Heavy Metals and Plants – a complicated relationship

→ Heavy metal resistance

Heavy metal-hyperaccumulation in the Wild West

modified from: http://strangematter.sci.waikato.ac.nz/
Variability of metal contents from deficiency to toxicity (I): A decisive factor for biodiversity

Plant communities in low metal habitats
- Non-metalliferous alpine meadow
- Non-polluted site in the same region

Plant communities in high metal habitats
- Natural serpentine barren
- Antropogenic (mining) polluted site

Mount Hood (Oregon, USA), From: commons.wikimedia.org
Sao Domingos mine (Alentejo, Portugal), From: commons.wikimedia.org
Slate Mountain serpentine barren (North Carolina, USA), From: US forest service
Heavy metal uptake characteristics of plants

Plants with an unusual appetite: Heavy metal hyperaccumulation

(a) Effects of Ni addition on hyperaccumulator plant growth and Ni concentration in shoots.

(b) Graph showing Ni concentration in shoots with different plant species.

Cadmium deficiency in the Cd/Zn hyperaccumulator *Noccaea* (formerly *Thlaspi*) *caerulescens*

With 10 µM cadmium in the nutrient solution
--> healthy plants

Without cadmium in the nutrient solution
--> damage due to attack of insects

→ Various studies by many labs have shown that hyperaccumulation serves as defence against pathogens and herbivores

Küpper H, Kroneck PMH (2005) MIBS 44 (Sigel et al., eds), chapter 5
Metalloid detoxification: overview of mechanisms proposed for arsenic

Briat J PNAS 2010;107:20853-20854
Metal detoxification by complexation
General Resistance-Mechanisms

Heavy metal detoxification with strong ligands

Phytochelatins (PCs)

- Bind Cd$^{2+}$ with very high affinity also As(III) and AS(V), but many other heavy metal ions with low affinity
- Specially for Cd$^{2+}$-binding synthesized by phytochelatin-synthase
- PC synthase activated by blocked thiols of glutathion and similar peptides

**General Resistance-Mechanisms**

**Heavy metal detoxification with strong ligands**

**Phytochelatins (PCs)**

- They are the main Cd-resistance mechanism in most plants (except hyperaccumulators) and many animals.
- PCs bind Cd$^{2+}$ in the cytoplasm, then the complex is sequestered in the vacuole.
- Phytochelatin-Cd-aggregates are formed in vacuoles.
Speciation of arsenic in a non-accumulator plant

- Higher Cd concentration → more different PCs
  + much higher amount of PC 2-4
- Threshold concentration specific for each PC
  - Most prominent: PC3 at 20nM
- PCs induced → no role in homeostasis, reported presence at „0“ metal(loid) stress most likely due to trace contaminations of chemicals

Andresen et al., 2013, Metallomics, 5, 1377-1386
Metallothionins

- MTs of type I and 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.

Glutathionin

- Also glutathione itself, the building block of phytochelatins, can bind and thus detoxify heavy metals - the *in vivo* relevance is questionable.
Speciation of hyperaccumulated metals revealed by EXAFS: Cd in the Cd/Zn-hyperaccumulator *T. caerulescens* and Cu in the Cu-hyperaccumulator *C. helmsii*

Hyperaccumulated metals are stored in weakly bound form, i.e. ideal for **defence**


Speciation of cadmium and zinc hyperaccumulated by *Thlaspi caerulescens* (Ganges ecotype) revealed by EXAFS of frozen-hydrated tissues

Differences in ligands between hyperaccumulated and non-hyperaccumulated metals: zinc, cadmium and copper in the Cu-sensitive Cd/Zn-hyperaccumulator T. caerulescens

- Hyperaccumulated metals are stored in weakly bound form, i.e. ideal for **defence**
- Non-hyperaccumulated metals in hyperaccumulator plants are stored in strongly bound form

Speciation of copper in the Cu-sensitive CdZn-hyperaccumulator *T. caerulescens* Analysed by XAS

- **Cu(II)-oxalate** (moolooite) structure from Michalowicz et al. (1979) Inorg Chem 18, 3004-310

- **Cu(I)-metallothioneins & phytochelatins**
  - Cu(II)-Nicotianamine
  - Cu(II)-aquo and Cu(II)-malate

- **Cu(I)-MT EXAFS** from Sayers et al. (1993) Eur J Biochem 212, 521-528

**Fe(III)-Nicotianamine**, structure from vonWiren et al. (1999) Plant Physiol 119

Speciation of copper in the Cu-sensitive CdZn-hyperaccumulator *T. caerulescens* comparison of sensitive vs. resistant individuals

Copper bio-mineralisation as hardly soluble Cu-oxalate serves as additional detoxification in Cu-resistance

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 Molybdemum binding (detoxification or storage?)

