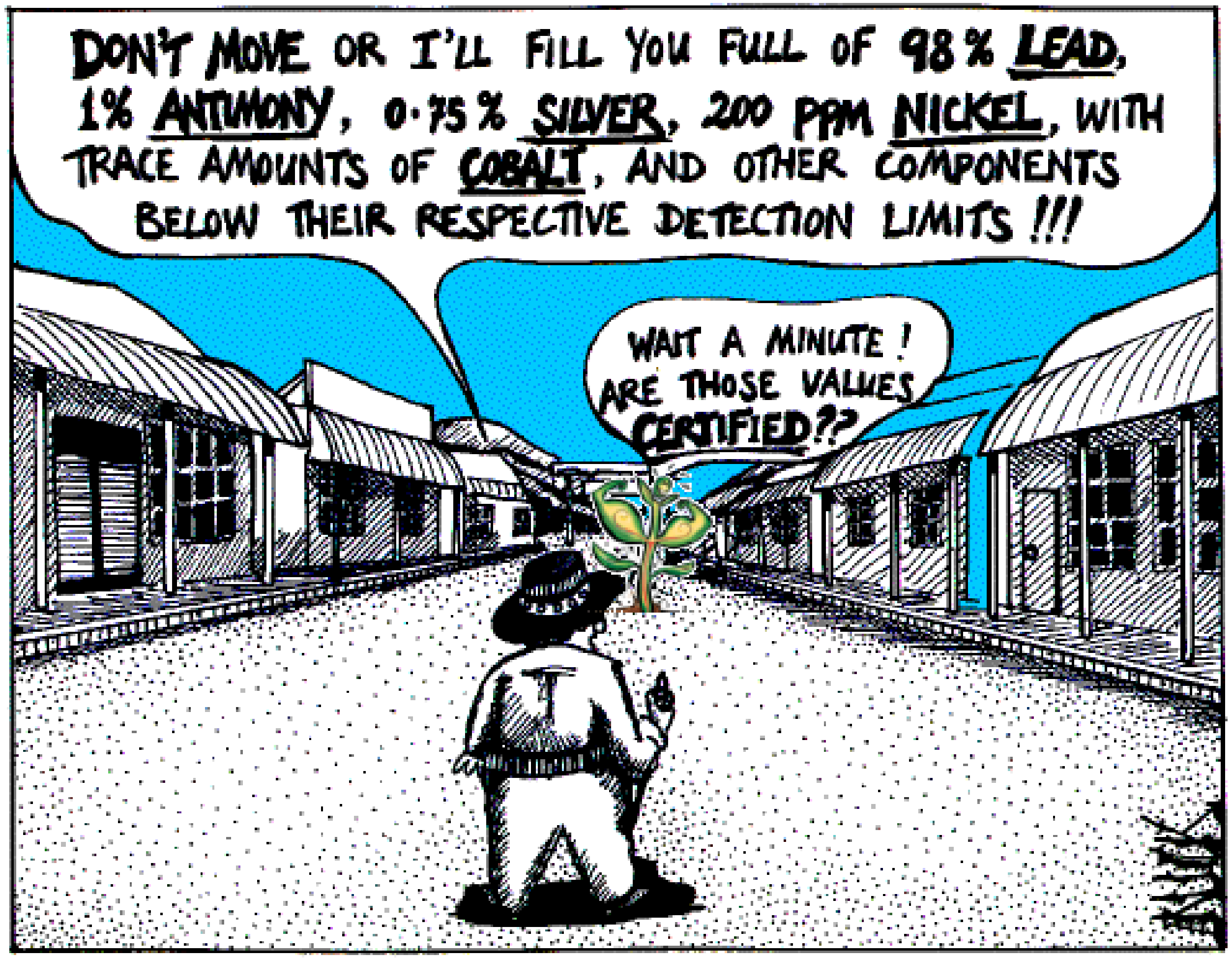
Heavy Metals and Plants - a complicated relationship

→ Cadmium toxicity



Heavy metal-hyperaccumulation in the Wild West

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

Cadmium

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 Fl [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]		

www.webelements.com

Cadmium in the environment

- Rather rare element in Earth‘s crust (0.1 - 0.5 ppm)
- Some natural sites, associated with ZnS
- Anthropogenic contamination, e.g. ore mining, some fertilizers, car traffic, cigarette smoke, industrial waste, NiCd-batteries

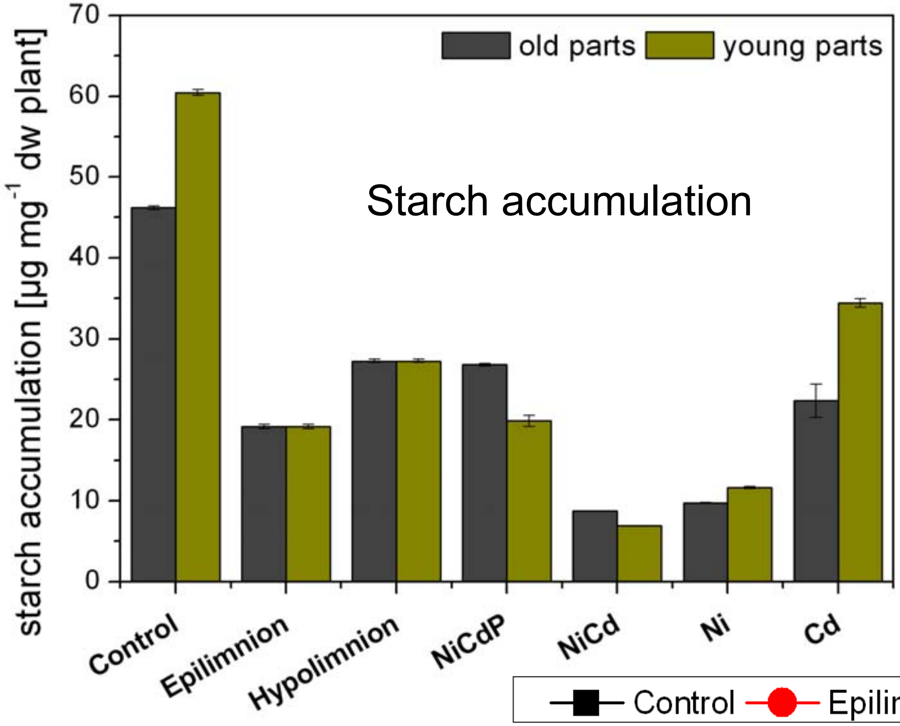
www.wikipedia.com

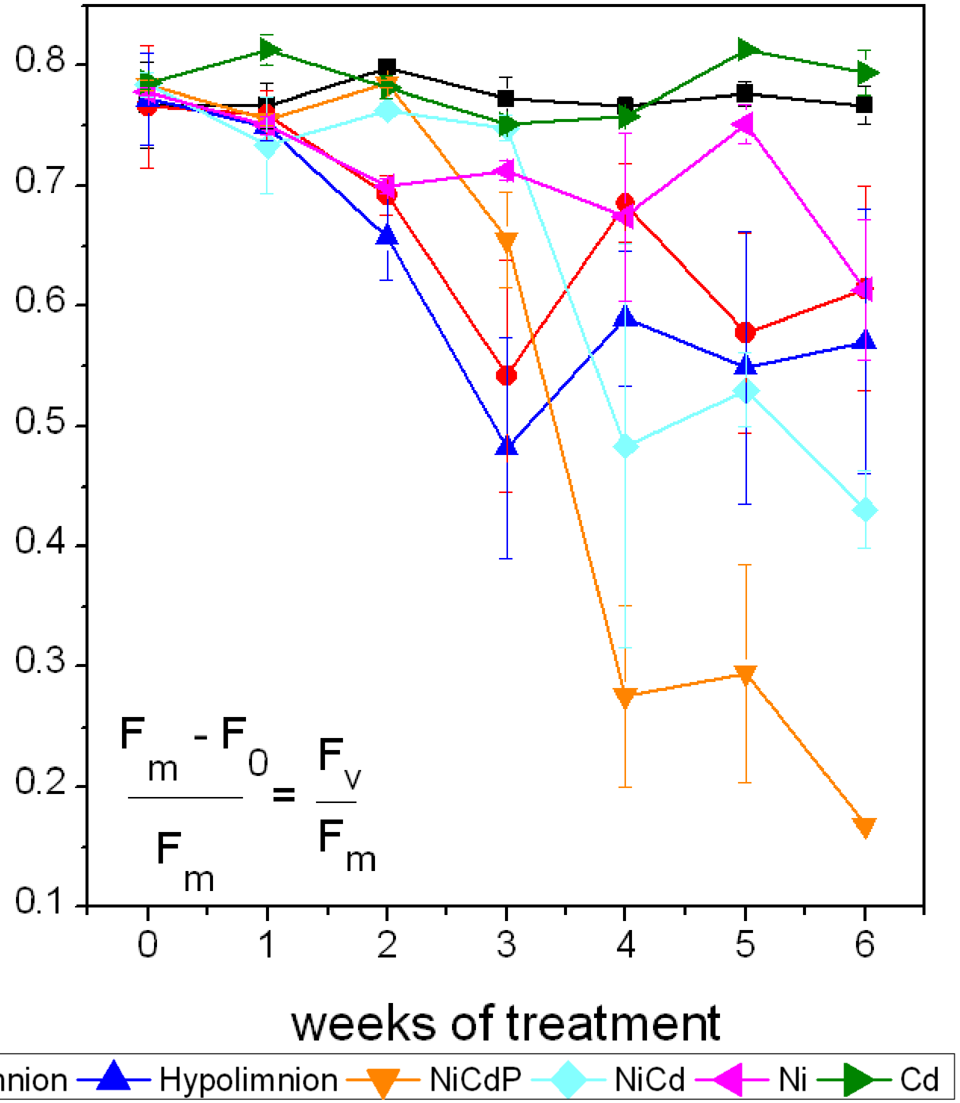
Cd toxicity – prominent diseases

- **Itai-itai disease** (japanese ouch-ouch sickness)
- 1 of the 4 big pollution diseases in Japan
- Mass cadmium poisoning in Japan, cadmium release into rivers by mining
- Severe pains in joints and spine, softening of the bones, kidney failure
- The mining companies were successfully sued for the damage

Heavy metal toxicity induced inhibition of photosynthesis at nanomolar concentrations

