2018) Journal of Experimental Botany 69, 909-954



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)

Energetics and variants of metal transport



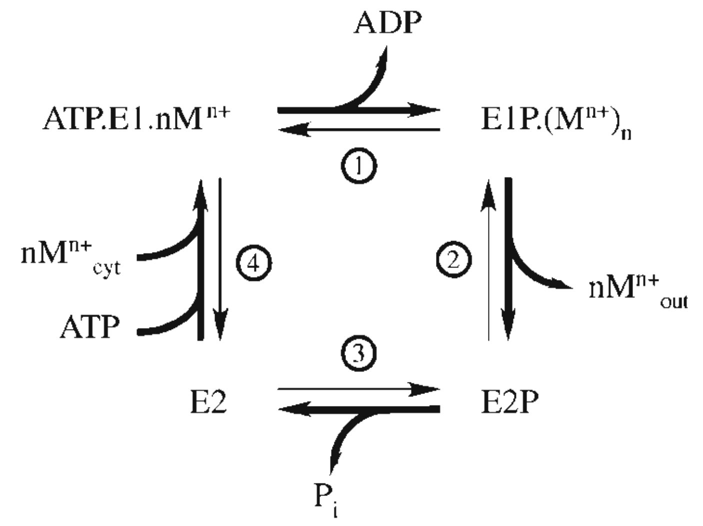
$$\Delta G = n_{Ionen} * R * T * \ln (c_{inside} / c_{outside}) + 3F (\varphi_{outside} - \varphi_{inside})$$

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

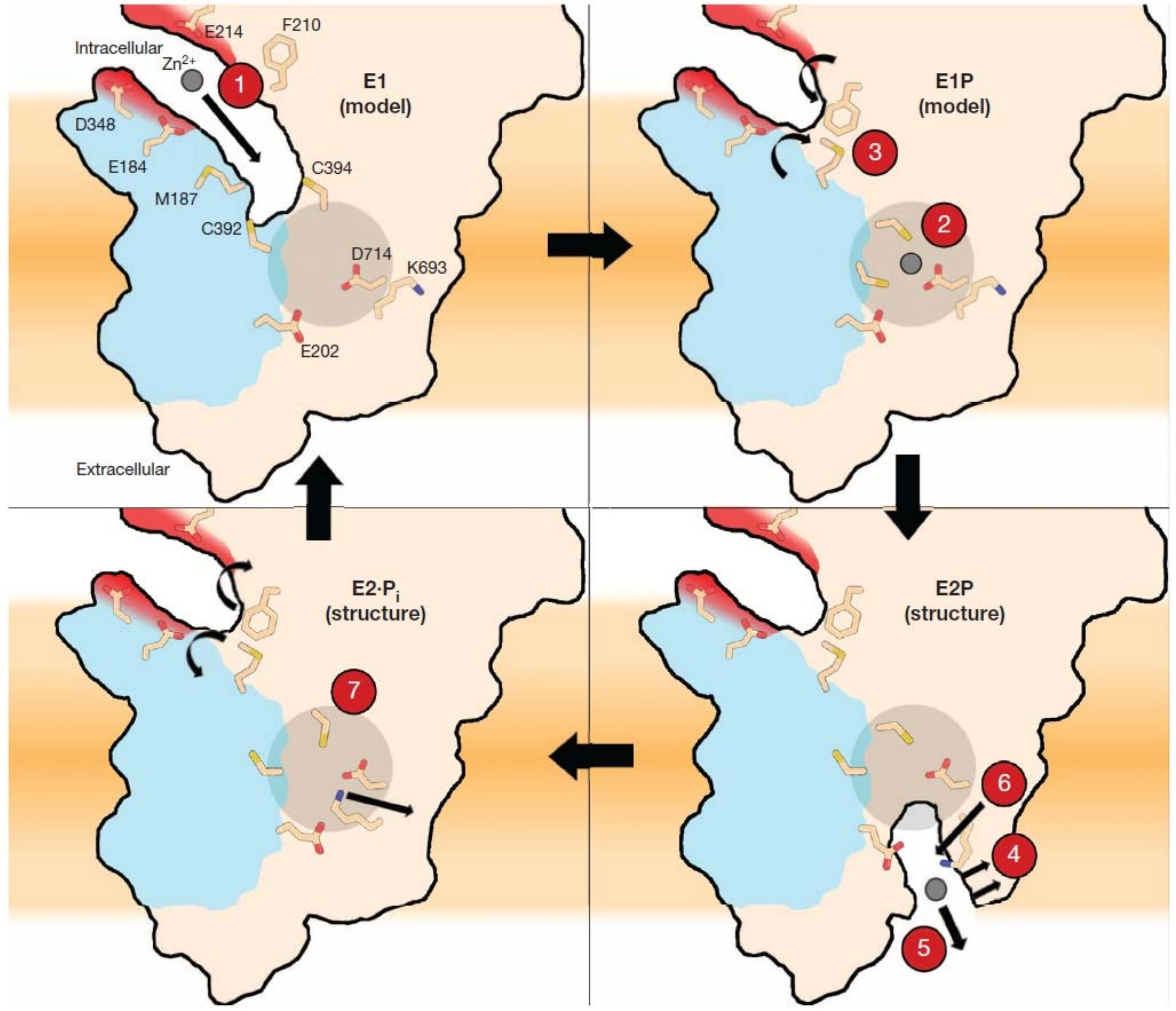
Mechanisms of metal uptake+compartmentation in plants (I)
CPx-type (=P$_{1B}$-type) ATPases

Mechanism

- Zn is guided into binding pocket by negatively charged residues
- binding pocket closes after ATP binding
- pore opens on other side of protein, release of Zn^{2+}
- pore closes after ATP hydrolysis

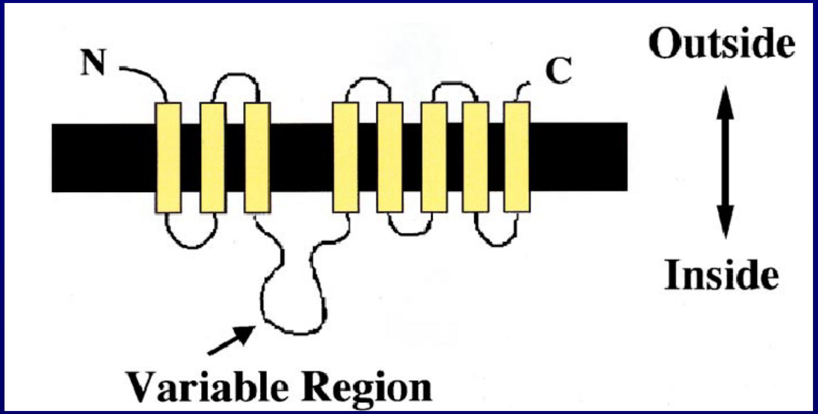


From: Argüello JM et al., 2007, Biometals, DOI 10.1007/s10534-006-9055-6

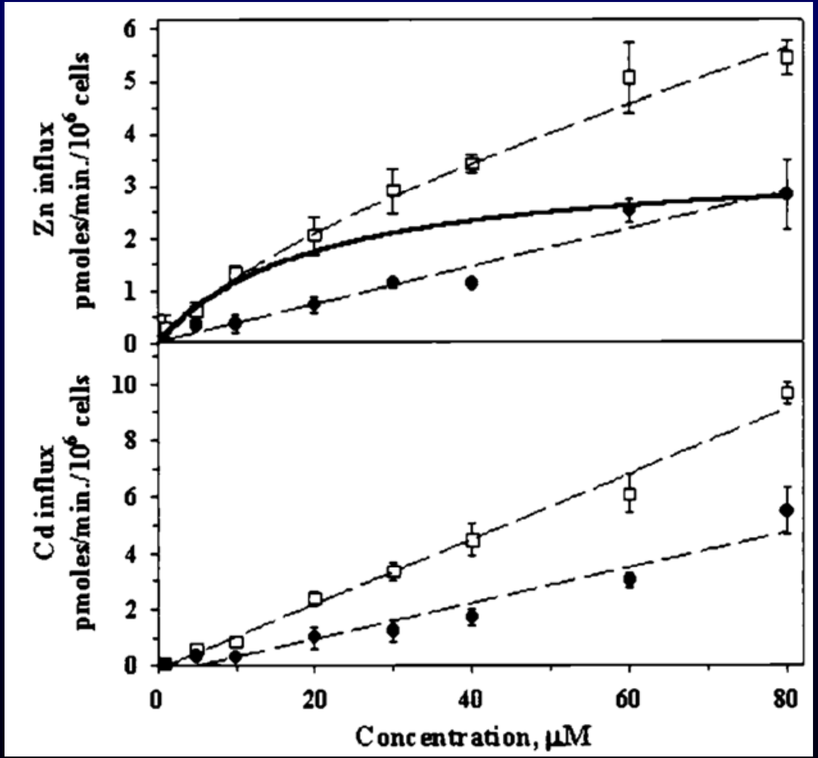


From: Wang K et al, 2014, Structure and mechanism of Zn-transporting P-type ATPases. Nature 514, 518-

Mechanisms of metal uptake+compartmentation in plants (II) ZIP-transporters



From:Guerinot ML, 2000, BBA 1465, 190-8



From:Pence NS et al., 2000, PNAS 97, 4956-60

Functions suggested by expression studies)

- uptake of metals into cells over the cytoplasmatic membrane
- abundant in all eucaryotes, incl. humans, plants and fungi

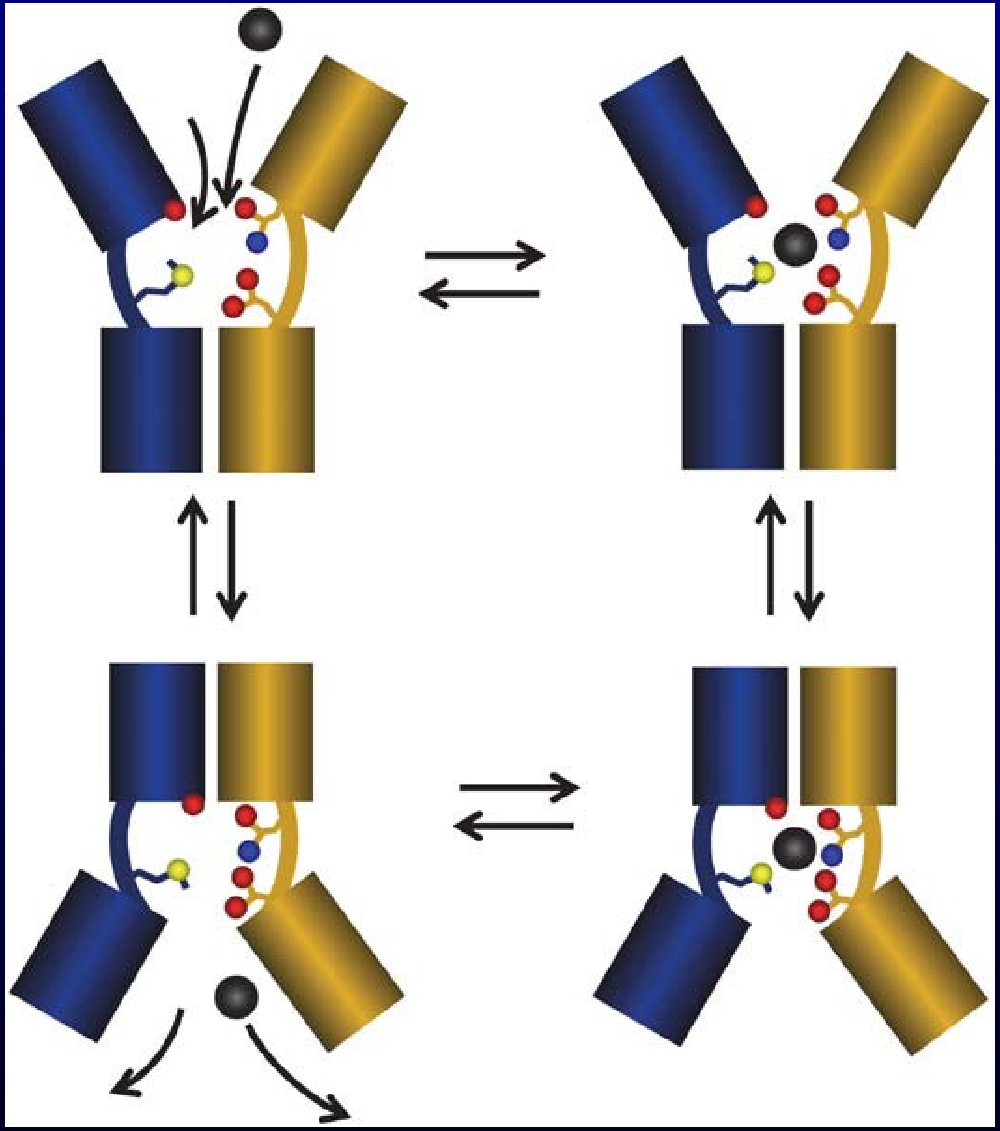
Structure predicted by sequence

- usually 8 transmembrane helices, one long variable region, predicted to be in the cytoplasm
- 309-476 amino acids
- still no complete 3D structure available

