

Heavy Metals and Plants - a complicated relationship

→ Cadmium toxicity



Heavy metal-hyperaccumulation in the Wild West

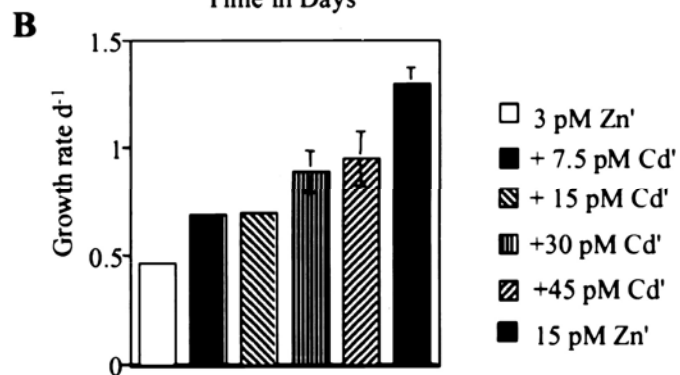
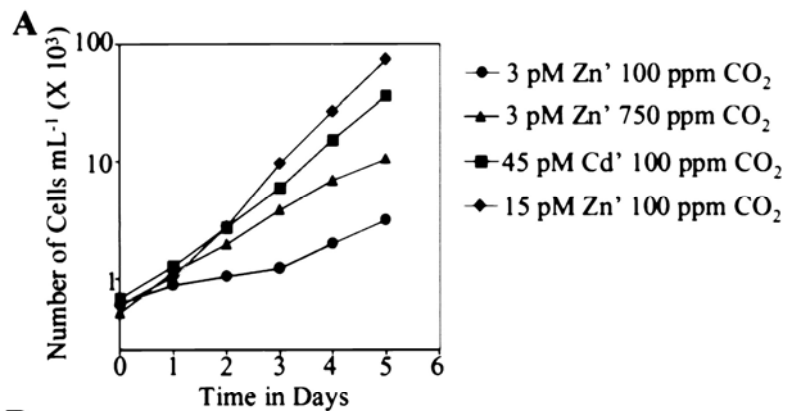
modified from: <http://strangematter.sci.waikato.ac.nz/>

Cadmium in nature

- Rather rare element in Earth's crust (0.1 - 0.5 ppm)
- Some natural sites, associated with ZnS

| | | | | | | | | | | | | | | | | | | |
|--------------|-----------------------------|-----------------------------|---------------------------------|------------------------------|------------------------------|------------------------------|-----------------------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|-------------------------------|------------------------------|-------------------------------|------------------------------|-----------------------------|-----------------------------|
| 1 | 1 H 1.008 | | | | | | | | | | | | | | | | | 2 He 4.0026 |
| 2 | 3 Li 6.94 | 4 Be 9.0122 | | | | | | | | | | 5 B 10.81 | 6 C 12.011 | 7 N 14.007 | 8 O 15.999 | 9 F 18.998 | 10 Ne 20.180 | |
| 3 | 11 Na 22.990 | 12 Mg 24.305 | | | | | | | | | | 13 Al 26.982 | 14 Si 28.085 | 15 P 30.974 | 16 S 32.06 | 17 Cl 35.45 | 18 Ar 39.948 | |
| 4 | 19 K 39.098 | 20 Ca 40.078 | 21 Sc 44.956 | 22 Ti 47.867 | 23 V 50.942 | 24 Cr 51.996 | 25 Mn 54.938 | 26 Fe 55.845 | 27 Co 58.933 | 28 Ni 58.693 | 29 Cu 63.546 | 30 Zn 65.38 | 31 Ga 69.723 | 32 Ge 72.63 | 33 As 74.922 | 34 Se 78.96 | 35 Br 79.904 | 36 Kr 83.798 |
| 5 | 37 Rb 85.468 | 38 Sr 87.62 | 39 Y 88.906 | 40 Zr 91.224 | 41 Nb 92.906 | 42 Mo 95.96 | 43 Tc [97.91] | 44 Ru 101.07 | 45 Rh 102.91 | 46 Pd 106.42 | 47 Ag 107.87 | 48 Cd 112.41 | 49 In 114.82 | 50 Sn 118.71 | 51 Sb 121.76 | 52 Te 127.60 | 53 I 126.90 | 54 Xe 131.29 |
| 6 | 55 Cs 132.91 | 56 Ba 137.33 | * 71 Lu 174.97 | 72 Hf 178.49 | 73 Ta 180.95 | 74 W 183.84 | 75 Re 186.21 | 76 Os 190.23 | 77 Ir 192.22 | 78 Pt 195.08 | 79 Au 196.97 | 80 Hg 200.59 | 81 Tl 204.38 | 82 Pb 207.2 | 83 Bi 208.98 | 84 Po [208.98] | 85 At [209.99] | 86 Rn [222.02] |
| 7 | 87 Fr [223.02] | 88 Ra [226.03] | ** 103 Lr [262.11] | 104 Rf [265.12] | 105 Db [268.13] | 106 Sg [271.13] | 107 Bh [270] | 108 Hs [277.15] | 109 Mt [276.15] | 110 Ds [281.16] | 111 Rg [280.16] | 112 Cn [285.17] | 113 Uut [284.18] | 114 Ff [289.19] | 115 Uup [288.19] | 116 Lv [293] | 117 Uus [294] | 118 Uuo [294] |
| *Lanthanoids | | | * 57 La 138.91 | 58 Ce 140.12 | 59 Pr 140.91 | 60 Nd 144.24 | 61 Pm [144.91] | 62 Sm 150.36 | 63 Eu 151.96 | 64 Gd 157.25 | 65 Tb 158.93 | 66 Dy 162.50 | 67 Ho 164.93 | 68 Er 167.26 | 69 Tm 168.93 | 70 Yb 173.05 | | |
| **Actinoids | | | ** 89 Ac [227.03] | 90 Th 232.04 | 91 Pa 231.04 | 92 U 238.03 | 93 Np [237.05] | 94 Pu [244.06] | 95 Am [243.06] | 96 Cm [247.07] | 97 Bk [247.07] | 98 Cf [251.08] | 99 Es [252.08] | 100 Fm [257.10] | 101 Md [258.10] | 102 No [259.10] | | |

Cadmium as a micronutrient: the exception...



With 10 μM cadmium in the nutrient solution
--> healthy *N. caerulea* plants

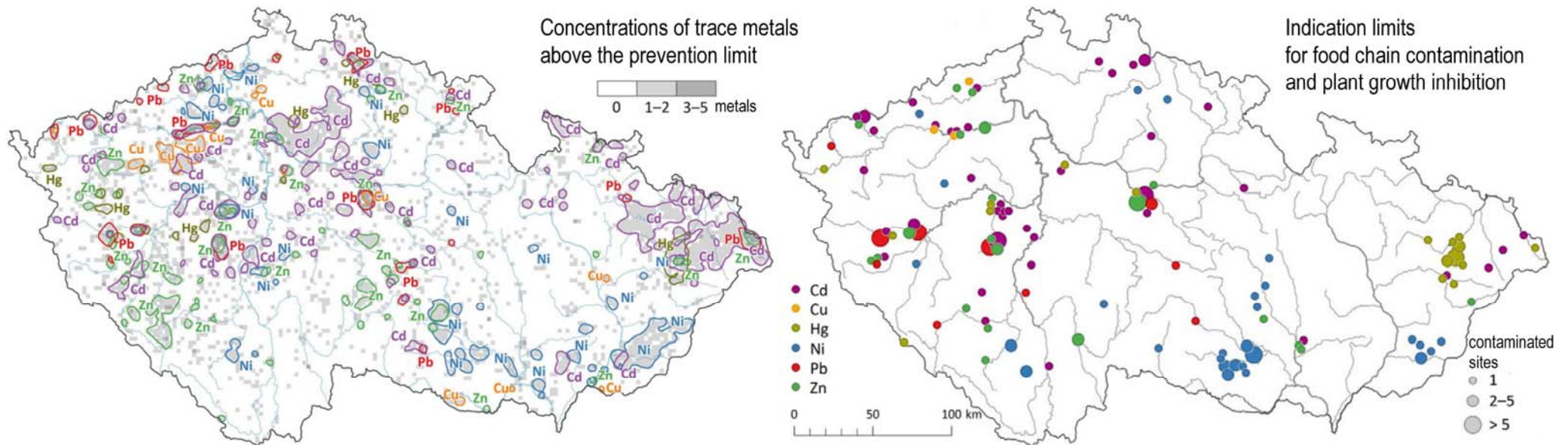
Without Cd in the nutrient solution
--> damage by insects

Cadmium as Plant-micronutrient in *Thalassiosira weissflogii*. A, B: growth of the algae. (Lane and Morel, 2000, PNAS97)

Küpper H, Kroneck PMH (2004) MIBS 44 (Sigel et al., eds), chapter 5

Cadmium pollution in the environment

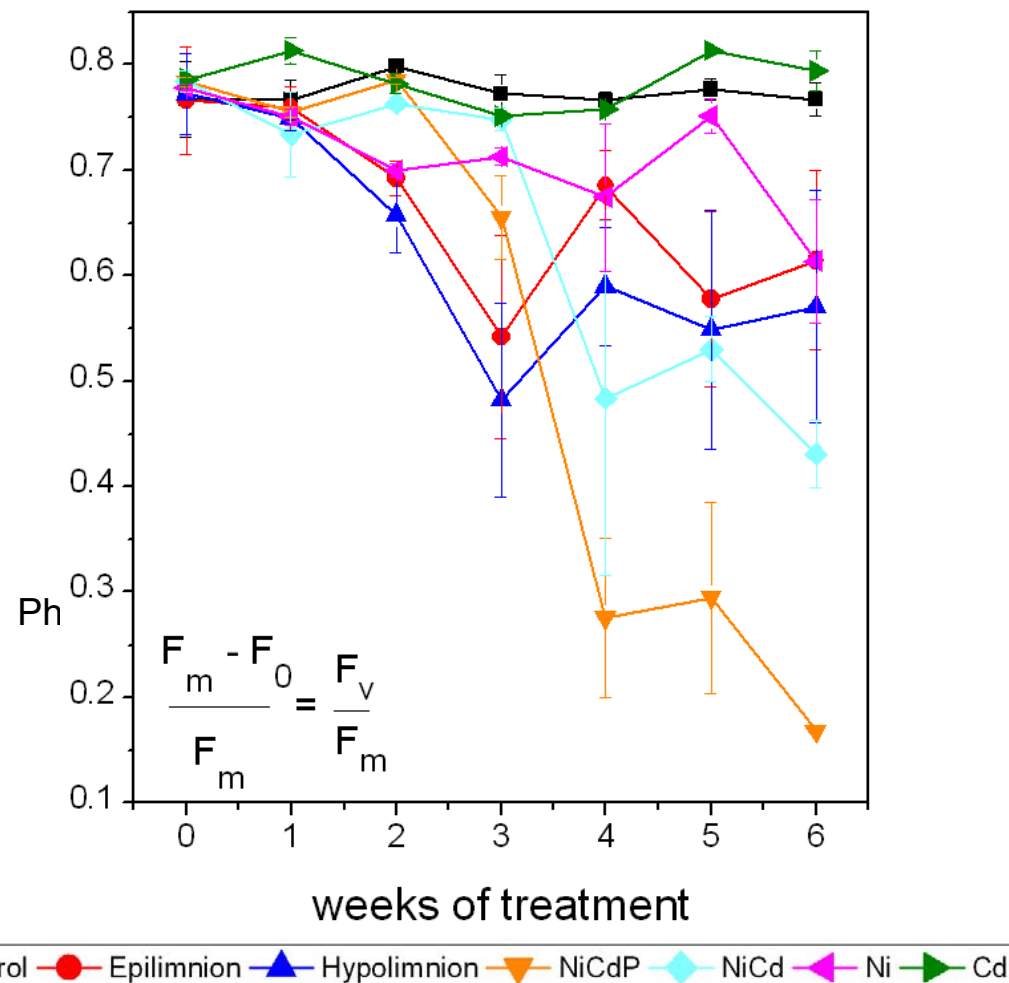
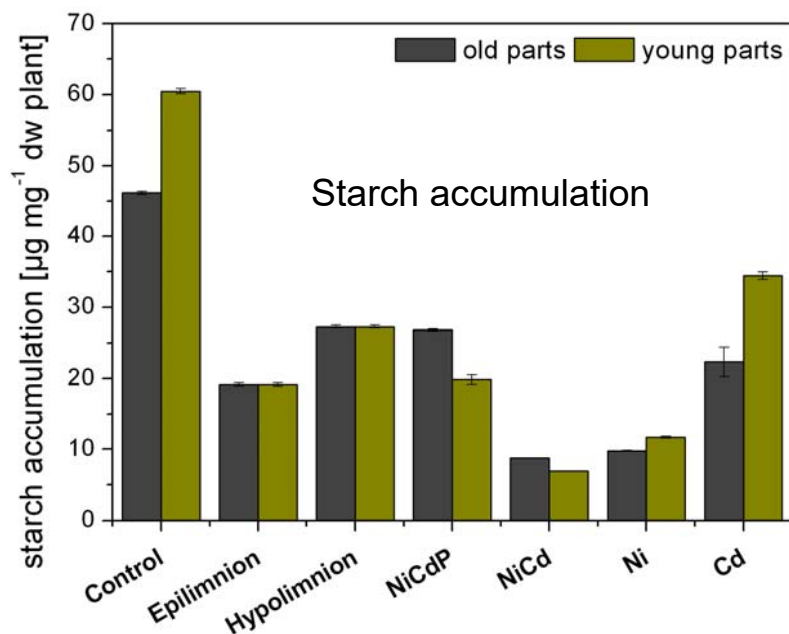
- Itai-itai disease (japanese ouch-ouch sickness), 1 of the 4 big pollution diseases in Japan
- Mass cadmium poisoning in Japan: severe pains in joints and spine, softening of the bones, kidney failure
- Cadmium release into rivers by mining, the mining companies were successfully sued for the damage
- In Europe: Cd from contamination of fertilisers in Agriculture
- Mechanistic knowledge of metal(loid) toxicity is required for realistic **environmental risk assessment**
- Revealing toxicity mechanisms is essential for **targeted breeding of plants** that are less vulnerable to stress



