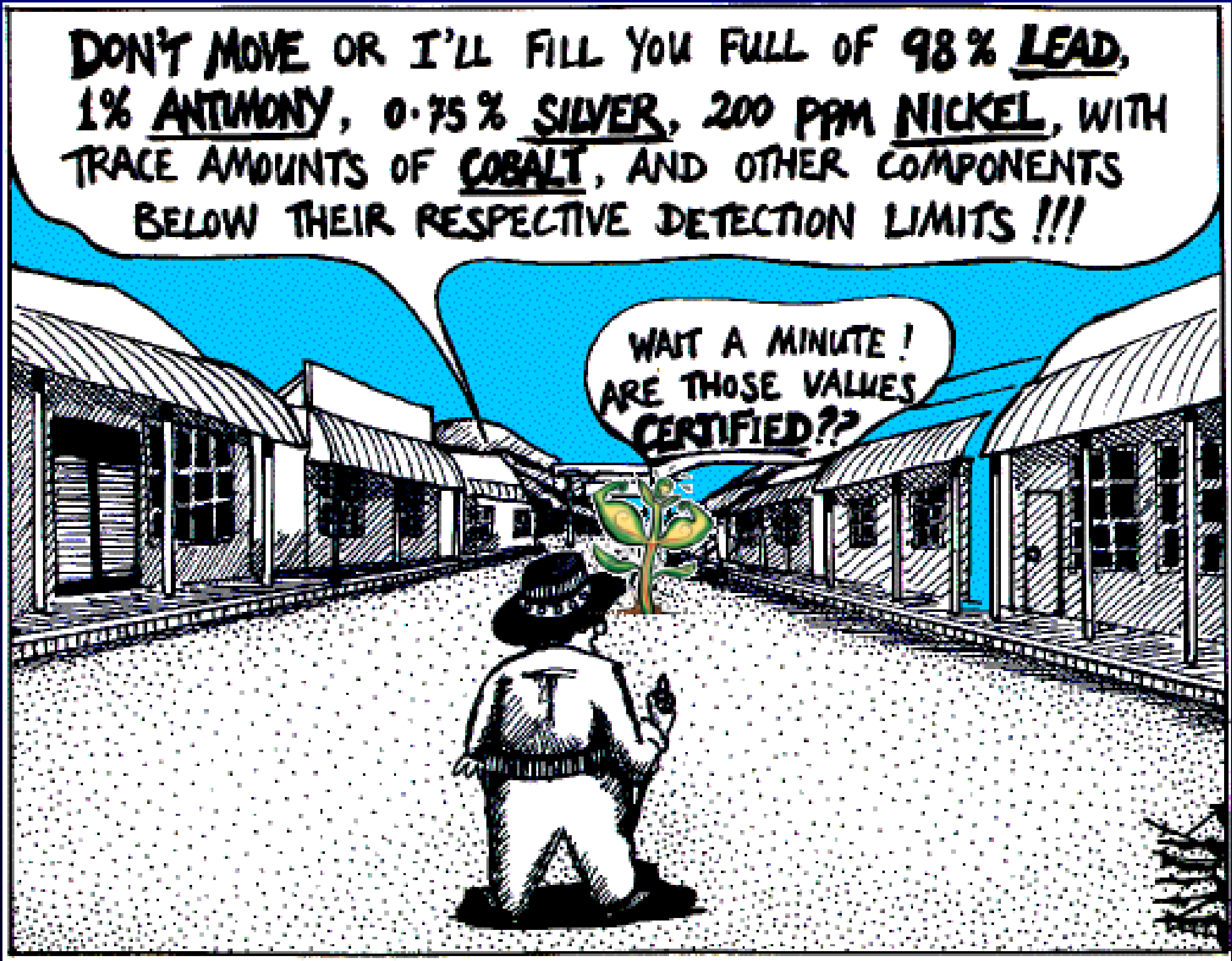
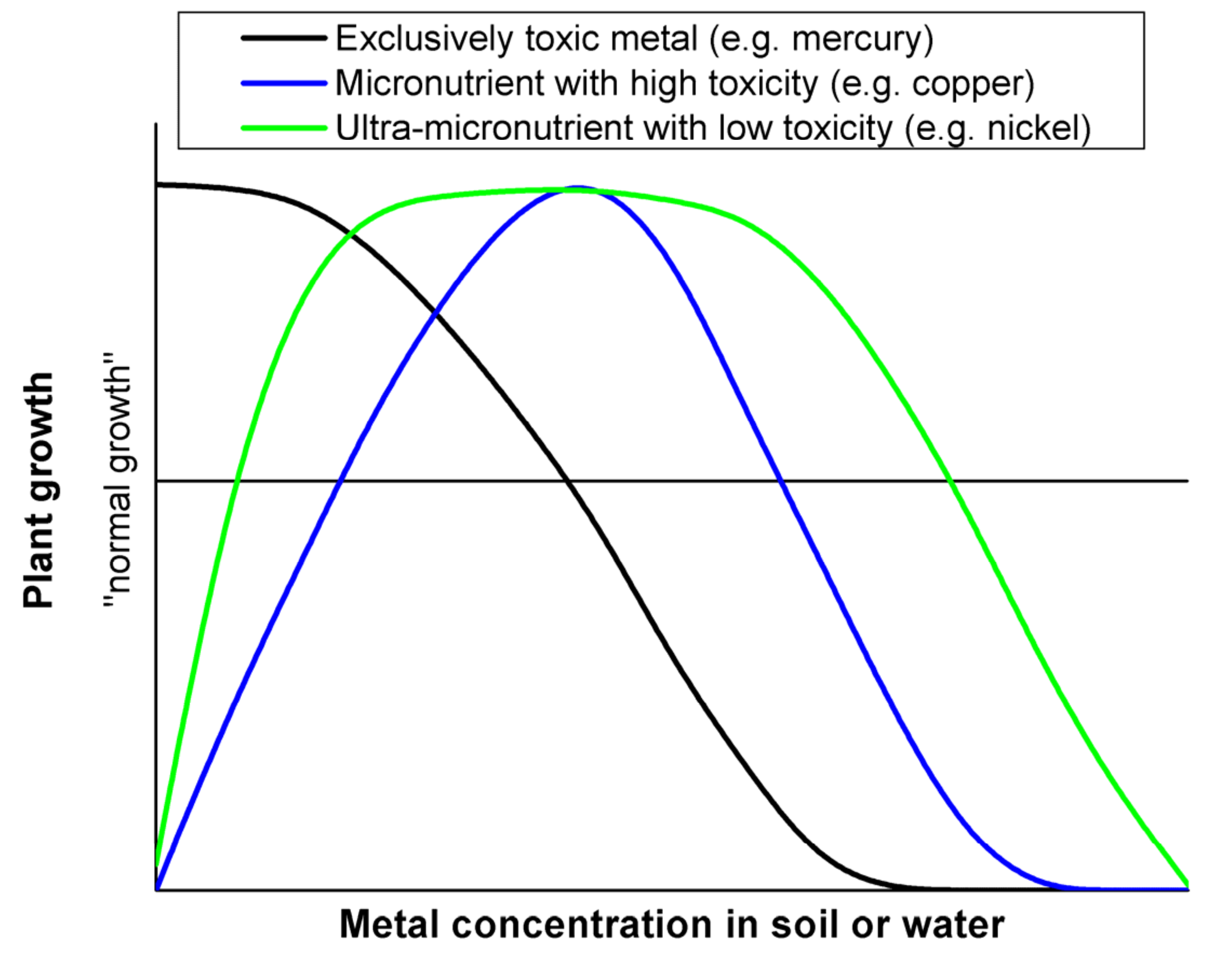
# Transition („Heavy") Metals and Plants - a complicated relationship → Metal stress




Heavy metal-hyperaccumulation in the Wild West

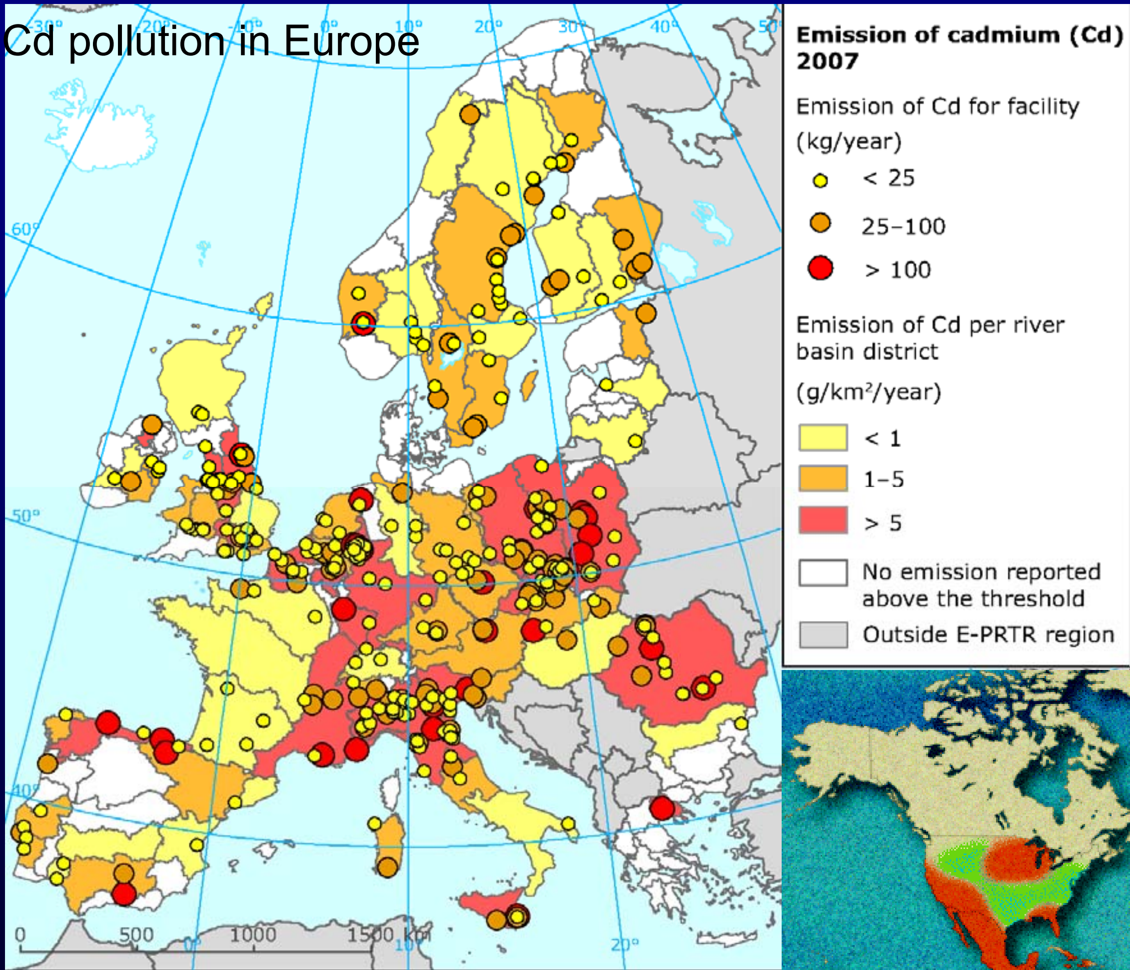
modified from: http://strangematter.sci.waikato.ac.nz/

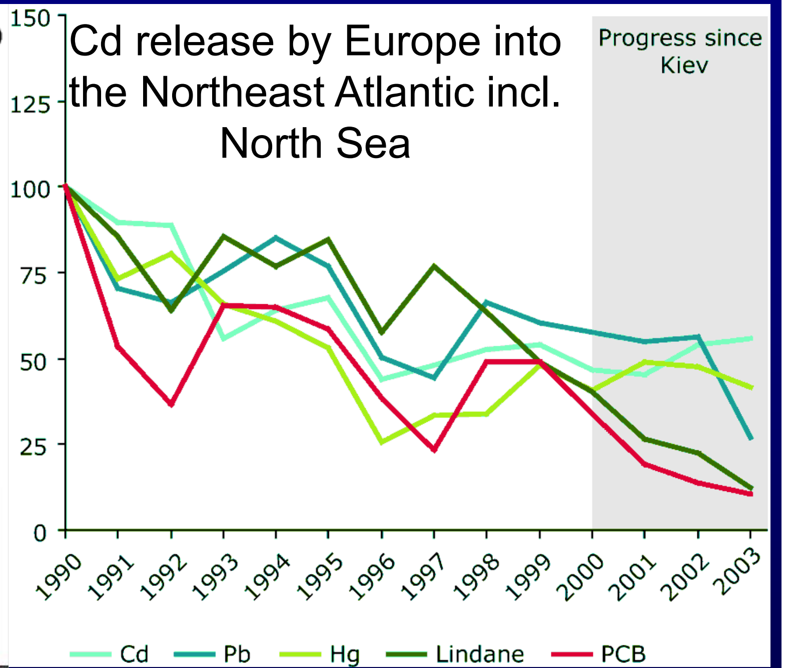
# Dose-Response principle for heavy metals

