



How plants cope with metal stress, from exclusion to hyperaccumulation

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Headlines:

- nutrient and non-nutrient metal elements
- *metal stress*: the “mechanisms of action” of metal ions into the plant cell
- strategy enacted by plants to counteract metal stress
- a particular adaptation to metal-rich soils: *hyperaccumulators*

Response to cadmium in higher plants

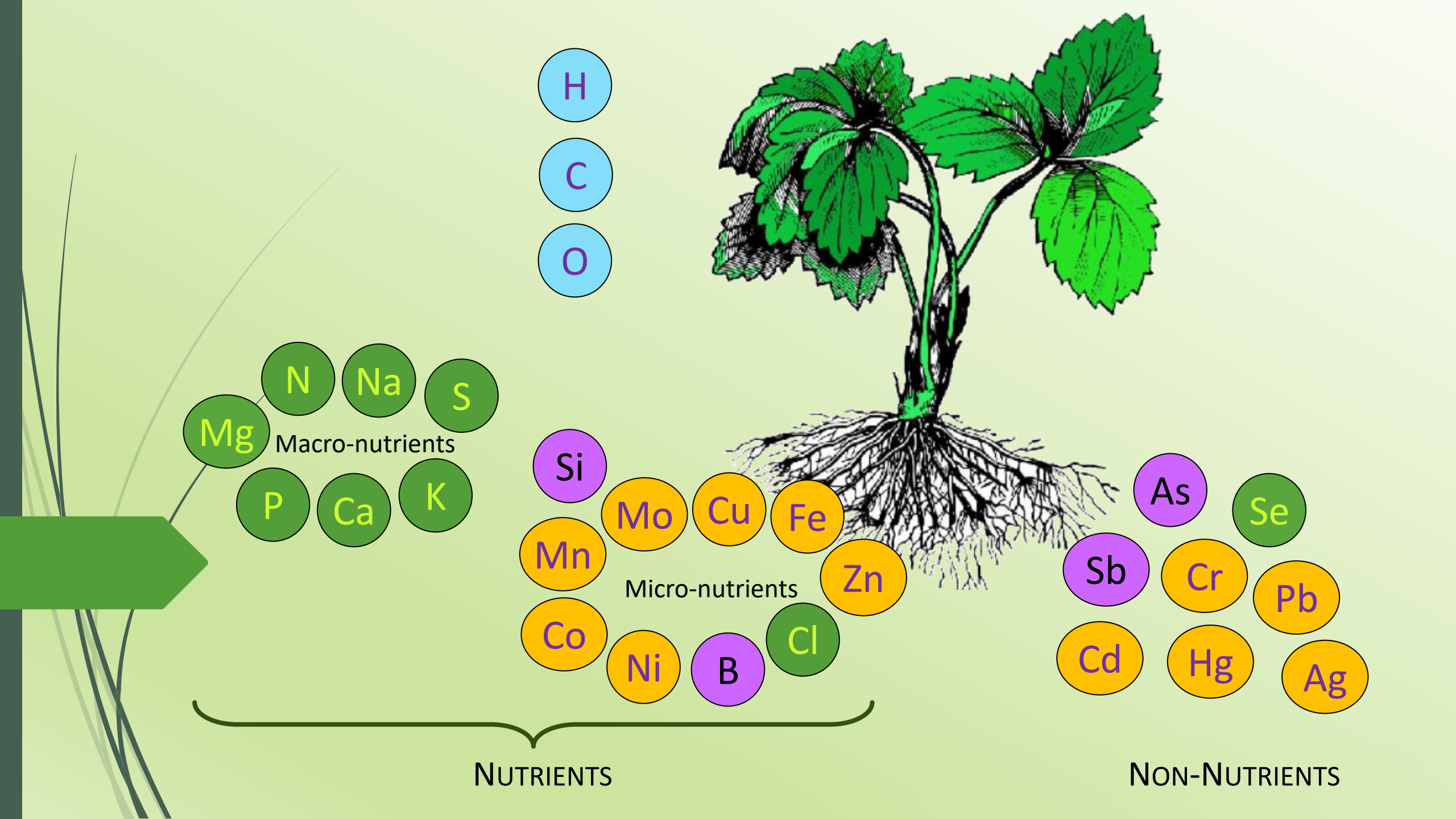
L. Sanità di Toppi *, R. Gabbrielli

1. Introduction

Heavy metals—a group of metals with density higher than 5.0 g cm^{-3} —such as cadmium (Cd), chromium (Cr), mercury (Hg), lead (Pb), aluminium (Al), silver (Ag), tin (Sn) etc., are impor-

But, to include also the metalloids **As** (d: 5.73) or **Sb** (d: 6.69), and nonmetal **Se** (d 4.79) we prefer the terms