Maps from Bednarova et al. (2016) Spatial distribution and risk assessment of metals in agricultural soils. *Geoderma* 284, 113-121

Metal toxicity induced inhibition of photosynthesis at synergistically acting nanomolar Cd+Ni concentrations

- Ceratophyllum demersum* plants treated with natural or simulated lake water containing **only 3 nM Cd²⁺ and 300 nM Ni²⁺** already show inhibition
- inhibition by Ni+Cd combination treatment much stronger than by the single metals → **synergistic effect!**



Andresen E, Opitz J, Thomas G, Stärk H-J, Dienemann H, Jenemann K, Chang C, Küpper H (2013)
 Aquatic Toxicology 142-143, 387-402

Growth inhibition

Before treatment start



After 1 week



After 2 weeks



After 3 weeks



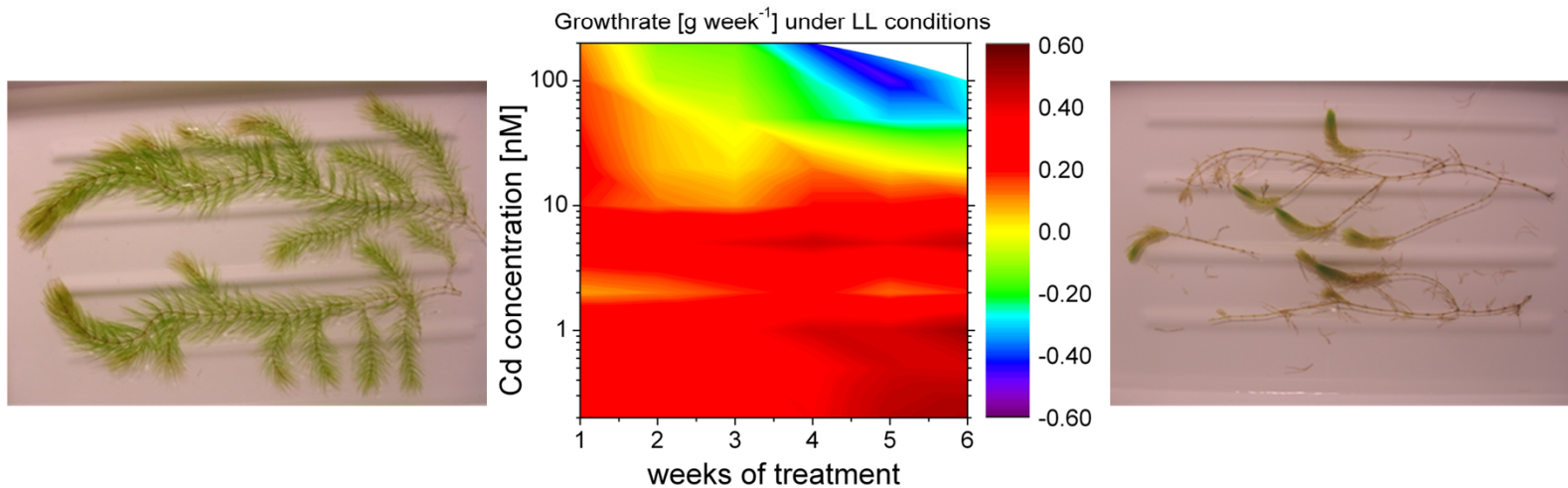
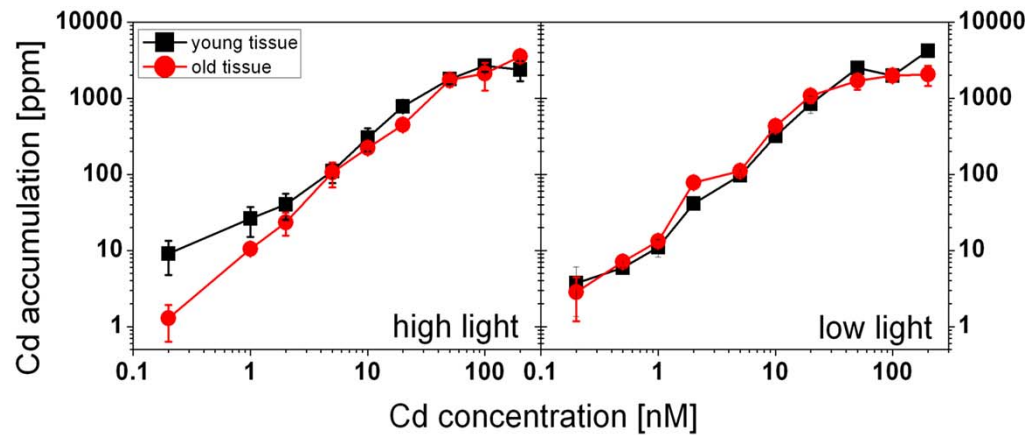
After 4 weeks



C. demersum treated with 200 nM Cd for 4 weeks

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.

Accumulation of Cd and growth



→ Already low nanomolar Cd is toxic for sensitive plants

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.

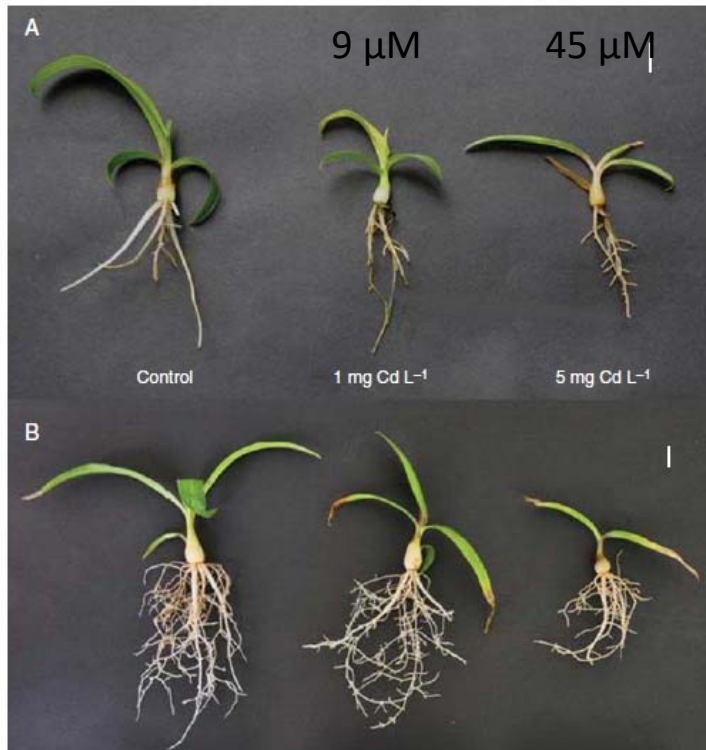
Cd toxicity in plants

- 1) Roots
- 2) Photosynthesis
- 3) Reactive oxygen species
- 4) Genotoxicity
- 5) Stress prevention

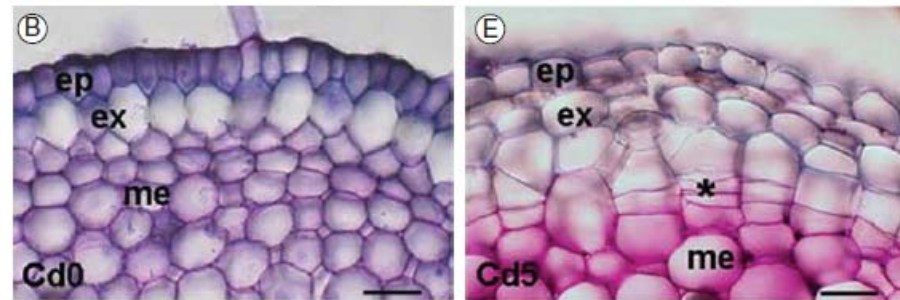
Cadmium toxicity in plants – 1: Roots

First organ which gets affected

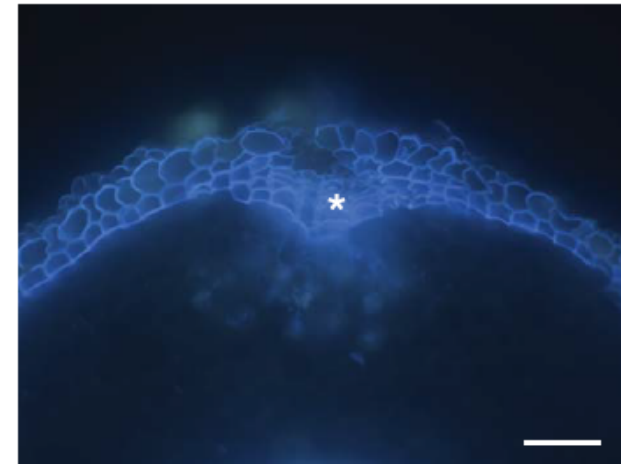
Reduced growth after Cd treatment



More layers of hypodermal periderm



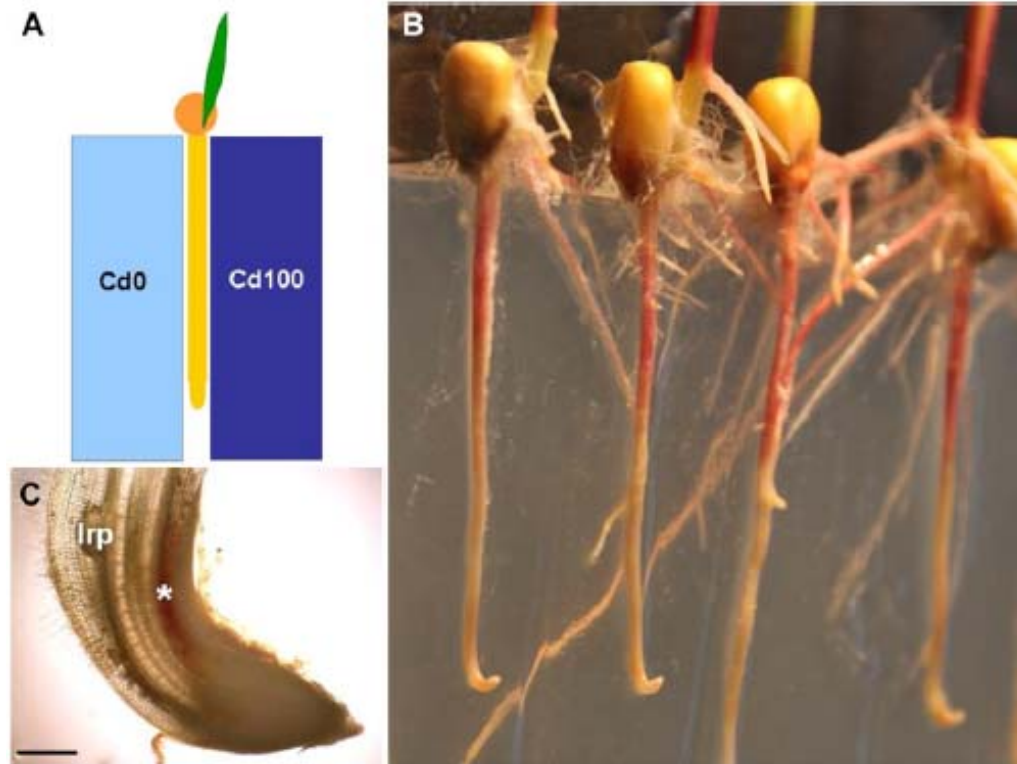
More layers & suberized cell walls (*) like after injury of root surface



- Maize seedlings with proper roots placed between 2 agar blocks
- one of which contained Cd (50 or 100 μ M), grown in phytochamber under nature-like conditions

