Heavy Metals and Plants - a complicated relationship

→ Heavy metal stress and heavy metal resistance

Heavy metal-hyperaccumulation in the Wild West

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

Presented by Hendrik Küpper for the VTK Plant Physiology 2011
Dose-Response principle for heavy metals

Küpper H, Kroneck PMH, 2005, Metal ions Life Sci 2, 31-62
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](image)
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. CuSO₄, 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 industries. 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.
Heavy-metal induced damage

--> Inhibition of root function
(⇒ KK 5th semester)

--> Genotoxicity

--> Direct inhibition of photosynthesis

--> Oxidative stress: direct and as a result of a malfunction of photosynthesis

--> Substitution of active centres in enzymes

--> Inhibition of respiration and other relatively insensitive processes
Comparison of photosynthesis and respiration changes caused by Cr- and Cu-stress in *Euglena gracilis*

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
  - Relevant 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 genotoxicity...

Genotoxicity

Mechanisms: Point Mutations and Homologous recombinations

Genotoxicity

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

- Abnormal spindle
- C-metaphase
- Tripolar anaphase
- Star anaphase
- Anaphase with a pair of lagging chromosomes
- Micronucleus (MCN) formation

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 heavy metal concentrations oft 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 concentrations.

Mechanisms generating reactive oxygen species during heavy metal stress

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

\[ Mn^+ + H_2O_2 \rightarrow M^{(n+1)+} + \cdot OH + OH^- \]

• Indirect: malfunktion 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.
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: en.wikipedia.org
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!
Environmental relevance of heavy metal induced inhibition of photosynthesis:

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

Metal sites in photosynthetic proteins

Fe^{3+/2+}

Mg^{2+}

FNR

Antenna Chl-protein complexes, main protein: LHCII

P680*
Phe

P680

4 h·ν

Chl

excitation energy transfer

WSC

4 e-

P700*

Q_A, Q_B

PQ
electron transport

Cyt b6/f complex

P700

Chl

excitation energy transfer

EET

Ca^{2+}

4 e-

O_2 + 4 H^+

2 H_2O

Cu^{+/2+}

Mn^{3+/4+}

Ca^{2+}

A_G, A_1, F_A, F_B

Fd
Heavy metal induced inhibitions of photosynthesis:
suggested targets

Heavy metals

Antenna Chl-protein complexes, main protein: LHCII

Excitation energy transfer

Chl

P680*

Pheo

QA

QB

PQ

FeS/Rieske

Electron transport

PC

P700*

A0

A1

Chl

Chl

Chl

2 H₂O

WSC

Excitation energy transfer

4 h·ν

4 e⁻
Inhibition of PSI vs. PSII

Comparison of PSI and PSII Activity

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.

Why are heavy metal chlorophylls unsuitable for photosynthesis?

- shift of absorbance/fluorescence bands --> less energy transfer
- unstable singlet excited state --> “black holes“ for excitons
- different structure --> proteins denature
- do not readily perform charge separation when in reaction centre.
Static fluorescence microscopy of metal-stressed *Elodea*

transmittant light observation

1 µM Cu²⁺ control                       100 µM Zn²⁺

red (650-700nm) chlorophyll fluorescence

Photosynthesis activity: Sun- vs. Shade-reaction

Cd-stress in the Zn-/Cd-hyperaccumulator *T. caerulescens*:
Spectral changes of PSII activity parameters

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

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

General Resistance-Mechanisms

Heavy metal detoxification with strong ligands

Phytochelatins

• Bind Cd\(^{2+}\) with very high affinity, but other heavy metal ions with low affinity

• Specially for Cd\(^{2+}\)-binding synthesized by phytochelatin-synthase

• They are the main Cd-resistance mechanism in most plants (except hyperaccumulators) and many animals

• Phytochelatin synthase becomes activated by (e.g. via Cd-binding) blocked thiols of glutathion and similar peptides

• Phytochelatins bind Cd\(^{2+}\) in the cytoplasm, then the complex is sequestered in the vacuole.

• In the vacuole large phytochelatin-Cd-aggregates are formed
General Resistance-Mechanisms
Heavy metal detoxification with strong ligands

Glutathion

- Also glutathione itself, the building block of phytochelatins, can bind and thus detoxify heavy metals - the *in vivo* relevance is questionable

Metallothionins

- MTs of type I und II bind Cu\(^+\) with high affinity and seem to be involved in its detoxification.
- BUT: Main role of MTs in plants seems to be Metal-distribution during the normal (non-stressed) metabolism
General Resistance-Mechanisms
Heavy metal detoxification with strong ligands

Other Ligands

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

• Cell wall

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

Hale et al. 2001, Plant Physiol 126, 1391-1402
Heavy metal detoxification by compartmentation

Mechanisms

- Generally: aktive transport processes against the concentration gradient → transport proteins involved.