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

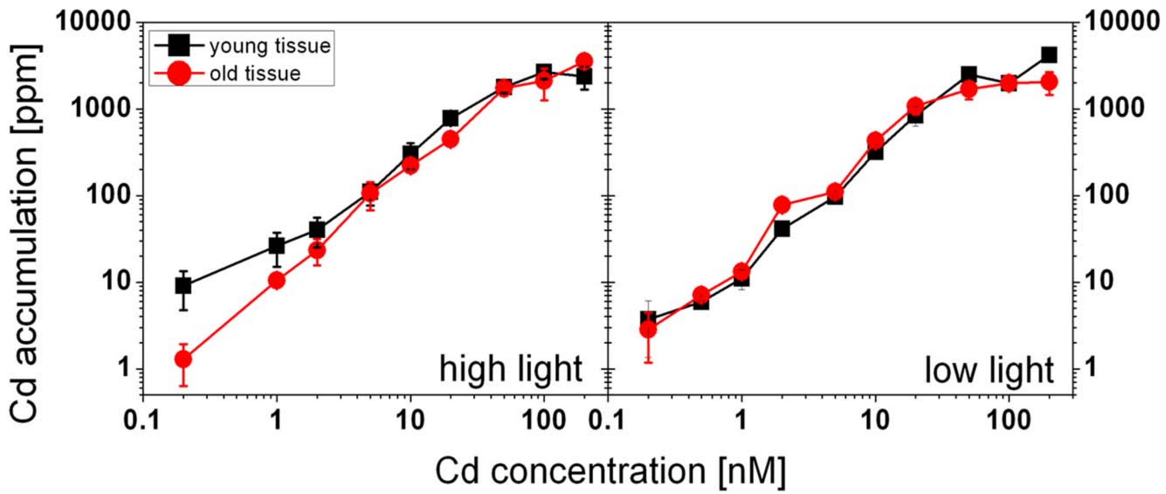
After 3 weeks

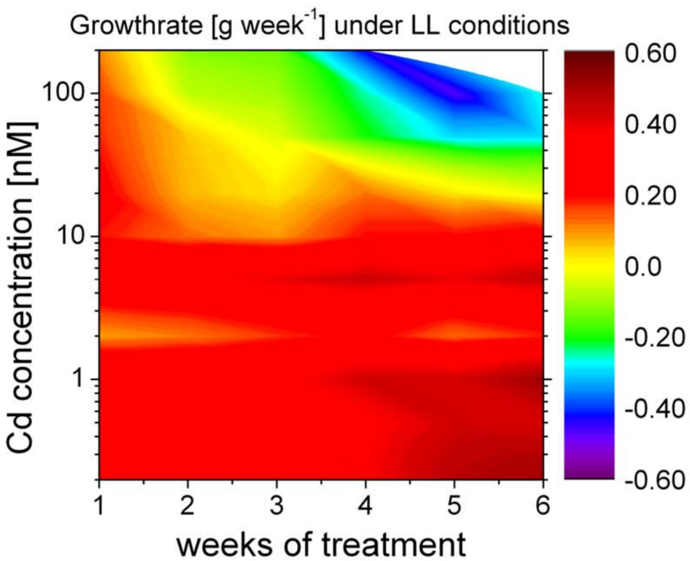
After 4 weeks

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 in plants

Toxicity

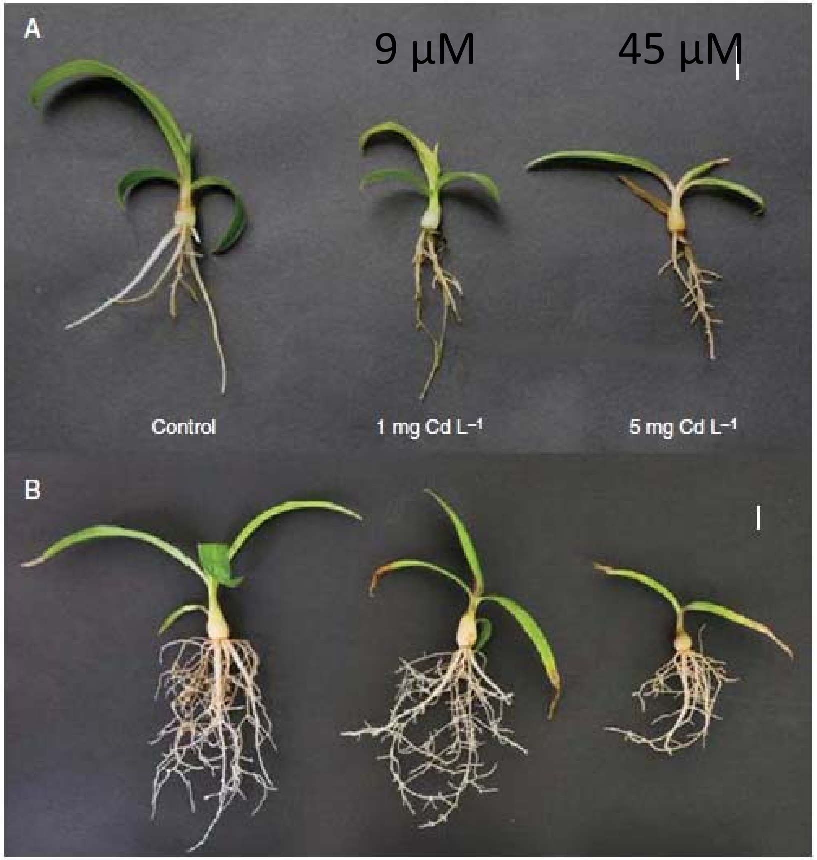
- 1) Roots
- 2) Photosynthesis
- 3) Reactive oxygen species
- 4) Genotoxicity
- 5) Stress prevention

Beneficial effects ?!