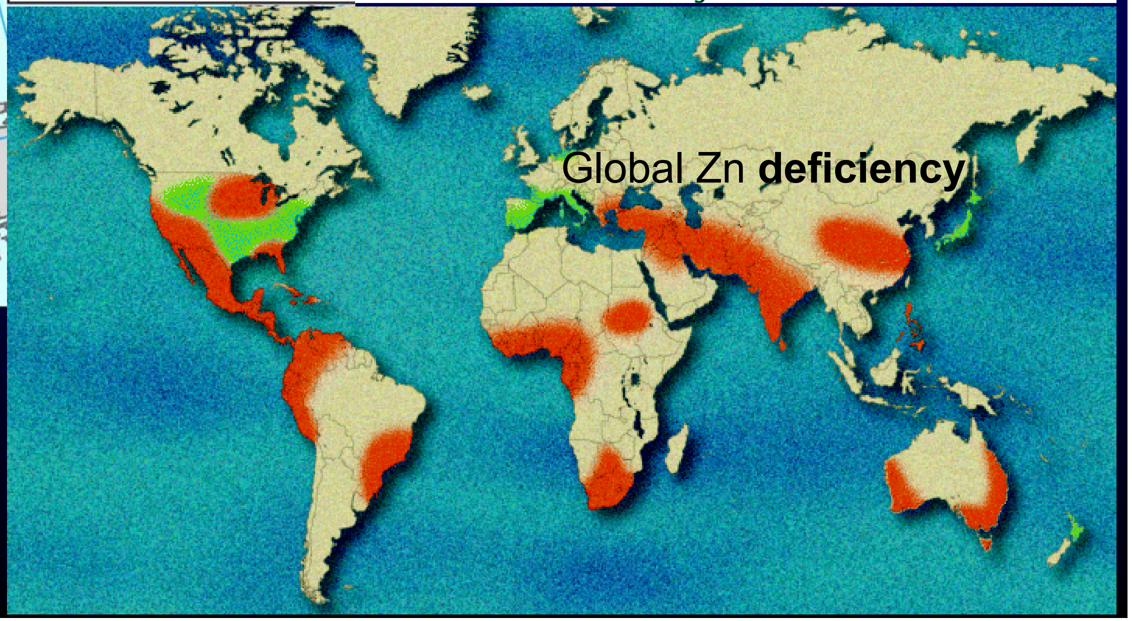



Küpper H, Kroneck PMH, 2005, Metal ions Life Sci 2, 31-62

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



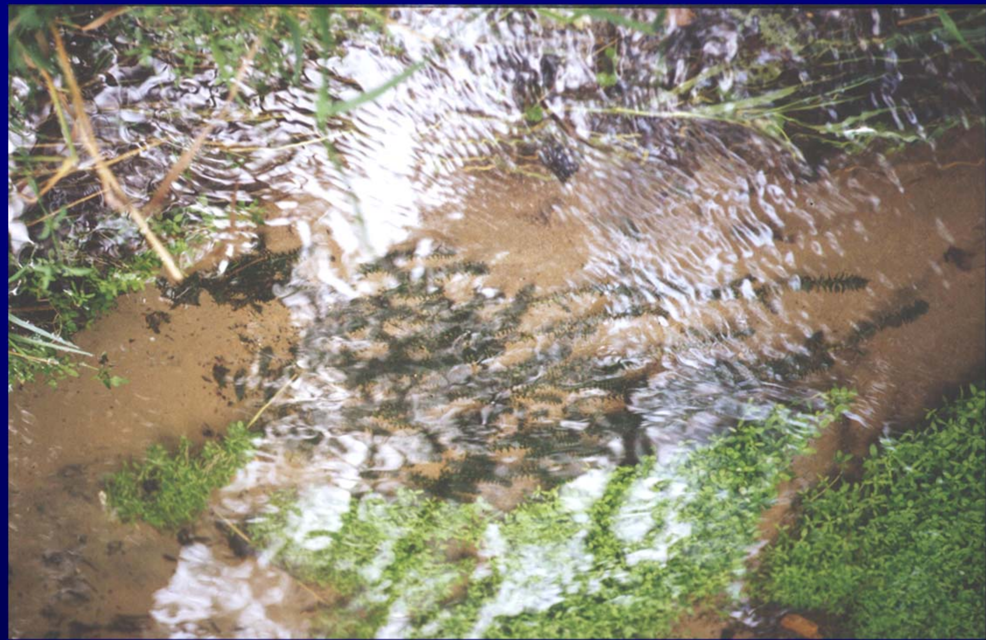



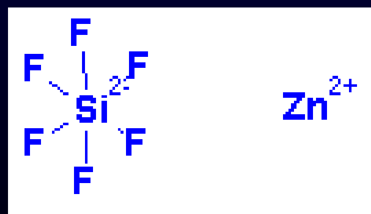

Cd map and trend from http://www.eea.europa.eu (European Environment Agency)

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

# 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

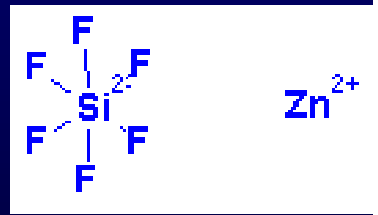
Küpper H, Küpper F, Spiller M (1996) Journal of Experimental Botany 47 (295), 259-66

# 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

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

# 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

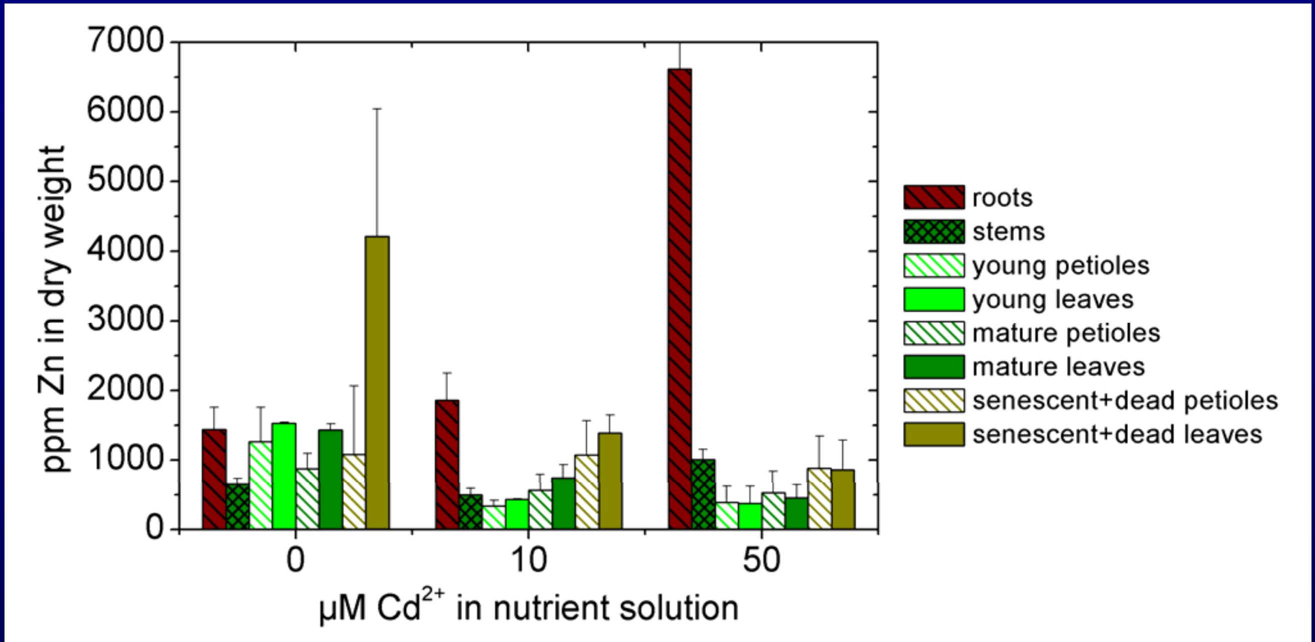
# 1. Inhibition of root function

**Why roots?**

- In terrestrial plants the root is generelly the first organ that comes into contact with the heavy metals.
- In the case of heavy metals with typically low mobility, e.g. copper, also the highest metal accumulation is found in the roots

# 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

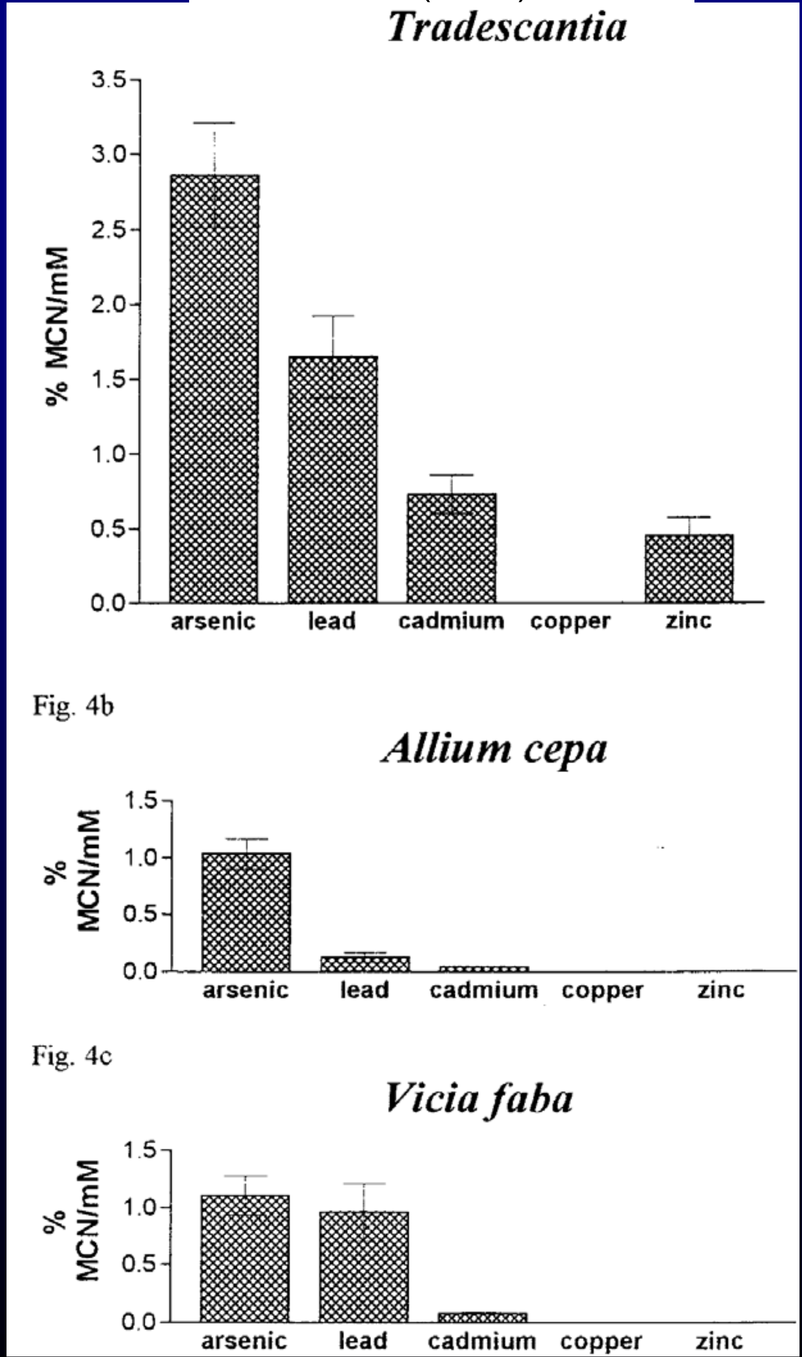
• For lead, it is not very efficient, but other mechanisms are even less efficient because the metal is generally NOT very toxic for plants! → Pb toxicity in general NOT environmentally relevant !

➢ Also depends on the plant species!

➢ Also depends on the type of genotoxity...

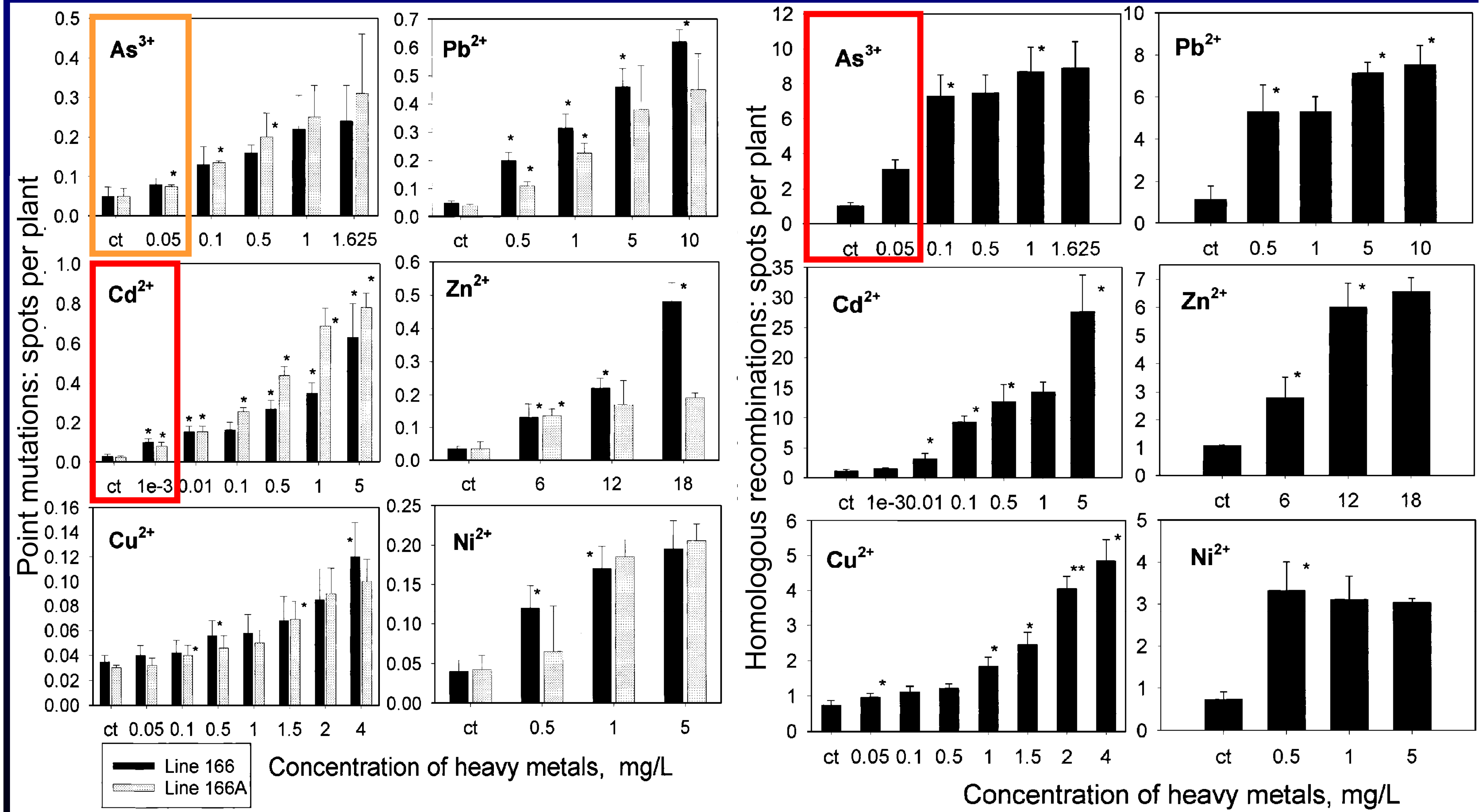
From: Steinkellner H, et al., 1998, Env.Mol.Mutag. 31, 183-191

Micronucleus (MCN) formation



# Genotoxicity

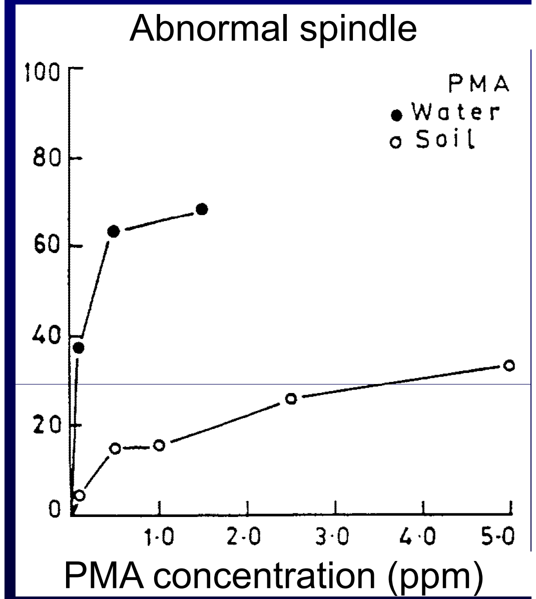
**Mechanisms:** Point Mutations and Homologous recombinations