Characteristics revealed by yeast expression studies

- High affinity and saturable kinetics for selected metal (e.g. Zn in ZNT1)
- Lower affinity uptake for related metals (e.g. Cd in ZNT1)

Mechanisms of metal uptake in plants (III)
Natural resistance associated macrophage proteins (NRAMPs)

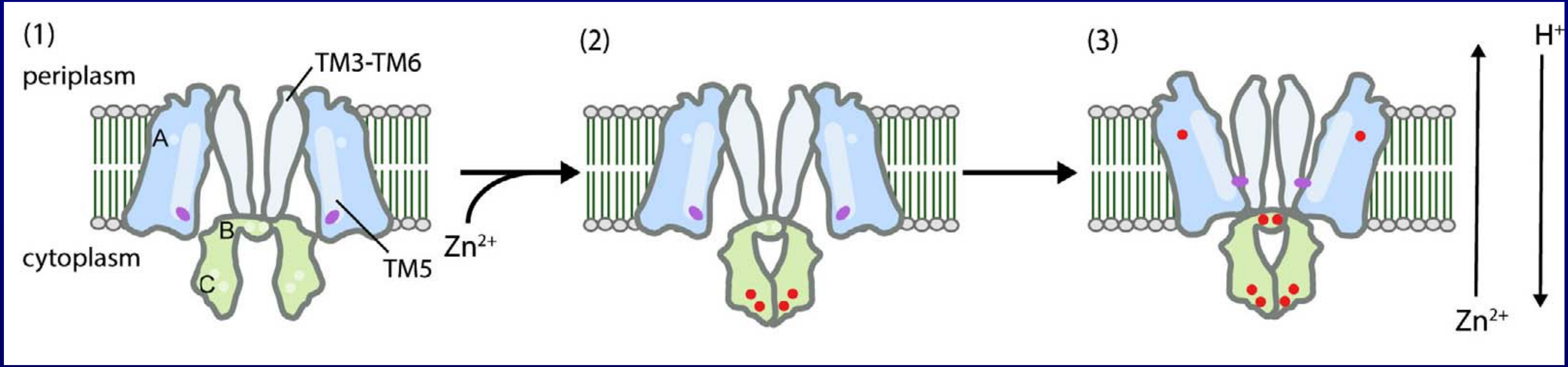


Mechanism deduced from crystal structure and enzyme kinetic studies

- proton symport with the electrochemical gradient drives metal translocation against the gradient
- binding of metal and proton induces a conformational change of the two halves of the helices 1 and 6 around a hinge in the metal binding site
- the conformational change closes the pore on the outer side and opens a pore on the inner side
- the opening of the intracellular pore releases metal and proton.

From: Ehrnstorfer IA, Geertsma ER, Pardon E, Steyaert J, Dutzler R. 2014. Nat Struct Mol Biol. 21, 990-6

Mechanisms of metal uptake in plants (IV): Cation diffusion facilitator (CDF)-transporters



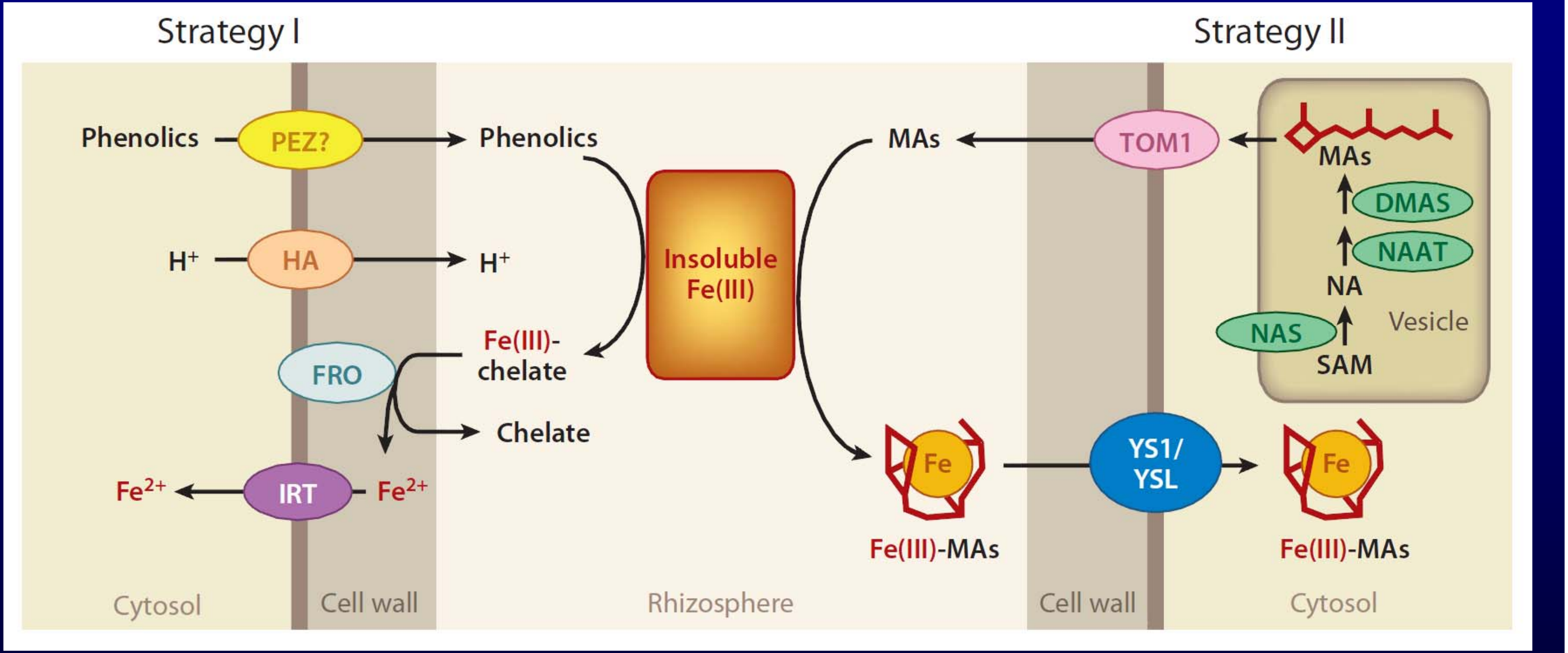
From: Kolaj-Robin O, Russell D, Hayes KA, Pembroke JT, Soulimane T. 2015. FEBS Lett. 589, 1283-95

Mechanism concluded from structure and kinetic studies

- Proton-metal antiport
- The exact movements are still discussed as the only available complete crystal structure is rather low resolution (3.8A)
- Metal binding causes a conformational change of the cytoplasmic domain
- The conformational change leads to release of the metals on the outside of the cell

Now let's have a look at a few plant micronutrients

Mechanisms of iron uptake in plants
Strategies of iron efficiency

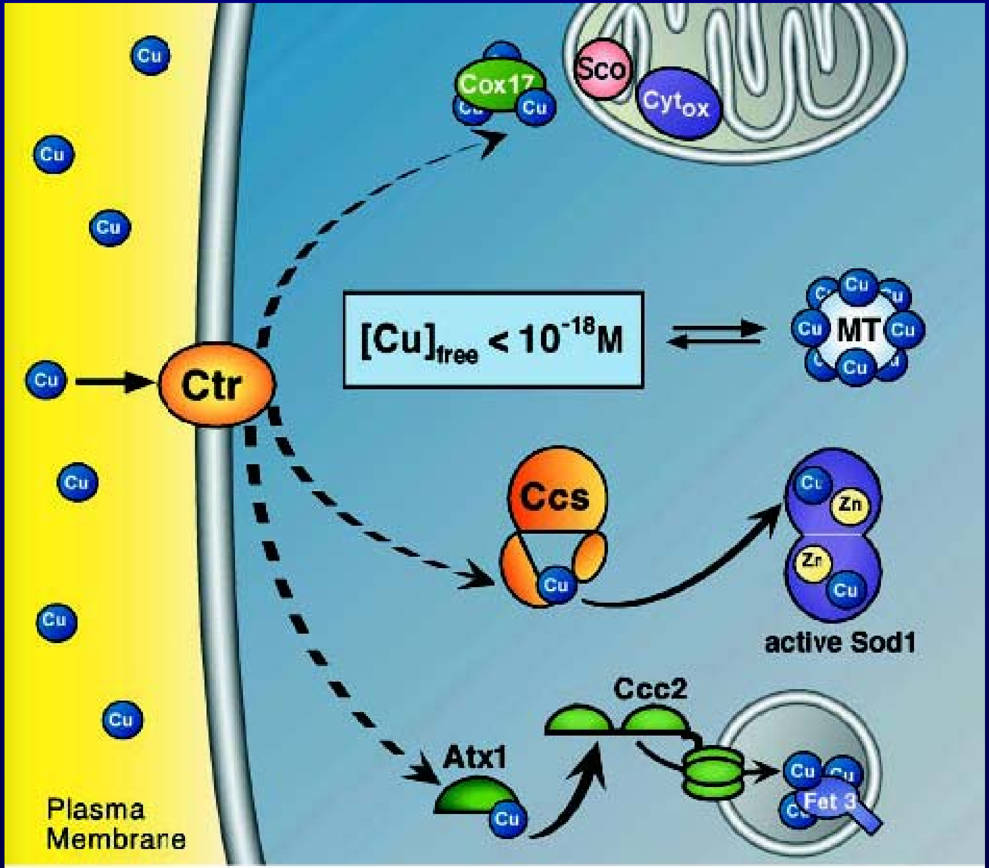


From: Kobayashi T, Nishizawa NK. 2012. Ann Rev Plant Biol 263, 131-152

Strategies of making insoluble Fe(III) bioavailable

- Strategy I (most plants): use mostly of soil acidification and iron reductase at root surface
- Strategy II (grasses): use of secreted iron ligand mugineic acid

Copper delivery inside cellular compartments

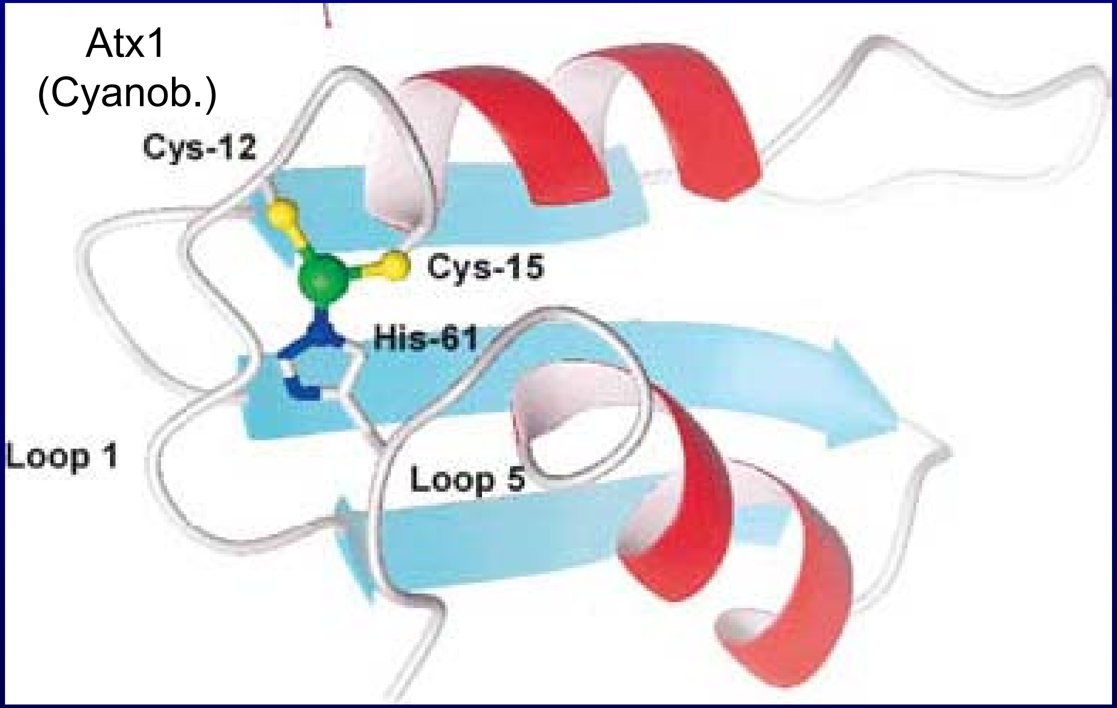


From: OHalloran TV, Culotta VC, 2000, JBC275, 25057-60

- confusing large number of names for homologous proteins in different organisms
- REALITY: just 3 really different (non-homologous) Cu-chaperones are well known, some more proteins are postulated to be Cu-chaperones