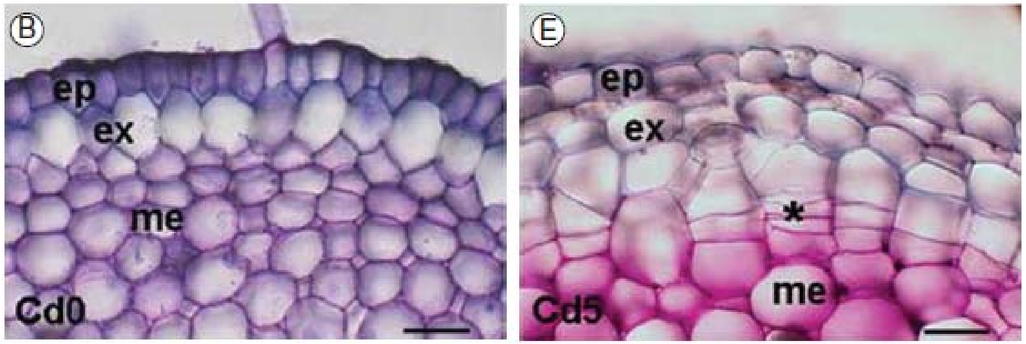
Cadmium toxicity in plants – 1: Roots

First organ which gets affected

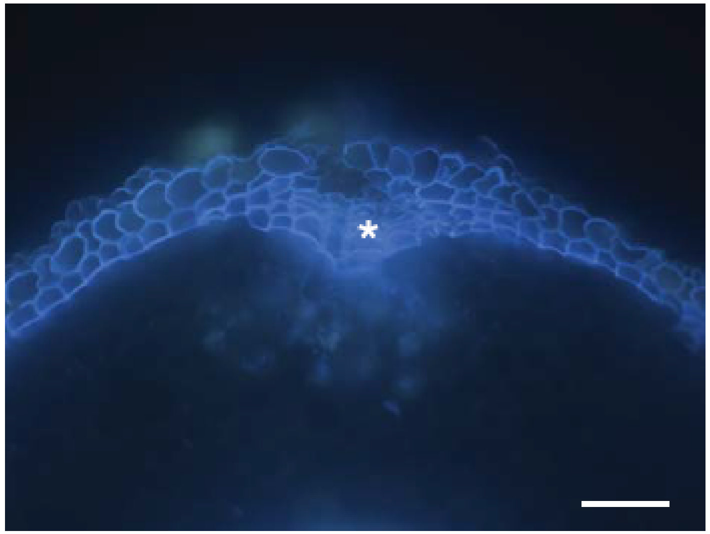
Reduced growth after Cd treatment



More layers of hypodermal periderm



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

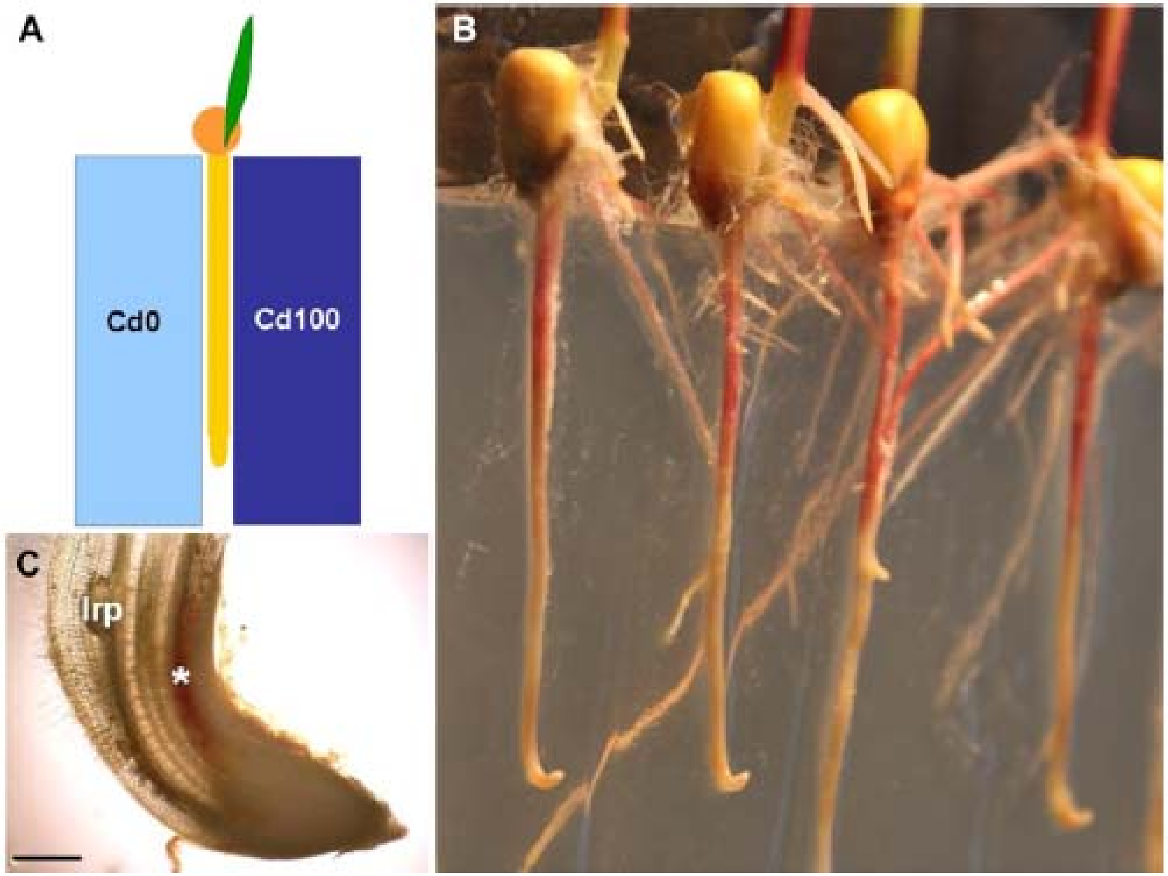


Lux et al., Annals of Botany 107:285-292, 2011

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

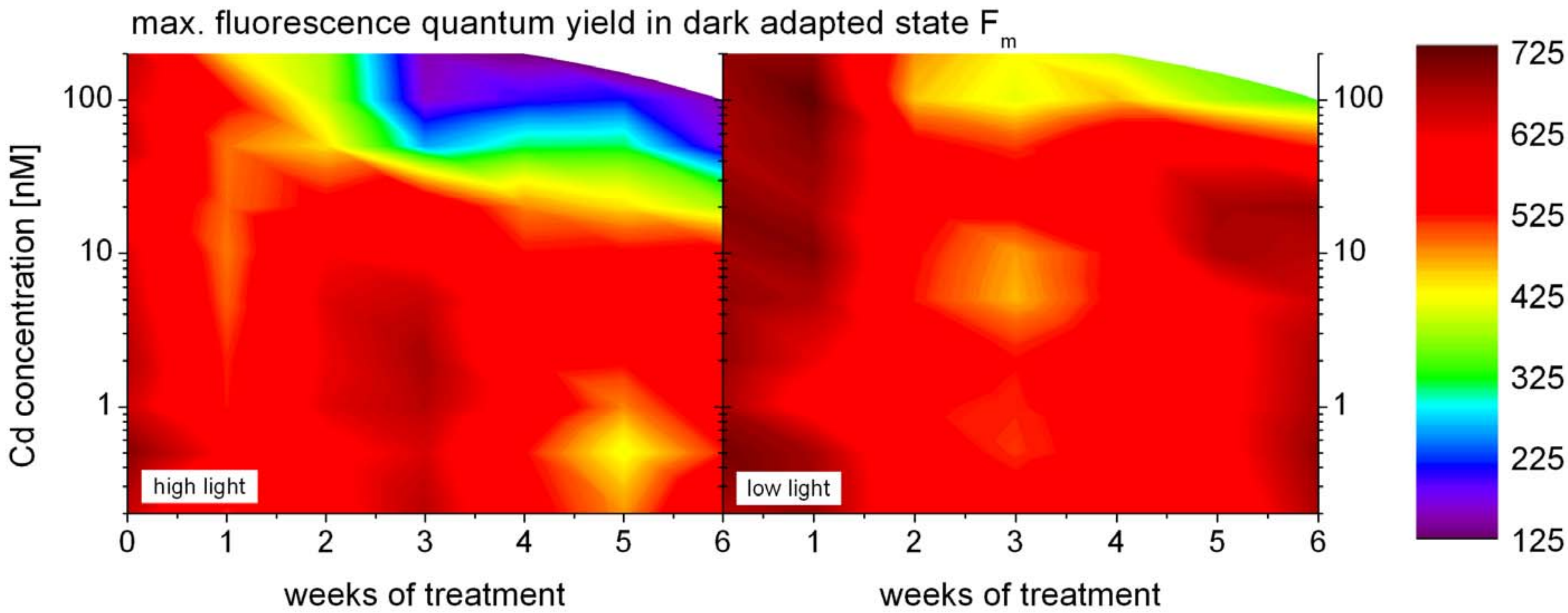


Lux et al., Journal of Experimental Botany 62(1): 21-37, 2011

Cadmium toxicity in plants – 2: Photosynthesis

- Indirect measurement: Growth, O_2 production / CO_2 consumption
  - Diminishing the Chl/pigment/protein content
- Direct: Photosynthetic paramters via Chlorophyll fluorescence measurement

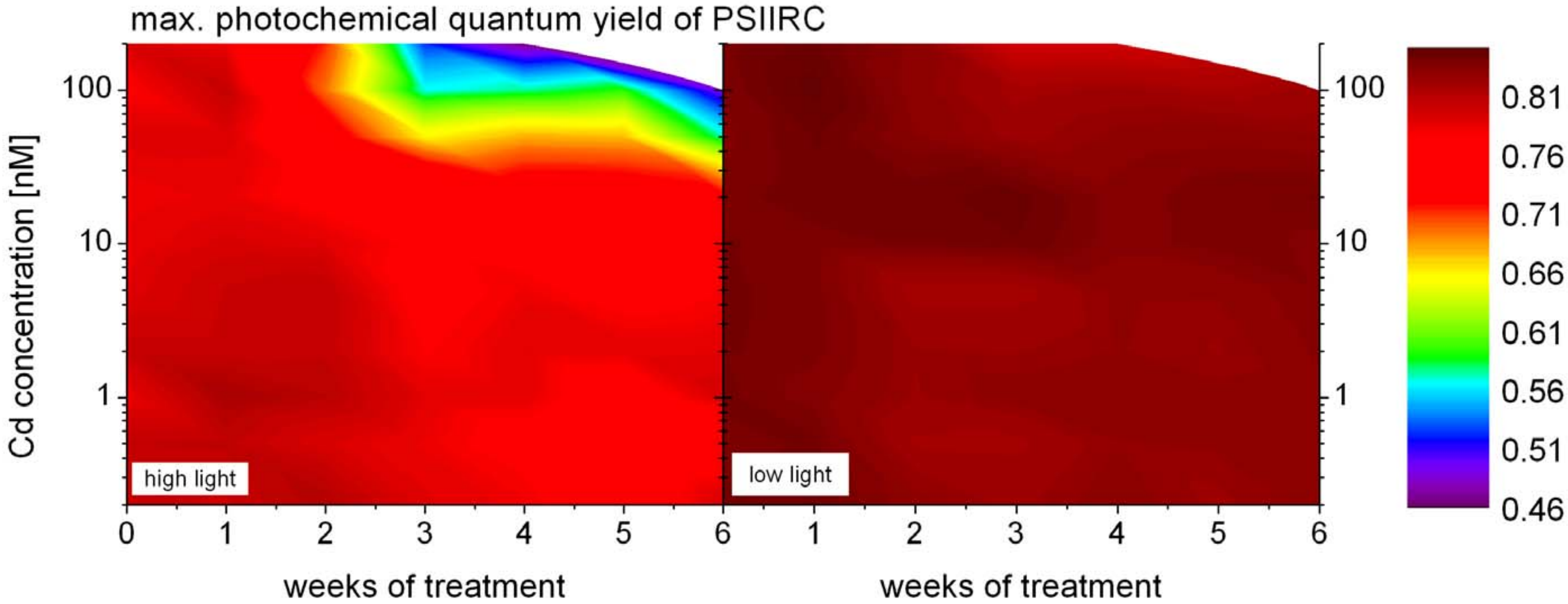
Cd affects Photosynthesis in *C. demersum*



- 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 Photosynthesis in *C. demersum*



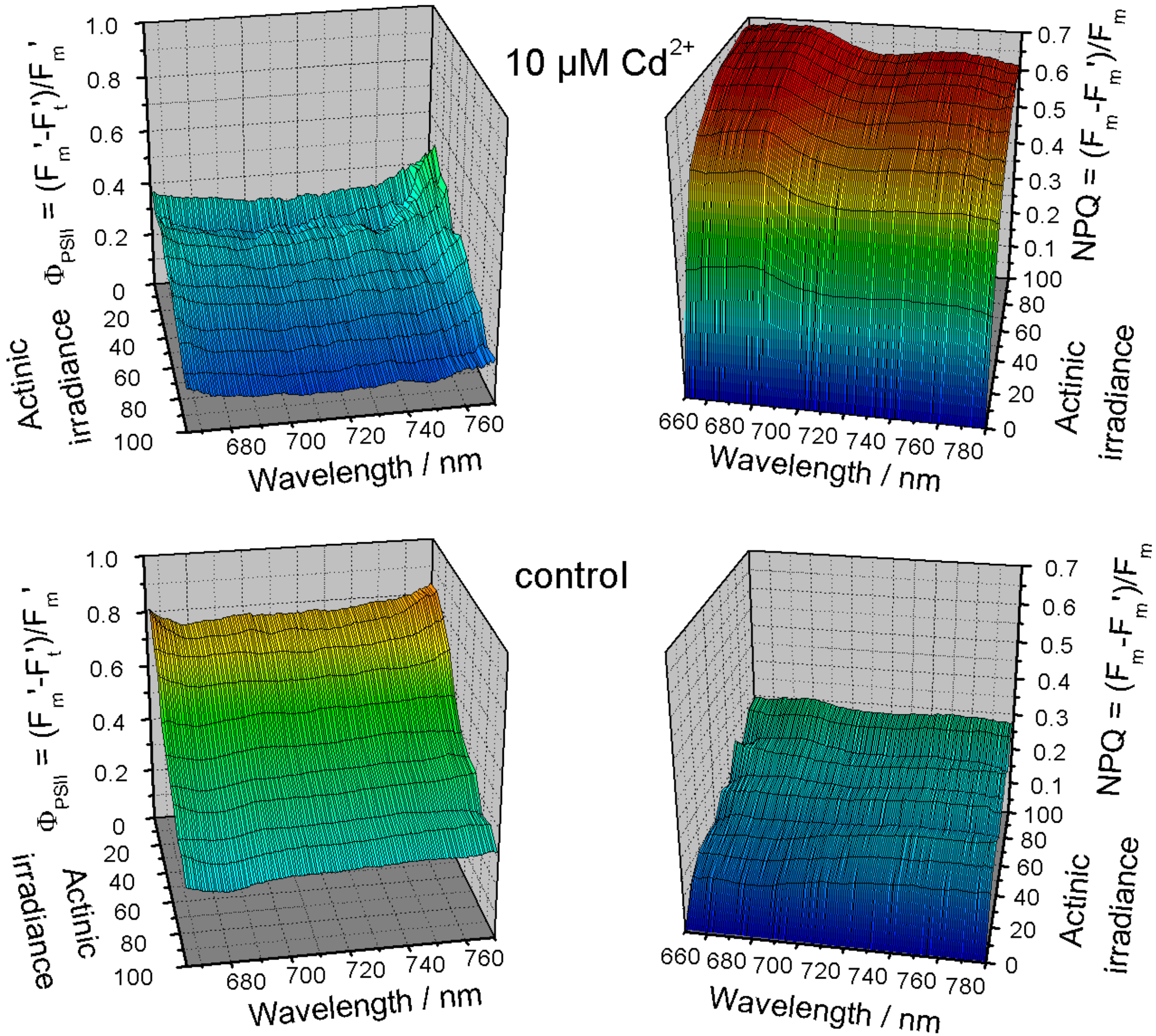
Reduced variable fluorescence $F_v/F_m = (F_m-F_0) / F_m$ → decreased activity of PS II towards higher concentrations and longer treatment duration

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.