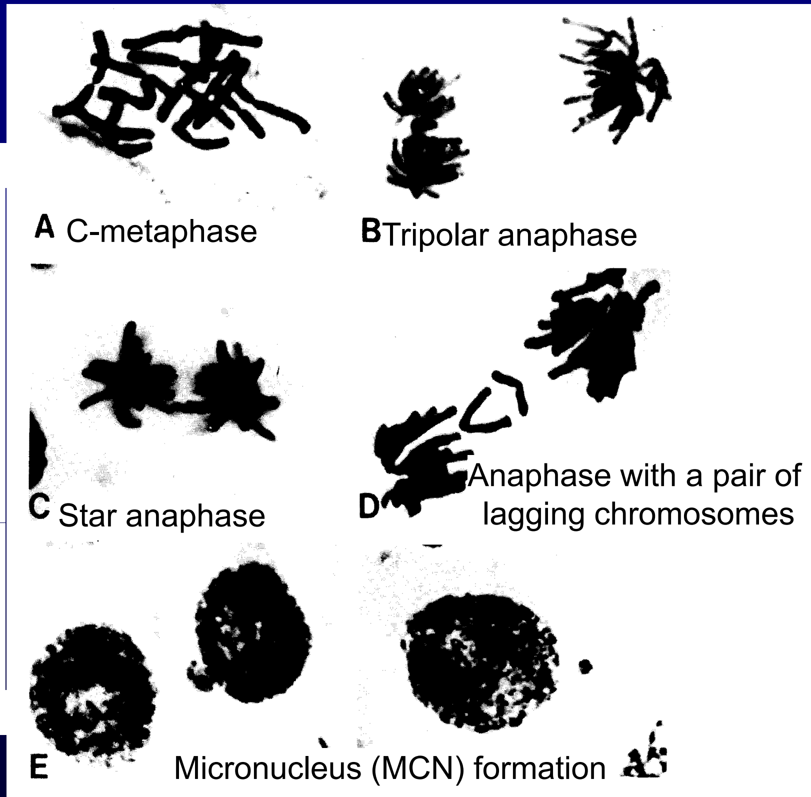


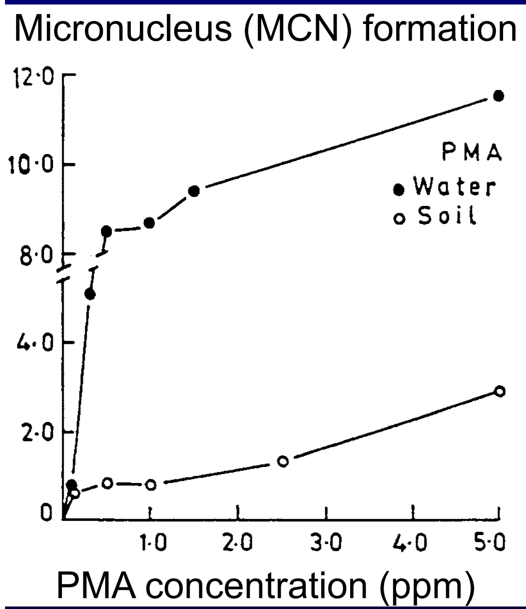

From: Kovalchuk O, Titov V, Hohn B, Kovalchuk I, 2001, Nature Biotechnol. 19, 568-72

# Genotoxicity

**Mechanisms:** Mitotic aberrations induced by phenyl mercuric acetate (PMA)




From: Dash S, Panda KK, Panda BB, 1988, Mutation Research 203, 11-21

# 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:

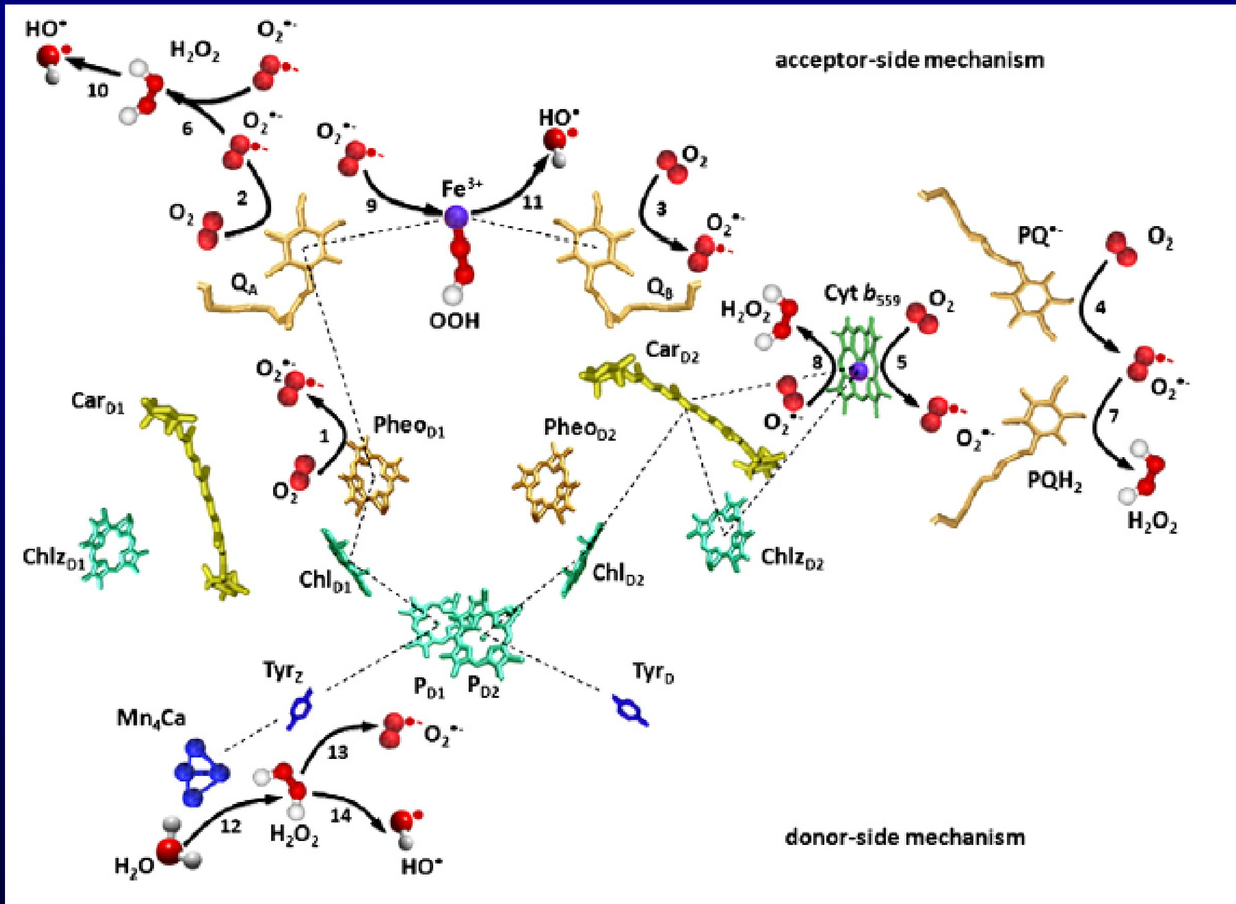
Never shown in vivo!

• 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

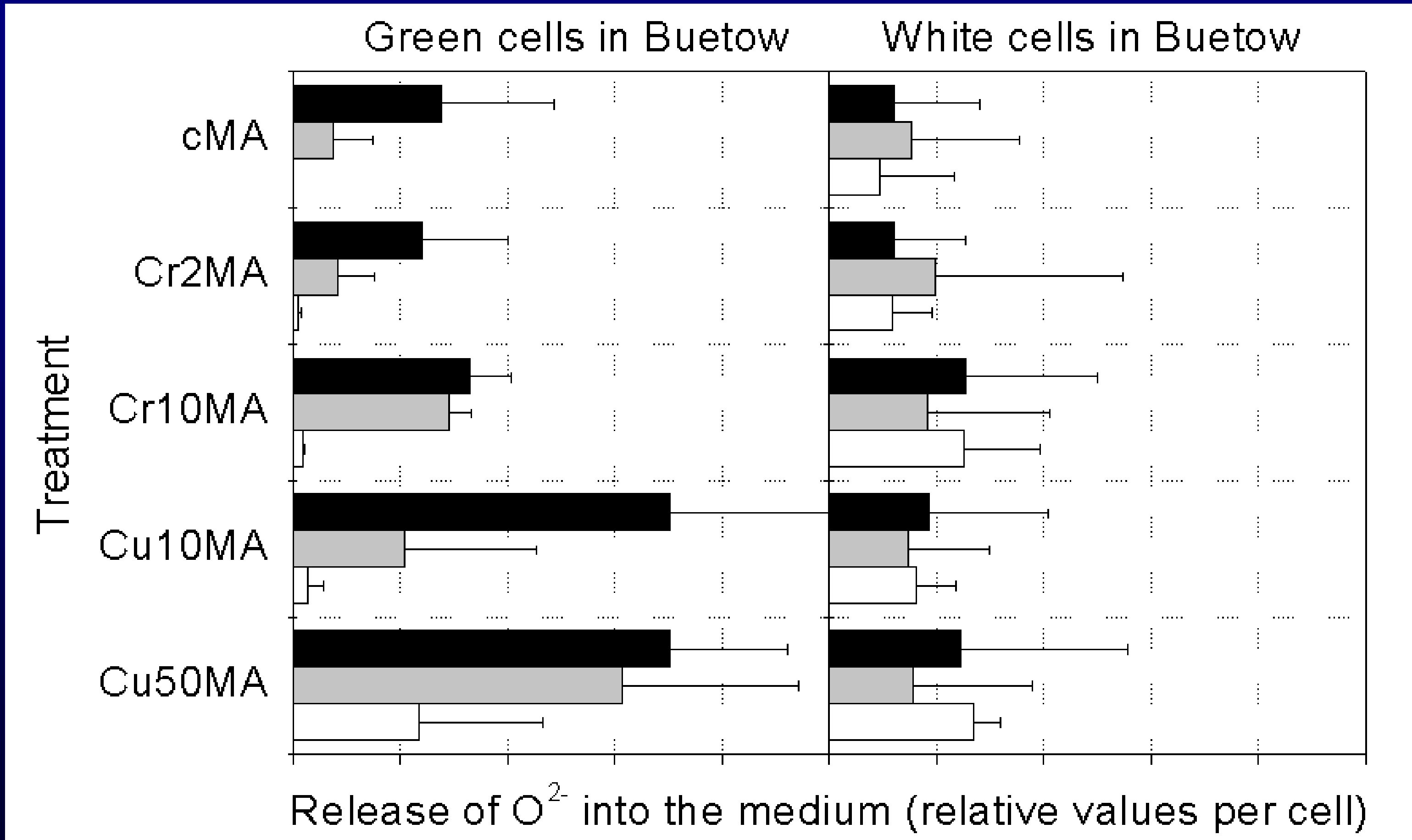
PS II – related ROS production




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

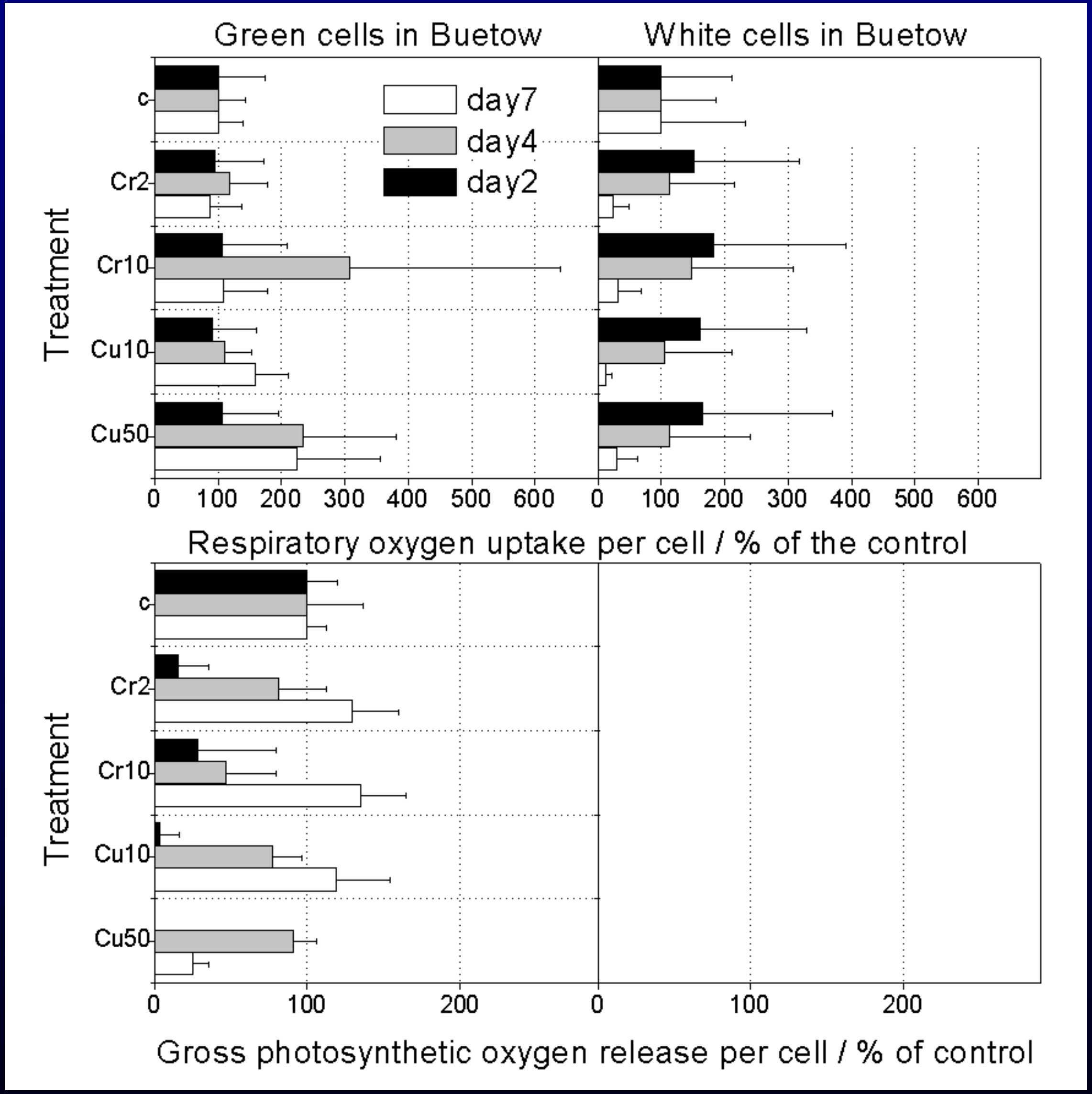
# Comparison of superoxide production during Cr- and Cu-stress in *Euglena gracilis*