- Exclusion from cells:
  - observed in brown algae
  - in roots

- Sequestration in the vacuole:
  - plant-specific mechanism (animals+bacteria usually don’t have vacuoles...)
  - very efficient, because the vacuole does not contain sensitive enzymes
  - saves the investment into the synthesis of strong ligands like phytochelatins
  - main mechanism in hyperaccumulators

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

- Reduction by reductases, e.g. $\text{Hg}^{2+} \rightarrow \text{Hg}_0$, $\text{Cu}^{2+} \rightarrow \text{Cu}^+$

- Precipitation of insoluble sulfides outside the cell (on the cell wall)

- Methylation, e.g. of arsenic

Rugh CL, et al, 1996, PNAS 93, 3182-3187
Root-specific resistance mechanisms

**Strategies**

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

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Resistance mechanisms against oxidative stress

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

- Changes in the cell membranes to make them more resistant against the attack of reactive oxygen species:
  - Lipids with less unsaturated bonds
  - Exchange of phosphatidyl-choline against phosphatidyl-ethanolamine as lipid-“head”
  - Diminished proportion of lipids and enhanced proportion of stabilising proteins in the membrane
Plants with an unusual appetite: Heavy metal hyperaccumulation

Effects of Ni$^{2+}$ addition on hyperaccumulator plant growth and Ni$^{2+}$ concentration in shoots

Speciation of hyperaccumulated metals revealed by EXAFS: Cd in the CdZn-hyperaccumulator *Thlaspi caerulescens* and Cu in the Cu-hyperaccumulator *Crassula helmsii*.


Speciation of zinc, cadmium and copper in the Cu-sensitive CdZn-hyperaccumulator *T. caerulescens*


Compartmentation of metals in leaves
Zn/Cd/Ni accumulation in epidermal vacuoles of *Thlaspi* and *Alyssum* species

![Graph showing concentrations of elements in leaf tissues](image)

Concentrations of elements in leaf tissues *Thlaspi caerulescens*

- **Zn**
- **Mg**
- **P**
- **S**
- **Cl**
- **K**

**Young leaves**

- Upper epidermis: [Graph]
- Upper mesophyll: [Graph]
- Lower mesophyll: [Graph]
- Lower epidermis: [Graph]

**Mature leaves**

- Upper epidermis: [Graph]
- Upper mesophyll: [Graph]
- Lower mesophyll: [Graph]
- Lower epidermis: [Graph]

*Zn Kα line scan and dot map of a *T. caerulescens* leaf*

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

**References**

Kompartimentierung von Metallen in Blättern
Korrelation zwischen Metallkonzentrationen
im Mesophyll von Arabidopsis halleri

Küpper H, Lombi E, Zhao FJ, McGrath SP (2000) Planta 212, 75-84
Mechanisms of Metal Uptake in plants
Root uptake and intracellular distribution in plants
example: iron and zinc transport in Brassicaceae

4 main families, all overexpressed in hyperaccumulators!
- P-type ATPases
- Cation diffusion facilitators (CDF-transporters)
- ZRT-/IRT-like proteins (ZIP-transporters)
- Natural resistance associated Macrophage proteins (Nramp-transporters)

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

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

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

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

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

↑ higher uptake rates in large metal storage cells compared to other epidermal cells are caused by higher transporter expression, NOT by differences in cell walls or transpiration stream.
Regulation of ZNT1 transcription analysed by quantitative mRNA in situ hybridisation (QISH) in a non-hyperaccumulating and a hyperaccumulating *Thlaspi* species

Quantitative cellular pattern of ZNT5 transcript abundance in young leaves of *Thlaspi carulescens* (Ganges ecotype)

→ judged by its expression pattern in the epidermis, ZNT5 may be a key player in hyperaccumulation of Zn

Regulation of ZNT5 transcription in young leaves of *Thlaspi carulescens* (*Ganges ecotype*) analysed by QISH

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

Purification and Characterisation of a Zn/Cd transporting P1B type ATPase from natural abundance in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*

Scheme from: Solioz M, Vulpe C (1996) TIBS 21_237-41


Plants grown on 100 μM Zn²⁺ crude membrane extract: 20mg roots per lane

![Image showing purification and characterisation process](image)

- Post-translational modification of TcHMA4
- $K_D$ of TcHMA in the high nanomolar to low micromolar range
EXAFS-analysis of TcHMA4 and another, so far unknown Cd-ATPase

Activation and substrate inhibition of TcHMA4

TcHMA4 Characterisation: 2D-Activity tests
Temperature dependence of substrate binding and influence of the substrate on the thermostability of TcHMA4

All slides of my lectures can be downloaded from my workgroup homepage

www.uni-konstanz.de → Department of Biology → Workgroups → Küpper lab,

or directly

http://www.uni-konstanz.de/FuF/Bio/kuepper/Homepage/AG_Kuepper_Homepage.html