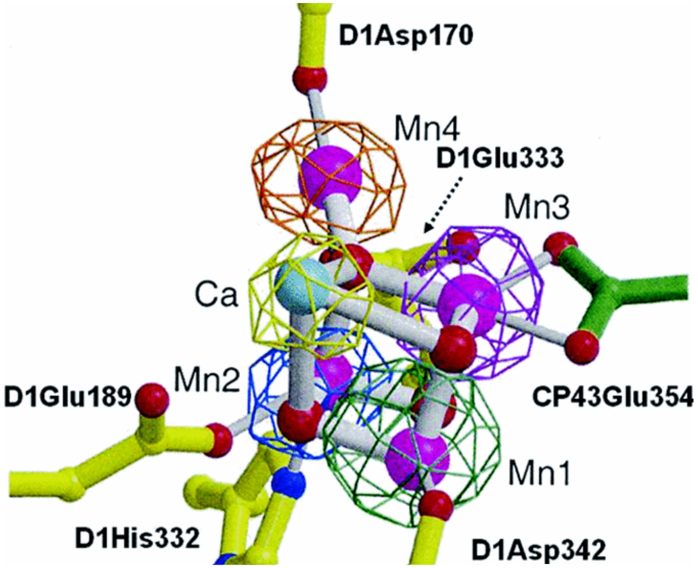
Cd-stress in the Zn-/Cd-hyperaccumulator *T. caerulescens:*

Spectral changes of PSII activity parameters

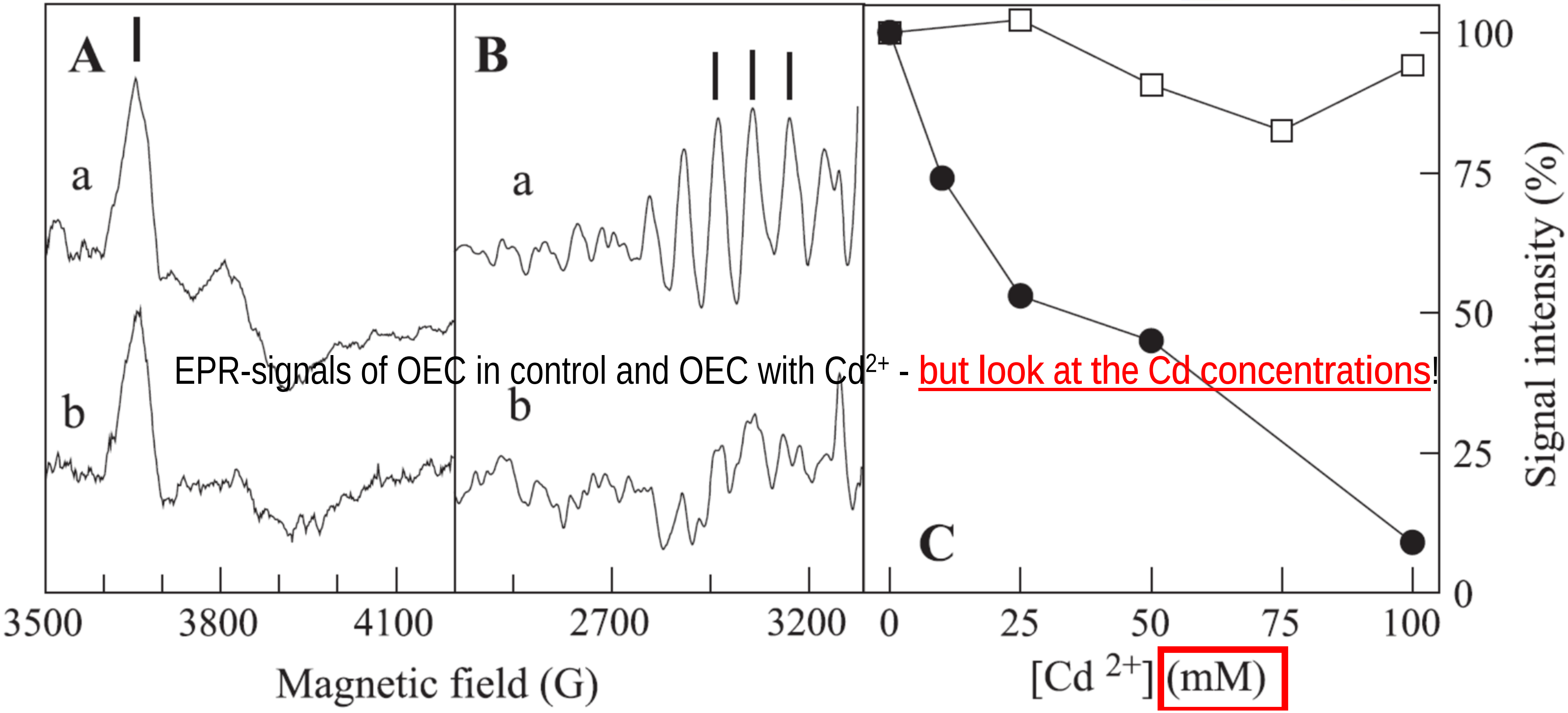


Küpper H, Aravind P, Leitenmaier B, Trtilek M, Šetlík I (2007) New Phytol175, 655-74

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

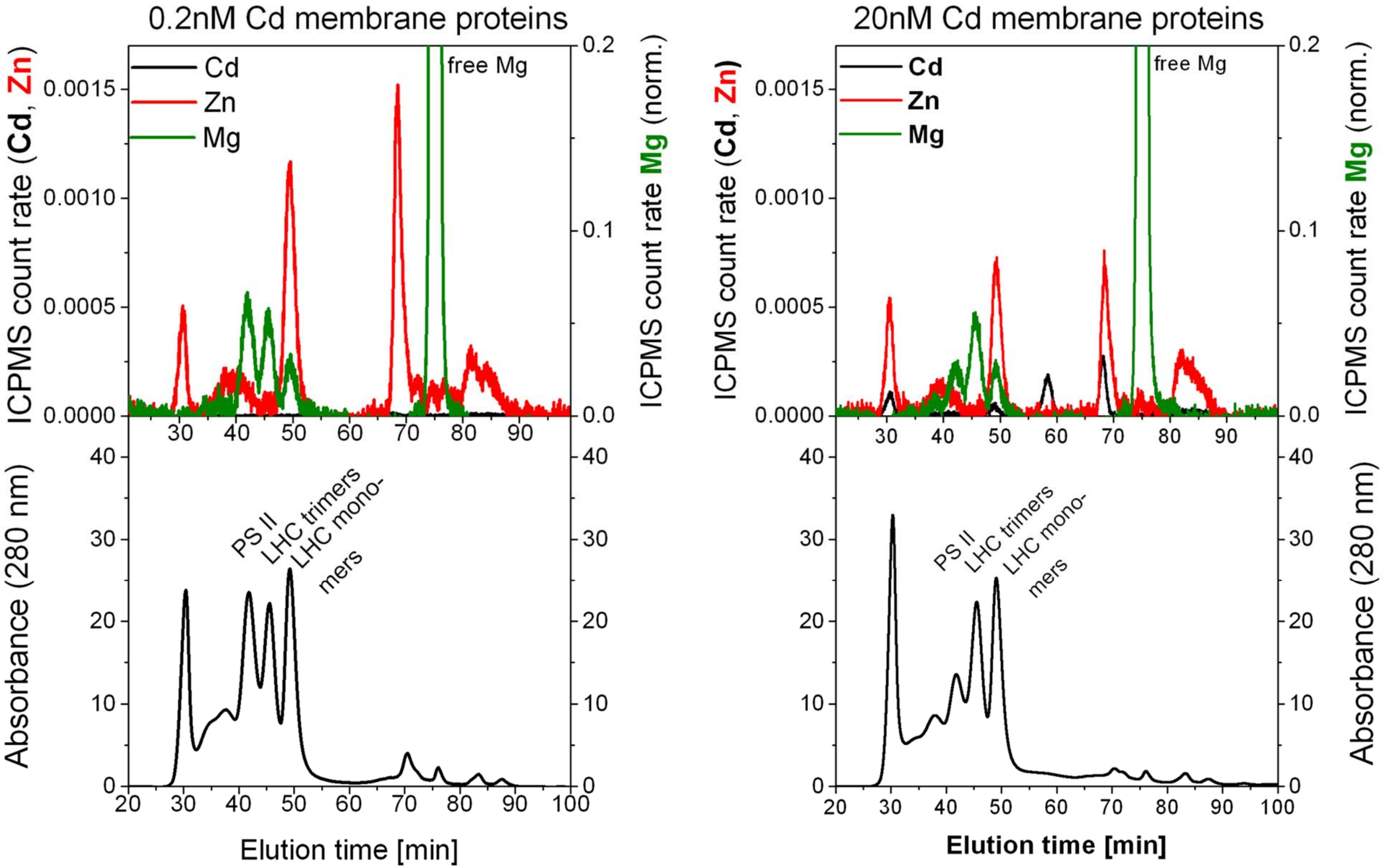


Sigfridsson KGV_et al (2004) BBA-Bioen1659, 19-31



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

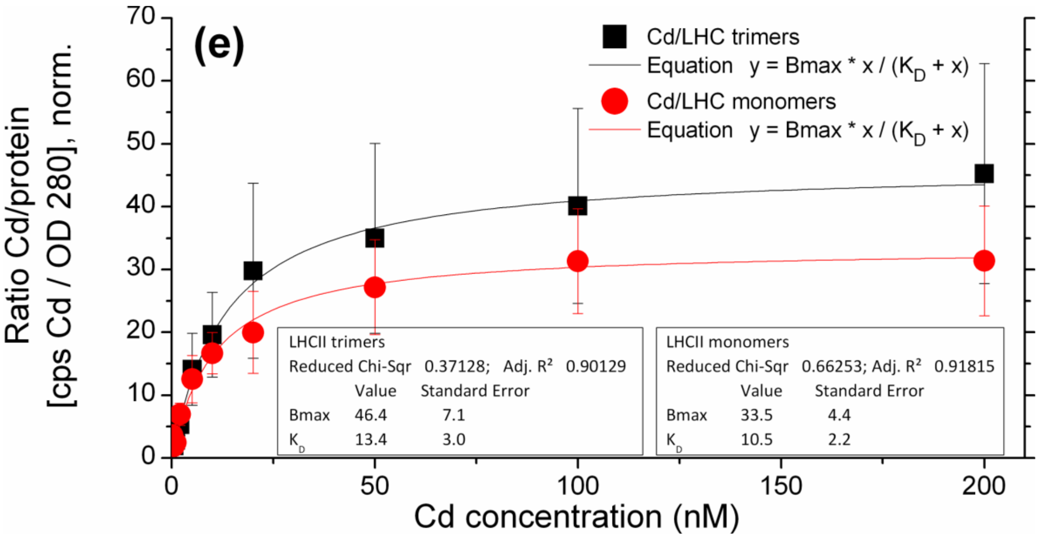
Cd stress in *C. demersum*: Incorporation of Cd into proteins in LL analysed by metalloproteomics via HPLC-ICP-MS