- *Trace elements*
- *Potentially toxic elements*



H

C

O

N Na S
Mg Macro-nutrients
P Ca K

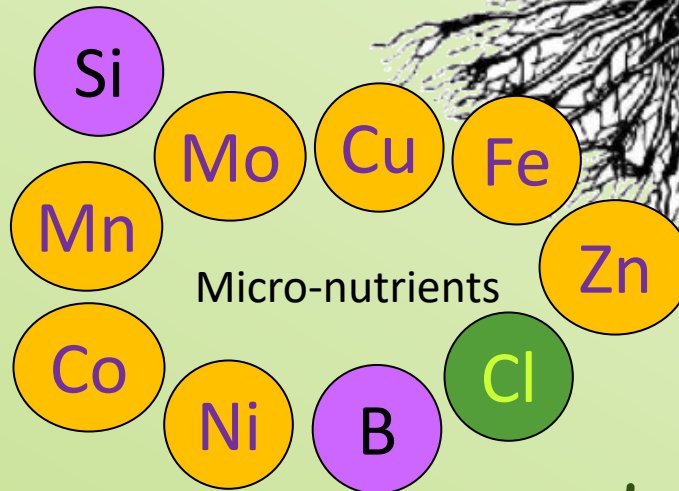
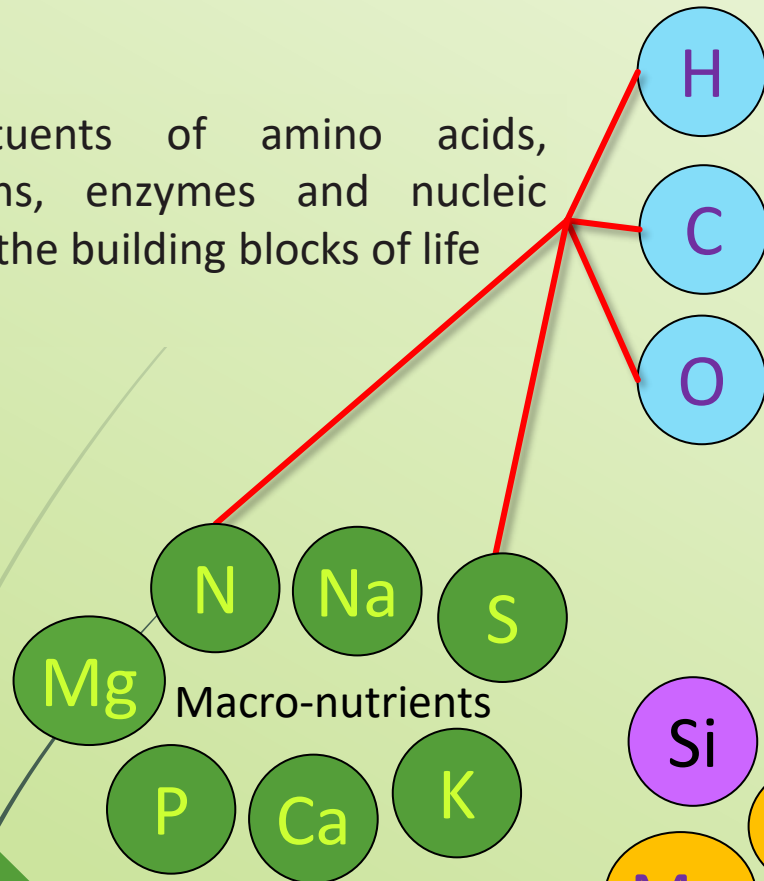
Si
Mn Mo Cu Fe
Micro-nutrients
Co Ni B Cl Zn

As Se
Sb Cr Pb
Cd Hg Ag

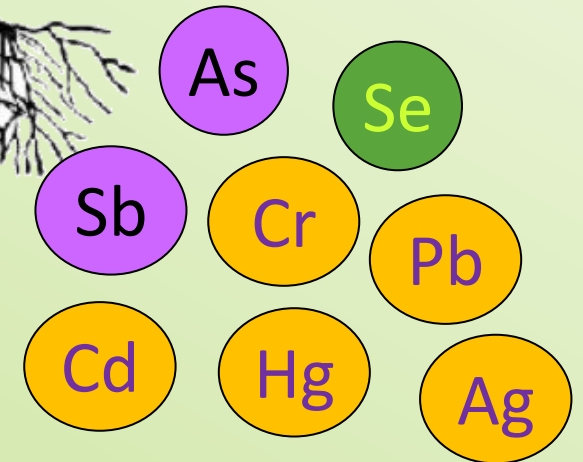
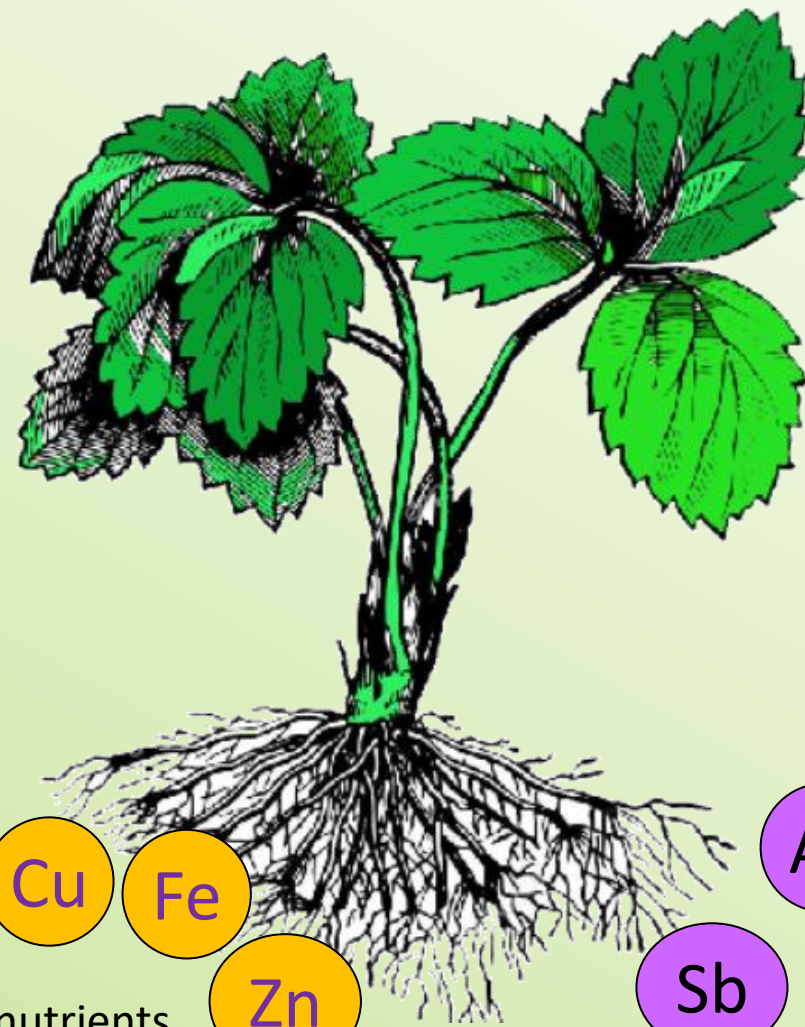
NUTRIENTS