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


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

# Comparison of photosynthesis and respiration changes caused by Cr- and Cu-stress in *Euglena gracilis*



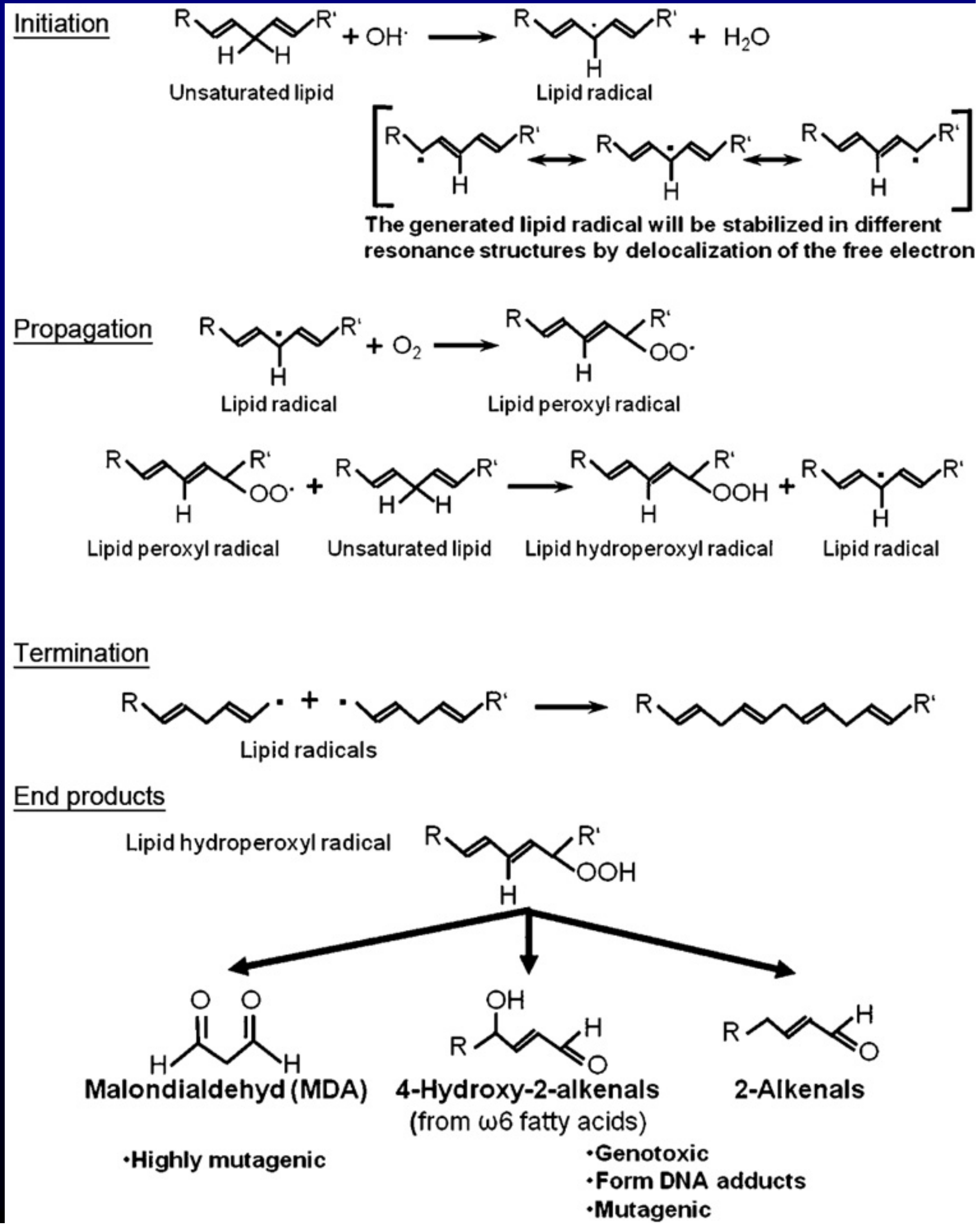

Rocchetta I, Küpper H (2009) New Phytologist 182, 405-420

# 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

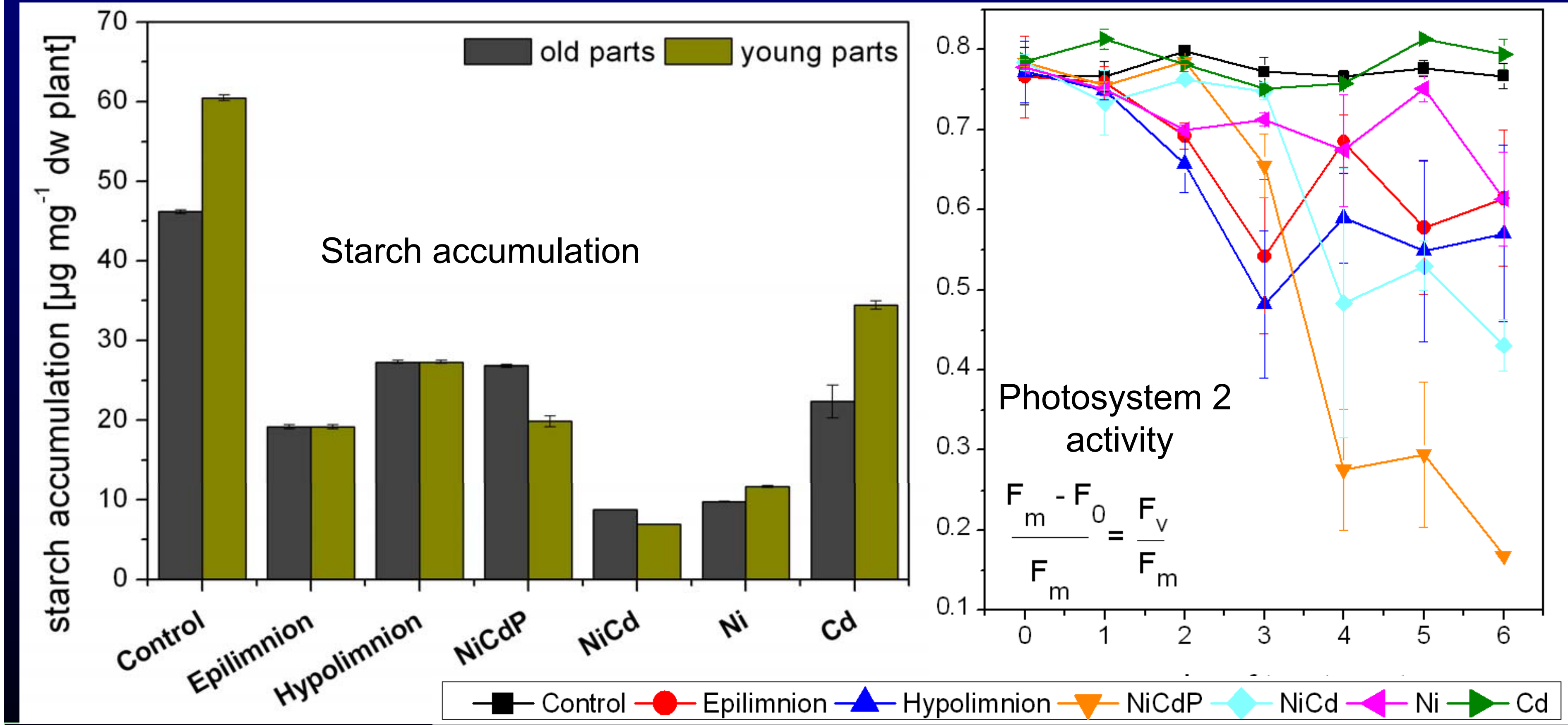



From: Henkel RR, Asian J Androl (2011) 13, 43–52

# Environmental relevance of heavy metal induced inhibition of photosynthesis: inhibitions of photosynthesis at nanomolar concentrations

-*Ceratophyllum demersum* plants treated with natural or simulated lake water containing up to 3 nM $Cd^{2+}$ and 300 nM $Ni^{2+}$ show inhibition

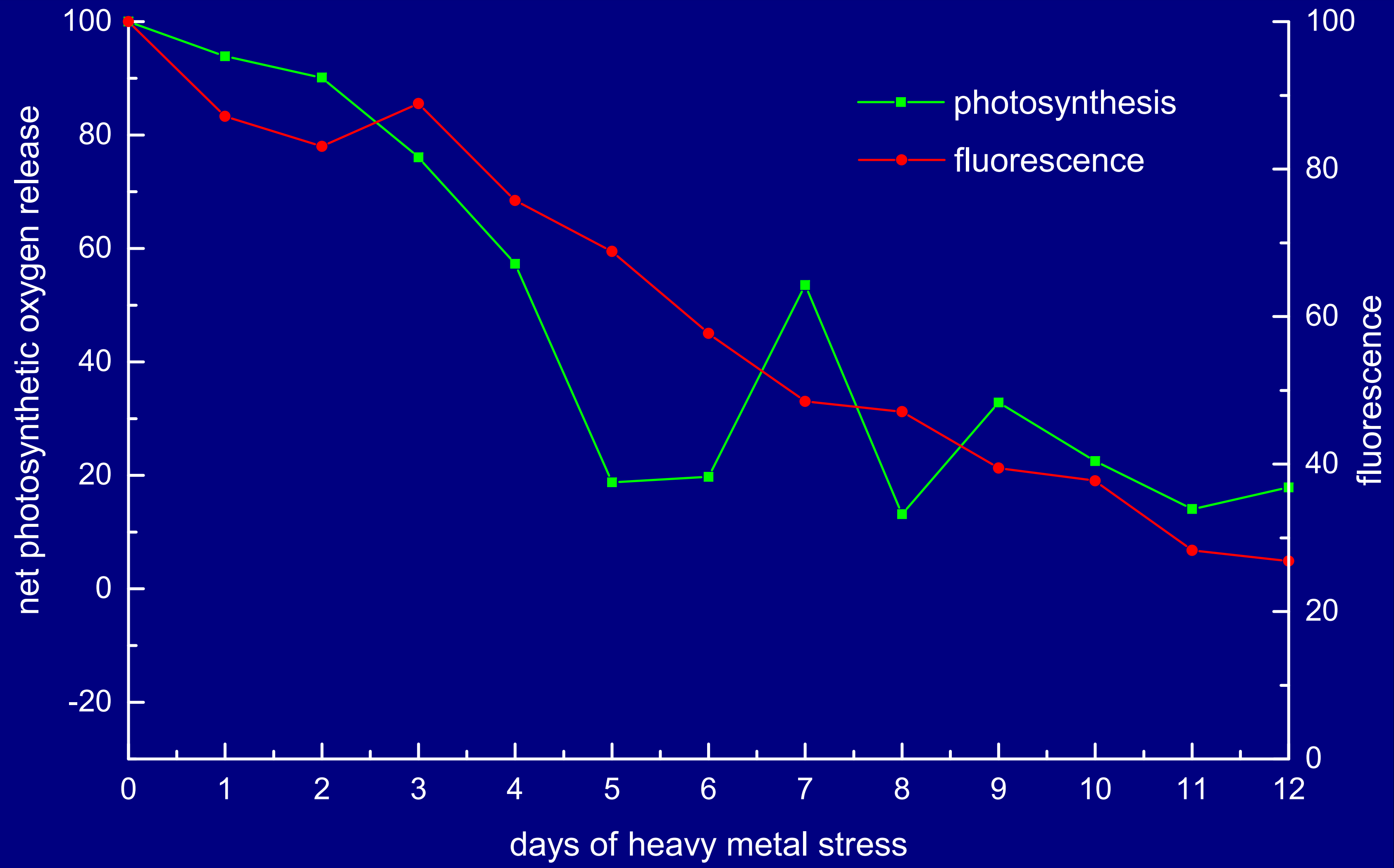
- inhibition by Ni+Cd combination treatment much stronger than by the single metals → synergistic effect!




Andresen E, et al., Küpper H (2013) Aquatic Toxicology 142-143, 387-402

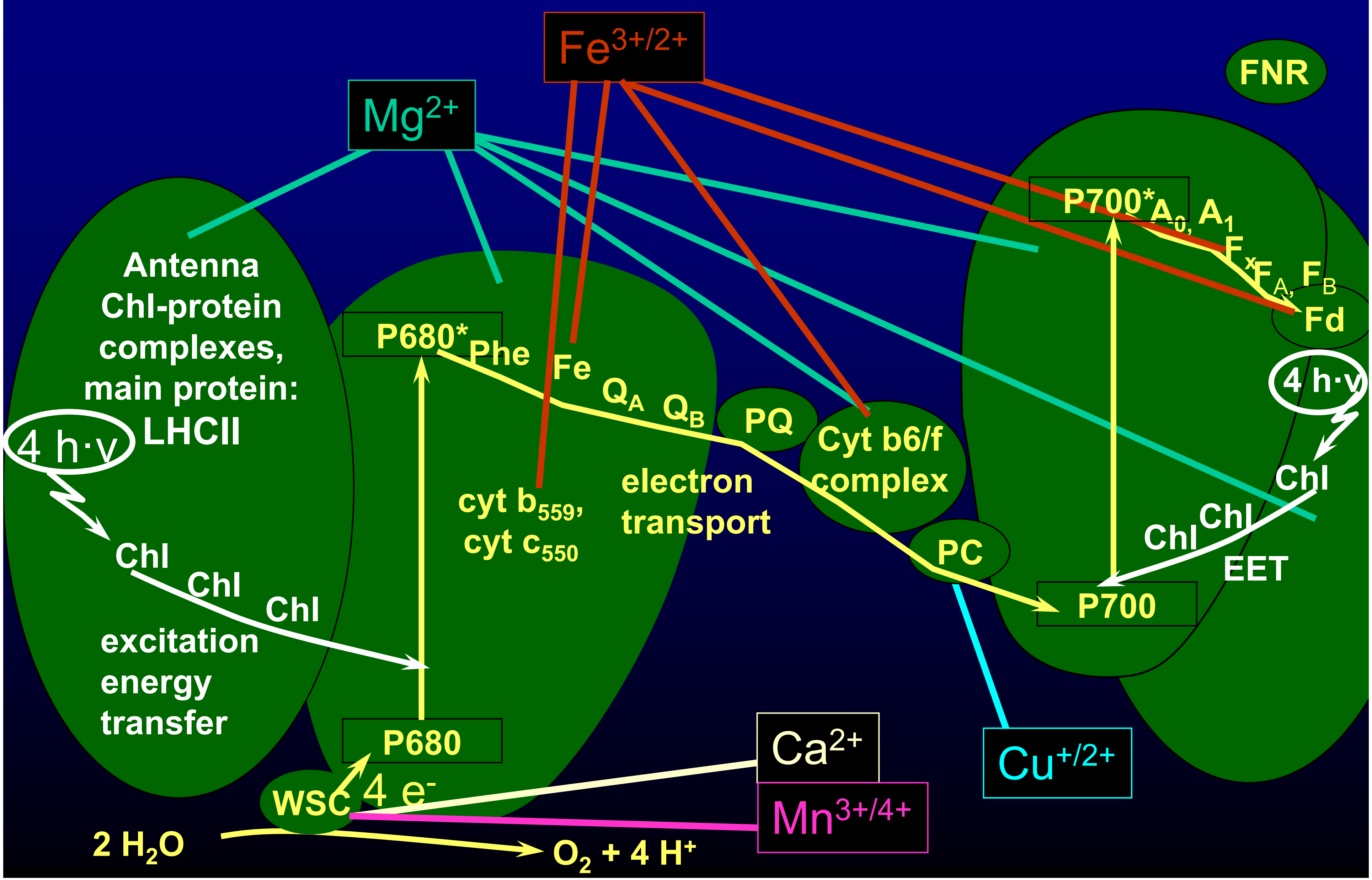
# Environmental relevance of heavy metal induced inhibition of photosynthesis:

*Elodea* stressed by 0.2 µM (= 0.013 ppm) $Cu^{2+}$

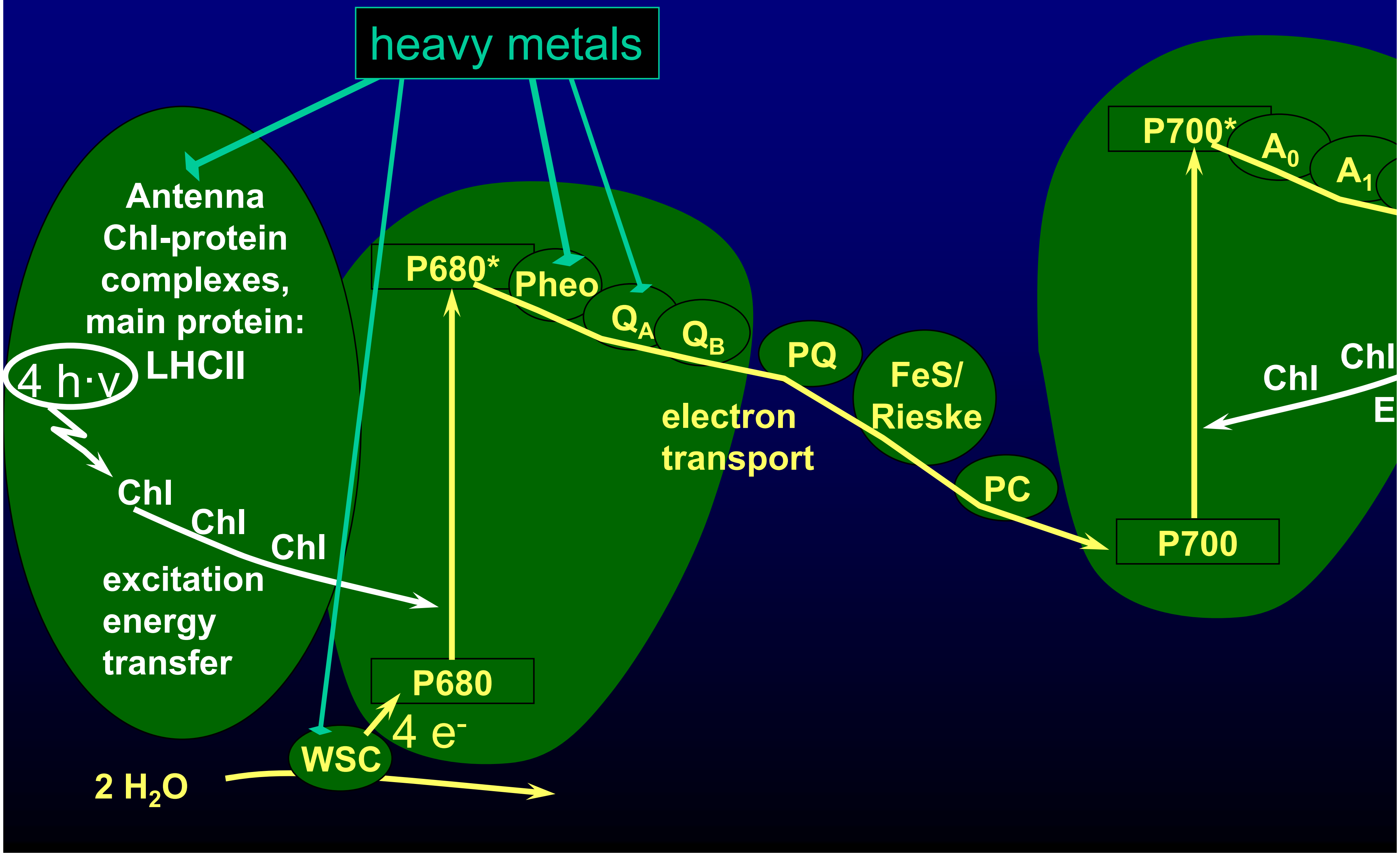



Küpper H, Küpper F, Spiller M (1996) Journal of Experimental Botany 47 (295), 259-66

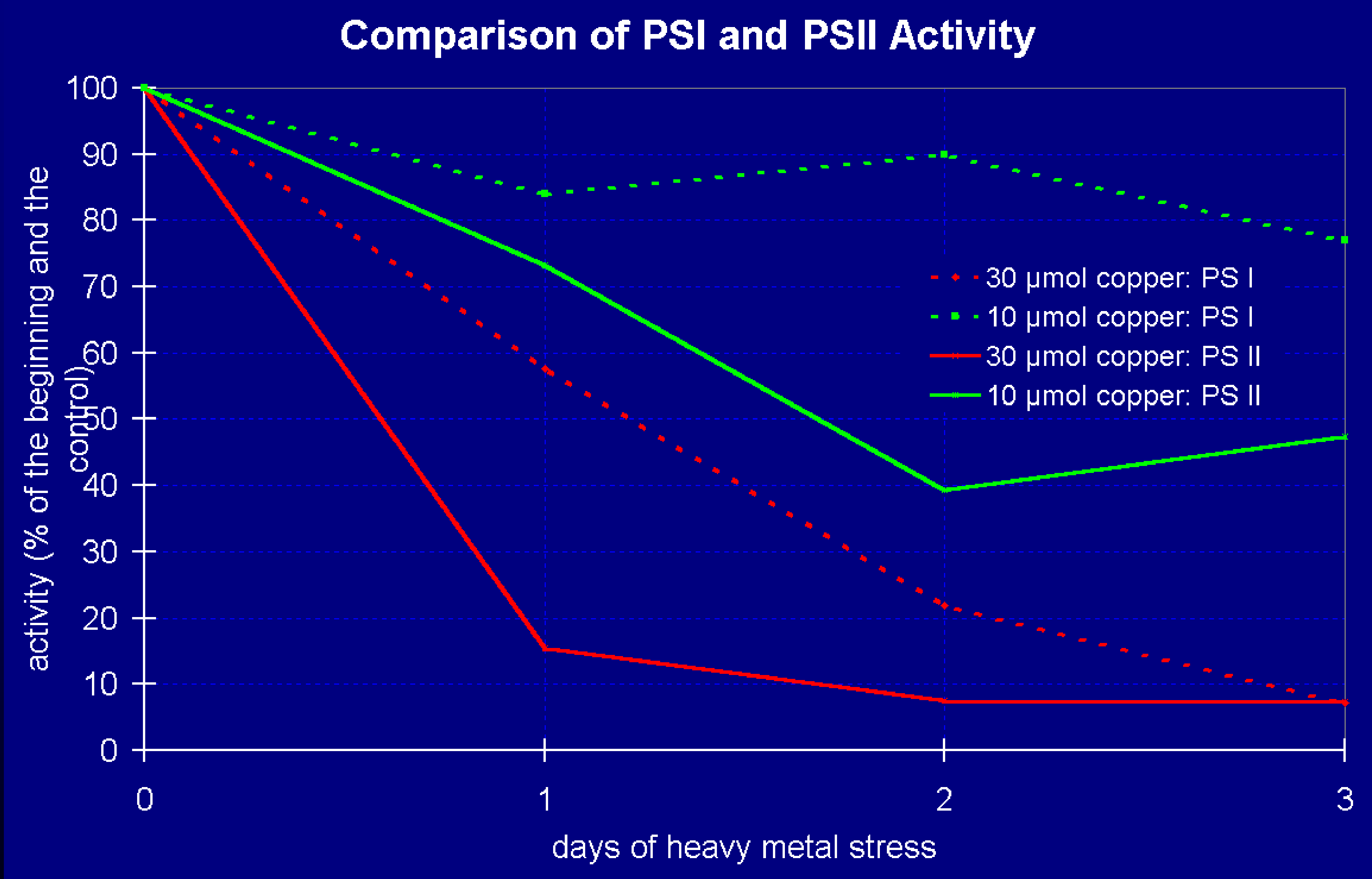
# Metal sites in photosynthetic proteins



# Heavy metal induced inhibitions of photosynthesis: suggested targets

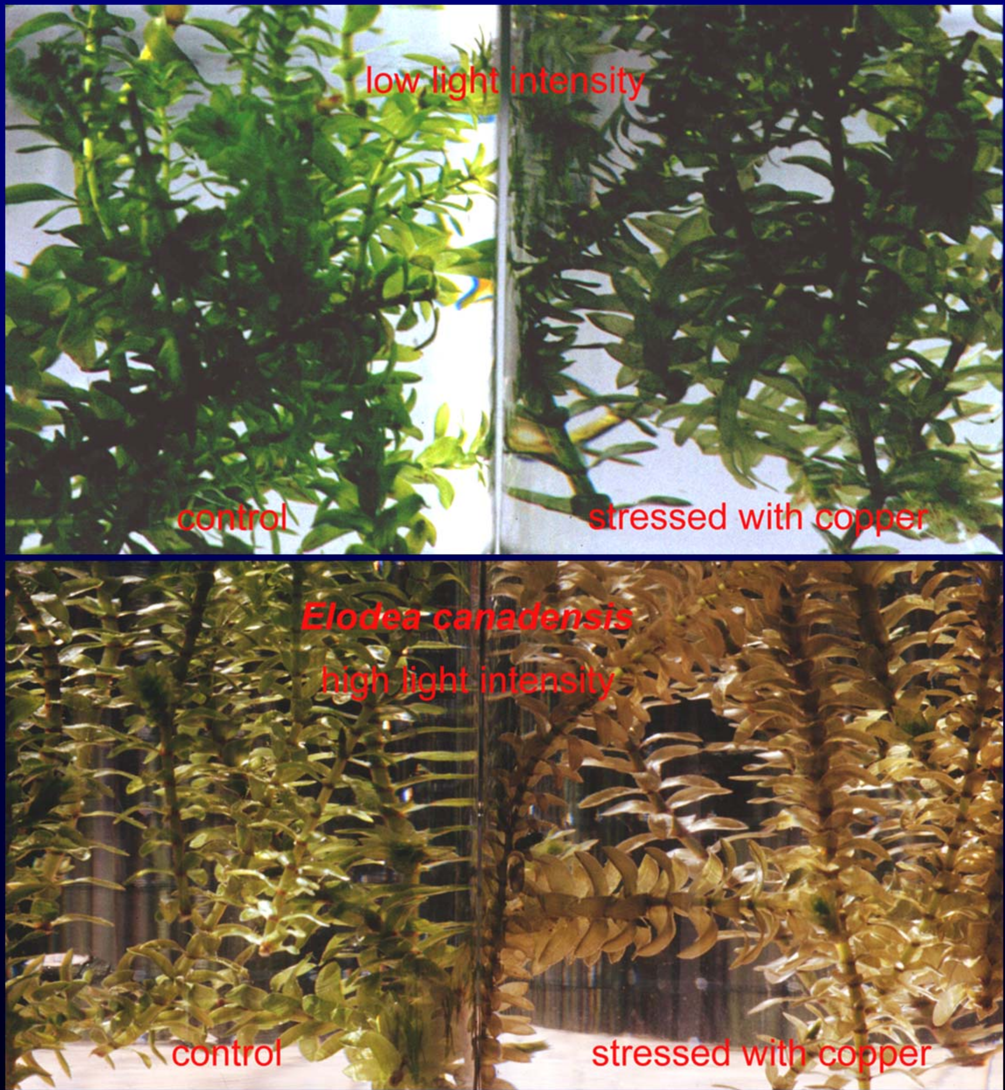


# Inhibition of PSI vs. PSII




Küpper H, Küpper F, Spiller M (1998) Photosynthesis Research 58, 125-33

# Macroscopically visible symptoms of heavy metal damage




**Shade Reaction**

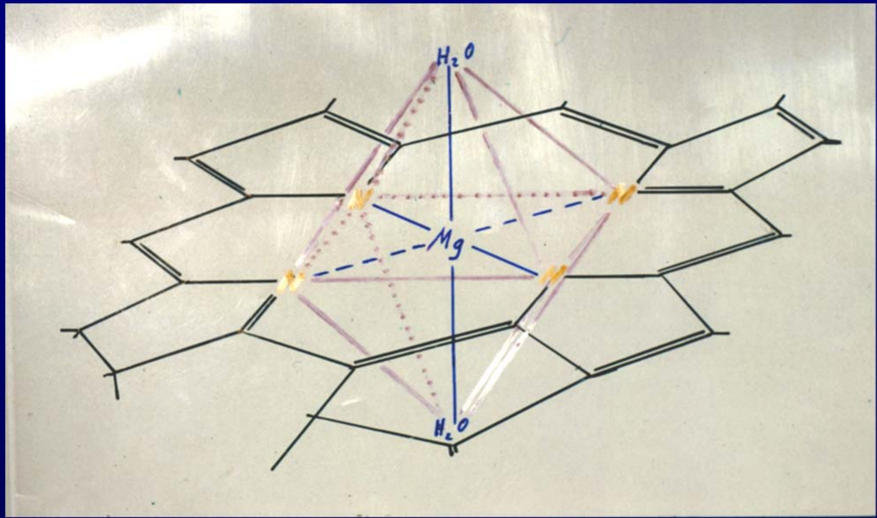
Under low irradiance conditions that include a dark phase, the majority of antenna (LHC II) chlorophylls is accessible to heavy metal Chl formation by substitution of the natural central ion of Chl, $Mg^{2+}$. If stable heavy metal Chls (e.g. Cu-Chl) are formed, plants remain green even when they are dead.