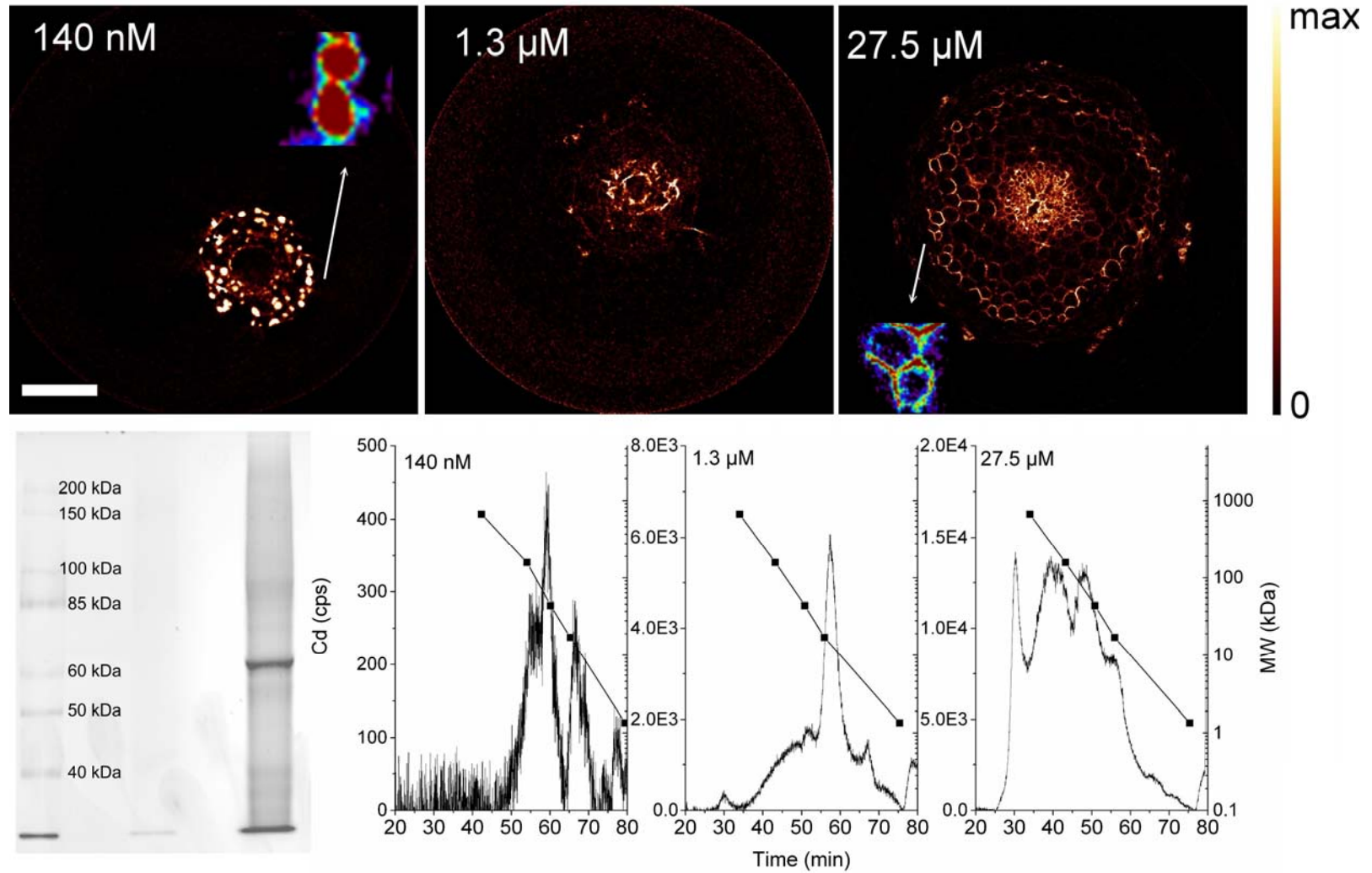
→ Roots bending towards the Cd-containing agar → due to growth stop on the Cd-side & continued growth on control-side

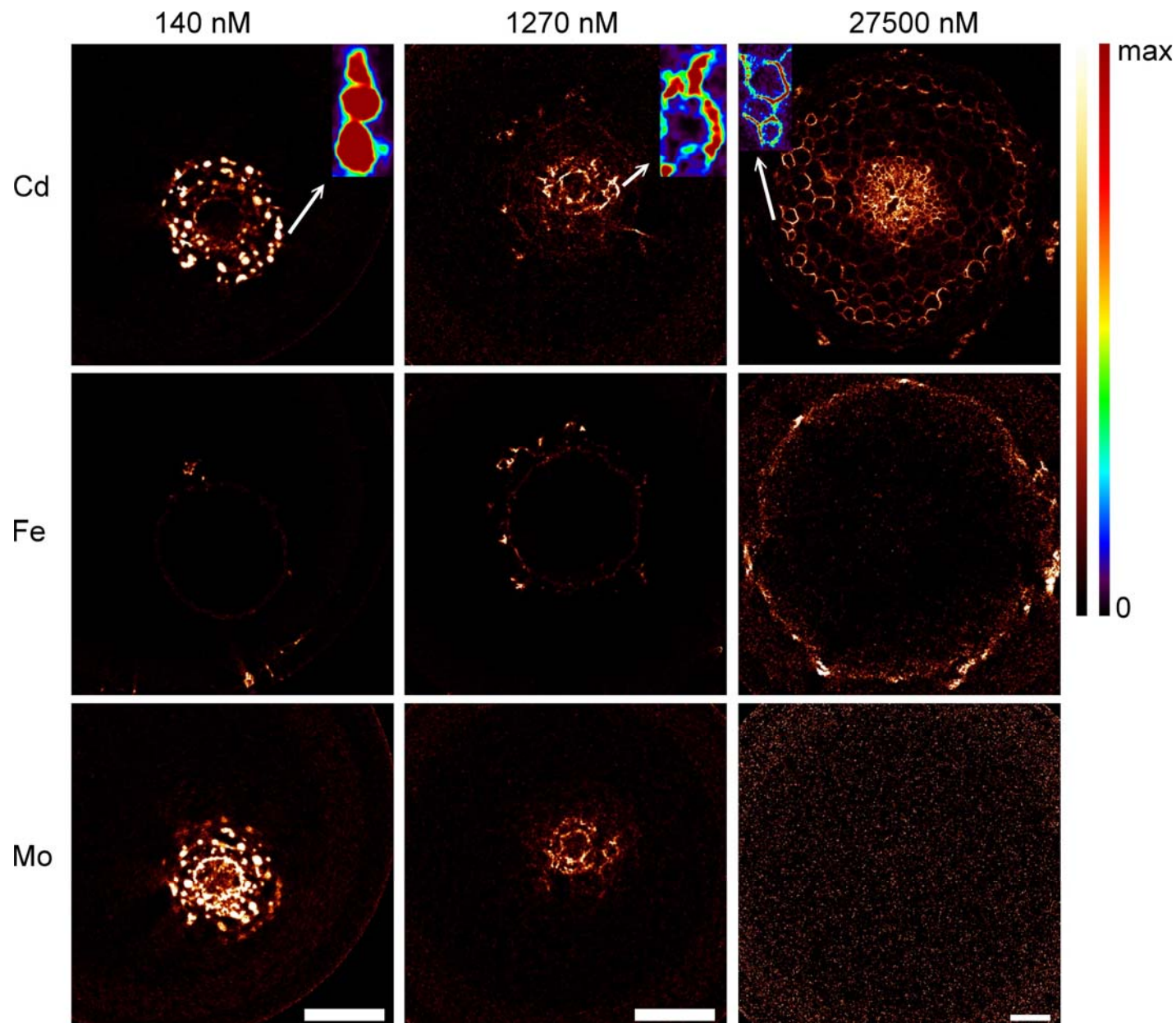
Lignification on Cd-exposed side
(*) and initiation of lateral root primordium (lrp)



Mechanisms of sublethal vs. lethal Cd toxicity in soybean roots

- 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!**





Where does the Cd go? Sub-cellular distribution (measured at Cd K α with 0.6 μ m resolution at beamline PETRA P6)

3- phase response to Cd toxicity

- A) Initially, at sublethal concentrations with low toxicity, Cd is sequestered into vacuoles of the root parenchyma
- B) At sublethal concentrations with high toxicity, stronger accumulation starts in cell walls in the vascular bundle, starting to block micronutrient (Fe, Mo) uptake
- C) At lethal concentration, Cd accumulates unspecifically in cell walls throughout the root diameter, and blocks transport of micronutrients (Fe & Mo)

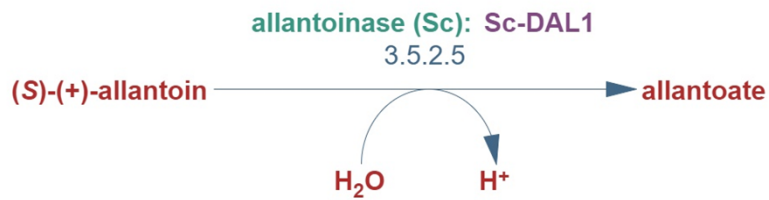
Andresen E, Flores-Sanchez IJ, Brückner D, Bokhari SNH, Falkenberg G, Küpper H (2023) Sublethal and lethal Cd toxicity in soybean roots specifically affects the metabolome, Cd binding to proteins and cellular distribution of Cd. *Journal of Hazardous Materials* 442, 130062
<https://doi.org/10.1016/j.jhazmat.2022.130062>

To which exact proteins does the Cd bind at sublethal concentrations?

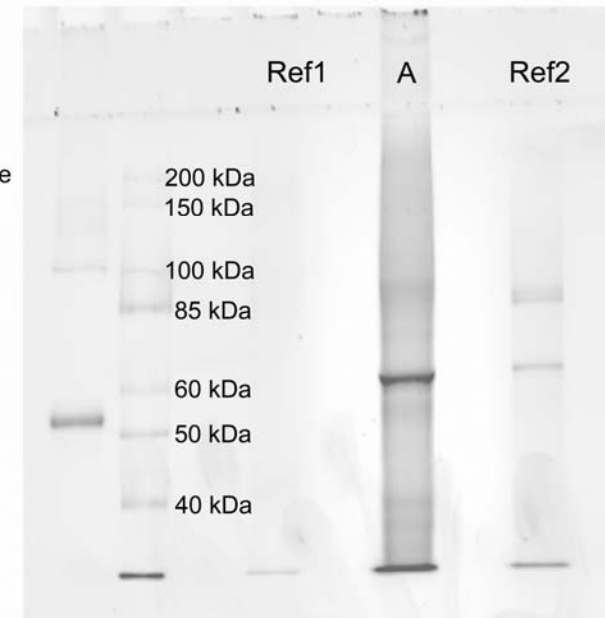
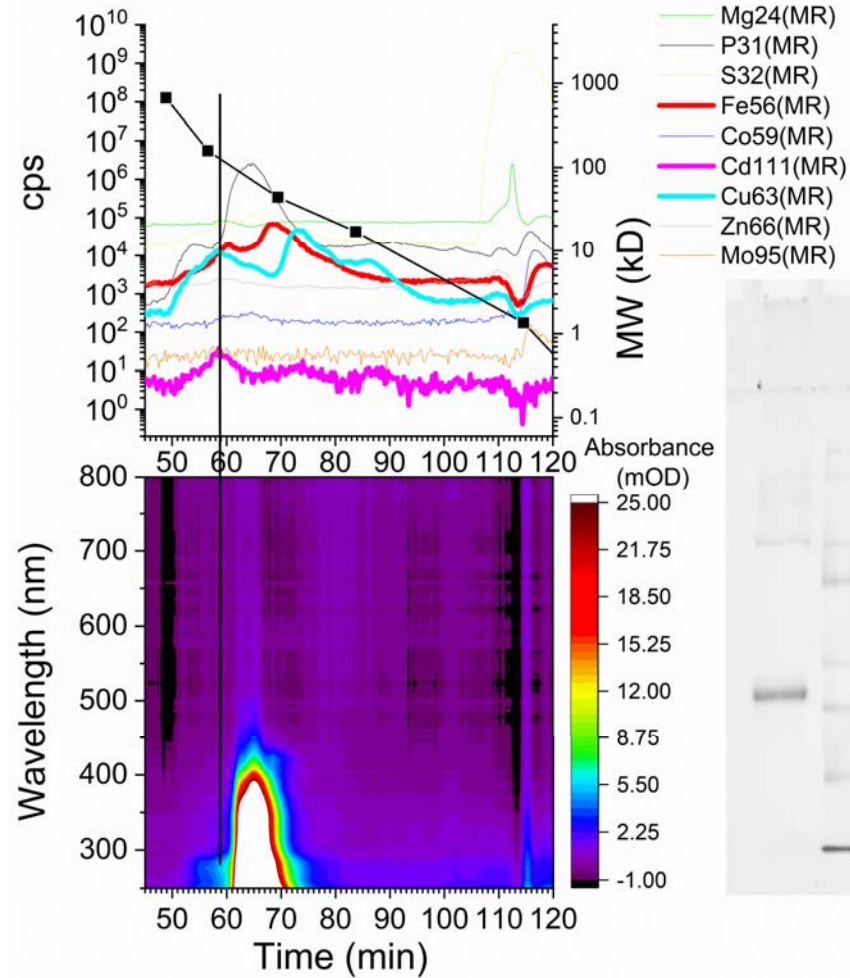
→ protein purification, identification and verification