NON-NUTRIENTS

constituents of amino acids,
proteins, enzymes and nucleic
acids, the building blocks of life



NUTRIENTS



NON-NUTRIENTS

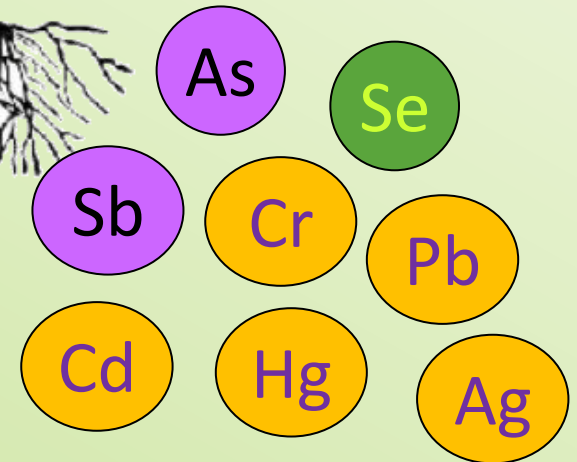
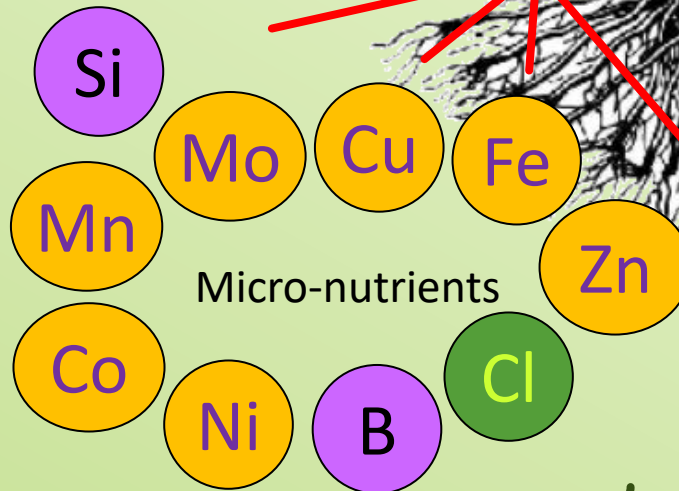
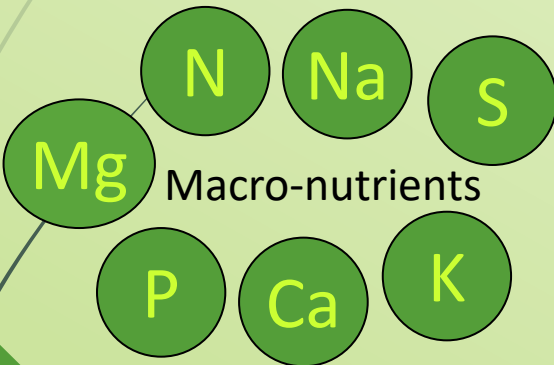
H

C

O



In chelated form in prosthetic groups of enzymes.
Some enable electron transport by valency change.