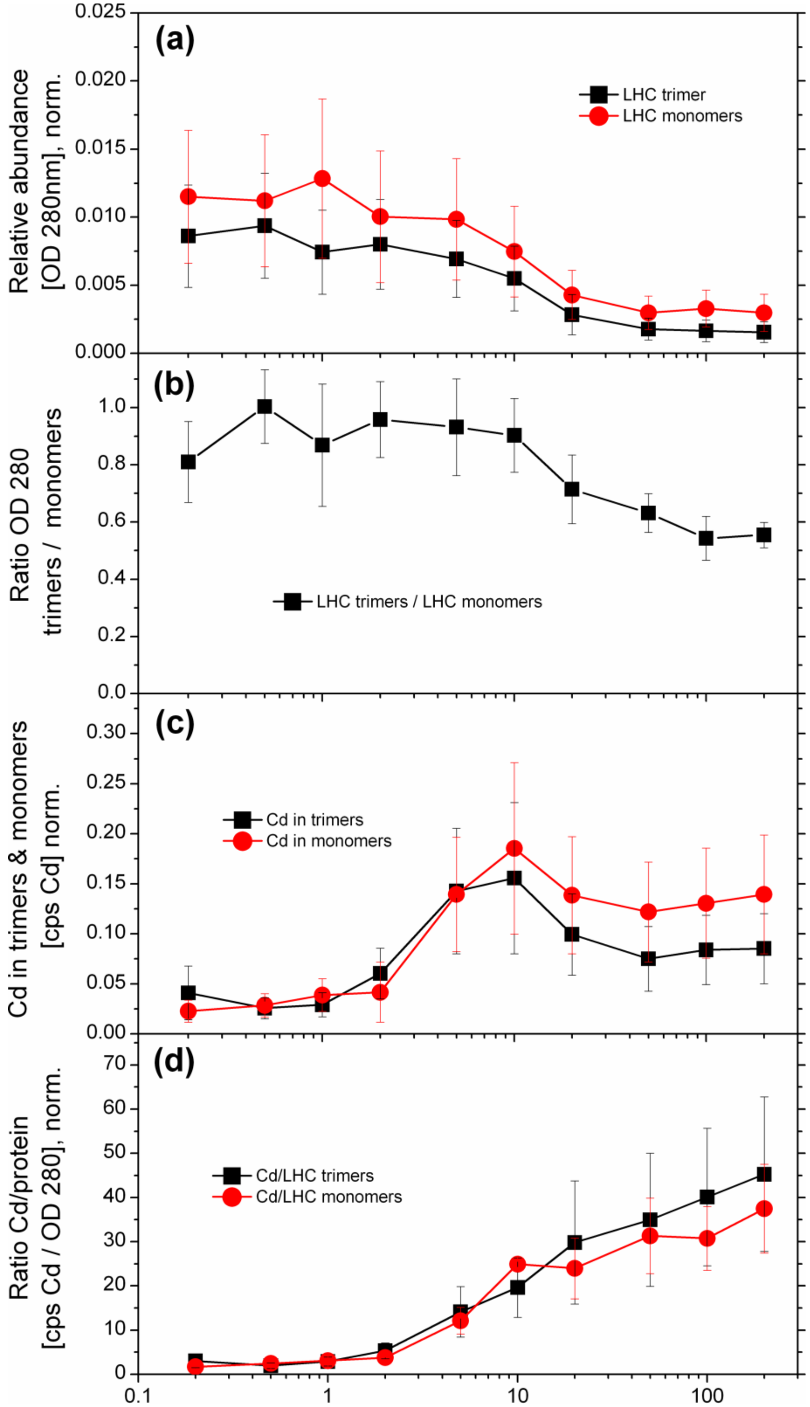


→ Cd binding to main light harvesting antenna LHCII in LL

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



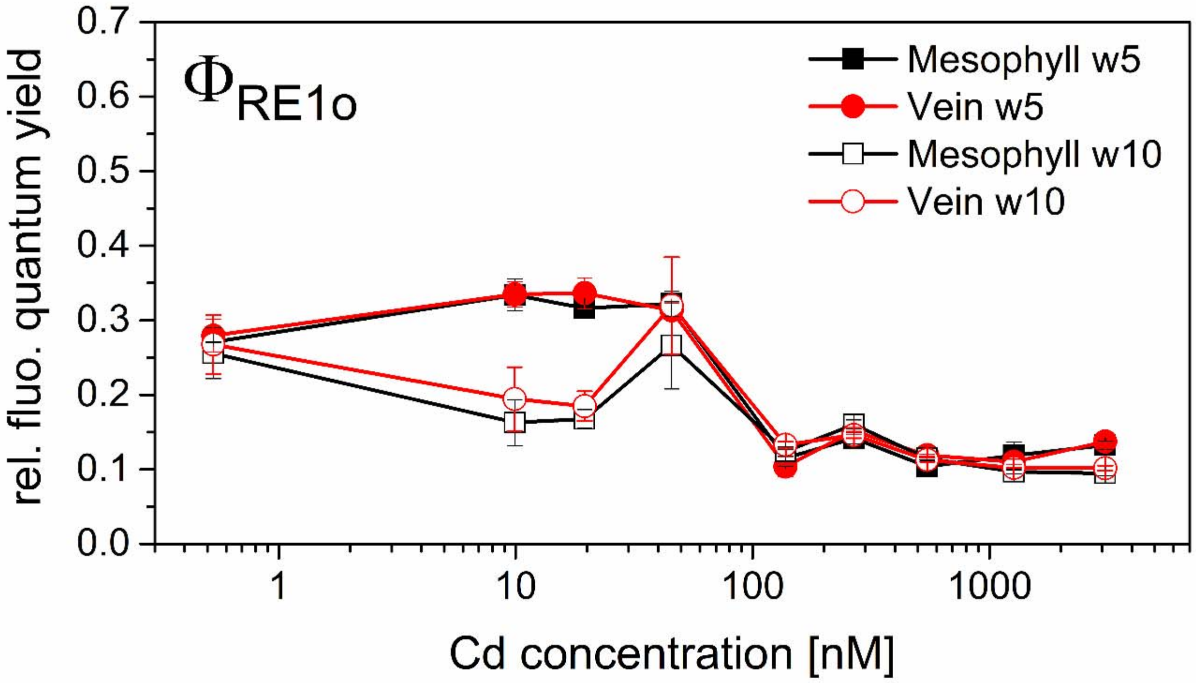


→ Cd binding to LHCII causes disintegration of trimers

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

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

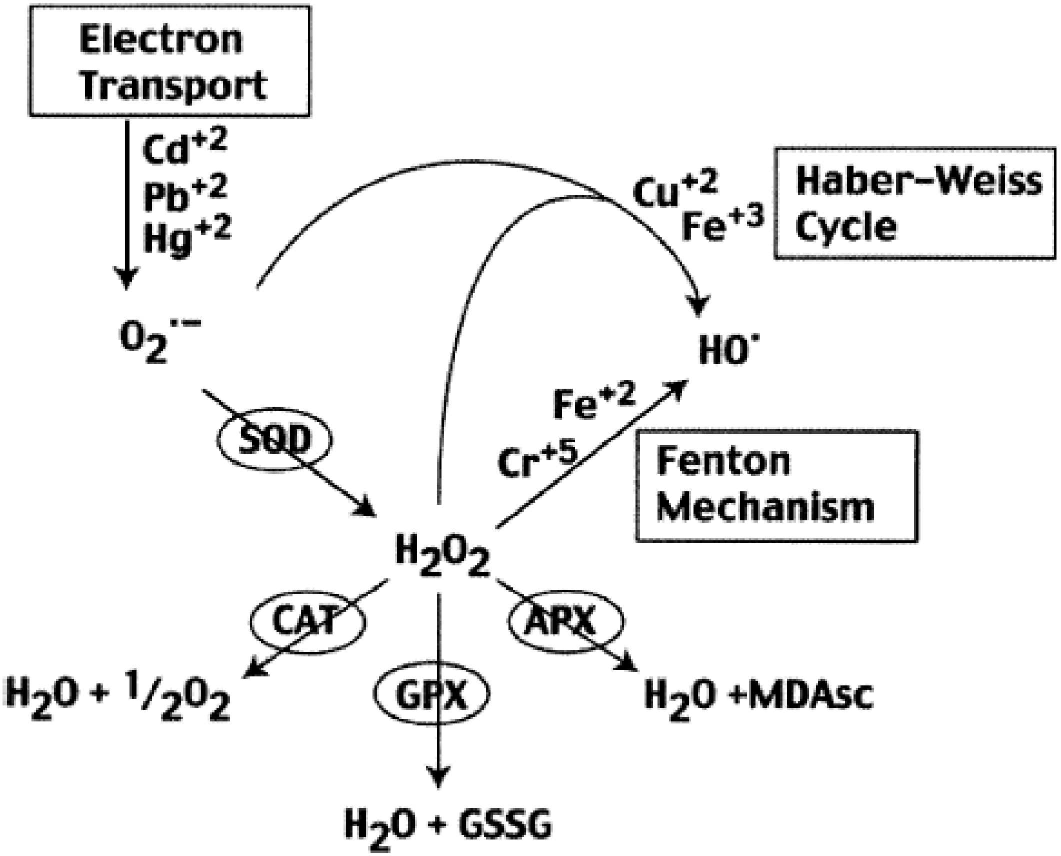


→ Cd inhibits electron flow to PSI already in the environmentally relevant nanomolar range

Andresen E, Lyubenova L, Hubáček T, Bokhari SNH, Matoušková Š, Mijovilovich A, Rohovec J, Küpper H (print: 2020, published online 24 November 2019) Journal of Experimental Botany 71, 1628-1644

ROS and Cadmium

- Cadmium redox inert → No Fenton reaction!



Fenton:
$Fe(II) + H_2O_2$ → →
$Fe(III) + R^* + OH^- + H_2O$

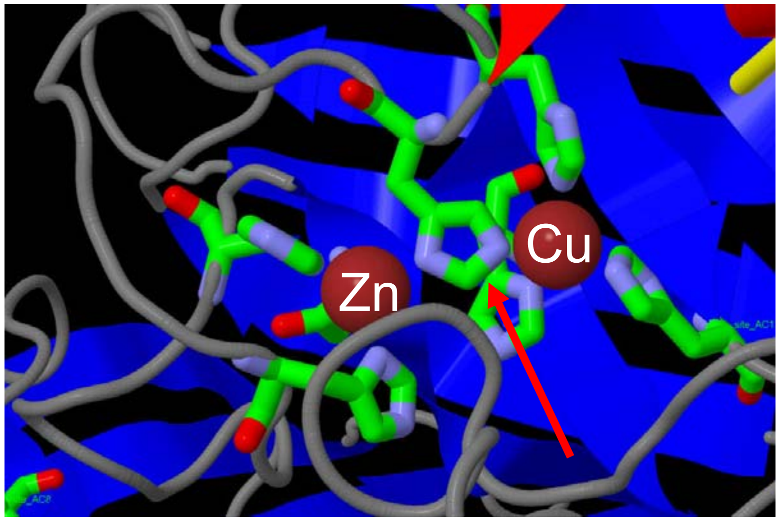
„Biggest source of ROS in animal cells“ …