→ highest affinity targets among soluble proteins in roots are allantoinase; protein disulfide isomerase-like proteins (PDI), aquaporin-like and peroxidase-like proteins,

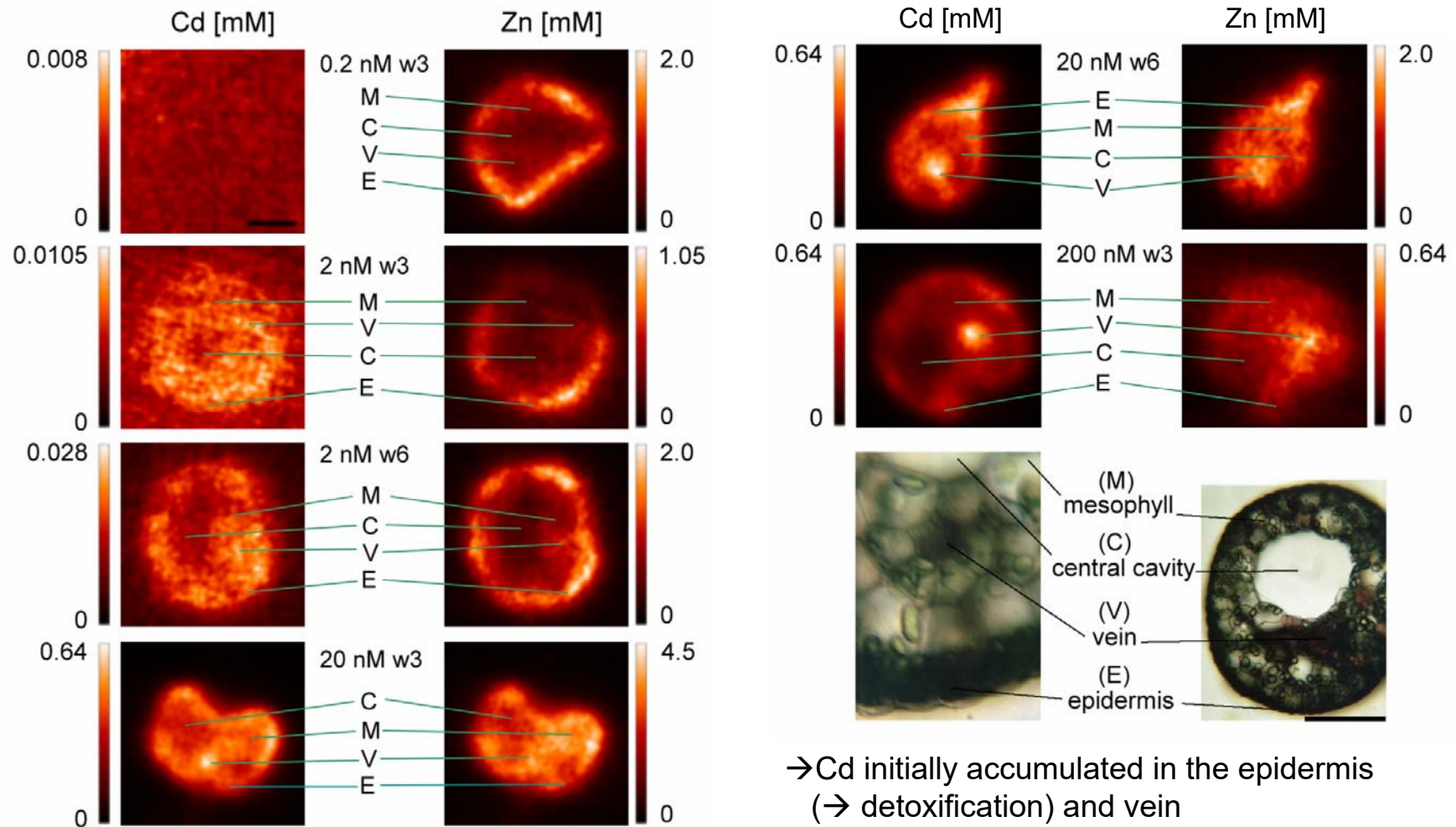
→ verified for allantoinase by metabolomics, there it most likely replaces the Zn^{2+} in the active centre



→ others to be investigated in future research



Distribution of Cd and its effect on Zn distribution in the non-accumulator shoot model *Ceratophyllum demersum*



→ Cd initially accumulated in the epidermis
 (→ detoxification) and vein
 → lethal Cd inhibited vein export. also of Zn

Changing distribution of Cd and micronutrients under Cd toxicity

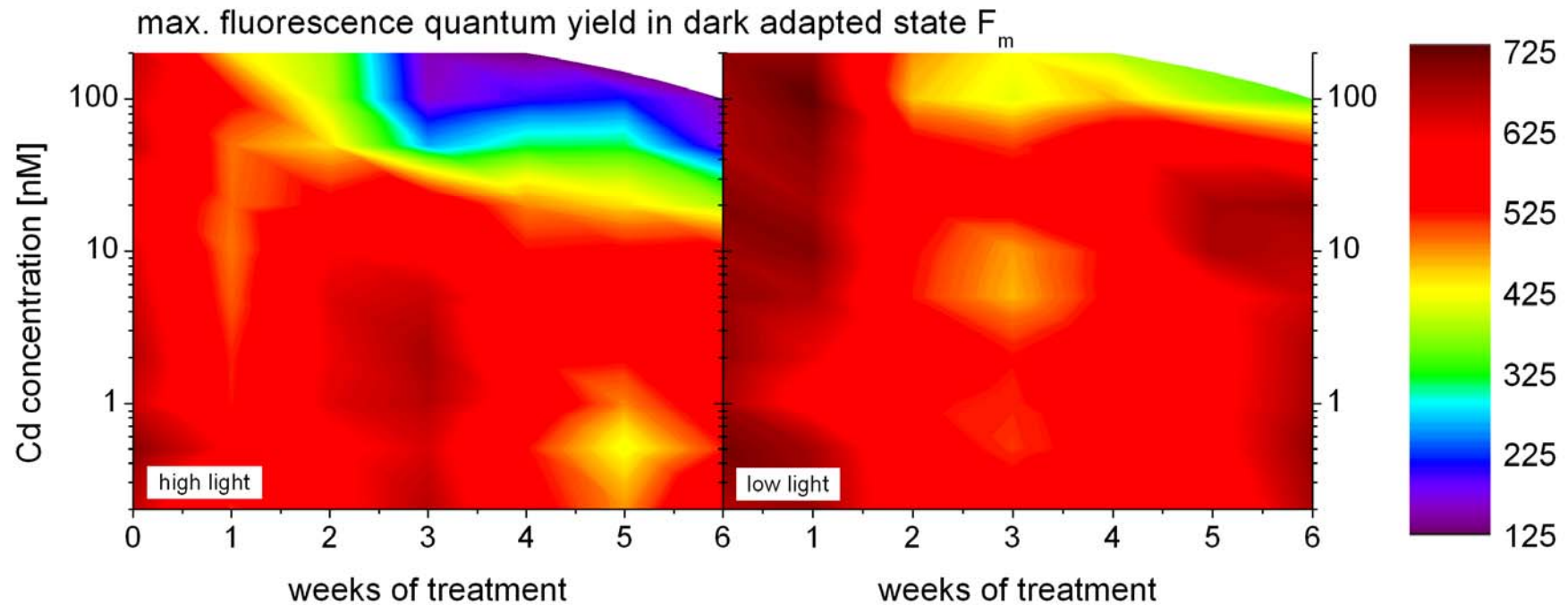
Increasing Cd:

- Roots: more aplastic Cd binding at high Cd
- Roots and leaves: more Cd in vein
- Roots and leaves: inhibition of micronutrient (Fe, Mo, Zn) transmembrane transport by Cd
- Leaves: Increased sequestration into non-photosynthetic tissues
- Leaves: re-distribution of Zn → inhibited export out of vein

Cadmium toxicity in plants – 2: Photosynthesis

- Indirect measurement:
 - Growth, O₂ production / CO₂ consumption: balance with O₂-consuming and CO₂ producing (photo-)respiration
 - Diminishing the Chl/pigment/protein content
- Direct: Photosynthetic parameters via Chlorophyll fluorescence measurement

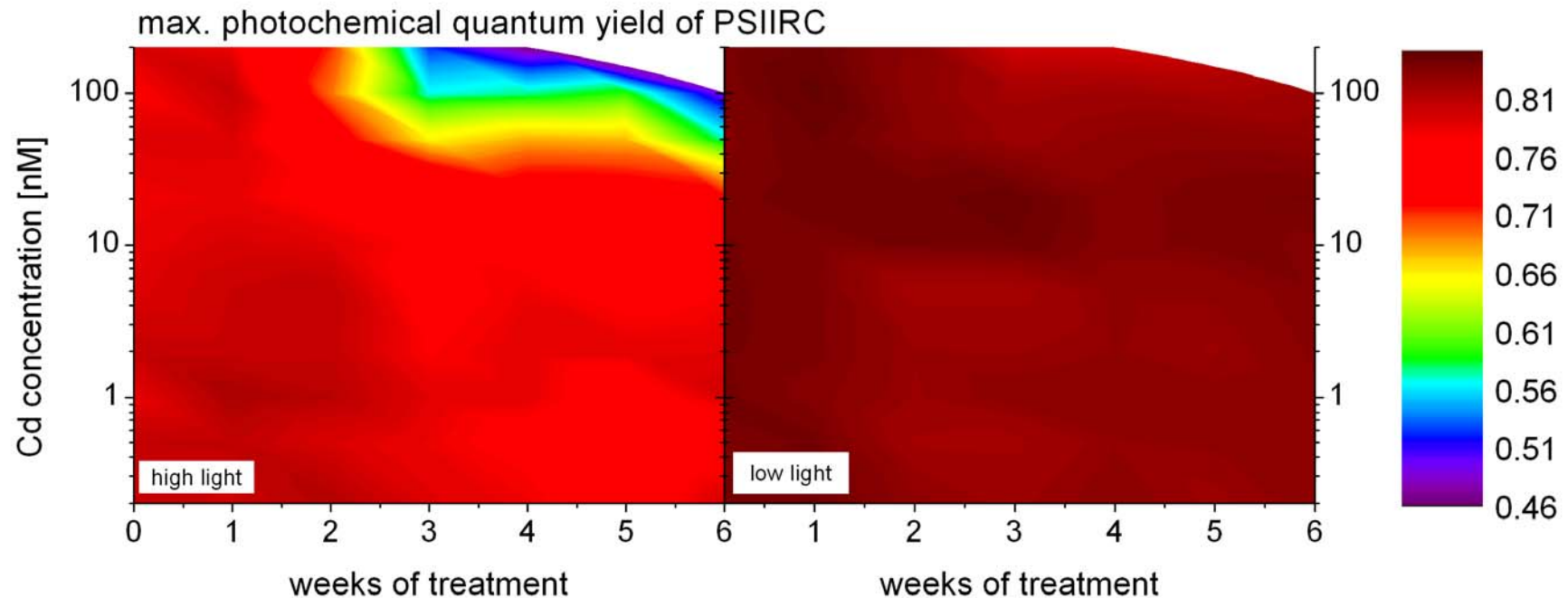
Cd affects Photosynthetic light reactions in *C. demersum*



- Max. fluorescent quantum yield of PSII → amount of fluorescent molecules → LL plants have bigger antenna systems
- Reduced F_m towards higher concentrations and longer treatment duration → decreased Chl content
- Reduction in week 1 due to acclimation to Cd + HL

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.