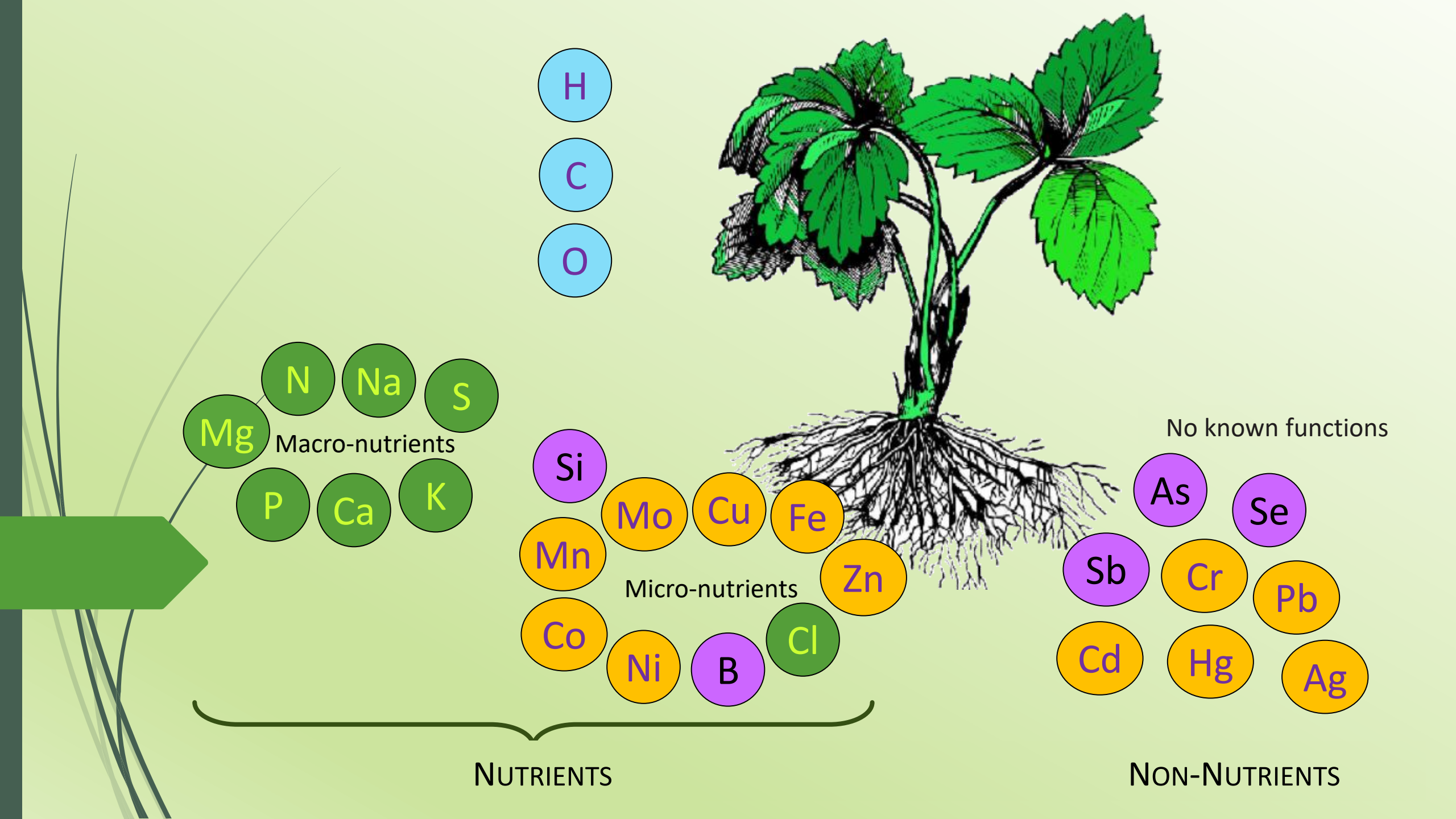


NUTRIENTS

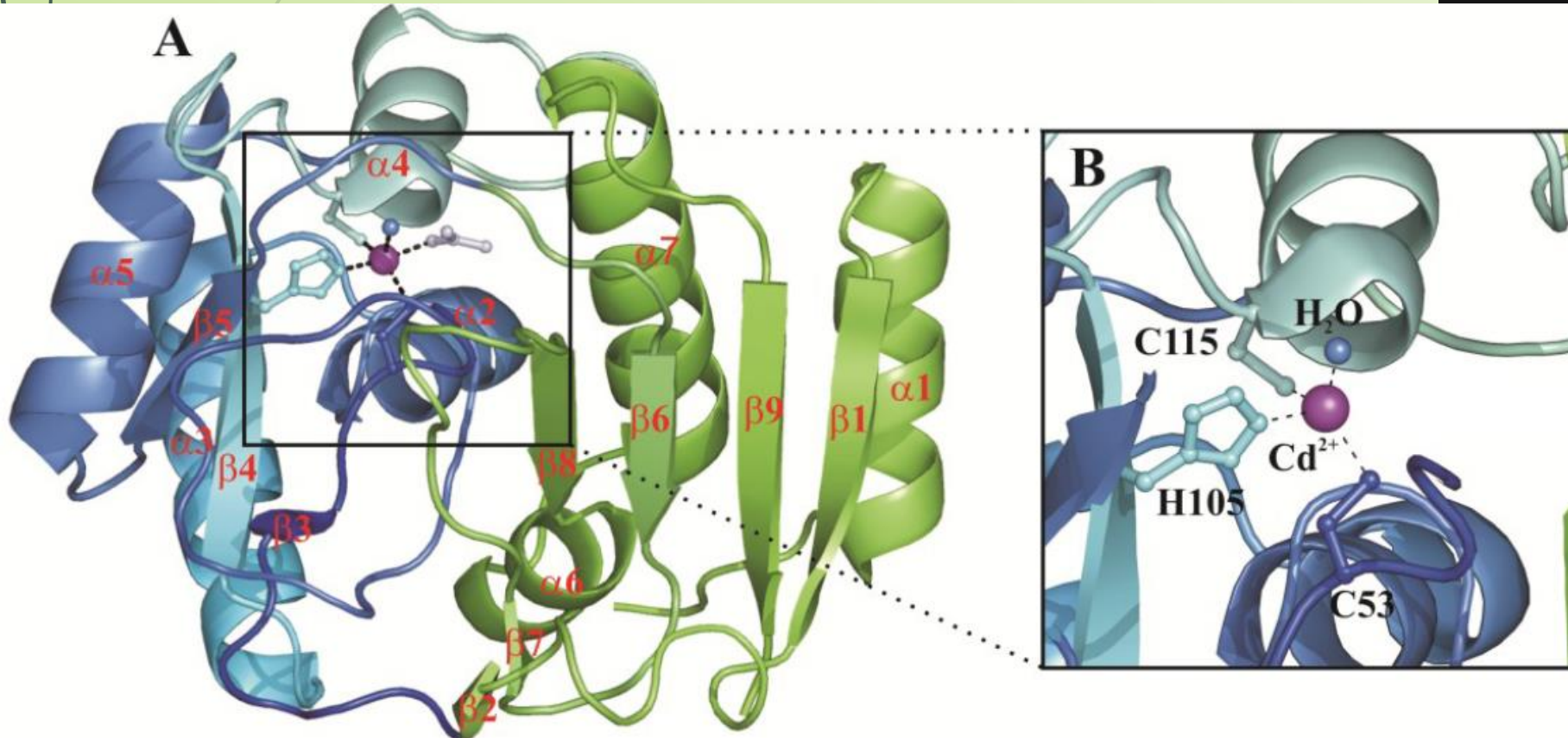
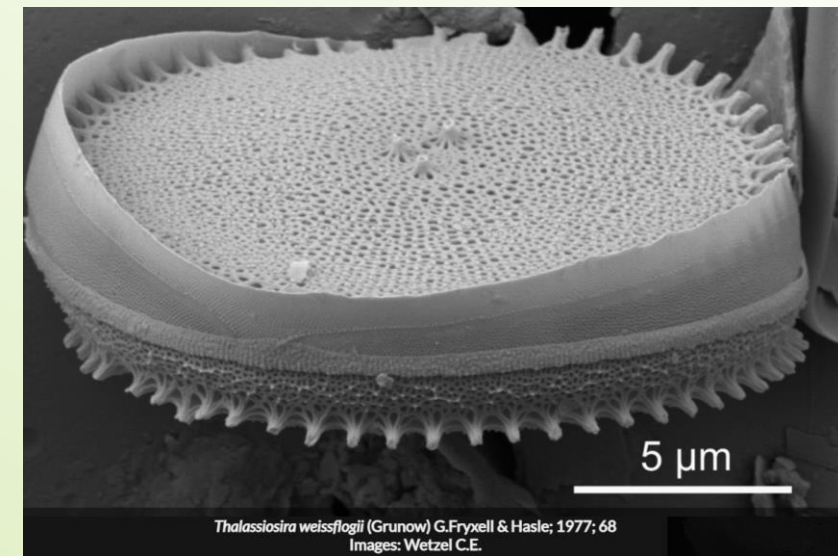
NON-NUTRIENTS

HMs

Element	Source	Role in the Plant
Carbon (C)	Air	Constituent of carbohydrates; necessary for photosynthesis.
Oxygen (O)	Air/Water	Constituent of carbohydrates: necessary for respiration.
Hydrogen (H)	Water	Maintains osmotic balance; important in many biochemical reactions, constituent of carbohydrates.
Nitrogen (N)	Air/Soil	Constituent of amino acids, proteins, chlorophyll, and nucleic acids.
Potassium (K)	Soil	Involved with photosynthesis, carbohydrates translocation, protein synthesis.
Phosphorous (P)	Soil	Constituent of proteins, coenzymes, nucleic acids, and metabolic substrates; important in energy transfer.
Magnesium (Mg)	Soil	Enzyme activator; component of chlorophyll.
Sulfur (S)	Soil	Component of certain amino acids and plant proteins.
Chlorine (Cl)	Soil	Involved with oxygen production and photosynthesis.
Iron (Fe)	Soil	Involved with chlorophyll synthesis and in enzymes electron transfer.
Manganese (Mn)	Soil	Controls several oxidation-reduction systems and photosynthesis.
Boron (B)	Soil	Important in sugar translocation and carbohydrates metabolism.
Zinc (Zn)	Soil	Involved with enzymes that regulate various enzymes.
Copper (Cu)	Soil	Catalyst for respiration; component of various enzymes.
Molybdenum (Mo)	Soil	Involved with nitrogen fixation and transforming nitrate to ammonium.
Nickel (Ni)	Soil	Necessary for proper functioning of urease and seed germination.