Copper delivery to the Golgi and thylakoid: ATX1 = HAH1 = ATOX1 = CopZ = Atx1

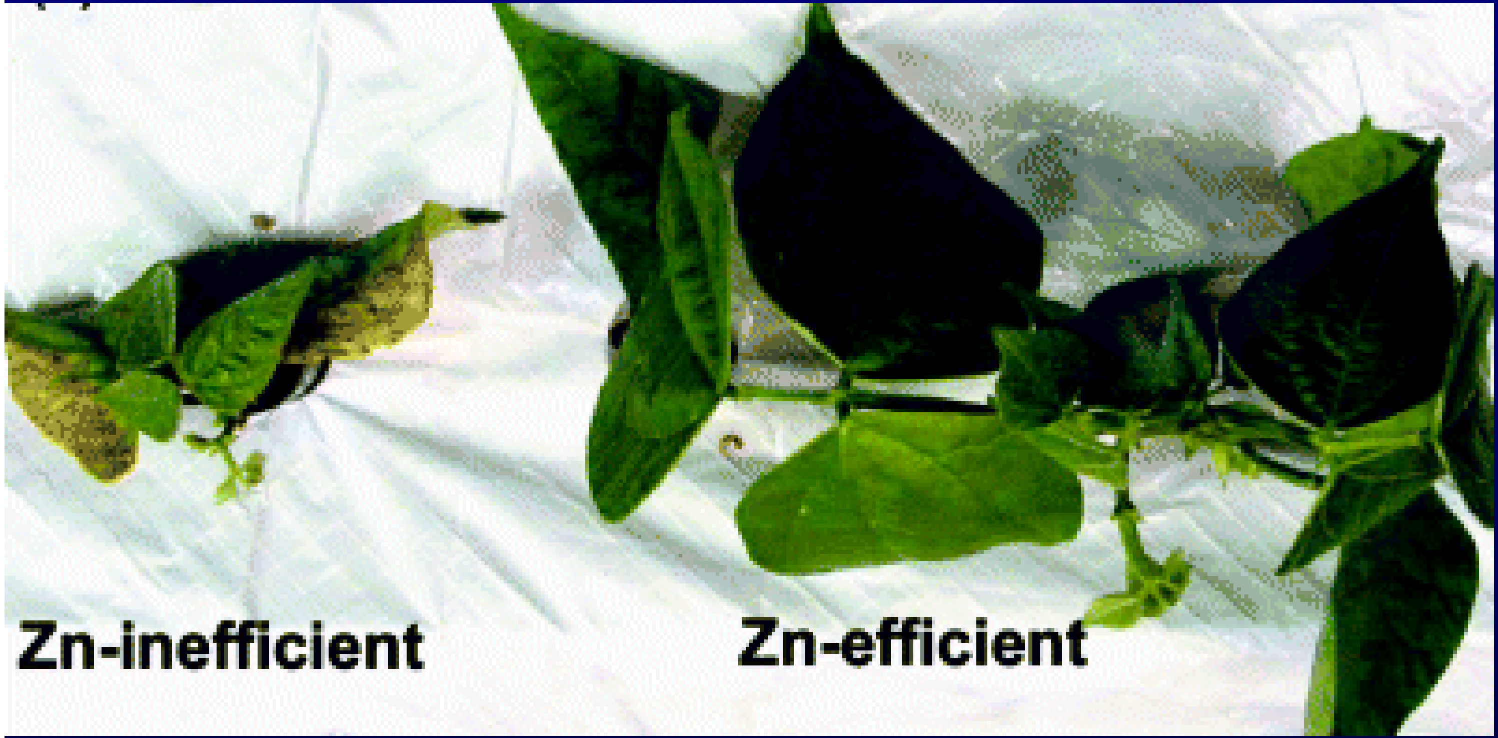
(c) Cu-binding in bacterial+cyanobacterial+plant version

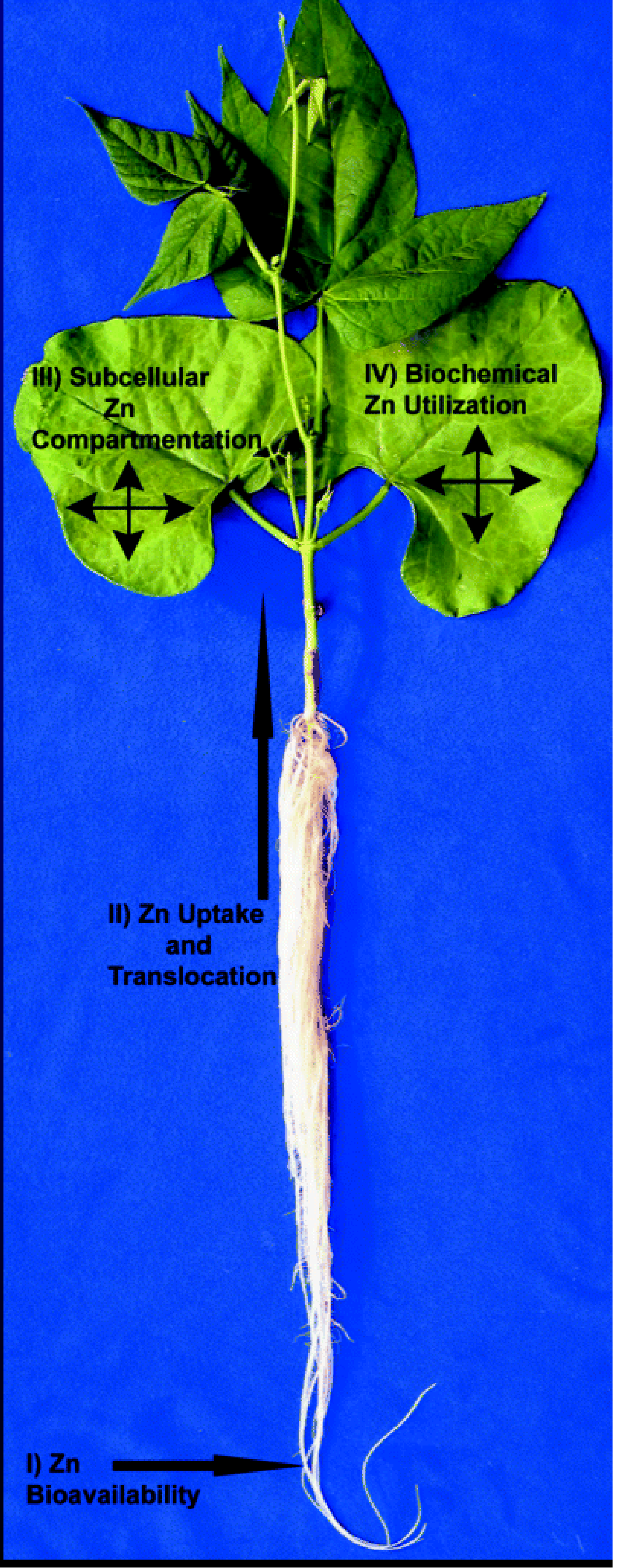


From: Borrelly GPM, et al., 2004, BiochemJ378, 293-7

- Atx1 binds a single Cu(I) ion like ATX1
- Atx1: like in the yeast+animal proteins, Cu-binding via two Cys in the sequence MT/HCXXC, **BUT** additional histidine61 from loop 5
- the additional histidine shifts Atx1 binding affinity towards CtaA by reducing affinity for PacS → trafficking of Cu(I) from one CtaA to the PacS
- other features like in yeast+animal proteins

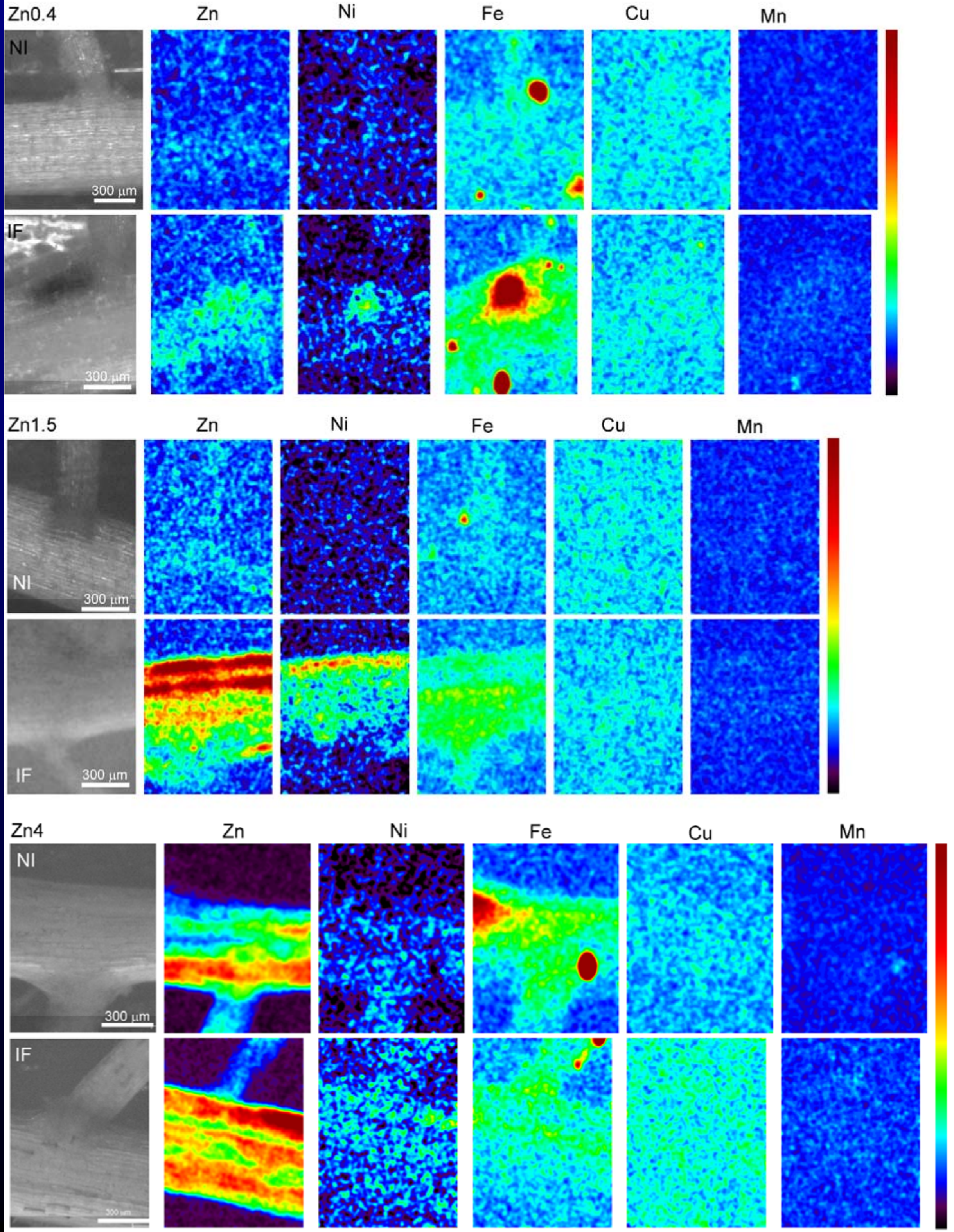
Zinc efficiency





From: Hacisalihoglu G, Kochian, LV.How do some plants tolerate low levels of soil zinc?
Mechanisms of zinc efficiency in crop plants.
New Phytologist 159 (2), 341-350.