Cd affects Photosynthetic light reactions in *C. demersum*



Reduced variable fluorescence $F_v/F_m = (F_m - F_0) / F_m \rightarrow$ decreased activity of PS II towards higher concentrations and longer treatment duration

HL much more affected than LL

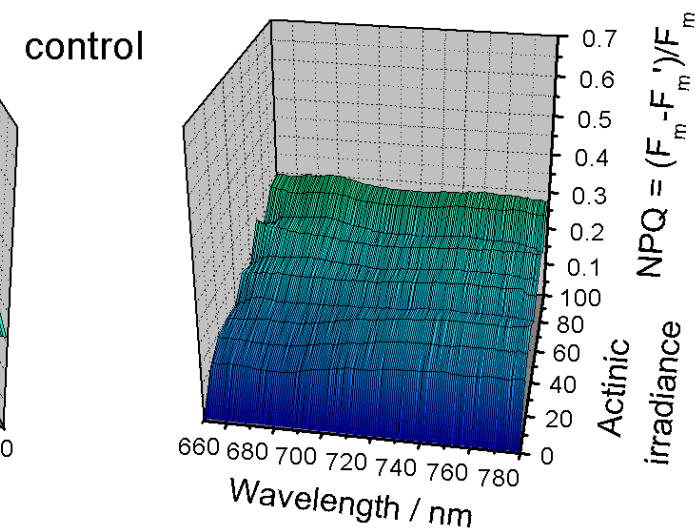
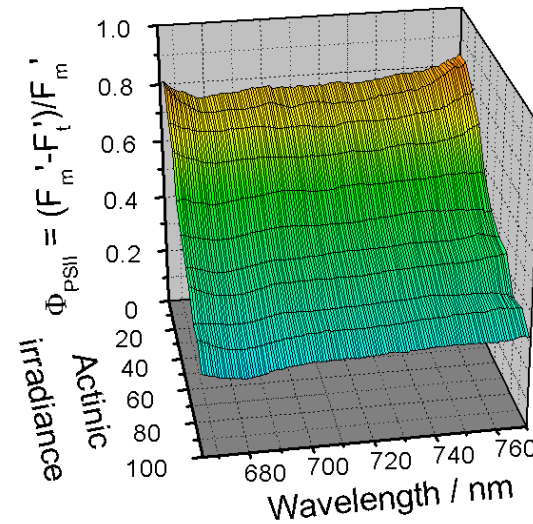
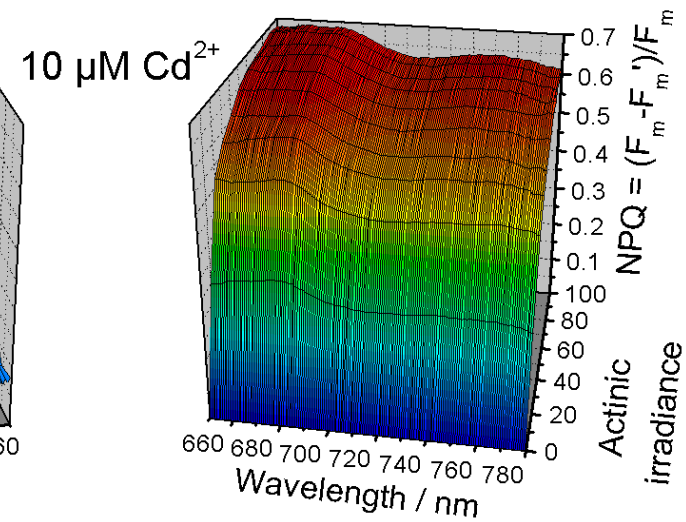
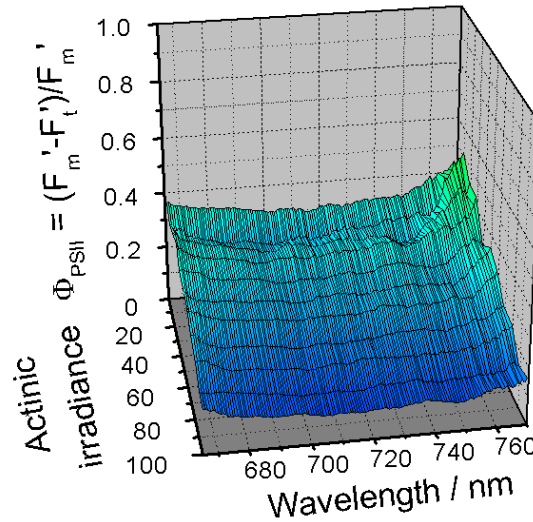
Andresen E, Kappel S, Stärk HJ, Riegger U, Borovec J, Mattusch J, Heinz A, Schmelzer CEH, Matoušková Š, Dickinson B, Küpper H (2016) New Phytologist 210, 1244-1258.

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

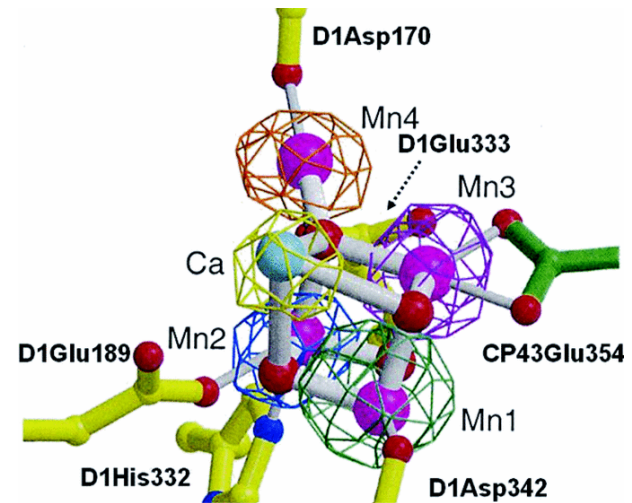
Spectral changes of PSII activity parameters:

- inhibition of electron flow visible over the whole excitation spectrum
→ RC-related

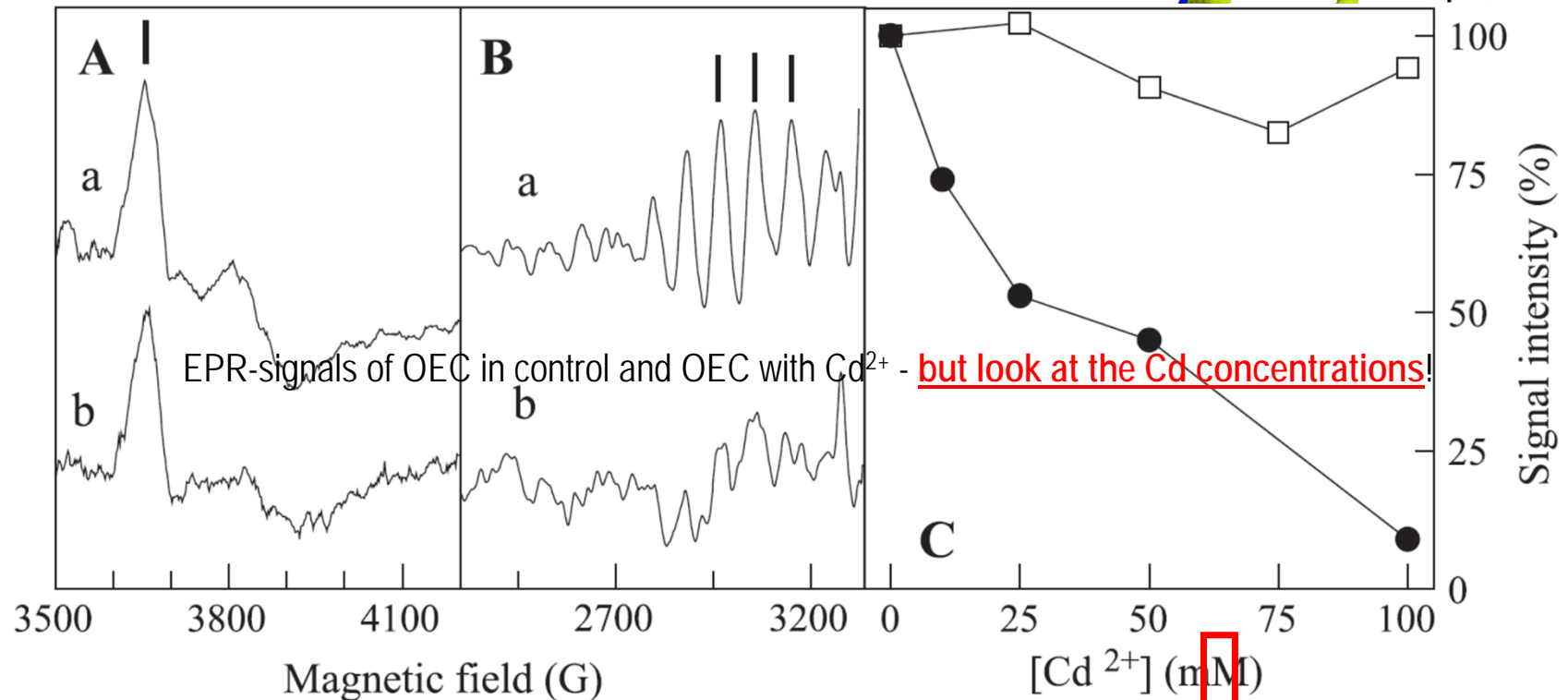
- increase of NPQ with peak at 680nm → Antenna-related



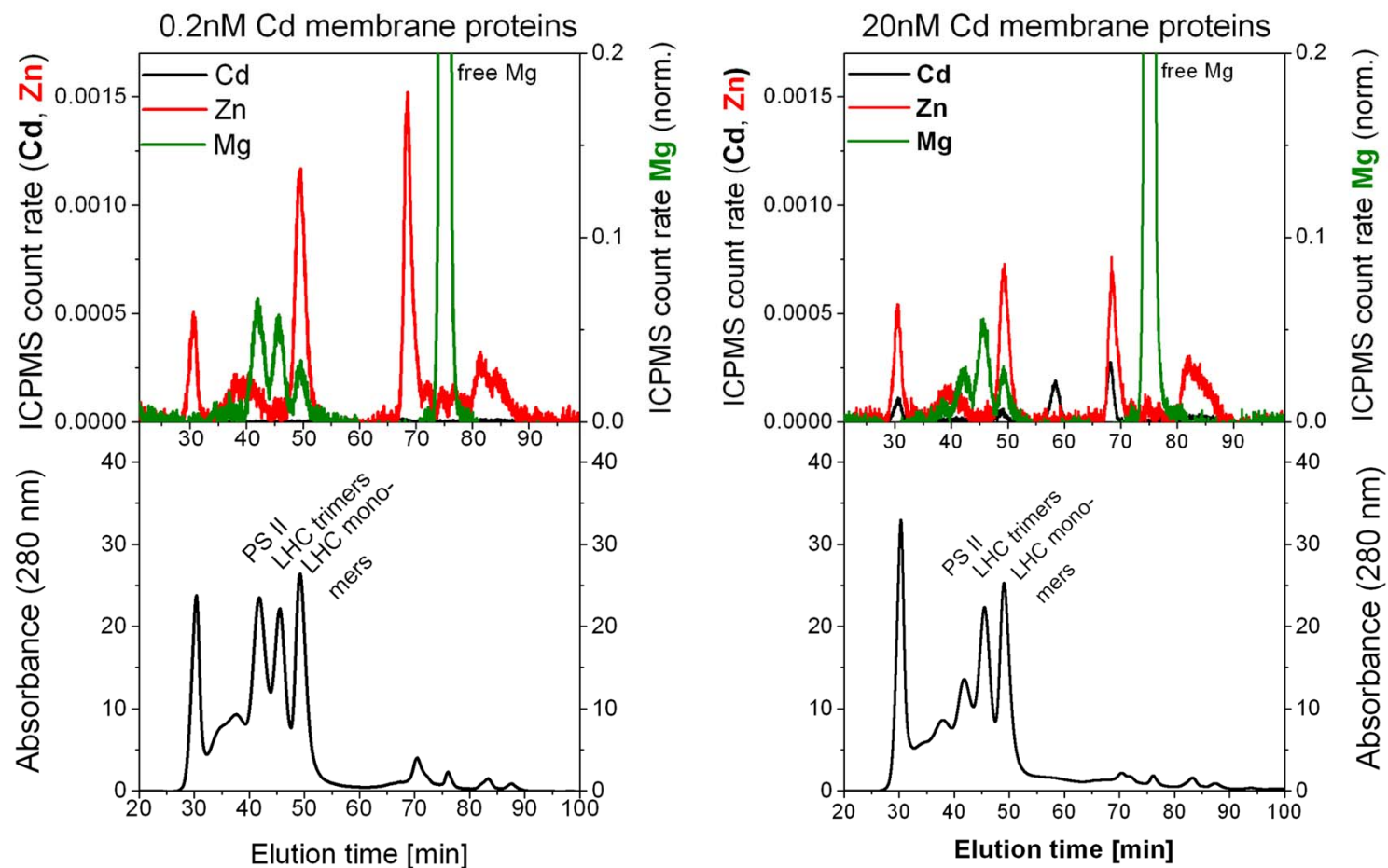
Substitution of Ca by Cd in water splitting complex of PSII would inhibit water splitting, if it would occur at relevant Cd concentrations



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



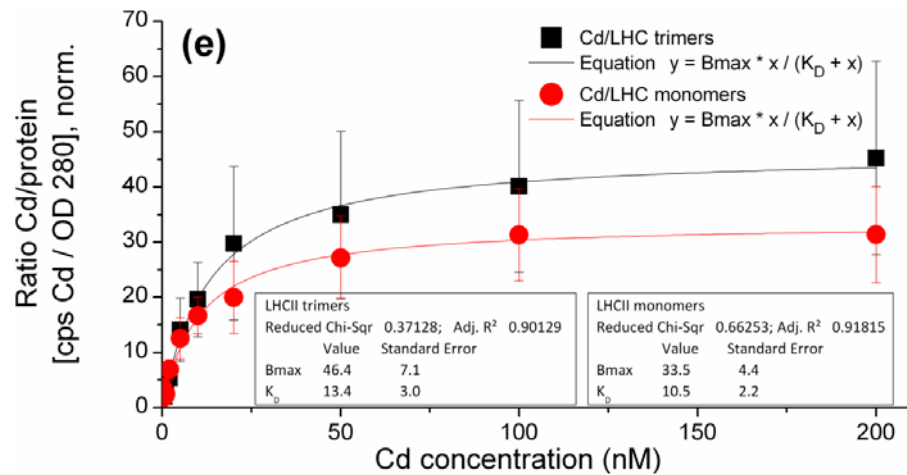
Cd stress in *C. demersum*: Incorporation of Cd into proteins in LL analysed by metalloproteomics via HPLC-ICP-MS



→ Cd binding to main light harvesting antenna LHCII in LL

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.