Cd: *Thalassiosira weissflogii*, where it builds up
the active center of a carbonic anhydrase



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doi:10.3390/md13041688

The concentration of metal nutrient elements is tightly controlled

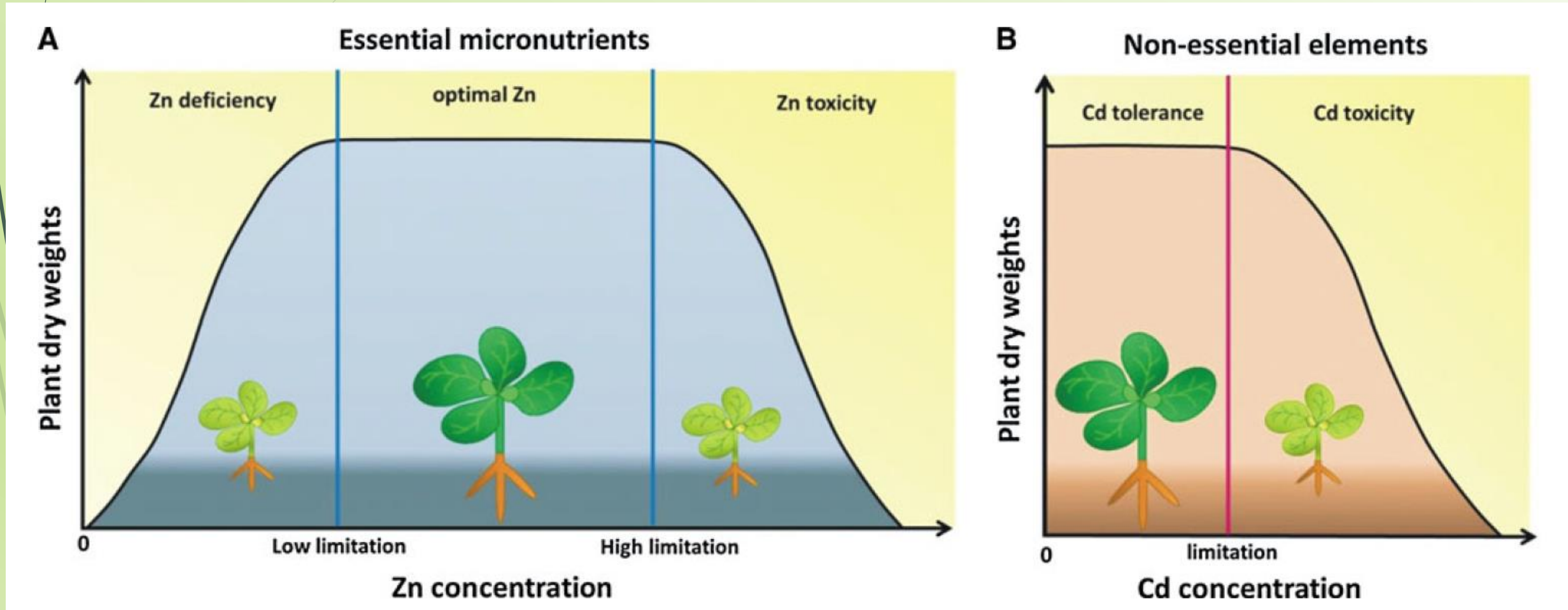
the toxicity of metals to plants depends on the external bioavailable metal concentration, the exposure time, the plant genotype, and the general condition of the plant.

TABLE 1.3 Average concentrations of mineral elements in plant shoot dry matter sufficient for adequate growth