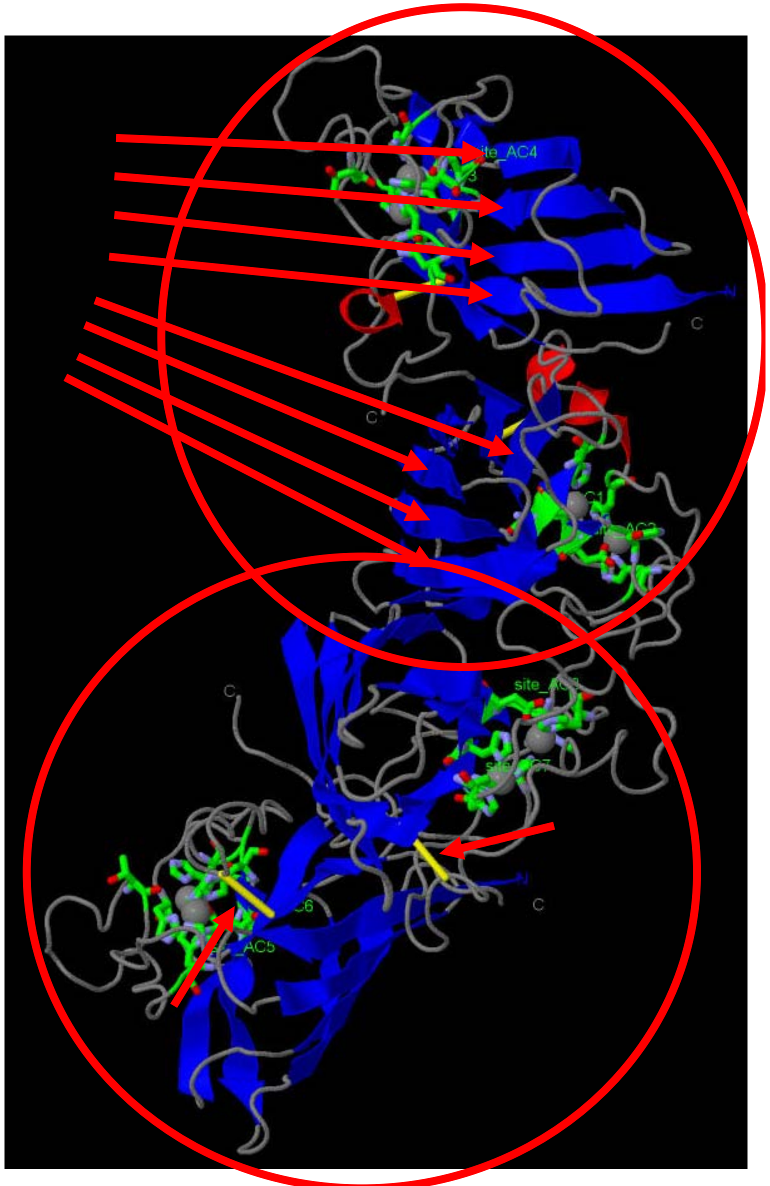
→ NEVER shown *in vivo*!

Pinto, Journal of Phycology 39:1008-1018, 2003

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

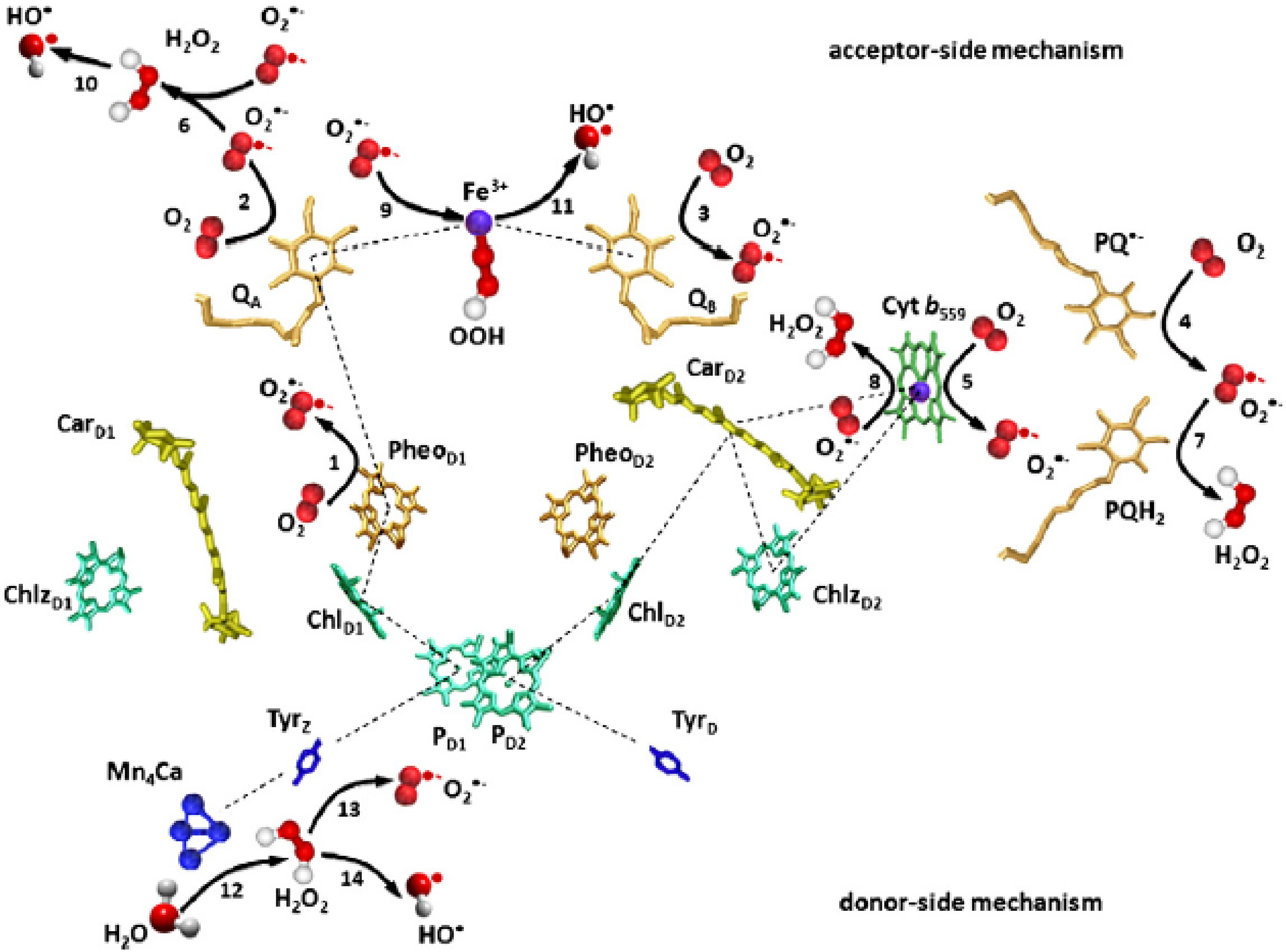
Substitution of Zn by Cd in SOD may contribute to oxidative stress during Cd toxicity





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 ↑

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

- In response antioxidant enzymes ↑

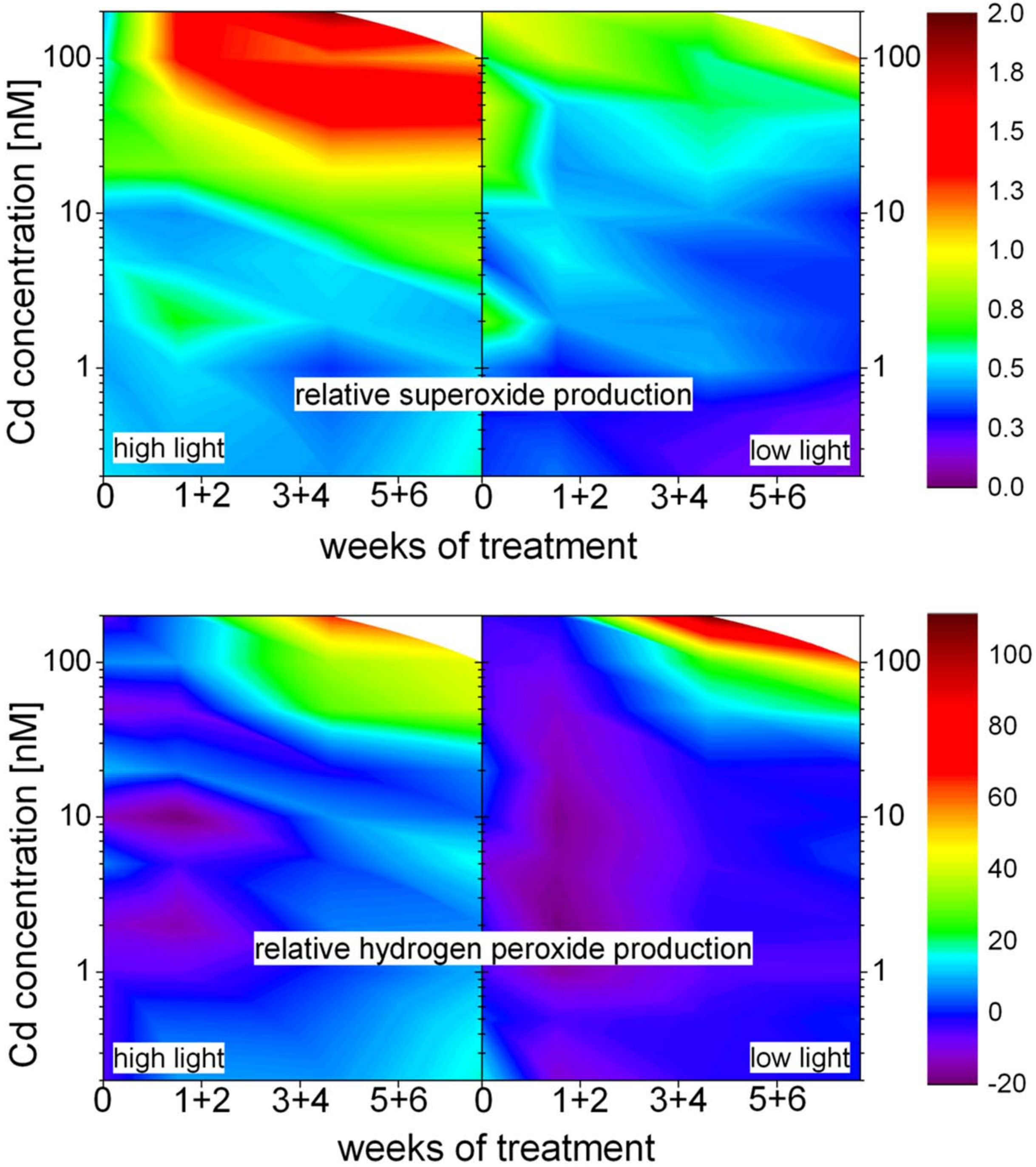
Removal of ROS ↓

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

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 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

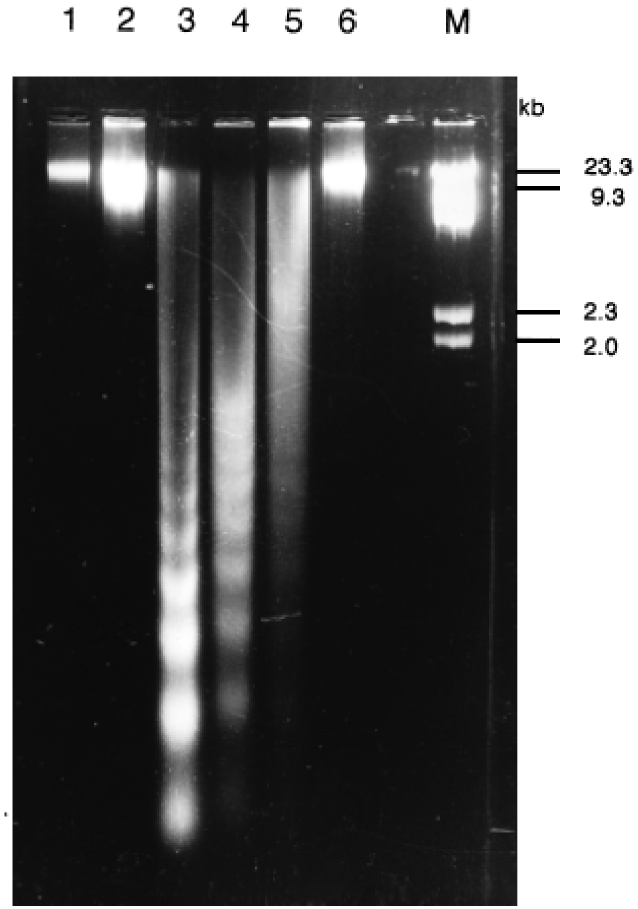
Cadmium toxicity in plants – 4. Genotoxicity