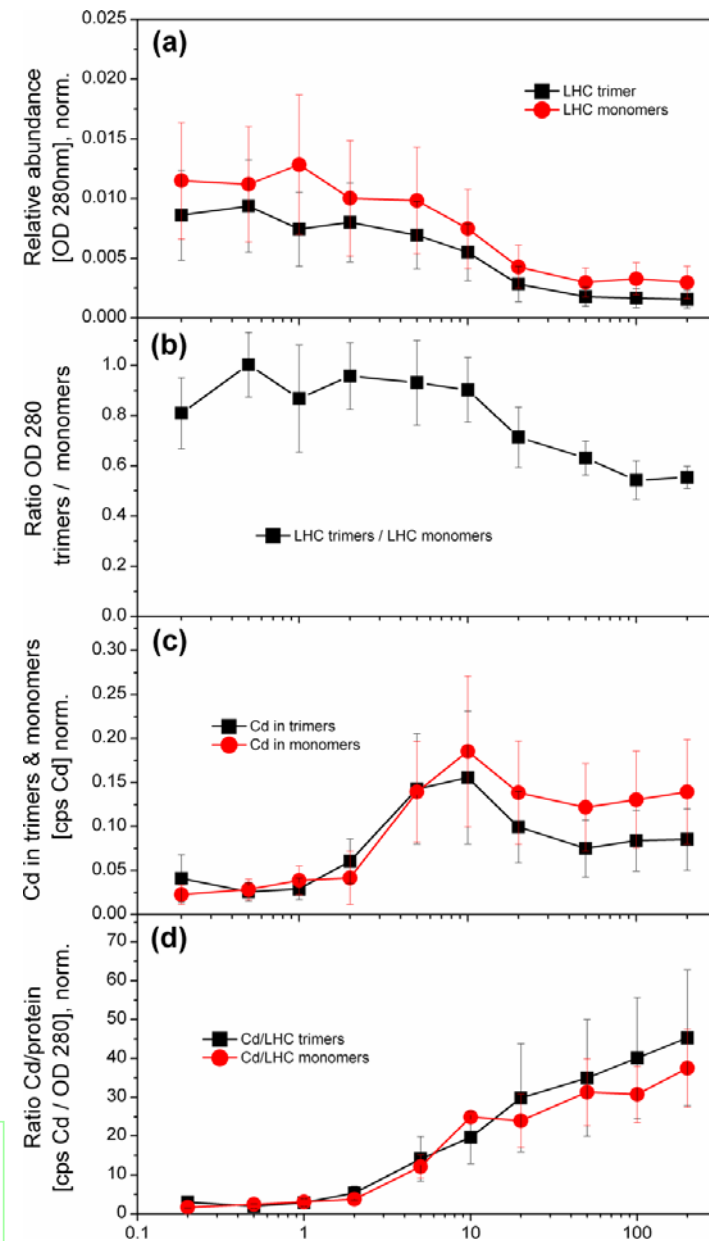
Example of metal toxicity in the nanomolar range in „normal“ plants: Incorporation of Cd into LHCII in LL



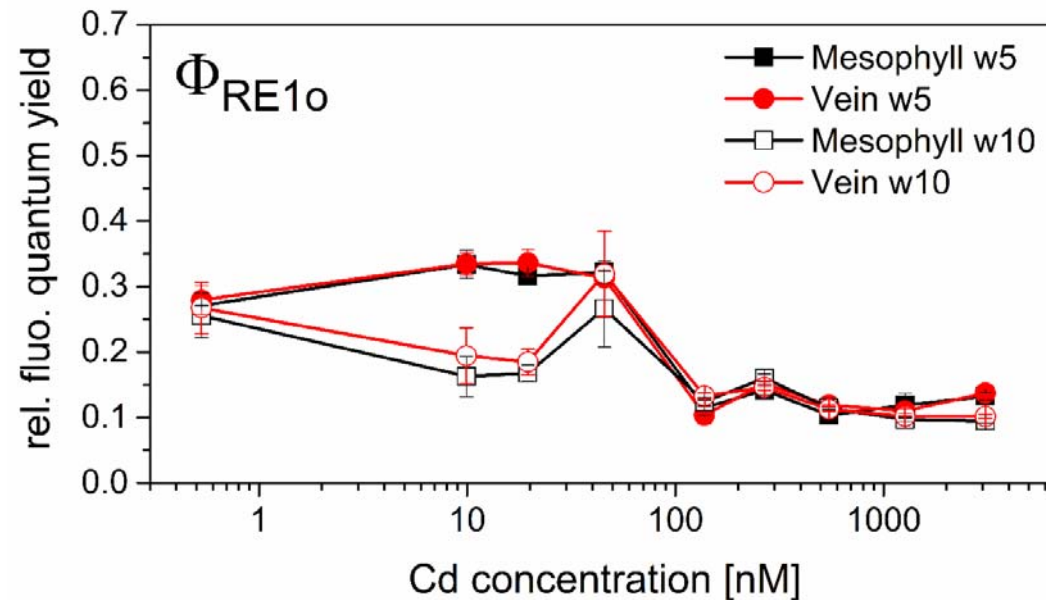
→ Cd binding to LHCII causes disintegration of trimers

→ Cd bind to LHCII with dissociation constants in the low nanomolar range
→ diminished photosynthesis despite functional 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.



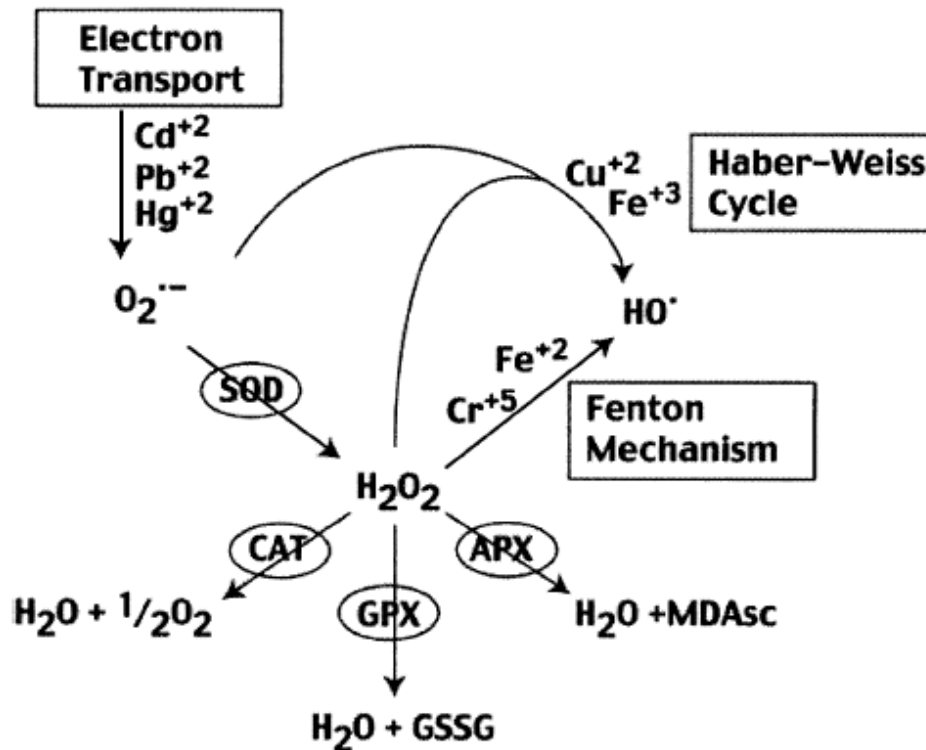
Chronic Cd toxicity in the nanomolar range in soybean plants: additional inhibition of electron transfer to PSI



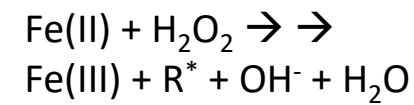
→ Cd inhibits electron flow to PSI already in the environmentally relevant nanomolar range

ROS and Cadmium

- Cadmium redox inert → No Fenton reaction!



Fenton:

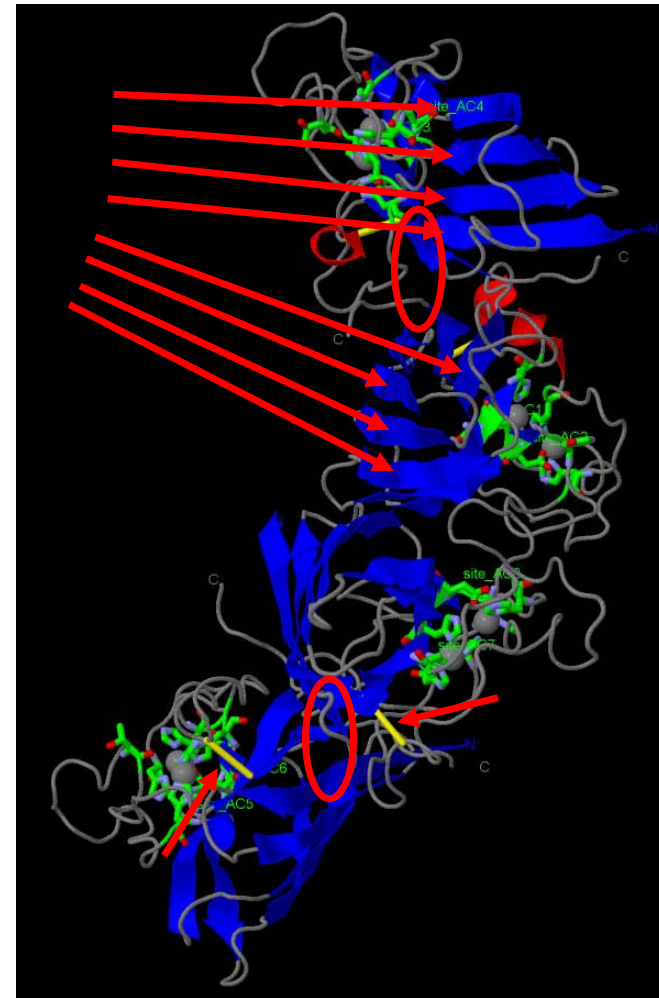
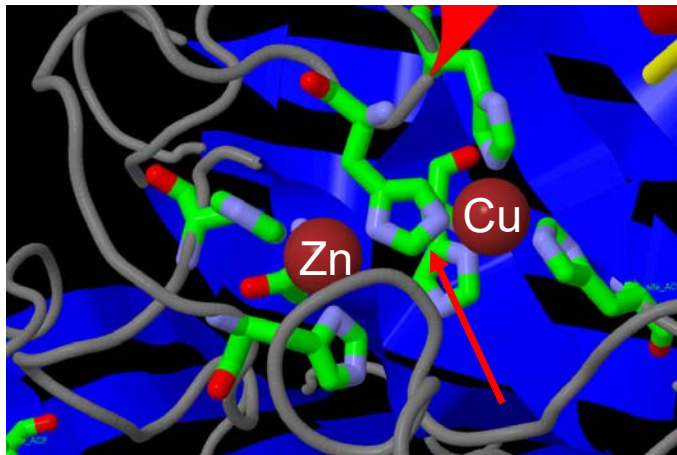


„Biggest source of ROS in animal cells“ ...

→ NEVER shown *in vivo*!