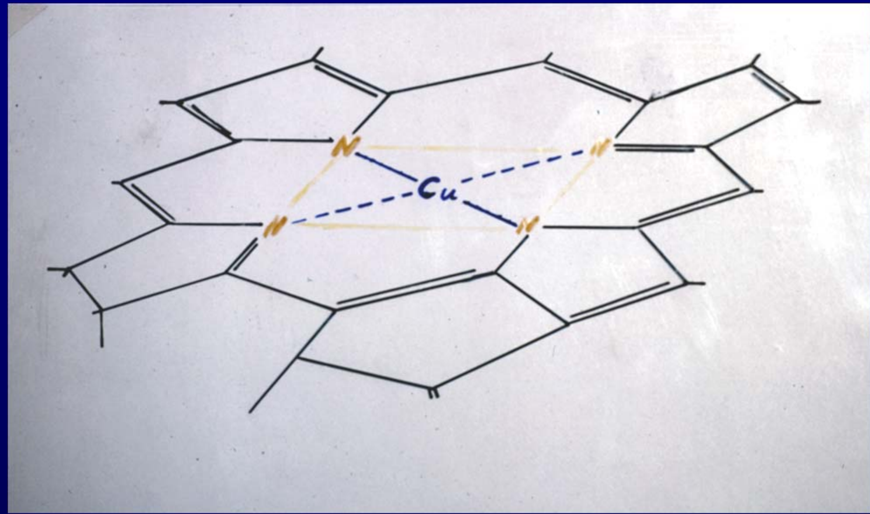
**Sun reaction**

In high irradiance, only a small fraction of the total Chl is accessible to heavy metal Chl formation, and direct damage to the PS II core occurs instead. The bulk of the pigments bleaches, in parallel to the destruction of the photosynthetic apparatus.

Küpper H, Küpper F, Spiller M (1998) Photosynthesis Research 58, 125-33

# Why are heavy metal chlorophylls unsuitable for photosynthesis?



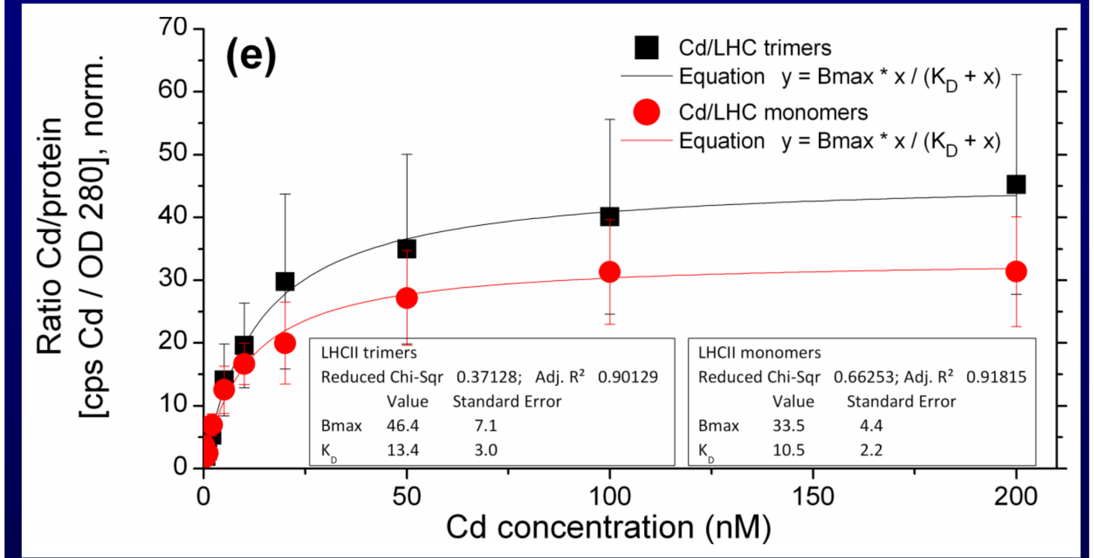

**Main reasons**

• 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.

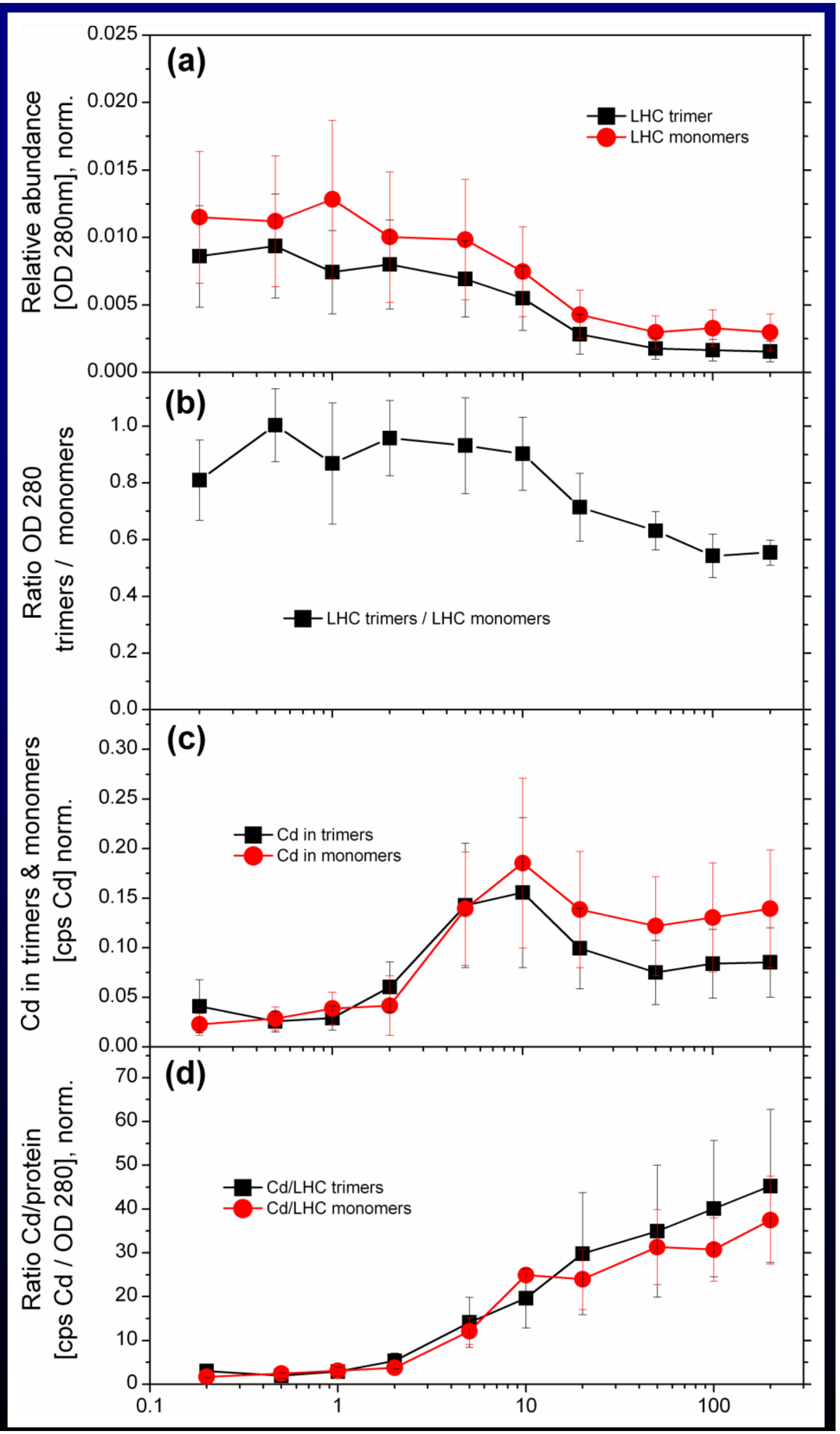
# Example of metal toxicity in the nanomolar range in „normal“ plants: Incorporation of Cd into LHCII in LL




→ Cd binding to LHCII causes disintegration of trimers

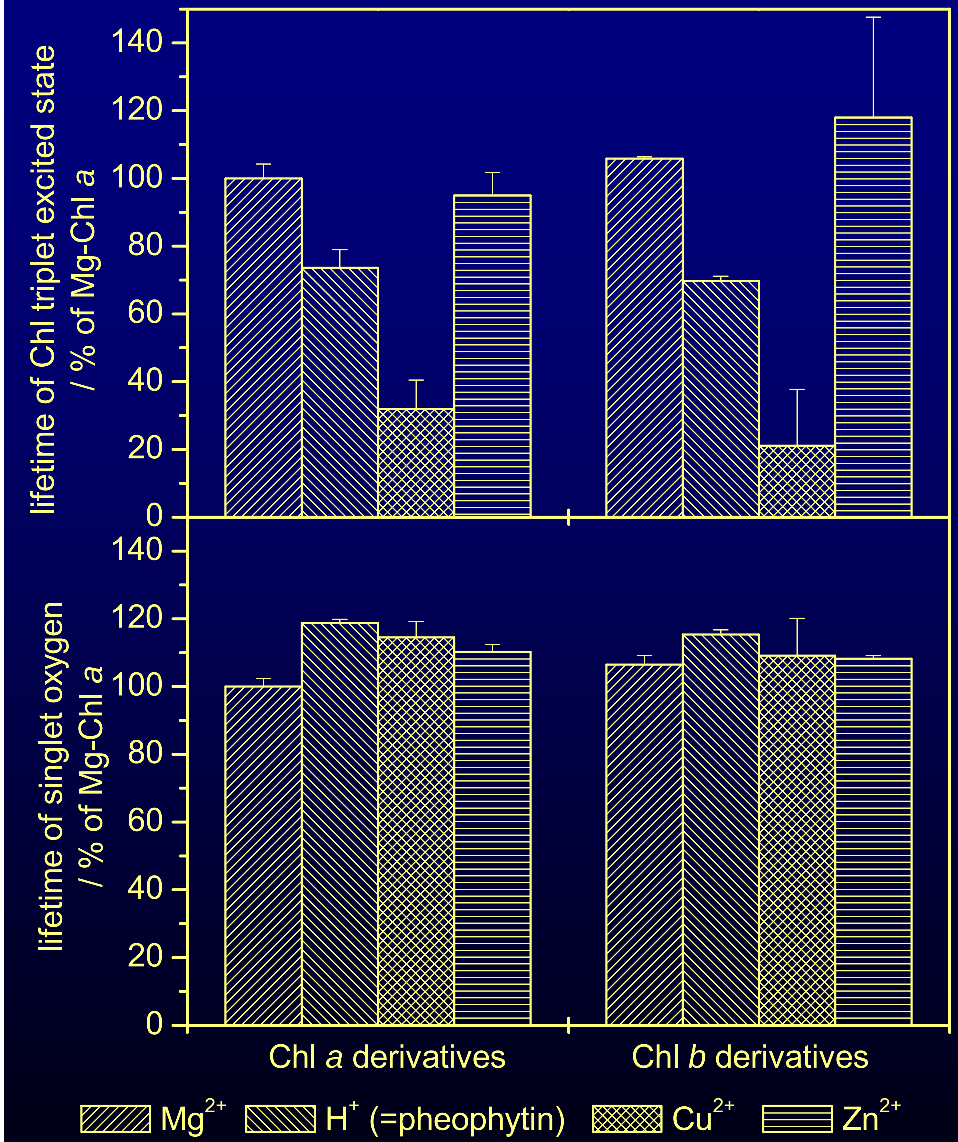
→ Cd bind to LHCII with dissociation constants in the low nanomolar range
→ diminished photosynthesis despite funtional reaction centres!

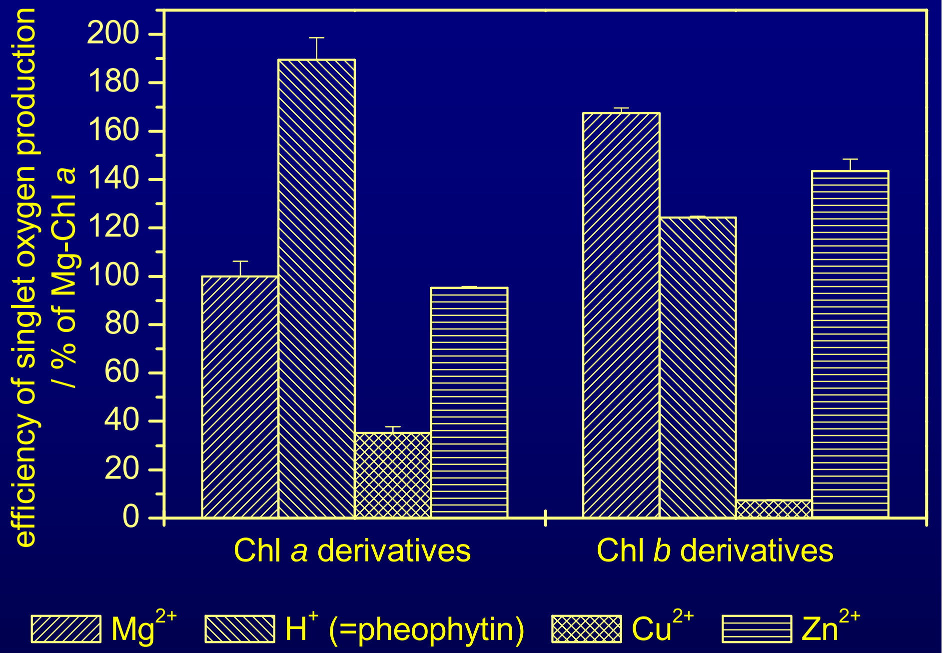
Andresen E, Kappel S, Stärk HJ, Riegger U, Borovec J, Mattusch J, Heinz A, Schmelzer CEH, Matoušková Š, Dickinson B, Küpper H (2016) New Phytologist 210, 1244-1258.