- 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

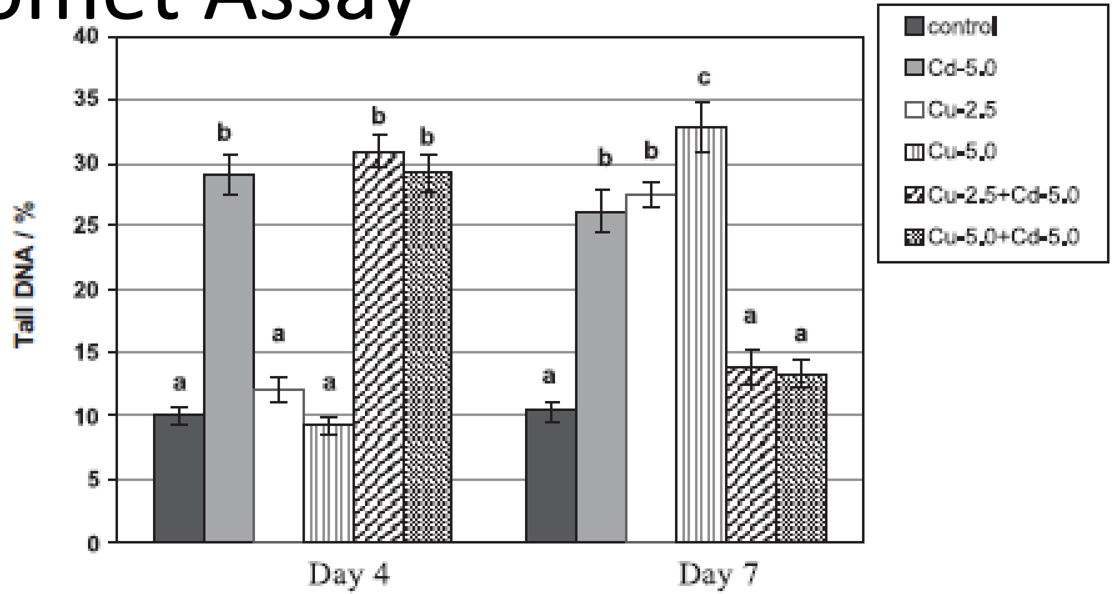
- DNA Analyses
  - Gelelectrophoresis and Comet Assay
  - Random amplification of polymorphism DNA (RAPD)
- DNA / Chromosome Analyses
  - Micronuclei formation
  - Sister chromatid exchange
  - Chromosomal aberrations
- Upregulation of DNA-related / repairing enzymes

DNA disruption – Gelelectrophoresis and Comet Assay



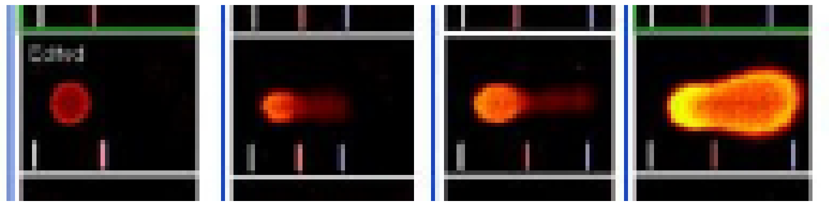
1: DNA from control plant, 2:-6: DNA from plants treated with Cd 10, 50, 75, 100, 1000 µM

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



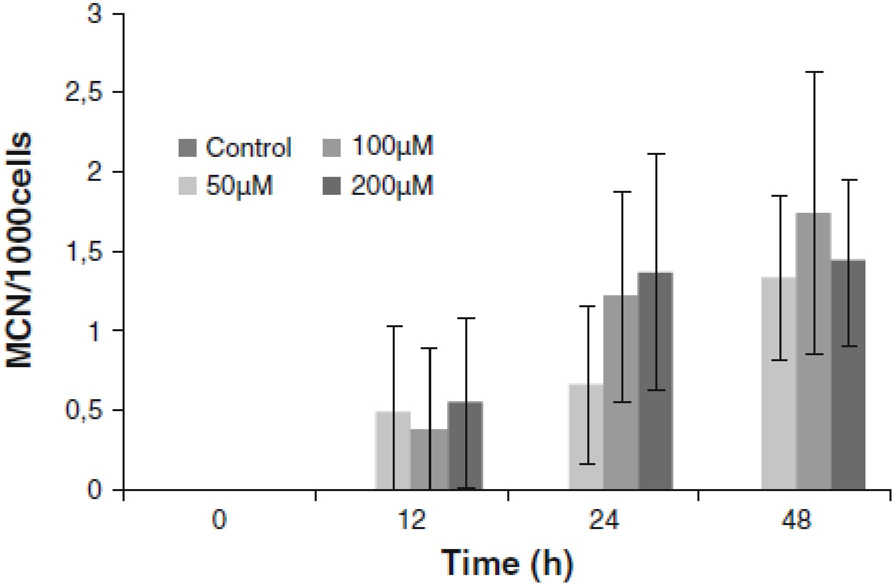
Lemna minor treated with µM concentrations of Cd and Cu. Tail DNA (Comet - length) increase due to metal treatment.

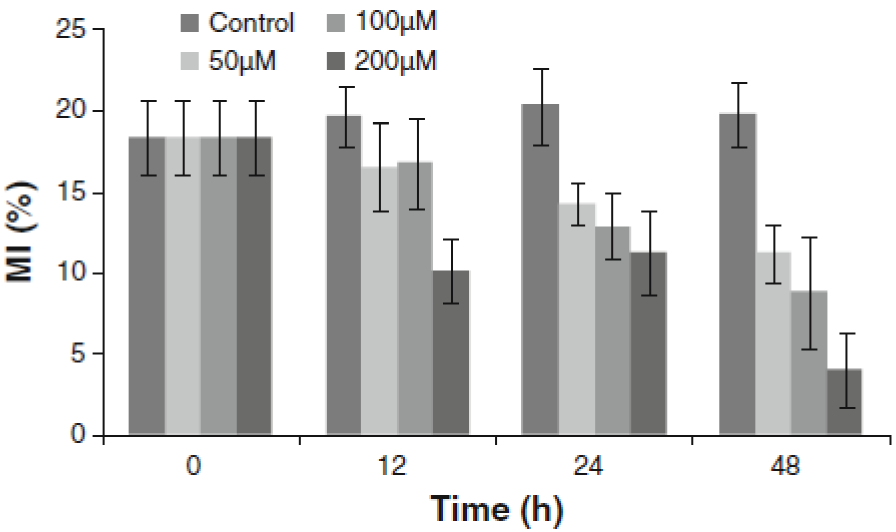
Cvjetko et al., Arh Hig Rada Toksikol 61:287-296, 2010



From the CometAssay Manual, Trevigen[R]

Micronuclei & Mitotic index

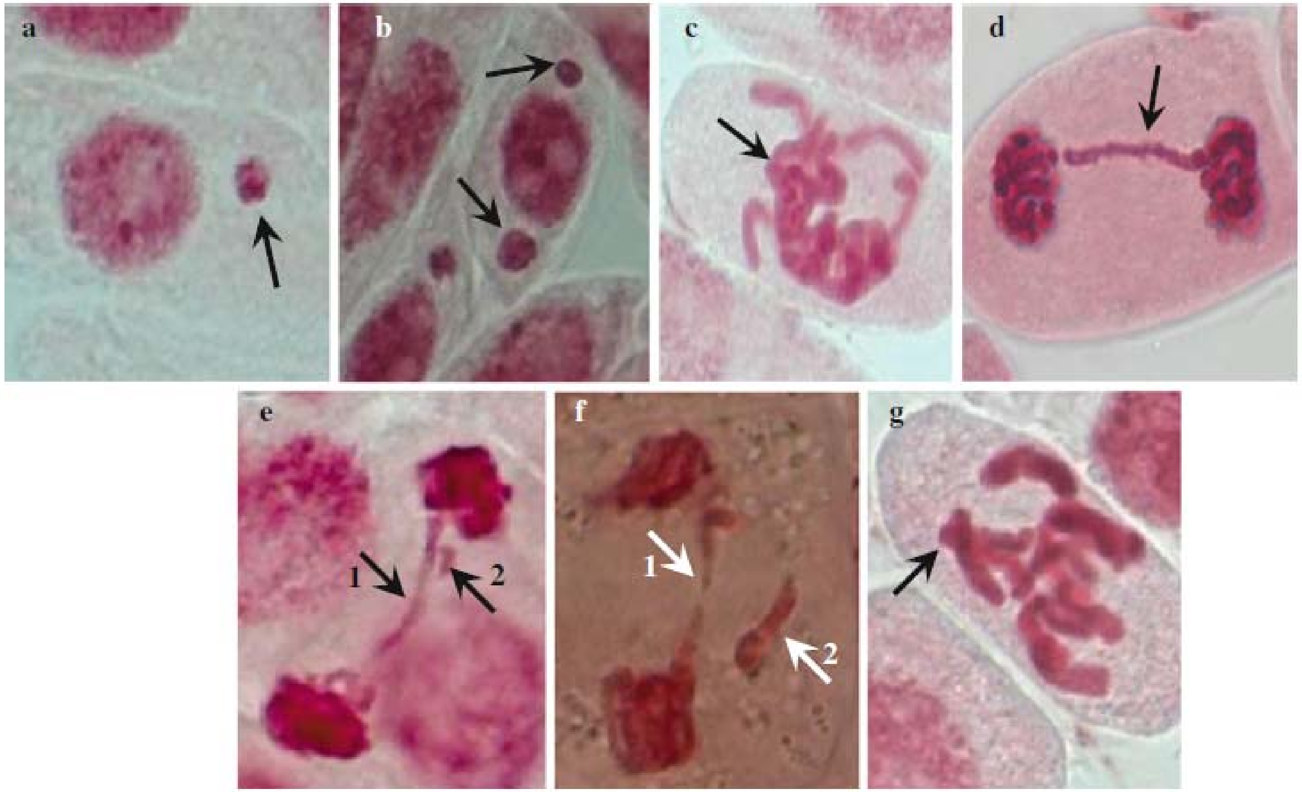




- *Vicia* root meristem cells; established assay
- Micronuclei formation due to malfunctioning cell division
- Dose and time dependent
  - Cd treatment increases MCN
- Mitotic index: ratio of cells in metaphase stage to all cells
  - Cd treatment reduces MI