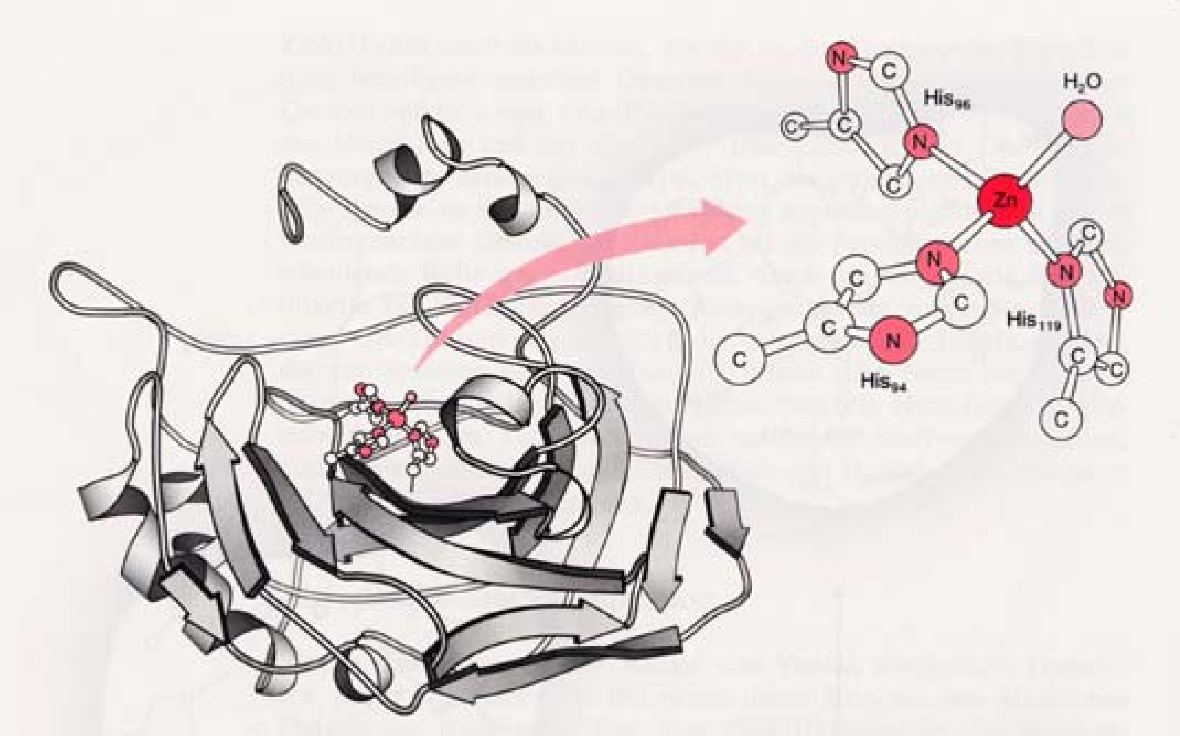
Emerging field: Role of trace metals in plant defence response to biotic stress



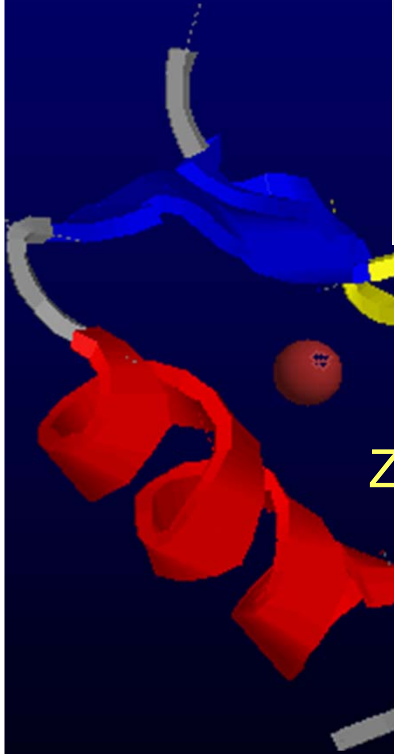
- ➢ Local Zn mobilization in response to pathogen Phomopsis in soybean roots revealed by µXRF imaging of living roots.
- ➢ Still unknown (ongoing work): regulatory mechanism, genes involved,...

Morina F, Mijovilovich A, Koloniuk I, Pecnik A, Novak O, Gruz J, Küpper H (2021) Journal of Experimental Botany DOI: doi.org/10.1093/jxb/erab052

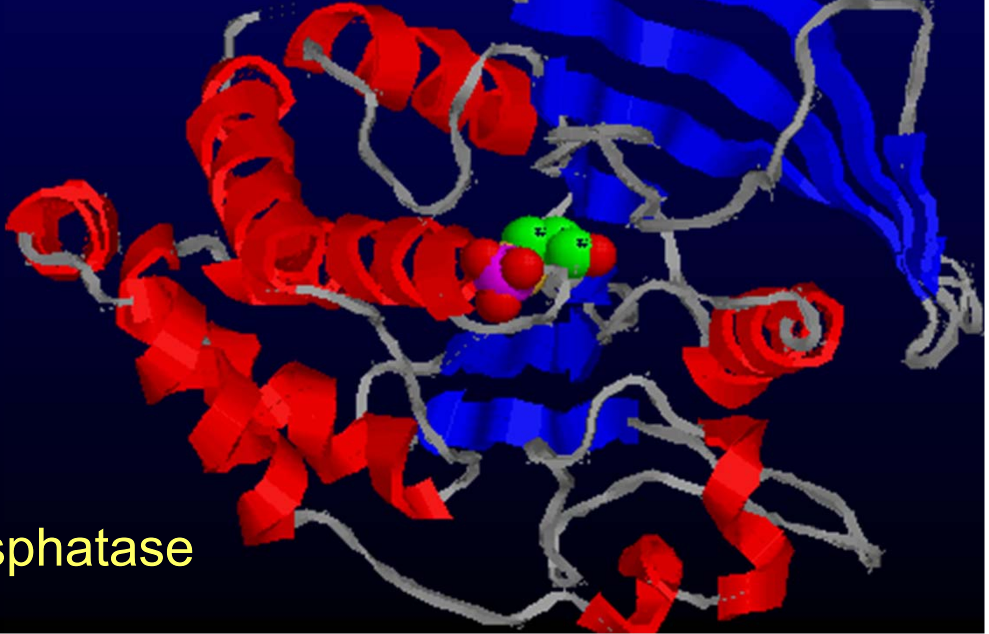
Selected important plant enzymes with zinc in their active centre



Carboanhydrase→ details in the lecture about photosynthesis related metal proteins



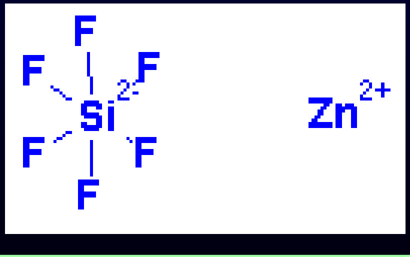
Zinc finger-motive



Tyrosin phosphatase

Environmental relevance of heavy metal toxicity

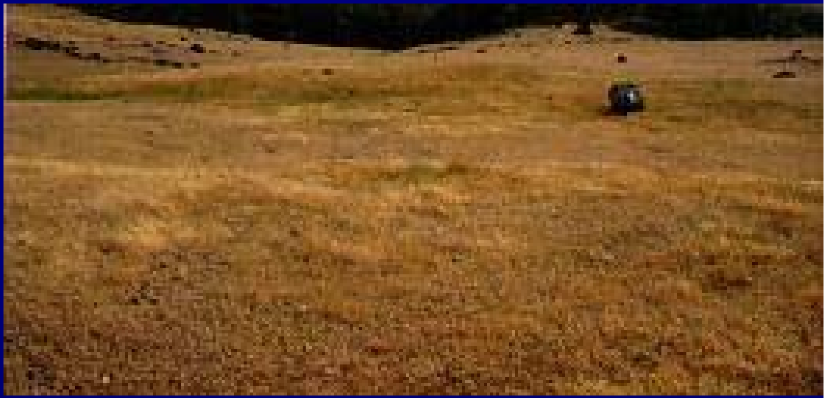
A seemingly intact, natural creek ...
However, the *Elodea canadensis* inside died from zinc stress that converted its chlorophyll to Zn-chlorophyll



Zn-Fluosilicate

Küpper H, Küpper F, Spiller M (1996) Journal of Experimental Botany 47 (295), 259-66

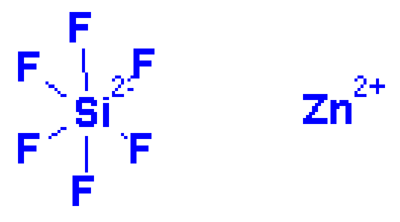
Environmental relevance of heavy metal toxicity



Where? How? Why?

- Naturally on heavy metal rich soils (Cu: e.g. in Zaire, Afrika; Zn/Cd: rel. frequent, incl. Europe; Ni: rel. frequent, serpentine soils e.g. in Africa, Australia, North and Middle Amerika): Heavy metal concentrations high enough for being toxic for most organisms.

- Naturally in copper-rich areas of the oceans (e.g. Sargasso sea): Cu-concentrations in the nanomolar range already inhibit some sensitive cyanobacteria.



- Anthropogenically due to the use of heavy metal salts (e.g. $CuSO_4$, z.B. Zn-phosphid, Zn-borate, Zn-fluosilicate): concentrations in the micromolar range are toxic for many plants, mainly water plants in neighbouring ponds and creeks

- Anthropogenically due to ore mining and refining, concentrations in the vicinity of mines, smelters and rubble dumps can be extremely high and toxic for all organisms.

- Anthropogenically due to the activities of other inductries. The longest river in Germany, the Rhine, contained up to 0.5 µM copper in the 1970‘s, which is lethal for sensitive water plants like *Stratiotes* or *Elodea*.

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

Plant communities in low metal habitats

Plant communities in high metal habitats

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

↑ Non-metalliferous alpine meadow

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

↑ Natural serpentine barren

Alentejo, Portugal, From: commons.wikimedia.org

↑ Non-polluted site in the same region

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

↑ Antropogenic (mining) polluted site

Metal deficiency & toxicity-induced damage

→ Uptake not sufficiently possible

→ Malfunction of gene regulation (→ e.g. Zn-fingers)

→ Lack of active centres leads to direct inhibition of photosynthesis

→ Oxidative stress as a result of a malfunction of photosynthesis and missing active centres in detoxifying enzymes

→ Interference with nutrient uptake: competitive or inhibitory

→ Genotoxicity (various mechanisms)