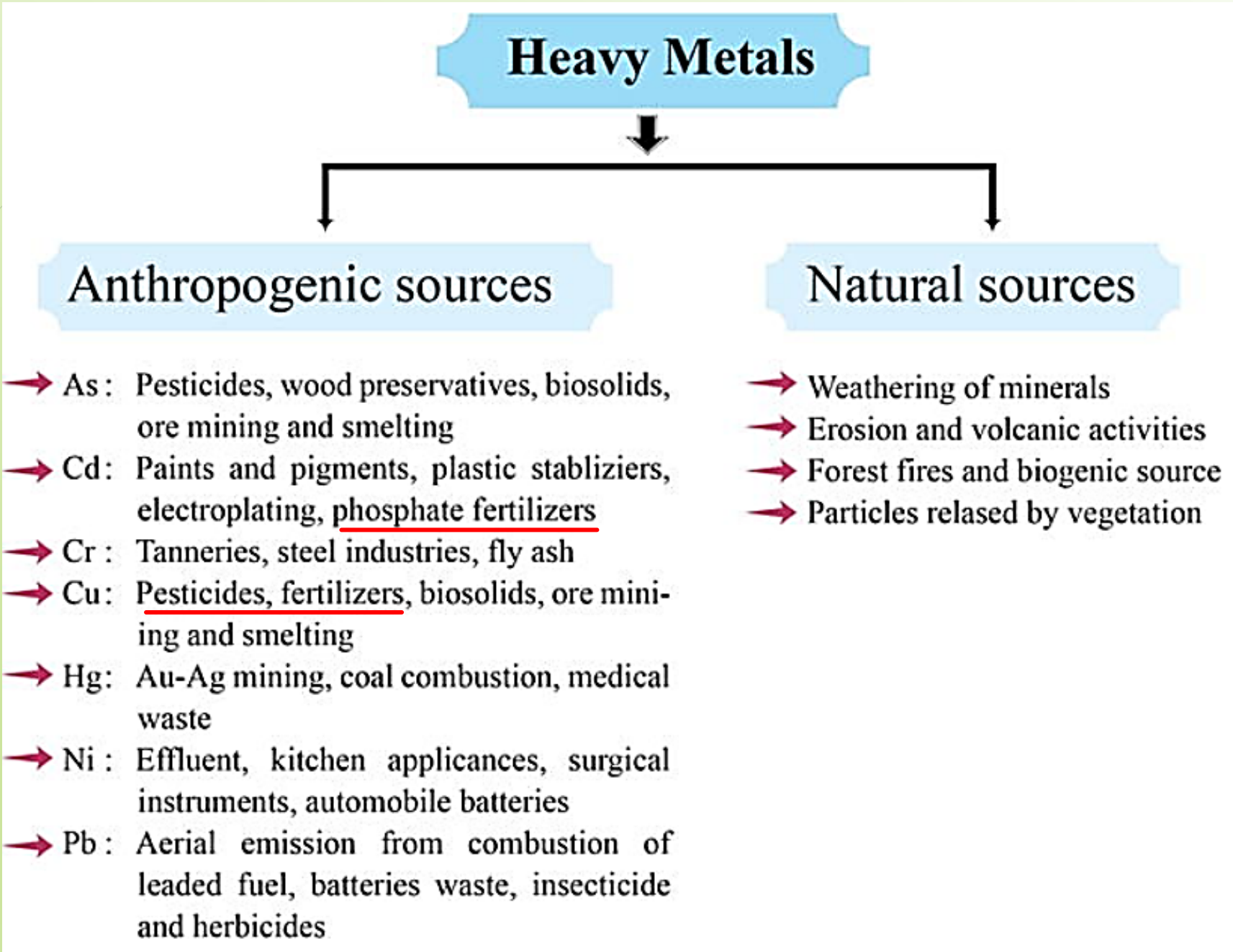
Element	Chemical symbol	$\mu\text{mol g}^{-1} \text{ dw}$	mg kg^{-1}
Molybdenum	Mo	0.001	0.1
Nickel	Ni	0.001	0.1
Copper	Cu	0.1	6
Zinc	Zn	0.3	20
Manganese	Mn	1.0	50
Iron	Fe	2.0	100
Boron	B	2.0	20
Chlorine	Cl	3.0	100
Sulphur	S	30	1,000
Phosphorus	P	60	2,000
Magnesium	Mg	80	2,000
Calcium	Ca	125	5,000
Potassium	K	250	10,000
Nitrogen	N	1,000	15,000

From Epstein (1965), Epstein and Bloom (2005), Brown *et al.* (1987b).

Dose–response curves to essential elements and non-essential elements are different



Heavy Metals



```
graph TD; A[Heavy Metals] --> B[Anthropogenic sources]; A --> C[Natural sources]; B --> B1[As: Pesticides, wood preservatives, biosolids, ore mining and smelting]; B --> B2[Cd: Paints and pigments, plastic stabilizers, electroplating, phosphate fertilizers]; B --> B3[Cr: Tanneries, steel industries, fly ash]; B --> B4[Cu: Pesticides, fertilizers, biosolids, ore mining and smelting]; B --> B5[Hg: Au-Ag mining, coal combustion, medical waste]; B --> B6[Ni: Effluent, kitchen appliances, surgical instruments, automobile batteries]; B --> B7[Pb: Aerial emission from combustion of leaded fuel, batteries waste, insecticide and herbicides]; C --> C1[Weathering of minerals]; C --> C2[Erosion and volcanic activities]; C --> C3[Forest fires and biogenic source]; C --> C4[Particles released by vegetation];
```

Anthropogenic sources

- As: Pesticides, wood preservatives, biosolids, ore mining and smelting
- Cd: Paints and pigments, plastic stabilizers, electroplating, phosphate fertilizers
- Cr: Tanneries, steel industries, fly ash
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- Hg: Au-Ag mining, coal combustion, medical waste
- Ni: Effluent, kitchen appliances, surgical instruments, automobile batteries
- Pb: Aerial emission from combustion of leaded fuel, batteries waste, insecticide and herbicides

Natural sources

- Weathering of minerals
- Erosion and volcanic activities
- Forest fires and biogenic source
- Particles released by vegetation