# NIR-luminescence study of excitation energy transfer

## between chlorophyll derivatives and singlet oxygen



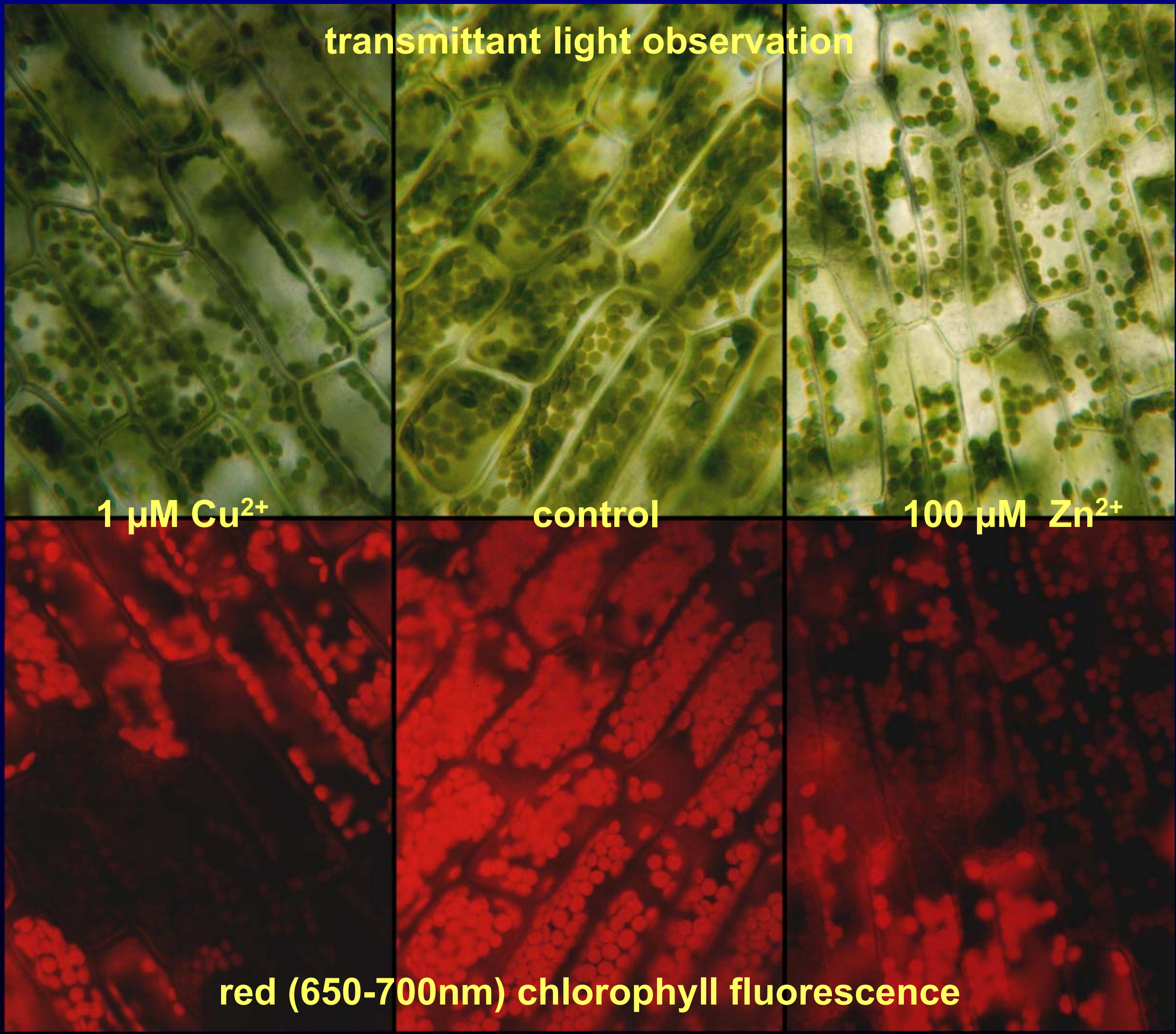




--> Hms-Chls have lower or equal quantum yields of singlet oxygen ($^1O_2$) production, but always lower yields of $^1O_2$ quenching compared to Mg-Chl. Phe has the most efficient $^1O_2$ production and least efficient quenching.

**--> Hms-Chl formation may indirectly lead to oxidative stress.**

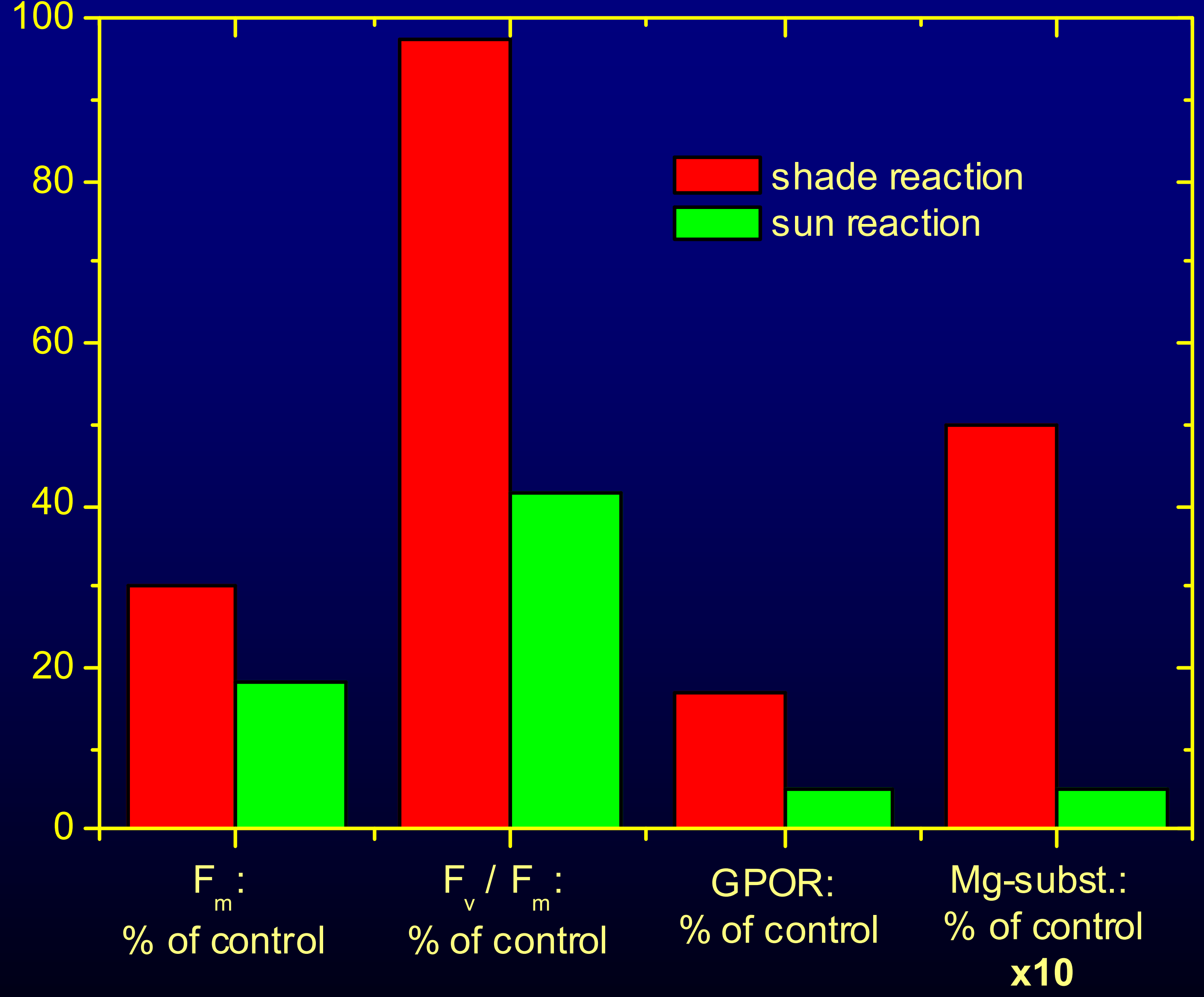
Küpper H, Dedic R, Svoboda A, Hála J, Kroneck PMH (2002) Biochim Biophys Act 1572, 107-113

# Static fluorescence microscopy of metal-stressed *Elodea*




Küpper H, Küpper F, Spiller M (1998) Photosynthesis Research 58, 125-33

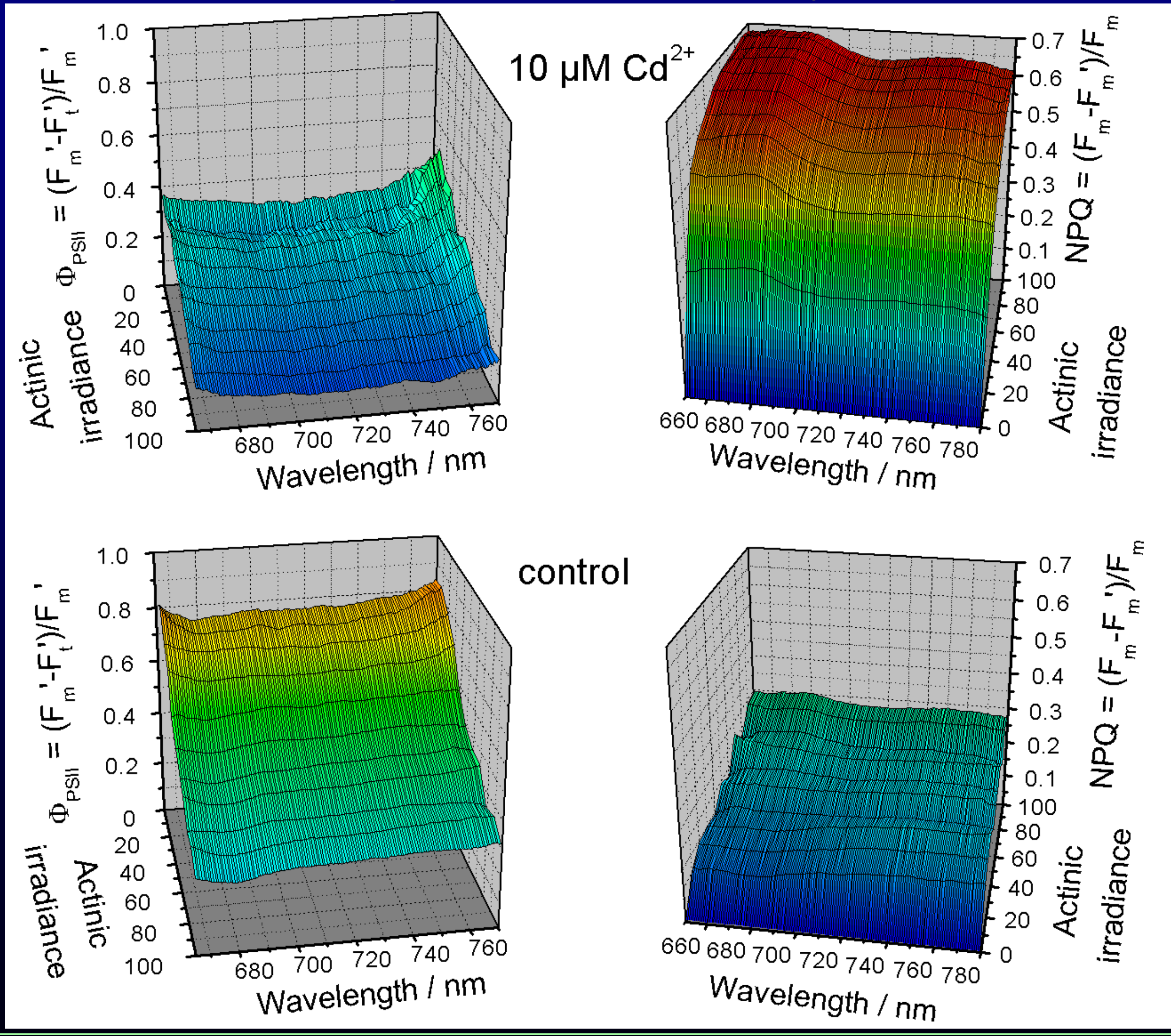
# Photosynthesis activity: Sun- vs. Shade-reaction




Küpper H, Šetlík I, Spiller M, Küpper FC, Prášil O (2002) Journal of Phycology 38(3), 429-441

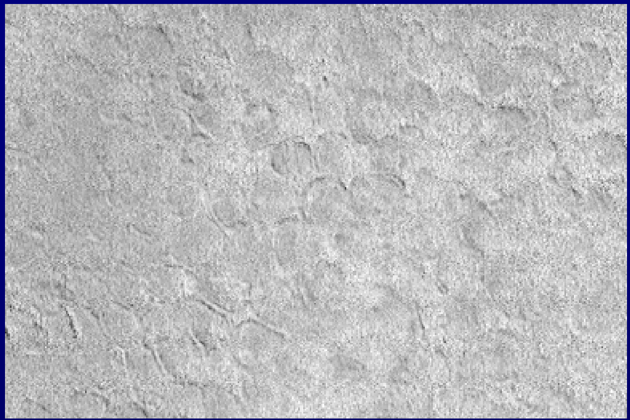
# **Cd-stress in the Zn-/Cd-hyperaccumulator *T. caerulescens:***

## Spectral changes of PSII activity parameters



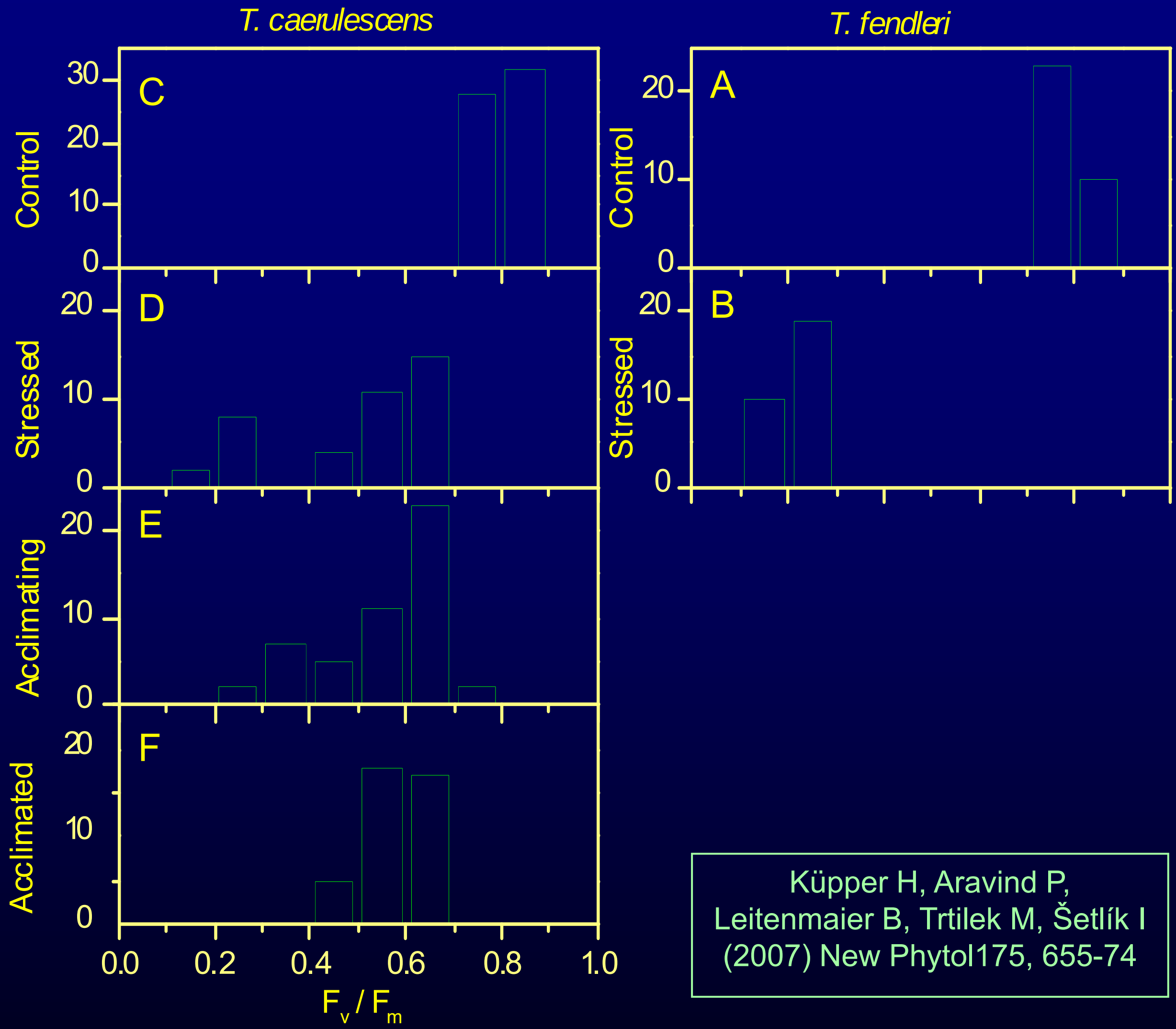

Küpper H, Aravind P, Leitenmaier B, Trtilek M, Šetlík I (2007) New Phytol175, 655-74