→ Replacement of active centres especially in photosynthesis

→ Oxidative stress: direct and as a result of a malfunction of photosynthesis

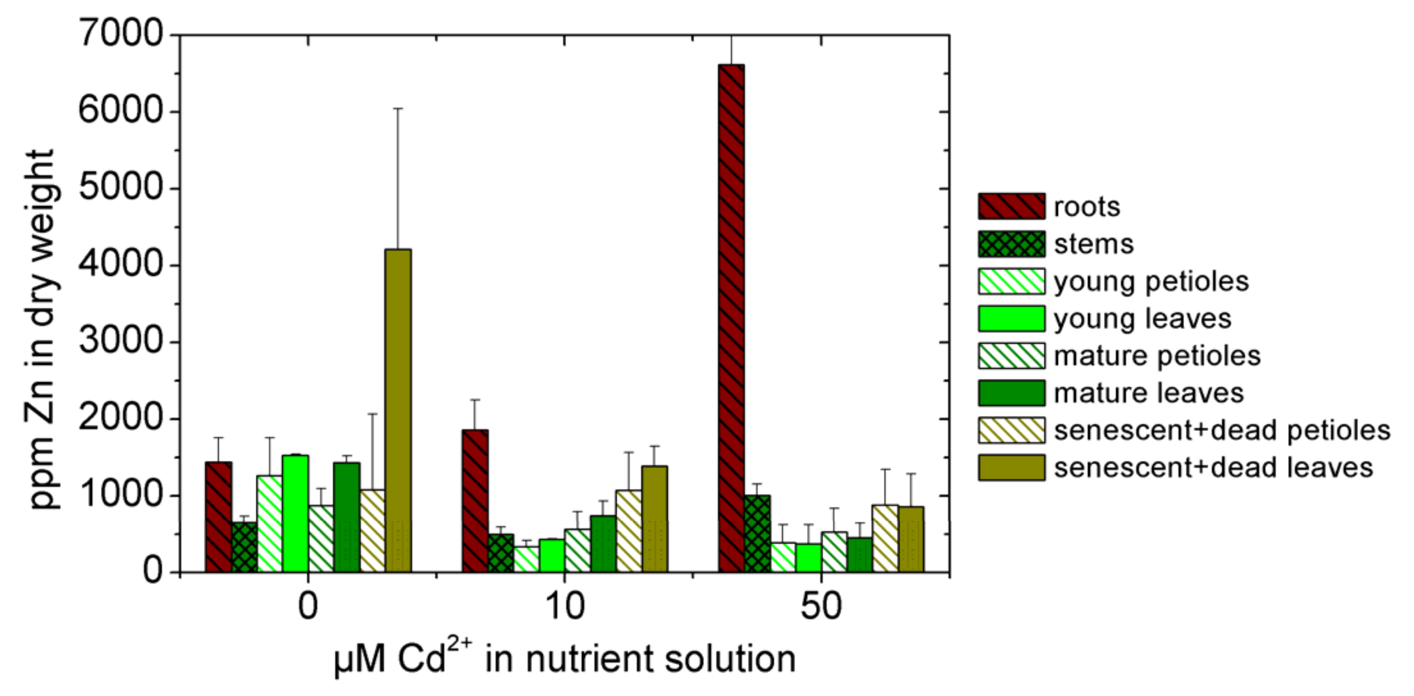
→ Inhibition of respiration and other relatively insensitive processes e.g. by binding to thiol groups of enzymes

Recent reviews:

Andresen E, Peiter E, Küpper H (2018) Trace metal metabolism in plants. Journal of Experimental Botany 69, 909-954
Küpper H, Andresen E (2016) Mechanisms of metal toxicity in plants. Metallomics 8, 269-285

1. Inhibition of root function and metal translocation

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



Mechanisms

- Competition in the uptake of less available essential micronutrients, which are sometimes transported by the same proteins
- Enhanced precipitation of essential micronutrients at the root surface
- Inhibition of transport proteins?
- Diverse relatively unspecific inhibitions of cytoplasmic enzymes
- Inhibition of cells division (relevance and mechanism unclear!)
- As a result of root toxicity, root tips and root hairs die off

Genotoxicity

Relevance

➢ Strongly DEPENDS on the metal applied:

• NOT relevant for copper and zinc toxicity, because other mechanisms (mainly photosynthesis inhibition) are MUCH more efficient

• Relavant for cadmium, because genotoxicity seems to be comparably efficient as photosynthesis inhibition

• For lead, it is not very efficient, but other mechanisms are even less efficient because the metal is generally NOT very toxic for plants! → Pb toxicity in general NOT environmentally relevant !

➢ Also depends on the plant species!

➢ Also depends on the type of genotoxity...

Micronucleus (MCN) formation

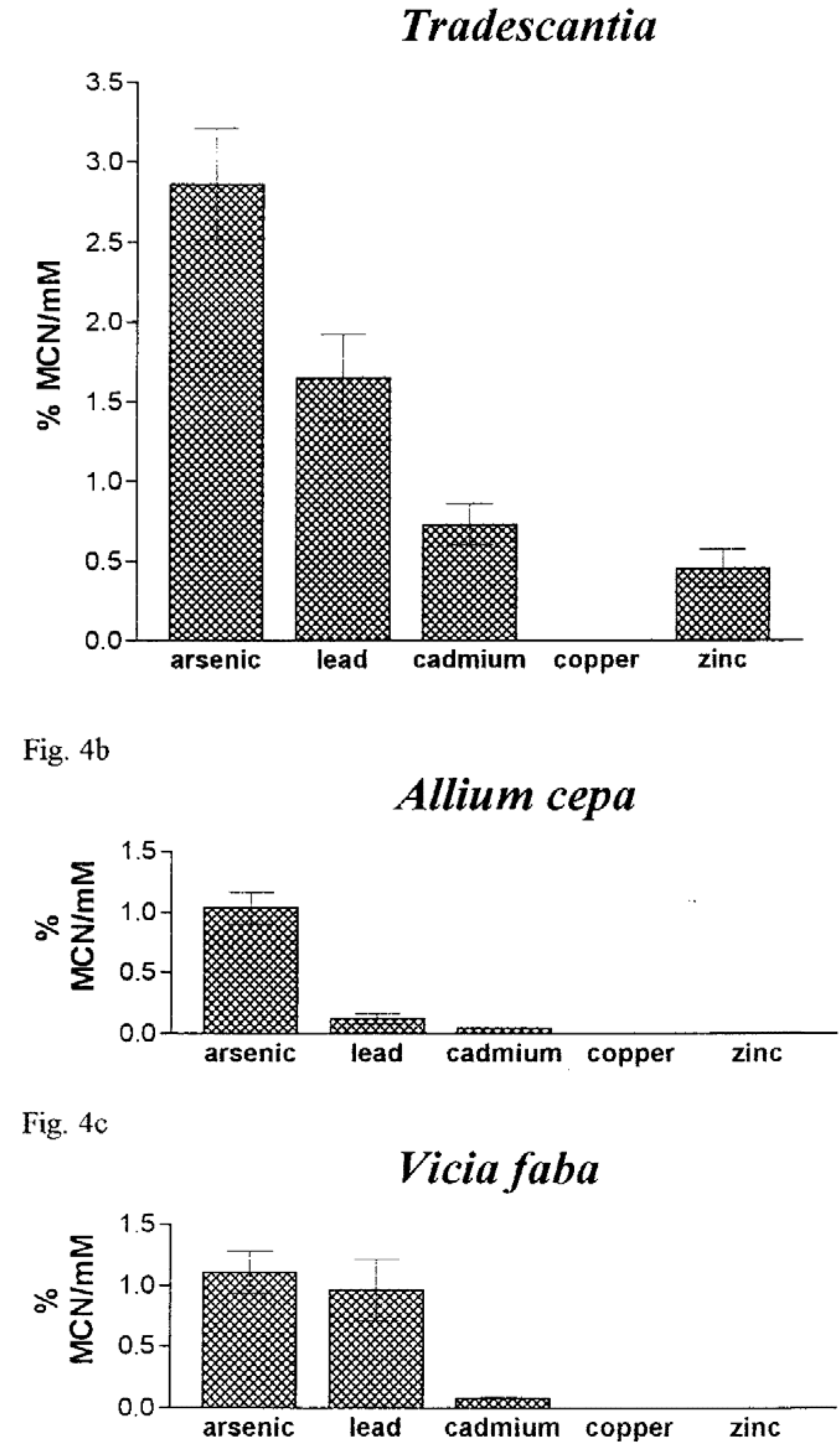


Fig. 4b

Fig. 4c

From: Steinkellner H, et al., 1998, Env.Mol.Mutag. 31, 183-191

Oxidative Stress

Relevance

- NOT clear: Studies with environmentally relevant realistic but still toxic metal(loid) concentrations often do NOT show oxidative stress! Almost all studies concluding that oxidative stress would be a major factor in heavy metal induced inhibition of plant metabolism were carried out using extremely high metal(loid) concentrations.

Mechanisms generating reactive oxygen species during heavy metal stress

- Direct: catalysed by redox-active metal(loid) ions (As^{3+}, Fe^{2+}, Cr^{3+}, Cu^{+}), hydrogen peroxide is converted to reactive oxygen radicals via the Fenton Reaction:

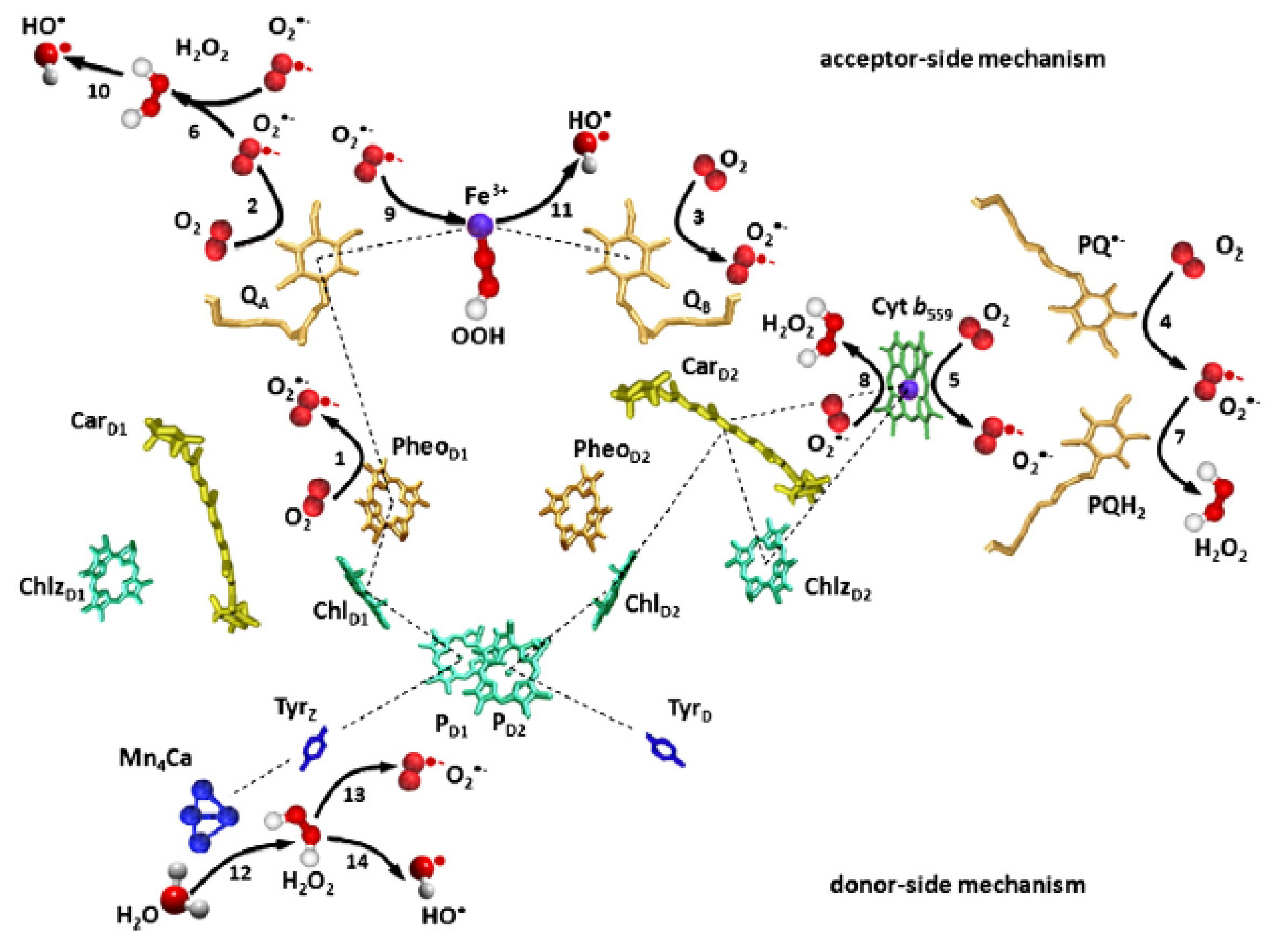
Never shown in vivo!

- Indirect: malfunction of photosynthesis and respiration can generate reactive oxygen species. Therefore, even *in vivo* redox-inert metal ions like Zn^{2+} and Cd^{2+} can cause oxidative stress.

Reviews:
Küpper H, Kroneck PMH, 2005, Metal ions Life Sci 2, 31-62;
Küpper H, Andresen E (2016) Mechanisms of metal toxicity in plants. Metallomics 8, 269-285.