• Cell wall

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

Hale et al., 2001, Plant Physiol 126, 1391-1402

Metal detoxification by sequestration within the plant
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)
Differences between species and on a cellular level: distribution of photosystem II activity parameters during Cd toxicity in the Zn/Cd-hyperaccumulator *T. caerulescens*

Cellular $F_v/F_m$ distribution in a control plant

Distribution of $F_v/F_m$ in a plant stressed with Cd$^{2+}$

Stress was applied as 10µM Cd$^{2+}$ in the nutrient solution that was continuously exchanged for 6 months.

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

Küpper H et al., 2001, J Exp Bot 52 (365), 2291-2300
Most common pattern of heavy metal detoxification by compartmentation in hyperaccumulators: Enhanced sequestration in epidermal vacuoles

- Sequestration in the vacuole: plant-specific mechanism (animals+bacteria usually don’t have storage vacuoles...)
- Sequestration in least sensitive tissues, e.g. the epidermis instead of the photosynthetically active mesophyll

Generally: active transport against the concentration gradient → transport proteins required!

Proposed mechanism of emergency defence against heavy metal stress

Normal: Sequestration in epidermal storage cells

Stressed: additional sequestration in selected mesophyll cells

Acclimated: Enhanced sequestration in epidermal storage cells

Heavy metal detoxification by compartmentation:
variations of the pattern as revealed by EDX

species-specific in *Arabidopsis halleri*

Accumulation of Zn mainly in the mesophyll instead of the epidermis, but highest concentrations (up to 1M) in epidermal trichomes (→ defence)

Küpper H, Lombi E, Zhao FJ, McGrath SP (2000) Planta 212, 75-84

metal-specific for Al in *Camellia sinensis* (tea)

Accumulation of Al in the cell walls instead of the vacuoles, but again in the epidermis (→ defence?)

Mechanisms of Metal transport proteins

\[ \Delta G = n I o n e n \times R \times T \times \ln \left( \frac{c_{\text{inside}}}{c_{\text{outside}}} \right) + 3F \left( \phi_{\text{outside}} - \phi_{\text{inside}} \right) \]

(R = gas constant, T = temperature, F = Faraday constant, \( \phi \) = electrochemical potential)
Mechanisms of metal uptake in Eucaryotes: Main families of metal transport proteins
example: manganese transport in yeast and plants

- **P-type ATPases**
- **cation diffusion facilitators (CDF-transporters)**
- **ZRT-/IRT-like proteins (ZIP-transporters)**
- **Natural resistance associated Macrophage proteins (Nramp-transporters)**

From: Pittman JK, 2005, NewPhytol167, 733-742
Mechanisms of metal uptake in plants:
Different transport steps require different transporters
Translocation. Root-to-shoot: Xylem, shoot-to-root: phloem
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).

Metal-dependent differences in energetics of NcHMA4

- Activation energy changes with the concentration and type of the metal to be pumped.
- Maximal activity after saturation of all high-affinity Cd binding sites
Different expression patterns of closely related Cd/Zn-pumping ATPases as revealed by Quantitative mRNA In Situ Hybridisation

**Arabidopsis halleri**
- mostly in mesophyll (final storage)
- HMA4 up-regulated in Zn-deficiency

**Noccaea caerulescens**
- HMA4 much stronger in bundle sheath, likely because final storage is in epidermis

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!
higher uptake rates in large metal storage cells compared to other cells are caused by higher transporter expression, NOT by differences in cell walls or transpiration stream.
Different expression patterns of closely related Zn-specific ZIP transporters as revealed by QISH

Expression of ZNT1 mainly in metabolically active cells, not metal storage cells

Expression of ZNT5 mainly in metal storage cells

judged by its expression pattern in the epidermis that matches known accumulation patterns for Zn and Ni, 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

HMA3 as a likely candidate for the vacuolar Cd sequestration in *T. caerulescens* and elevated Cd-accumulation in the Ganges vs. Prayon ecotype

HMA3 is much stronger expressed in T.c. Ganges

HMA3 is localised in the vacuolar membrane
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
Other mechanisms of metal resistance
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
Kompartimentierung von Metallen in Blättern
Korrelation zwischen Metallkonzentrationen im Mesophyll von Arabidospis halleri

Küpper H, Lombi E, Zhao FJ, McGrath SP (2000) Planta 212, 75-84
## Summary

<table>
<thead>
<tr>
<th>Low trace metal content in soil</th>
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### Reviews:

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