Souguir et al., Ecotoxicology 20:329-336, 2011

Chromosomal aberrations

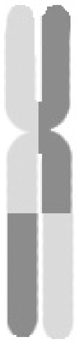


Vicia root meristem cells
a, b, e, f = 50 µM Cd
c, d, g= 200 µM Cd

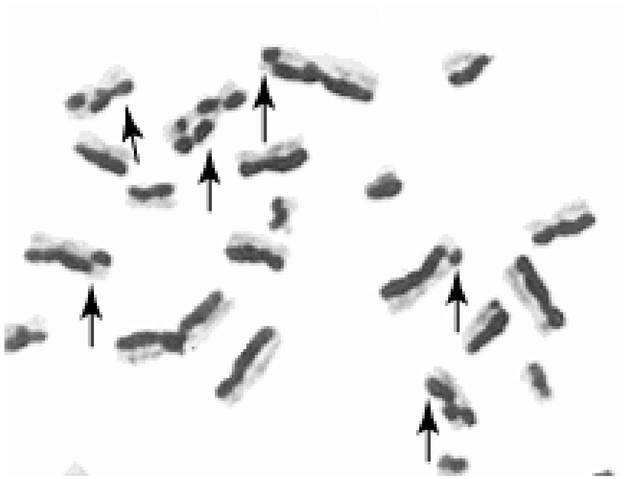
a & b = micronuclei
c = sticky chromosome
d = chromosome bridge
e = "" + break
f = "" +isolated chromosome
g = laggered chromosome in metaphase

Souguir et al., Ecotoxicology 20:329-336, 2011

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 (≠ crossing over)
-Happens in normal cells, but enhanced after treatment with toxic / radioactive substances



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

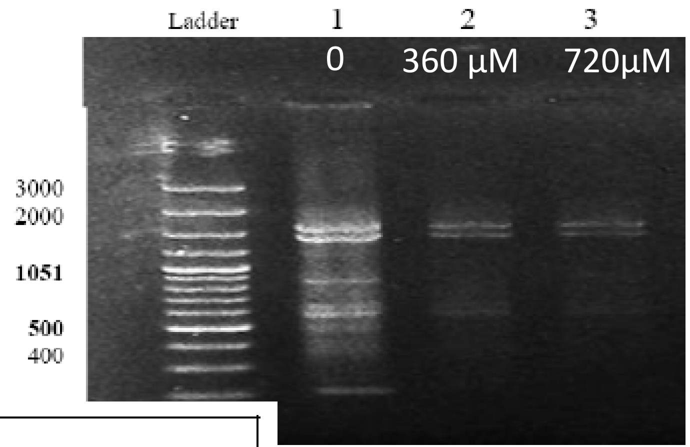
Test substance	Concentration	SCEs/metaphase (mean ± SE)	MI (mean ± SE)
Negative Control (Hoagland's nutrient Solution)	0	6.26 ± 0.29	11.80 ± 0.11
Cadmium nitrate (μM)	50	6.63 ± 0.3	9.70 ± 0.26***
	100	7.43 ± 0.04**	7.16 ± 0.32***
	200	7.90 ± 0.07***	1.16 ± 0.03***
Positive control (Cyclophosphamide,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

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

Ünyayar et al., Turk J Biol 34:413-422, 2010

Random amplification of polymorphism DNA analyses (RAPD)

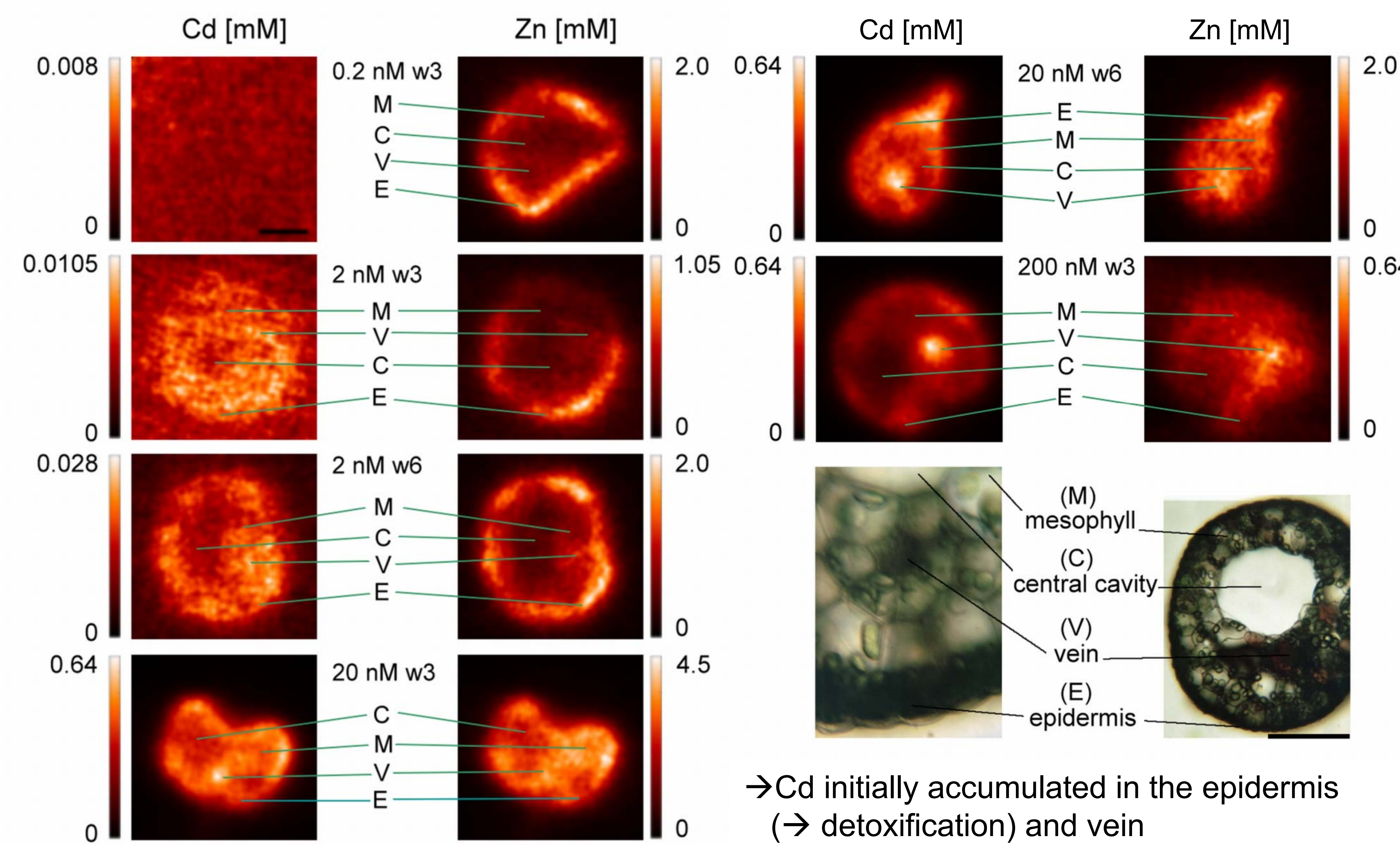
- Cd interacts with DNA / induces mutation
- New / disappearing primer binding sites
- → New / disappearing bands on gel



	Treatments				
		40 (mgL^{-1}) Cd concentration		80 (mgL^{-1}) Cd concentration	
Primers names	Total bands in control	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

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

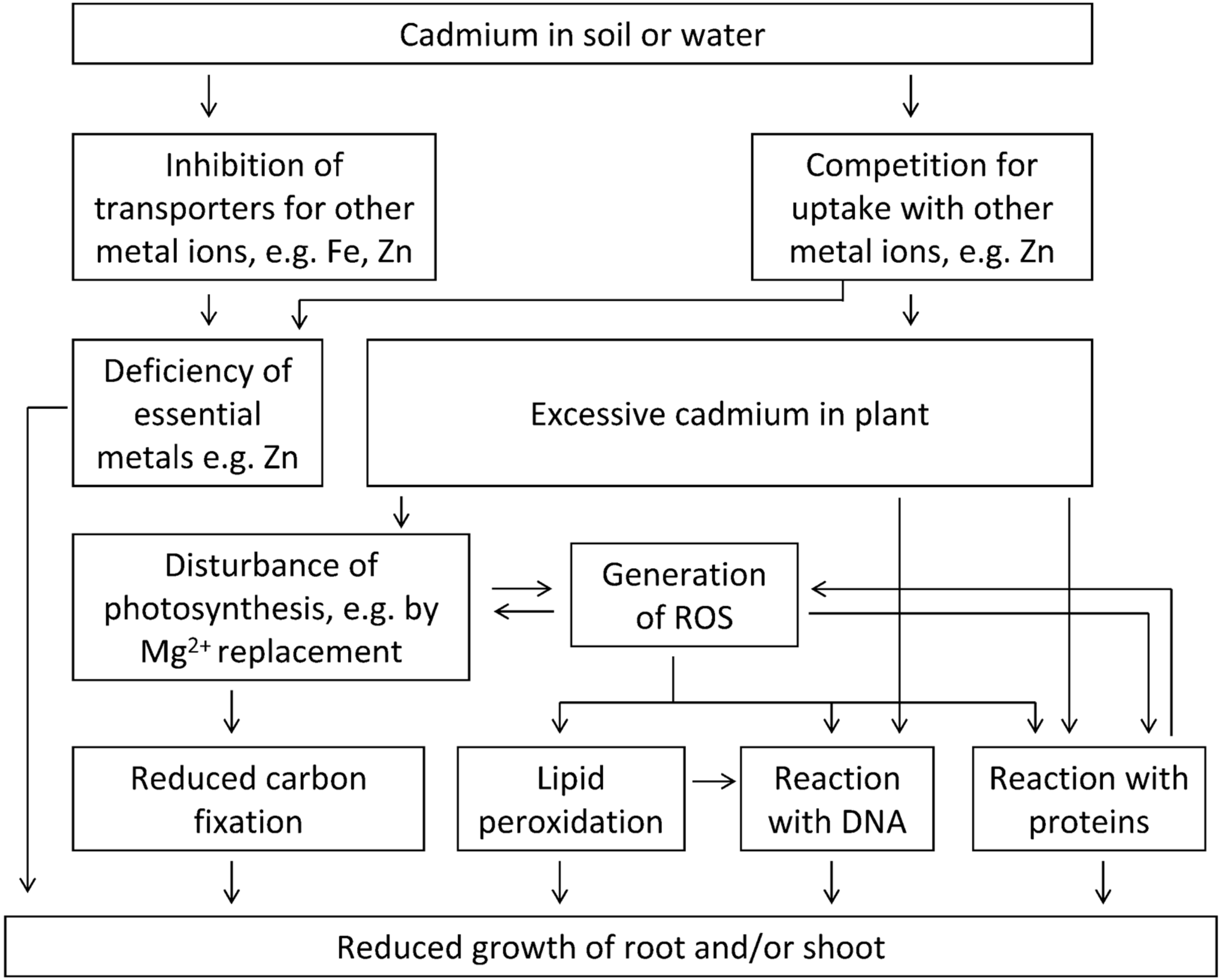
→ lethal Cd inhibited vein export. also of Zn

Andresen E, Mattusch J, Wellenreuther G, Thomas G, Abad UA, Küpper H (2013) Metallomics 5, 1377-1386

Changing distribution of Cd and Zn

Increasing Cd:

- Higher Cd concentration in leaves
- Increased sequestration into non-photosynthetic tissues
- Re-distribution of Zn → inhibited export out of vein



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 most toxic effects: 20nM
  - Most fluorescence parameters, growth, pigments
- First site of inhibition: photosynthetic apparatus
  - ROS follow reaction
- Direct inhibition of PSIIRC 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

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