Generation of oxidative stress in photosynthesis

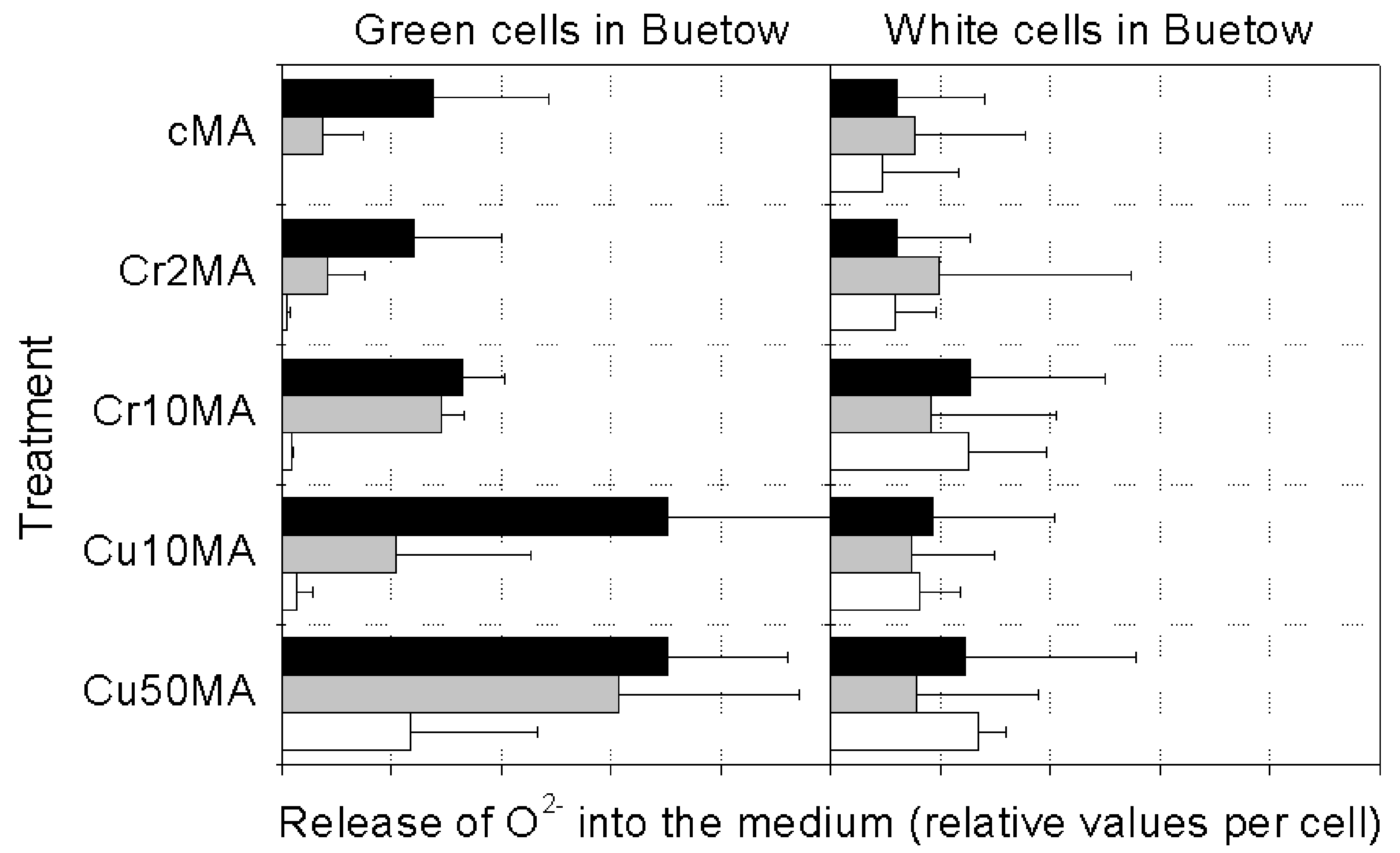
PS II – related ROS production



Pospisil, Biochim & Biophys Acta 1817:218-231, 2012

- Cadmium is redox inert → no direct reaction with oxygen
- Enhancement of ROS by Cd is due to malfunction of photosynthesis and respiration - Cd enhances malfunction

Comparison of superoxide production during Cr- and Cu-stress in *Euglena gracilis*



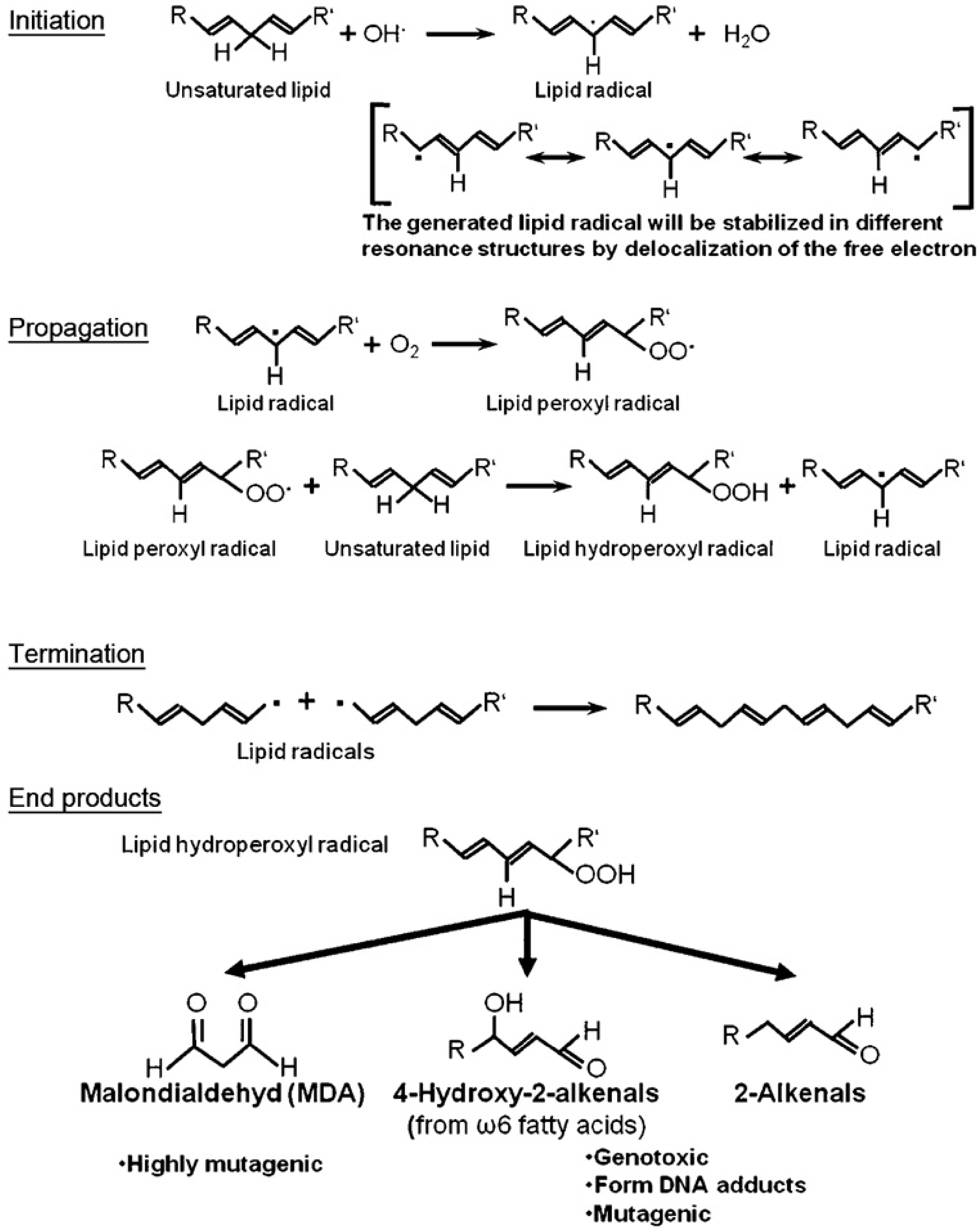
→ Increase in superoxide production under heavy metal stress is mainly caused by malfunctioning photosynthesis!

Rocchetta I, Küpper H (2009) New Phytologist 182, 405-420

Oxidative Stress

Mechanisms of damage caused by oxidative stress in plants

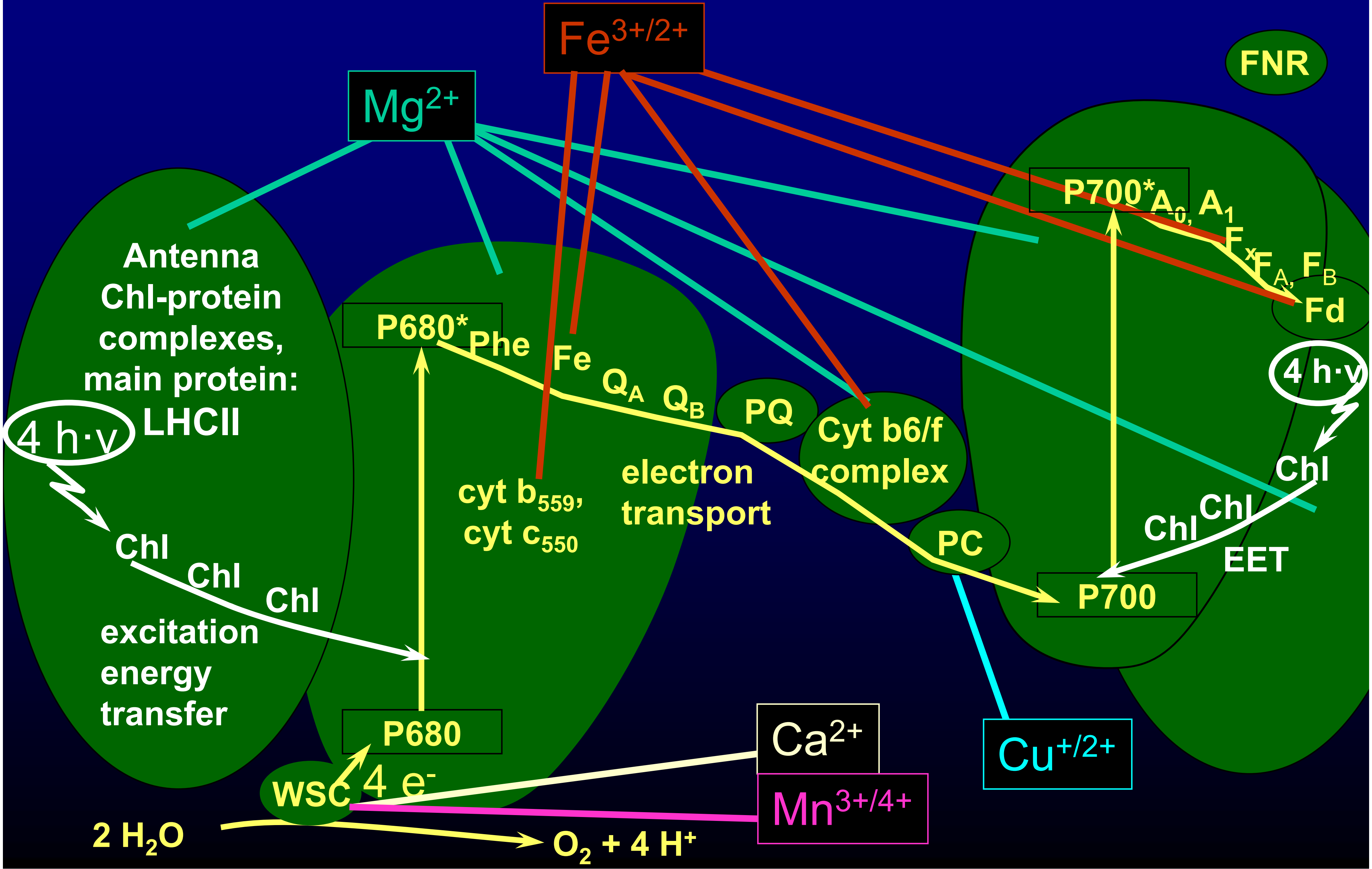
- Oxidative stress can lead to oxidation of Lipids in membranes and thus make them leaky. This is a popular but debated mechanism.
- Oxidation of proteins