# **Cd-stress in the Zn-/Cd-hyperaccumulator *T. caerulescens:***
## distribution of photosystem II activity parameters



Cellular $F_v/F_m$ distribution in a control plant

Distribution of $F_v/F_m$ in a Cd-stressed plant

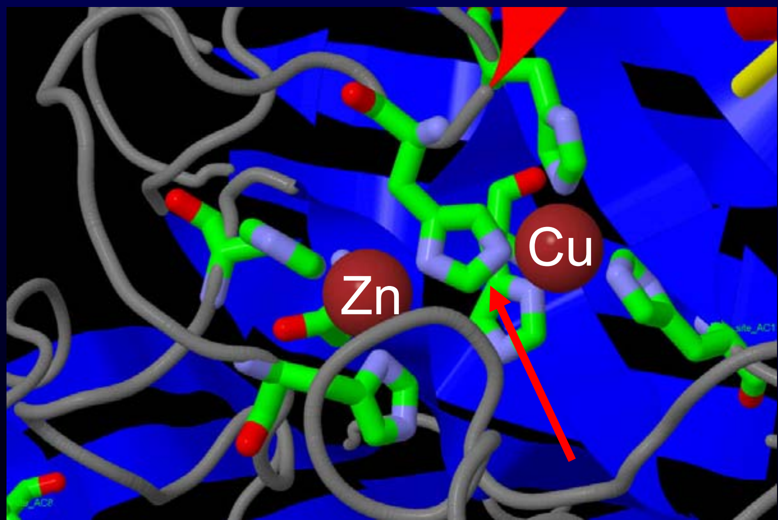


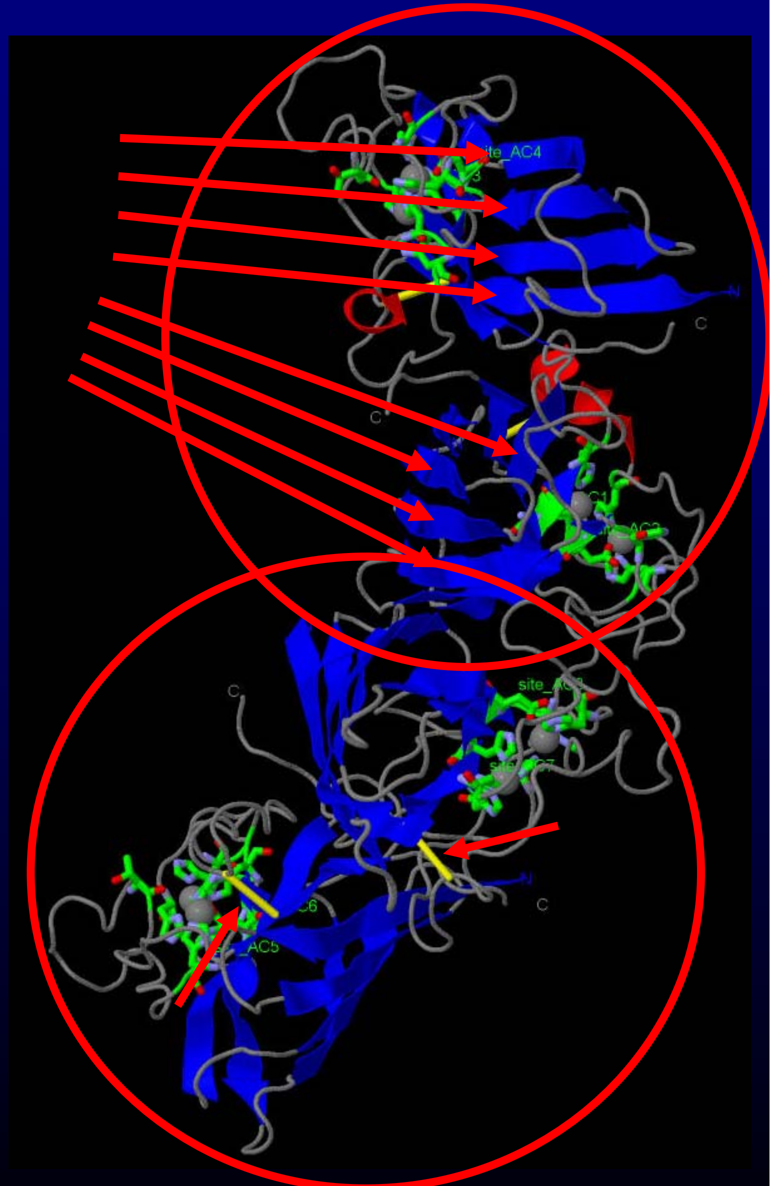

Küpper H, Aravind P, Leitenmaier B, Trtilek M, Šetlík I (2007) New Phytol175, 655-74

→ transient heterogeneity of mesophyll activity during period of Cd-induced stress

# Possible Target for Cd toxicity: Superoxide dismutase (SOD), in plants a Cu/Zn enzyme

Substitution of Zn by Cd in SOD may contribute to oxidative stress during Cd toxicity → Elisa's talk



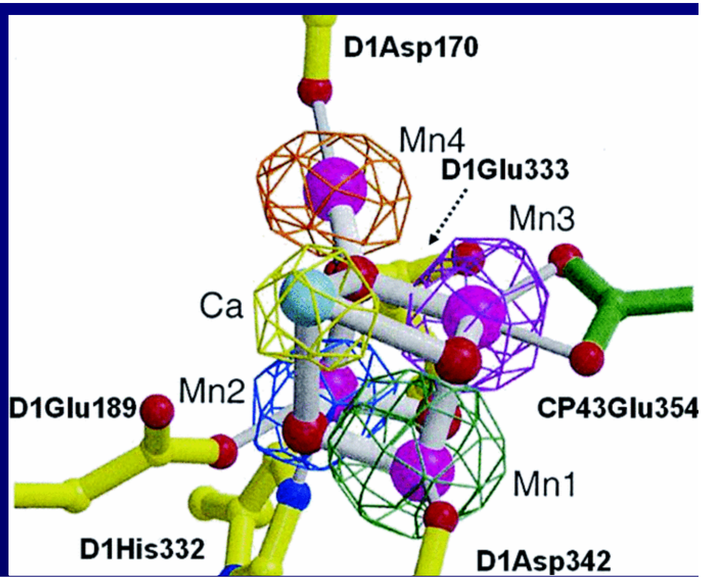



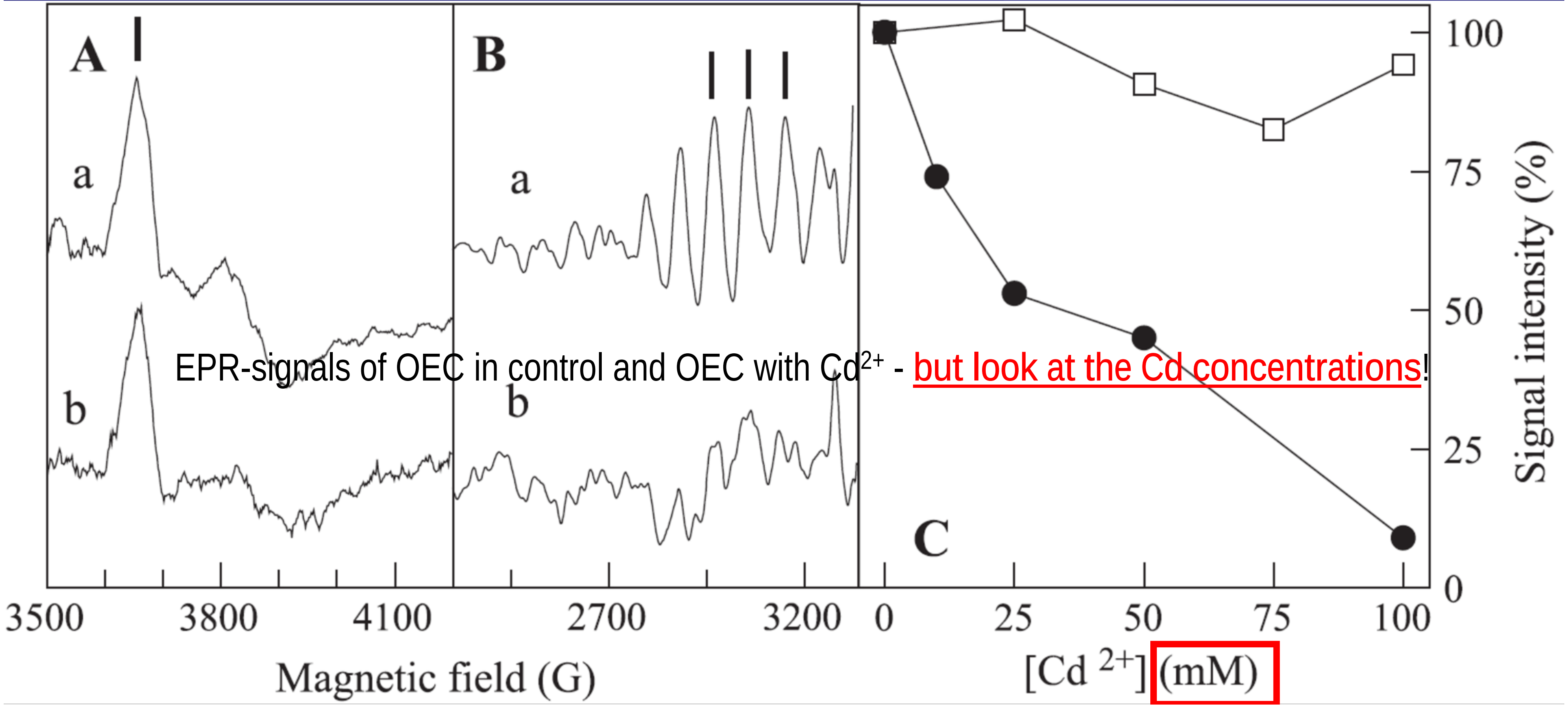
Spinach SOD, From: Kitagawa Y et al., 1991, J Biochem 109, 477-85, images generated with Jena 3D viewer

# Substitution of Ca by Cd in water splitting complex of PSII would inhibit water splitting, if it would occur at relevant Cd concentrations

→ details in Elisa's talk

Sigfridsson KGV_et al (2004) BBA-Bioen1659, 19-31




EPR-signals of OEC in control and OEC with $Cd^{2+}$ - but look at the Cd concentrations!

# Summary 1: Examples of Toxicity Mechanisms

## Copper toxicity

at high irradiance

>10nM Cu: Damage to the PSII reaction centre

→ decreased photochemical quantum yield ($F_v/F_m$)

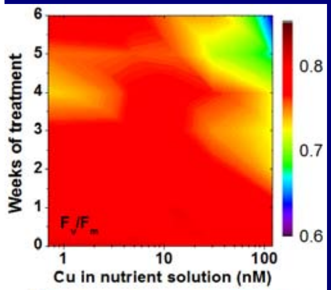
⇩

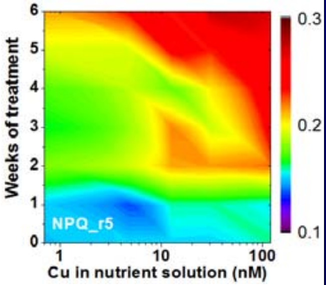
• Up-regulation of the dissipation of excitons as heat (NPQ)

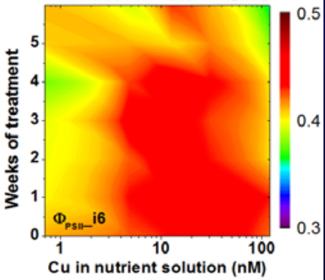
• Electron transport ($\Phi_{PSII}$) inhibited in addition to PSIIRC damage

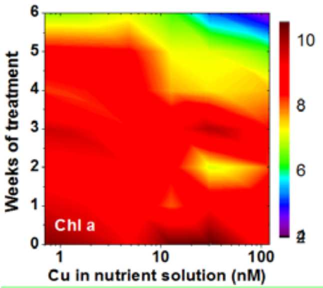
⇩

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

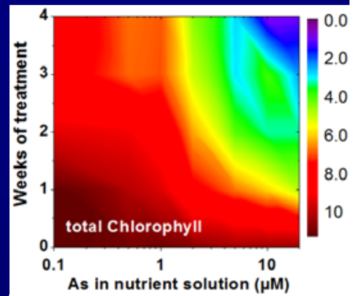
→ up-regulation of thermal exciton dissipation (NPQ)

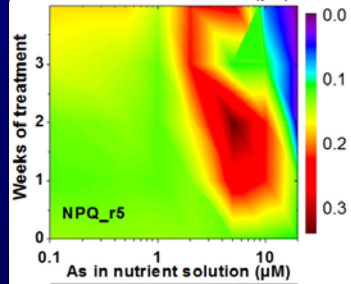
>2µM As: Electron transport ($\Phi_{PSII}$) inhibited

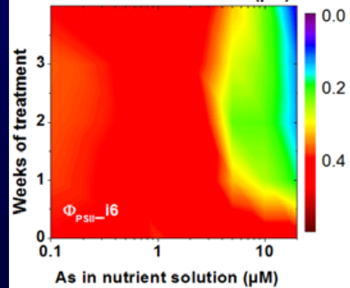
>5µM As: NPQ inhibition

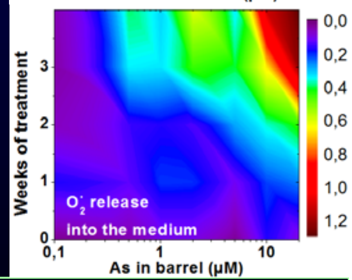
⇩

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**