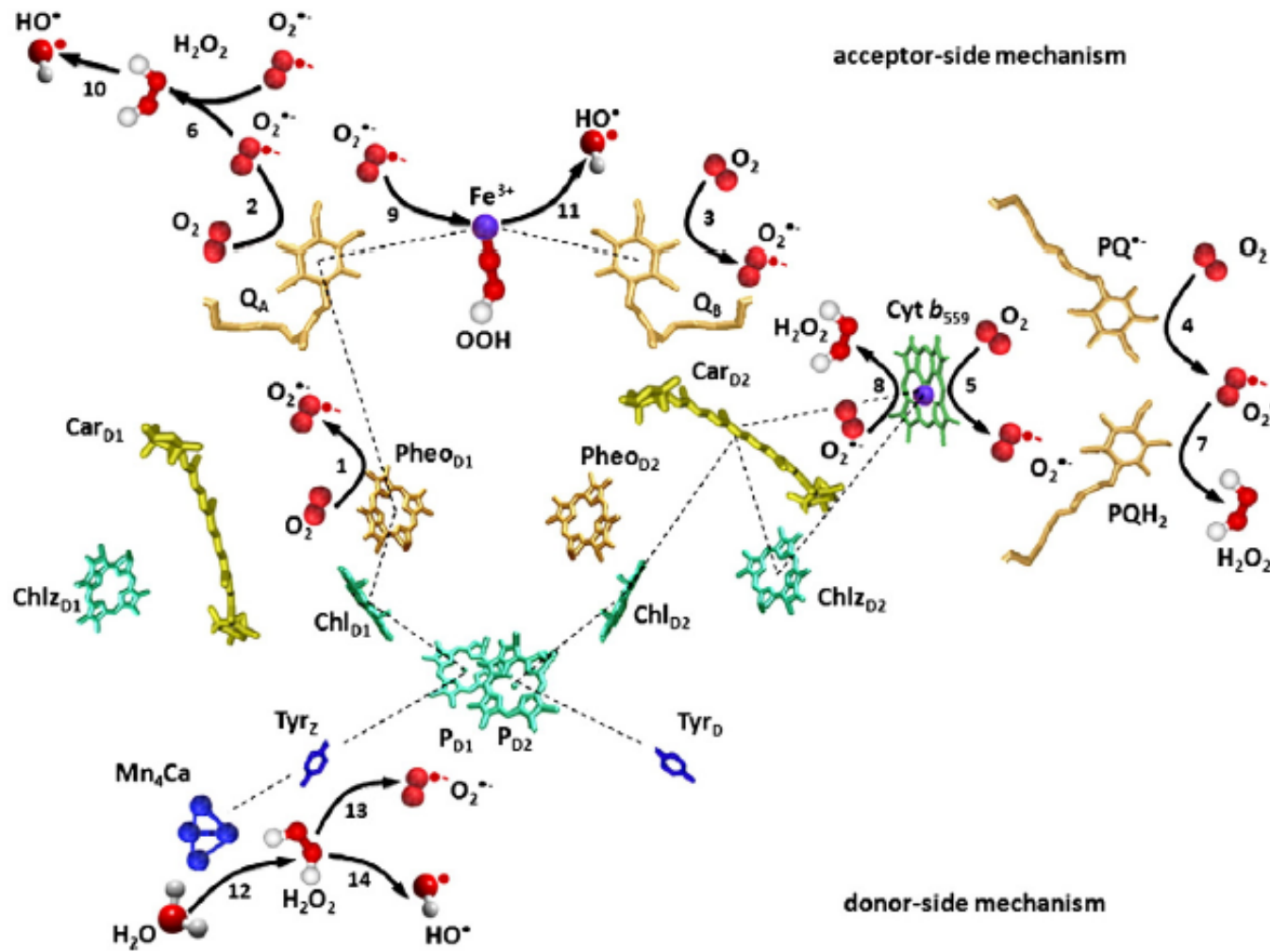
Possible target for Cd toxicity leading to ROS: Superoxide dismutase (SOD), in plants a Cu/Zn enzyme

Substitution of Zn by Cd in SOD may contribute to oxidative stress during Cd toxicity



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

Photosynthesis-related ROS



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

ROS and Cadmium

ROS production



Removal of ROS



- Cd interferes with photosynthesis / respiration → electrons transferred to O_2

- Cd replaces Zn in SOD (e.g.) → less functional SOD

- In response antioxidant enzymes

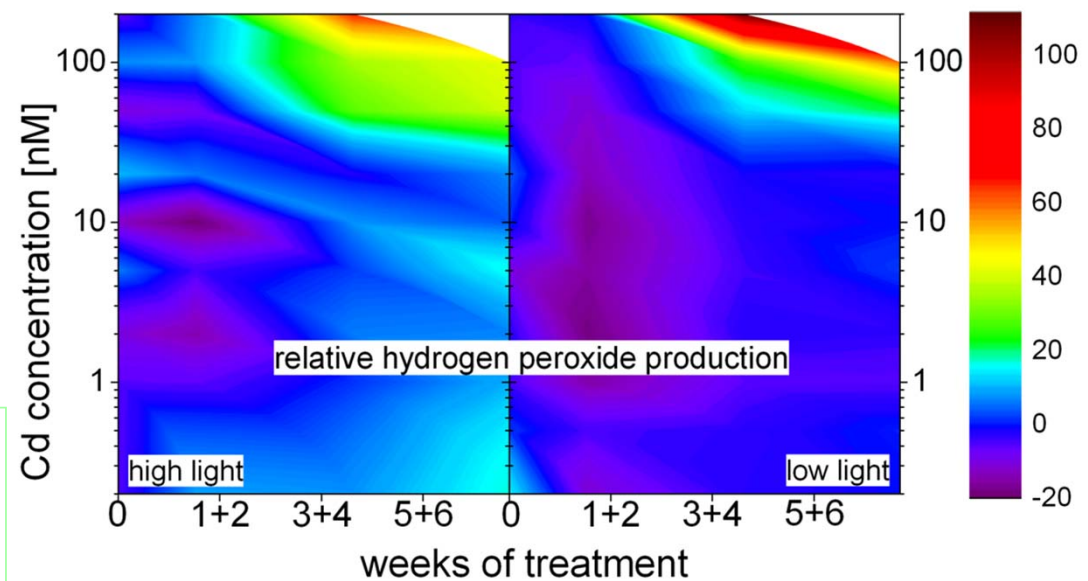
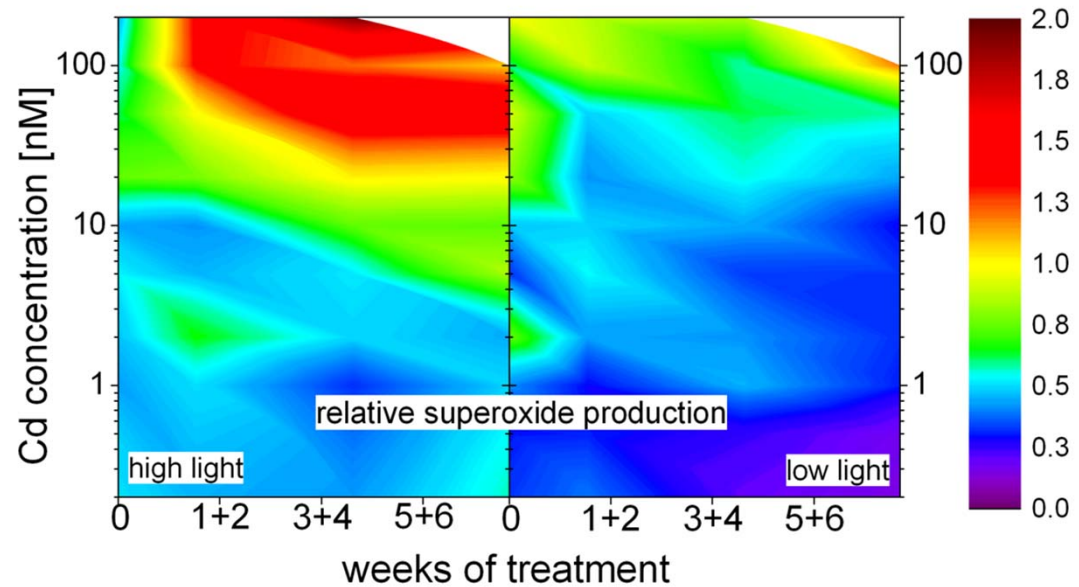


Production of reactive oxygen species (ROS) during Cd-stress in HL vs. LL

→ Cd-induced formation of ROS stronger in HL

→ superoxide formation starts at lower Cd concentrations than peroxide formation

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.



Influence of Cd on antioxidant enzymes

- **Lower** Cd concentrations and **shorter** treatment duration tend to increase the antioxidant system
- **Longer** exposure and **higher** Cd concentrations lead to decreased activity or content of the antioxidants

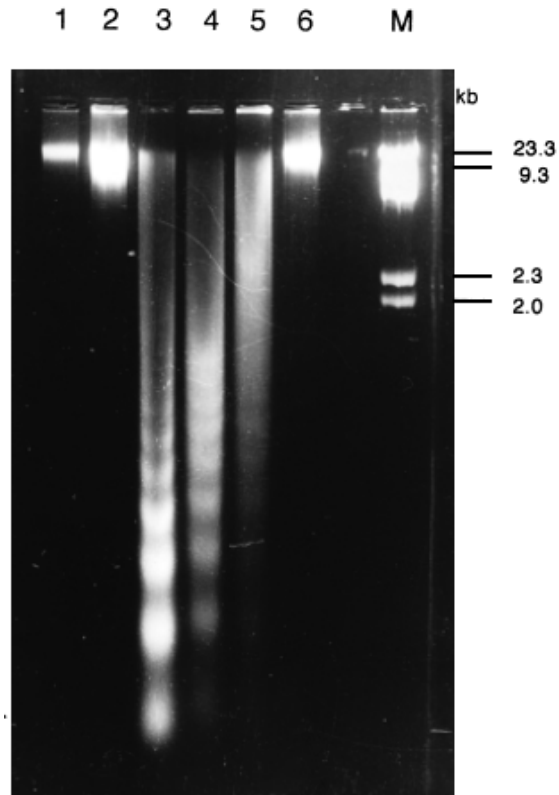
Cadmium toxicity in plants – 4. Genotoxicity

- Induction of DNA damage by
 - direct interaction with the nucleotides
 - modifications like base and sugar lesions, DNA strand breaks, destruction of DNA-protein crosslinks etc.
 - inhibiting DNA repairing enzymes
 - Induction of ROS, ROS lead to lipid peroxidation, which causes membrane damage and production of mutagenic aldehydes

Methods to detect Genotoxicity

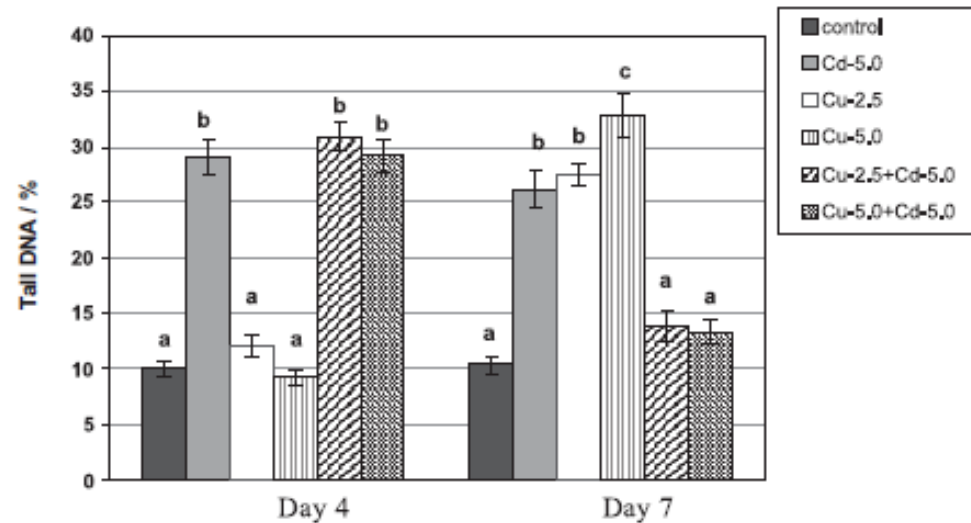
- DNA Analyses
 - Gelelectrophoresis and Comet Assay
 - Random amplification of polymorphism DNA (RAPD)
- DNA / Chromosome Analyses
 - Micronuclei formation
 - Sister chromatid exchange
 - Chromosomal aberrations
- Upregulation of DNA-related / repairing enzymes

DNA disruption – Gelelectrophoresis and Comet Assay



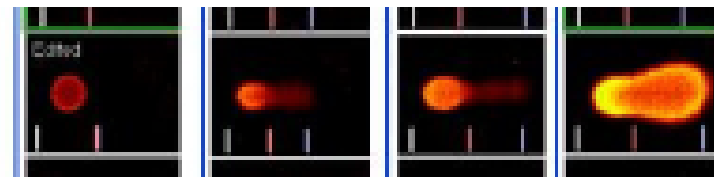
1: DNA from control plant, 2:-6: DNA from plants treated with Cd 10, 50, 75, 100, 1000 μM

Fojtova & Kovarik, Plant, Cell & Envir. 23:531-537, 2000



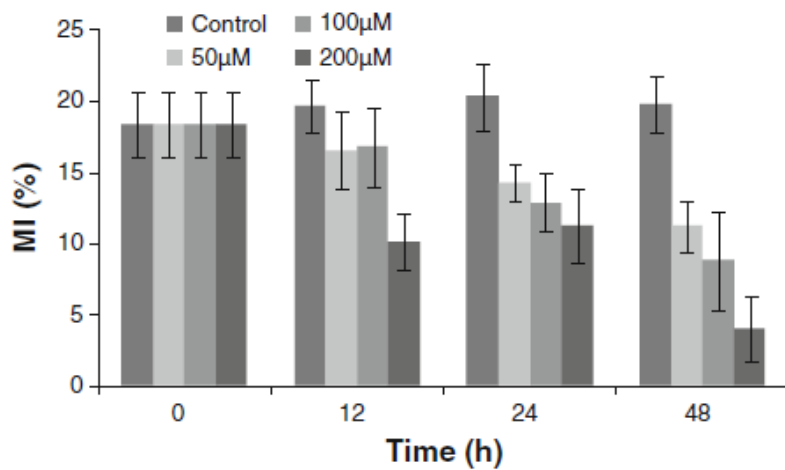
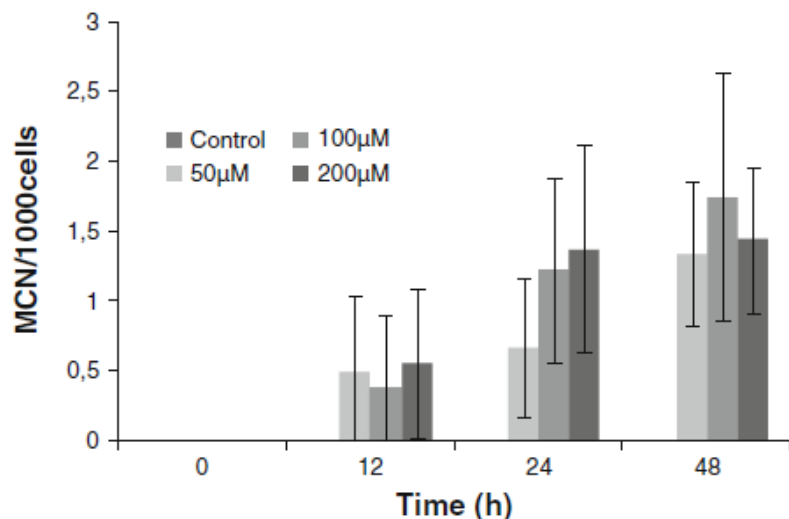
Lemna minor treated with μM concentrations of Cd and Cu. Tail DNA (Comet - length) increase due to metal treatment.