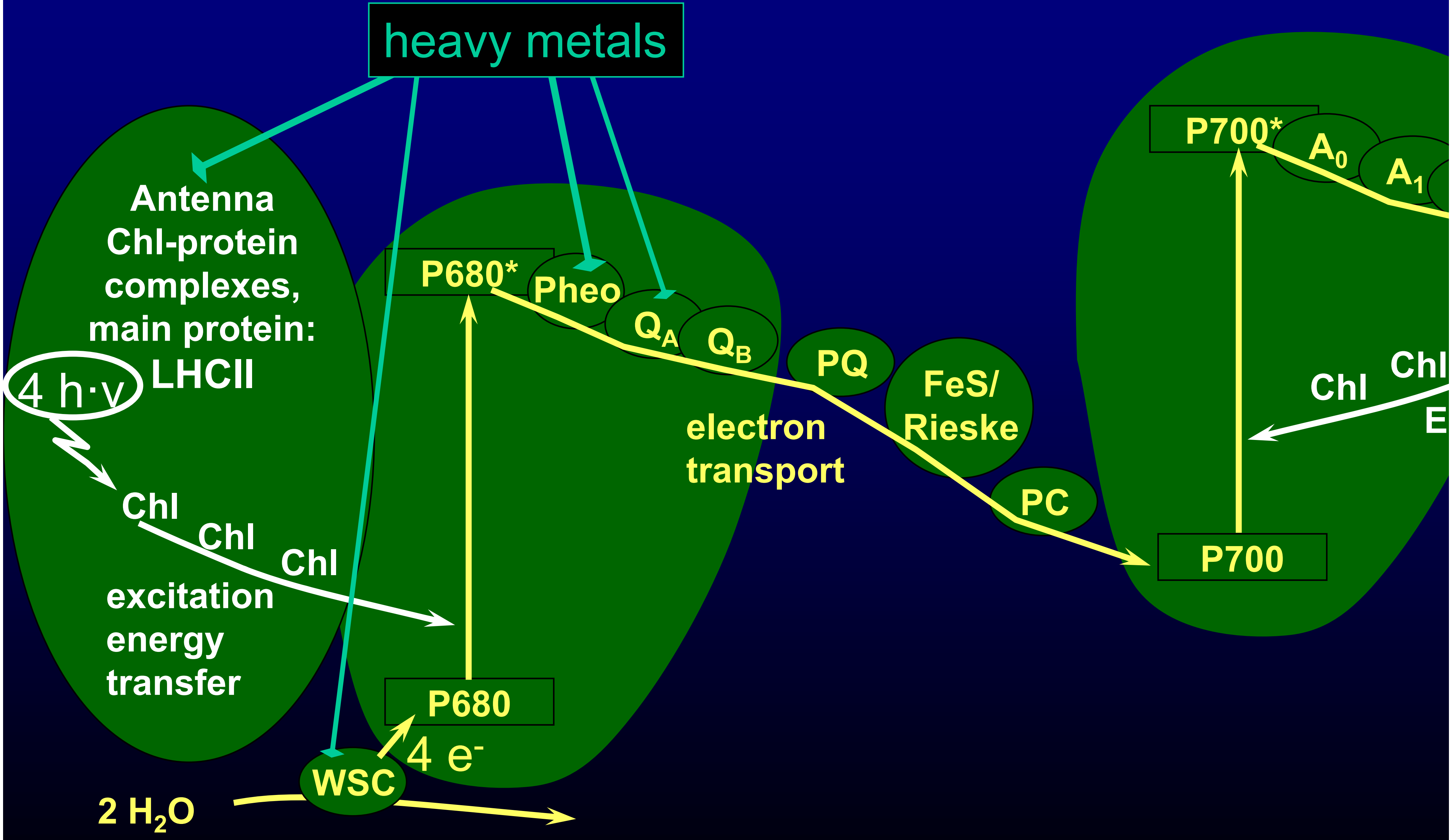
The generated lipid radical will be stabilized in different resonance structures by delocalization of the free electron

From: Henkel RR, Asian J Androl (2011) 13, 43–52

Metal sites in photosynthetic proteins



Heavy metal induced inhibitions of photosynthesis: suggested targets



heavy metals

Antenna Chl-protein complexes, main protein: LHCII

4 h·v

Chl

Chl

Chl

excitation energy transfer

P680*

Pheo

Q_A

Q_B

PQ

FeS/ Rieske

PC

electron transport

P680

WSC

4 e^-

2 H_2O

P700*

A_0

A_1

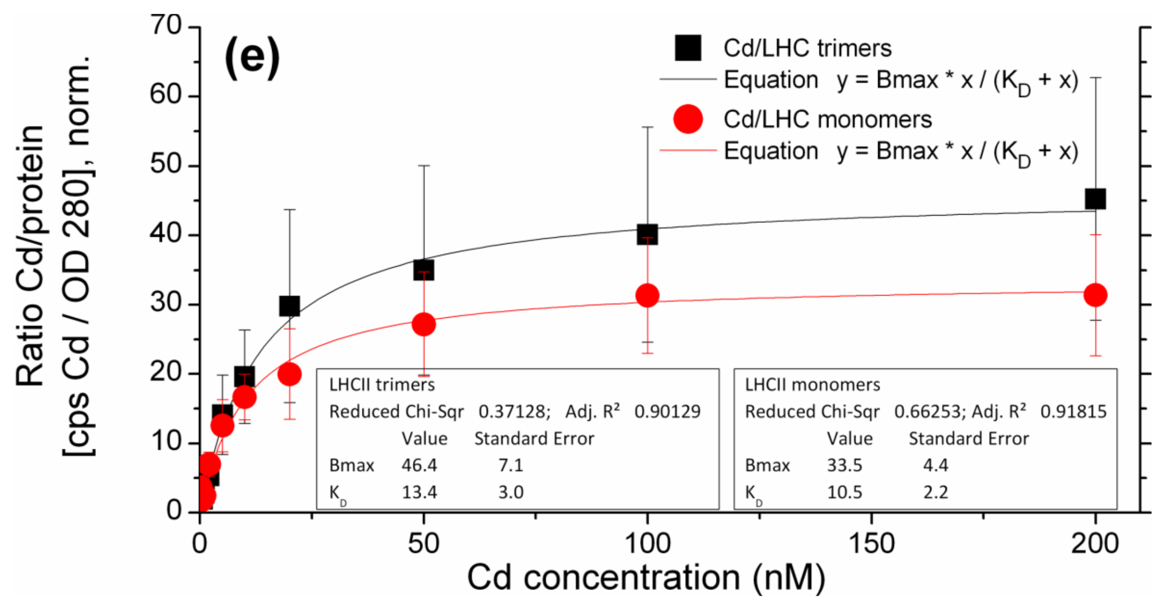
P700

Chl

Chl

E

Example of metal toxicity in the nanomolar range in „normal" plants: Incorporation of Cd into LHCII in LL



LHCII trimers

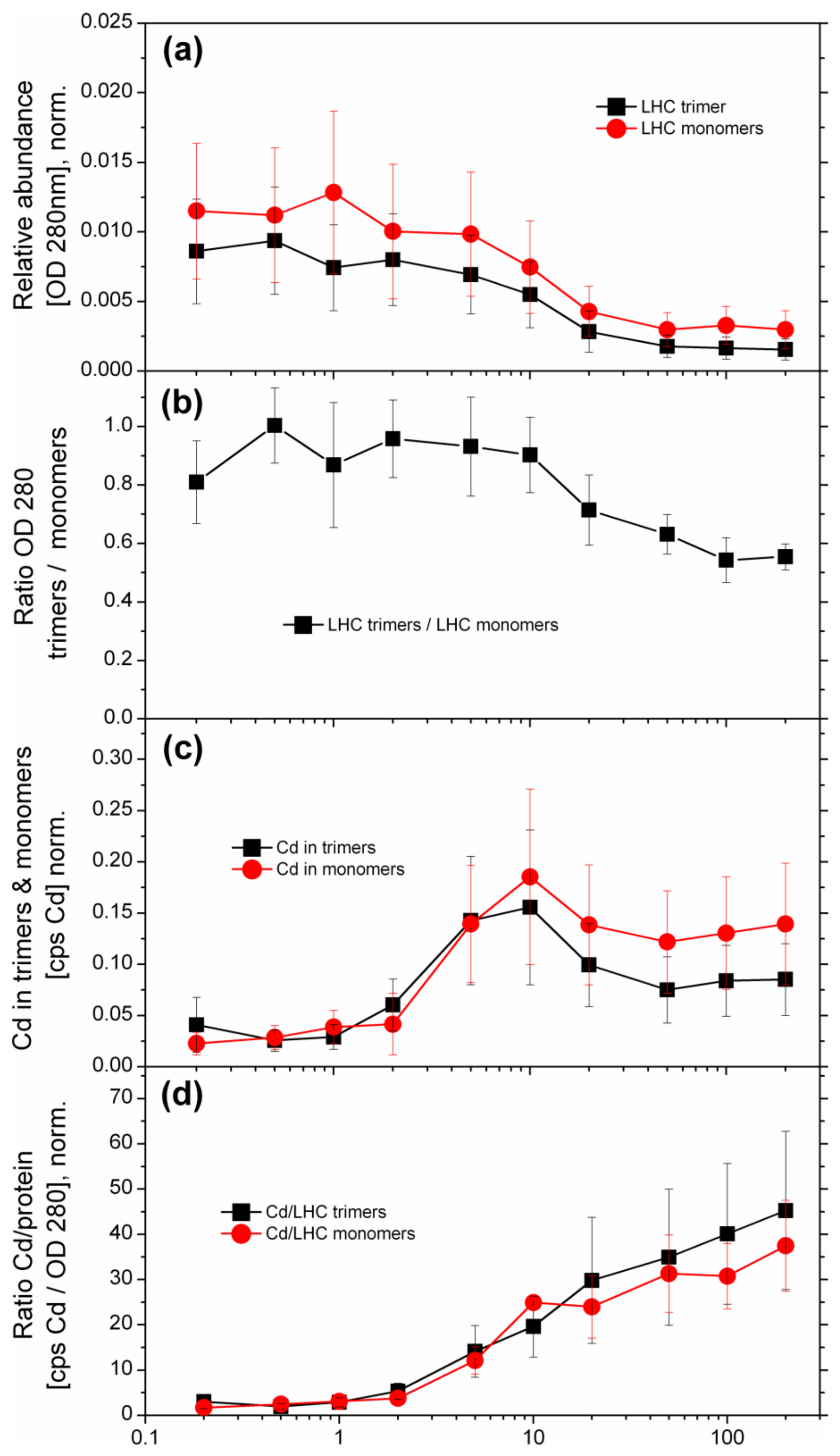
Reduced Chi-Sqr 0.37128; Adj. R^2 0.90129

	Value	Standard Error
Bmax	46.4	7.1
K_D	13.4	3.0

LHCII monomers

Reduced Chi-Sqr 0.66253; Adj. R^2 0.91815

	Value	Standard Error
Bmax	33.5	4.4
K_D	10.5	2.2



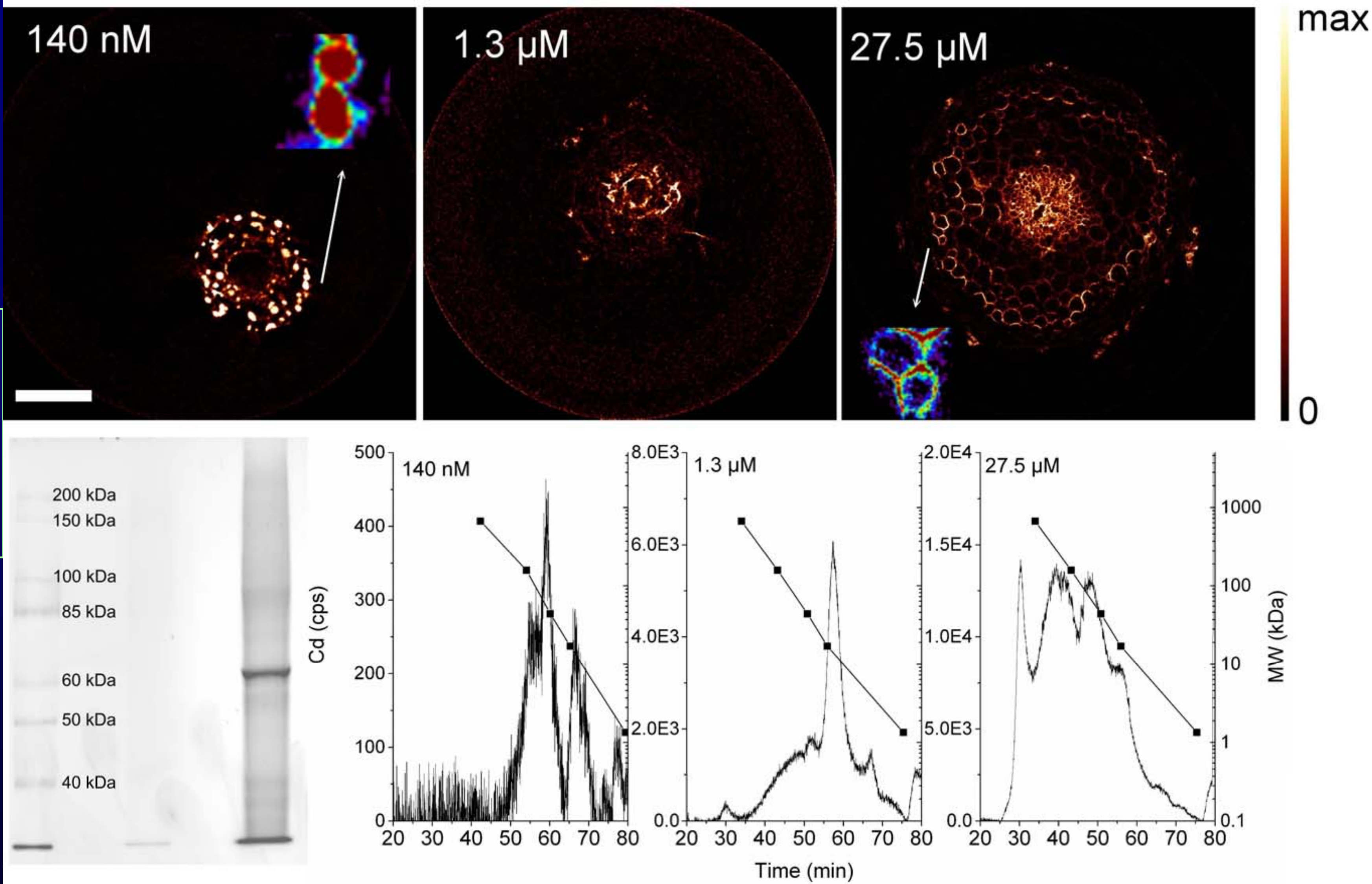
→ Cd binding to LHCII causes disintegration of trimers

→ Cd bind to LHCII with dissociation constants in the low nanomolar range

→ diminished photosynthesis despite funtional reaction centres!

Andresen E, Kappel S, Stärk HJ, Riegger U, Borovec J, Mattusch J, Heinz A, Schmelzer CEH, Matoušková Š, Dickinson B, Küpper H (2016) New Phytologist 210, 1244-1258.

Sublethal vs. lethal metal toxicity soybean roots



Andresen E, Flores-Sanchez IJ, Brückner D, Bokhari SNH, Falkenberg G, Küpper H* (2023) J Hazard Materials 442, 130062.

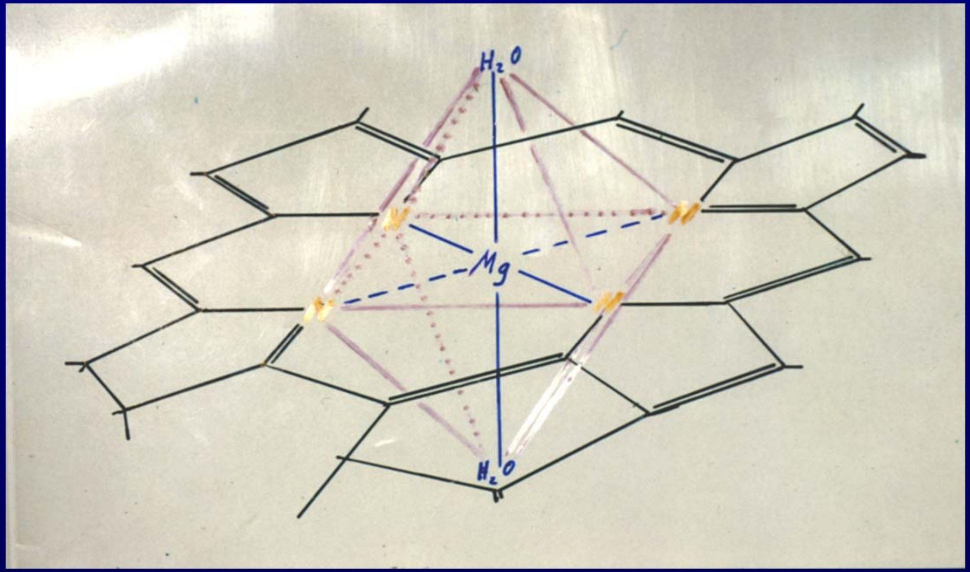
→ change of intracellular localisation: symplastic vs. apoplastic

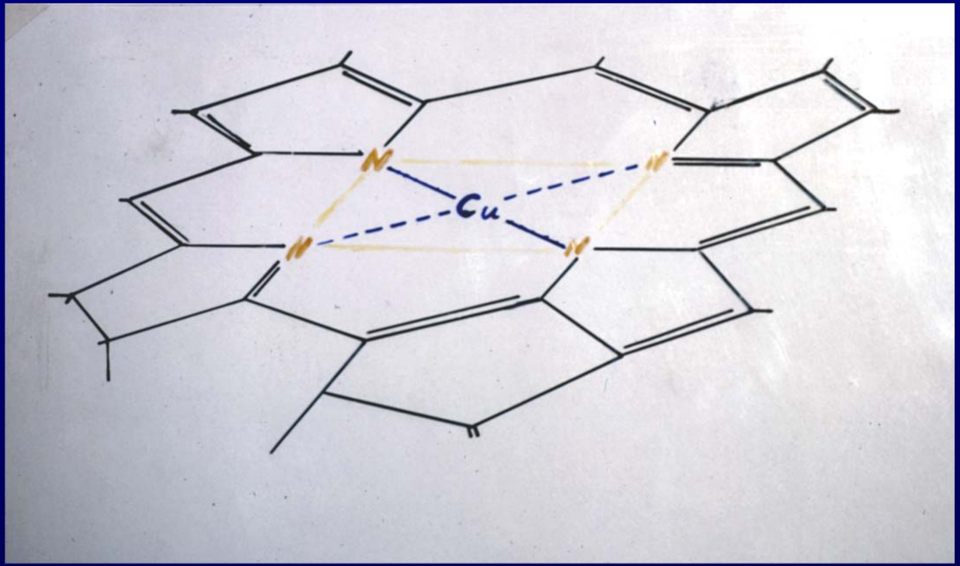
→ change of tissue localisation: central cylinder vs. unspecific

→ change of target proteins: few vs. unspecific

→ **Drastically different mechanism of toxicity at rarely studied sublethal vs. frequently studied lethal concentrations!**

Why are heavy metal chlorophylls unsuitable for photosynthesis?





Main reasons

- heavy metal chlorophylls bind axial ligands only weakly (Zn-Chl) or not at all (Cu-Chl)
  → light harvesting proteins denature

- unstable singlet excited state → relaxation of absorbed & transferred energy as heat
  → "black holes" for excitons

Review: Küpper H, Küpper FC, Spiller M (2006) [Heavy metal]-chlorophylls formed in vivo during heavy metal stress and degradation products formed during digestion, extraction and storage of plant material. In: Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications (B. Grimm, R. Porra, W. Rüdiger and H. Scheer, eds.), Vol. 25 of series "Advances in Photosynthesis and Respiration" (Series editor: Govindjee). Kluwer Academic Publishers, Dordrecht; pp. 67-77.

Summary 1: Examples of Toxicity Mechanisms

Copper toxicity

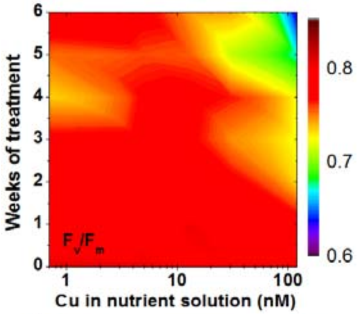
at high irradiance

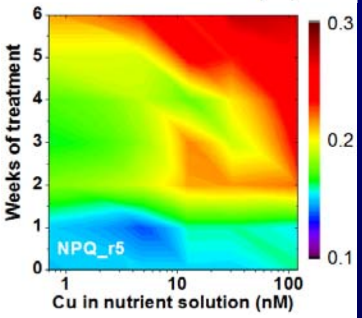
>10nM Cu: Damage to the PSII reaction centre

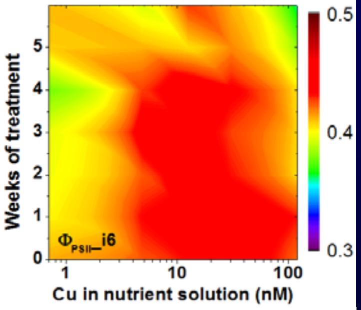
→ decreased photochemical quantum yield (F_v/F_m)

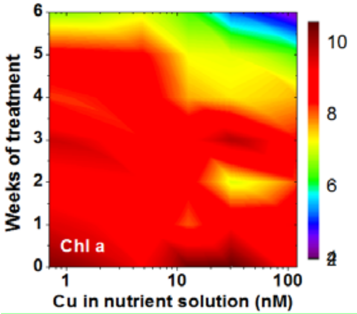
- Up-regulation of the dissipation of excitons as heat (NPQ)
- Electron transport (Φ_{PSII}) inhibited in addition to PSIIRC damage

Decrease of Chl during death of cells









Arsenic toxicity

>0.5µM As: inhibition of Chl biosynthesis

→ decreased light harvesting

> 1µM As: (1) As binding in nucleus

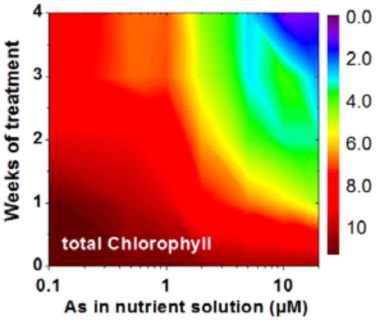
(2) decreased exciton transfer from the antenna to the RC

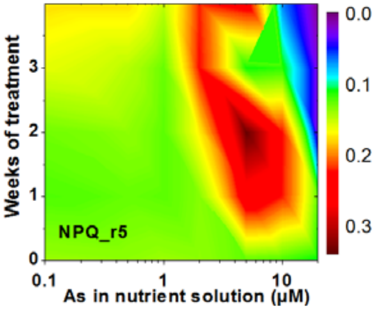
→ up-regulation of thermal exciton dissipation (NPQ)

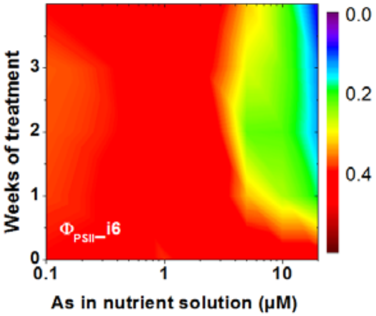
>2µM As: Electron transport (Φ_{PSII}) inhibited

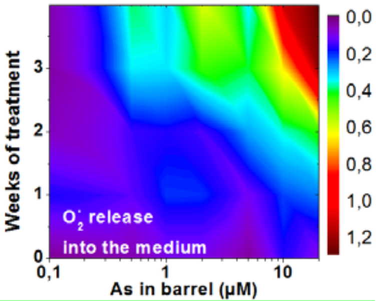
>5µM As: NPQ inhibition

Malfunctioning of photosynthesis leads to generation of ROS in addition to increased inhibitions









As: Mishra S, Stärk H-J, Küpper H (2014) Metallomics 6, 444-454

Cu: Thomas G, Stärk H-J, Wellenreuther G, Dickinson BC (2013) Aquatic toxicology 140-141, 27-36

Conclusions:

Mechanisms of heavy metal stress

- Damage clearly occurs even at nanomolar concentrations of heavy metals that are frequently found even in only slightly polluted waterbodies.

- Damage mechanisms show different dependence on the type of metal, its concentrations and environmental factors. This is because of differences in the chemistry of the metals and plant physiology, both of which is often ignored.

- Concentration dependence and kinetics and of many proposed damage mechanisms not known

- Many (most) studies were performed at far too high, environmentally not relevant heavy metal concentrations and/or other unphysiological experimental conditions (e.g. submerged seedlings of terrestrial plants, missing dark phase, rectangular light cycles, etc etc.)

→ Environmental relevance, kinetics and causal interdependence of various proposed damage mechanisms still unclear despite decades of research!

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