Cvjetko et al., Arh Hig Rada Toksikol 61:287-296, 2010



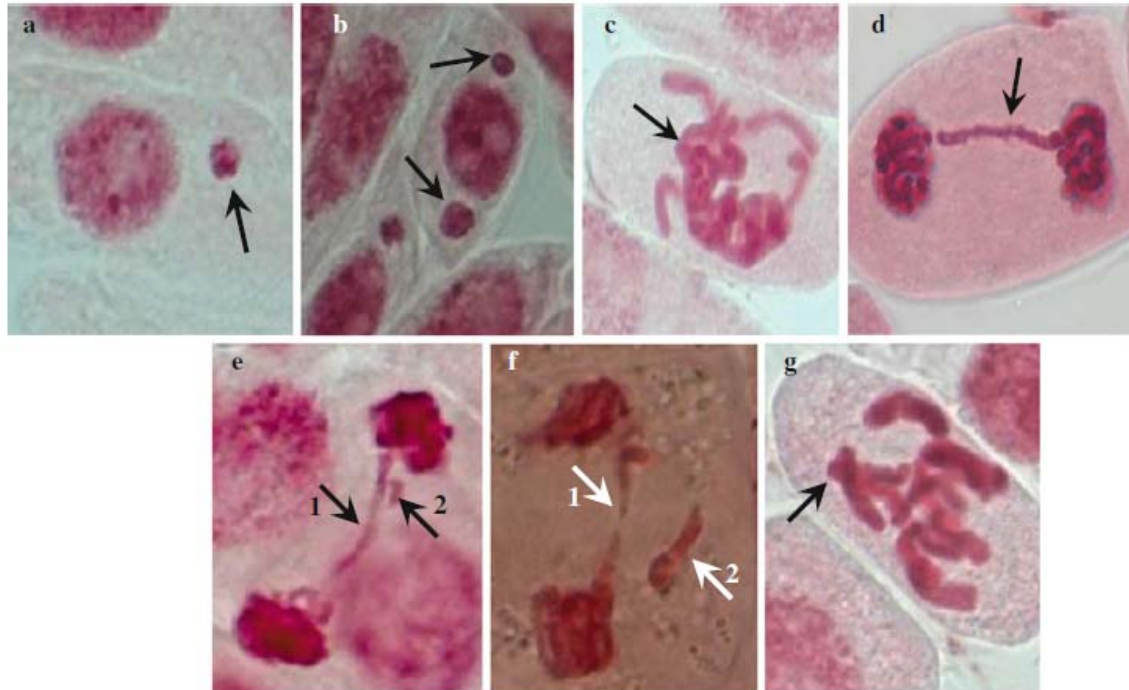
From the CometAssay Manual, Trevigen[®]

Micronuclei & Mitotic index



- *Vicia* root meristem cells; established assay
- Micronuclei formation due to malfunctioning cell division
- Dose and time dependent
 - Cd treatment increases MCN at high µM Cd
- Mitotic index: ratio of cells in metaphase stage to all cells
 - Cd treatment reduces MI at high µM Cd

Chromosomal aberrations



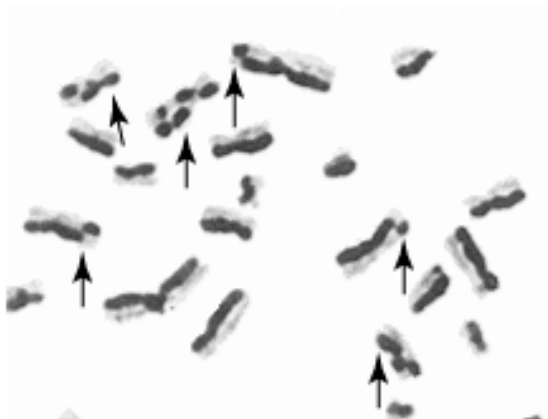
Vicia root meristem cells
a, b, e, f = 50 μ M Cd
c, d, g = 200 μ M Cd

a & b = micronuclei
c = sticky chromosome
d = chromosome bridge
e = " " + break
f = " " + isolated
chromosome
g = lagged chromosome
in metaphase

Sister chromatid exchange



- Exchange of identical parts of both sister chromatids in the same chromosome after / during DNA replication
- As DNA sequence identical, exchange does not lead to genetic information change (\neq crossing over)
- Happens in normal cells, but enhanced after treatment with toxic / radioactive substances



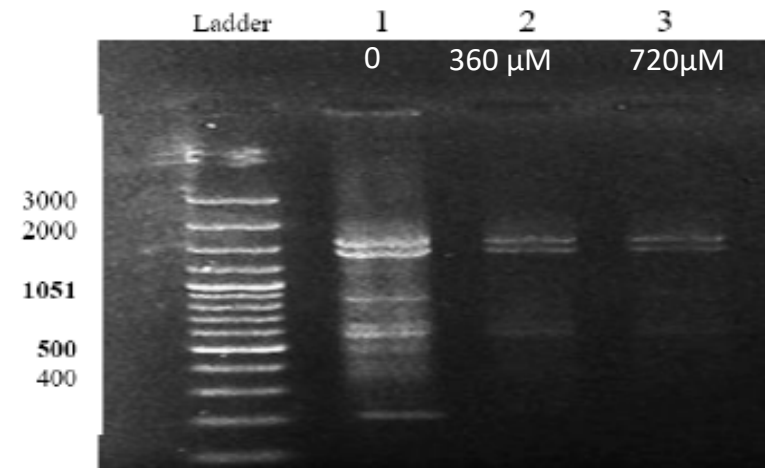
| Test substance | Concentration | SCEs/metaphase (mean \pm SE) | MI (mean \pm SE) |
|--|---------------|-----------------------------------|-----------------------|
| Negative Control (Hoagland's nutrient Solution) | 0 | 6.26 \pm 0.29 | 11.80 \pm 0.11 |
| Cadmium nitrate (μ M) | 50 | 6.63 \pm 0.3 | 9.70 \pm 0.26*** |
| | 100 | 7.43 \pm 0.04** | 7.16 \pm 0.32*** |
| | 200 | 7.90 \pm 0.07*** | 1.16 \pm 0.03*** |
| Positive control (Cyclophosphamide, μ g/mL) | 5 | 12.11 \pm 0.06*** | 2.36 \pm 0.31*** |

** (P < 0.01) and *** (P < 0.001) compared with negative control.

From: <http://www.siteklabs.com/GenTox/MammalianCellCytogenetics.html>

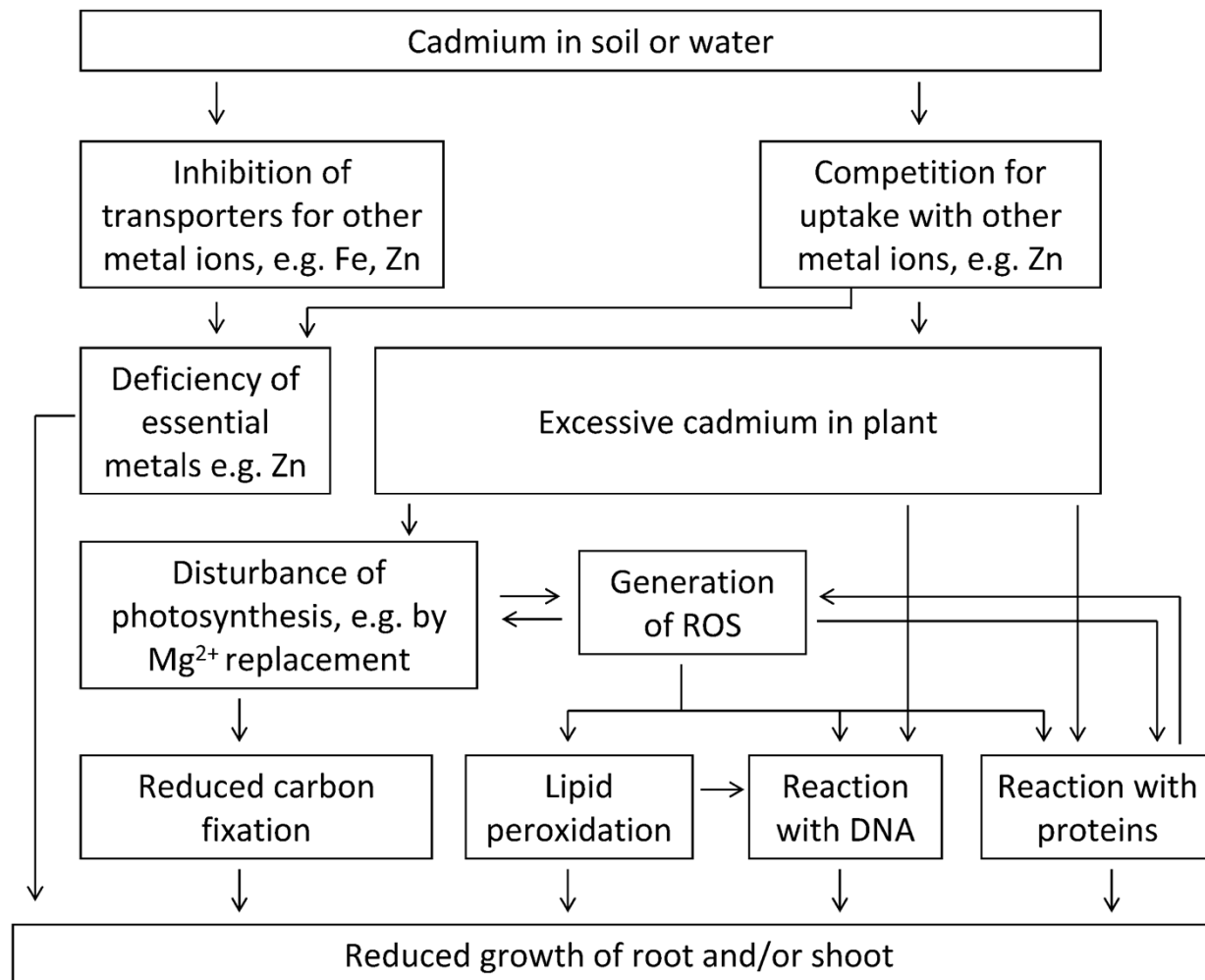
Random amplification of polymorphism DNA analyses (RAPD)

- Cd interacts with DNA / induces mutation
- New / disappearing primer binding sites
- → New / disappearing bands on gel
- But mostly seen at very high μM Cd!



| Primers names | Treatments | | | | |
|---------------|---|---|--------------------------------|---|--------------------------------|
| | Total bands in control | 40 (mgL^{-1}) Cd concentration | | 80 (mgL^{-1}) Cd concentration | |
| | | Appearance of new bands | Disappearance of control bands | Appearance of new bands | Disappearance of control bands |
| OPA-2 | 1679, 1500, 1205, 874, 657, 603, 513, 221 | | 1205, 874, 513, 221 | 900 | 1205, 874, 513, 221 |

Shahrtash et al., J of Cell & Molecular Research 2(1):42-48, 2010



Andresen E, Küpper H (2013) Cadmium Toxicity in Plants. In: Cadmium: From Toxicity to Essentiality, "Metal Ions in Life Sciences Vol. 11;

Summary Cd toxicity

- Threshold concentration for first toxic effects (*Ceratophyllum*, soybean): 20nM
 - Most fluorescence parameters, growth, pigments,
- First site of inhibition: photosynthetic apparatus
 - ROS generation due to photosynthesis malfunction
- Direct inhibition of PSIIIRC only pronounced in HL
- In LL binding of Cd to LHCII already below 20nM
- Toxicity more pronounced under HL conditions
 - Chl in LHCII acts as buffer under LL

- Slightly higher but still nanomolar concentrations:
 - selective Cd binding to few enzymes like allantoinase also in roots → disturbance of root metabolism

- High micromolar concentrations
 - many unselective effects, also genotoxicity

**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