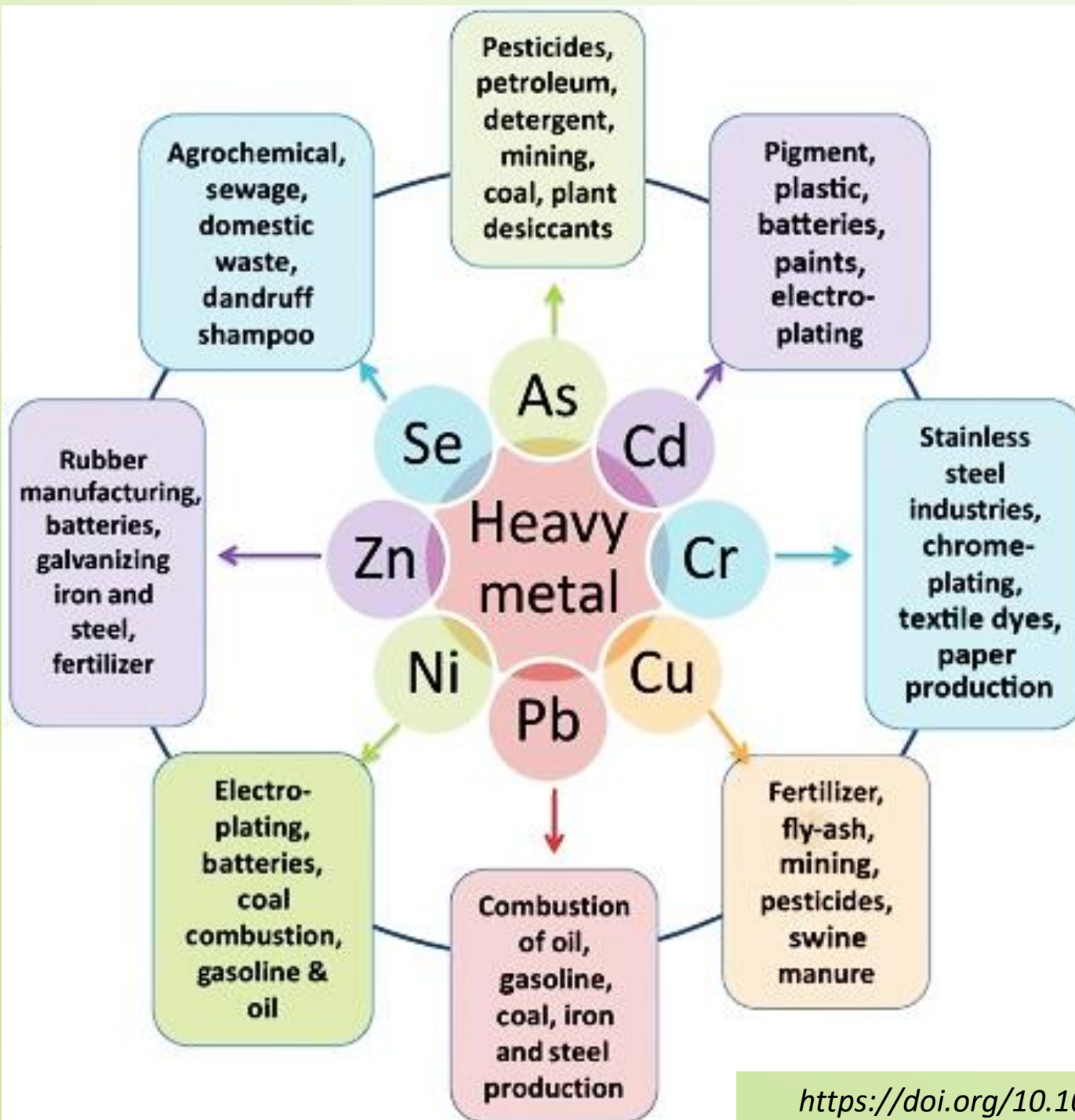
**NATURALLY
CONTAMINATED
SOILS**

SERPENTINE SOILS → derived from Fe- and Mg-rich ultramafic rocks that are enriched with Cr, Co and Ni

SELENIFEROUS SOILS → derived from Se-rich rock types

CALAMINE SOILS → enriched with Zn, Cd and Pb

Co- CU-CONTAINING SOILS → derived from argillites and dolomites



Soil contamination is still a concern!

Table 1 Average metal concentrations in mg kg⁻¹ in urban soils, contaminated areas and guide values for soil-clean ups

City/region	Cd	Cu	Fe	Hg	Ni	Zn	Ref.
Pittsburgh, USA	1.2	—	—	0.51	—	—	Carey 1980 ²³³
La Coruña, Spain	0.3	60	—	—	28	206	Cal-Prieto 2001 ²³⁴
Madrid, Spain	—	71.7	—	—	141	210	De Miguel 1998 ²³⁵
Aberdeen, Scotland (parkland soils)	—	27	18 469	—	14.9	58.4	Paterson 1996 ²³⁶
Aberdeen, Scotland (roadside soils)	—	44.6	18 116	—	15.9	113	Paterson 1996 ²³⁶
Hong Kong	2.18	24.8	—	—	—	168	Li 2001 ²³⁷
Palermo, Italy	0.82	75.5	—	1.85	18.8	149	Manta 2002 ²³⁸
Montreal Island, Canada, historic industry area, 3 rail yards	2.3–7.3	160–245	—	—	64–98	410–547	Ge 2000 ²³⁹
Baltimore, USA, historic industry area	1.06	45	23 495	—	27	141	Yesilonis 2008 ²⁴⁰
Guizhou, China (mining area) at smelter	60.5	202	—	—	24.1	2551	Li 2007 ²⁴¹
Guizhou, China (mining area) 15 km away	5.1	72.6	—	—	9.9	867	Li 2007 ²⁴¹
Sicily, Italy, unpolluted	1.3	34	—	0.066	—	122	Modified from Manta 2001 ²³⁸

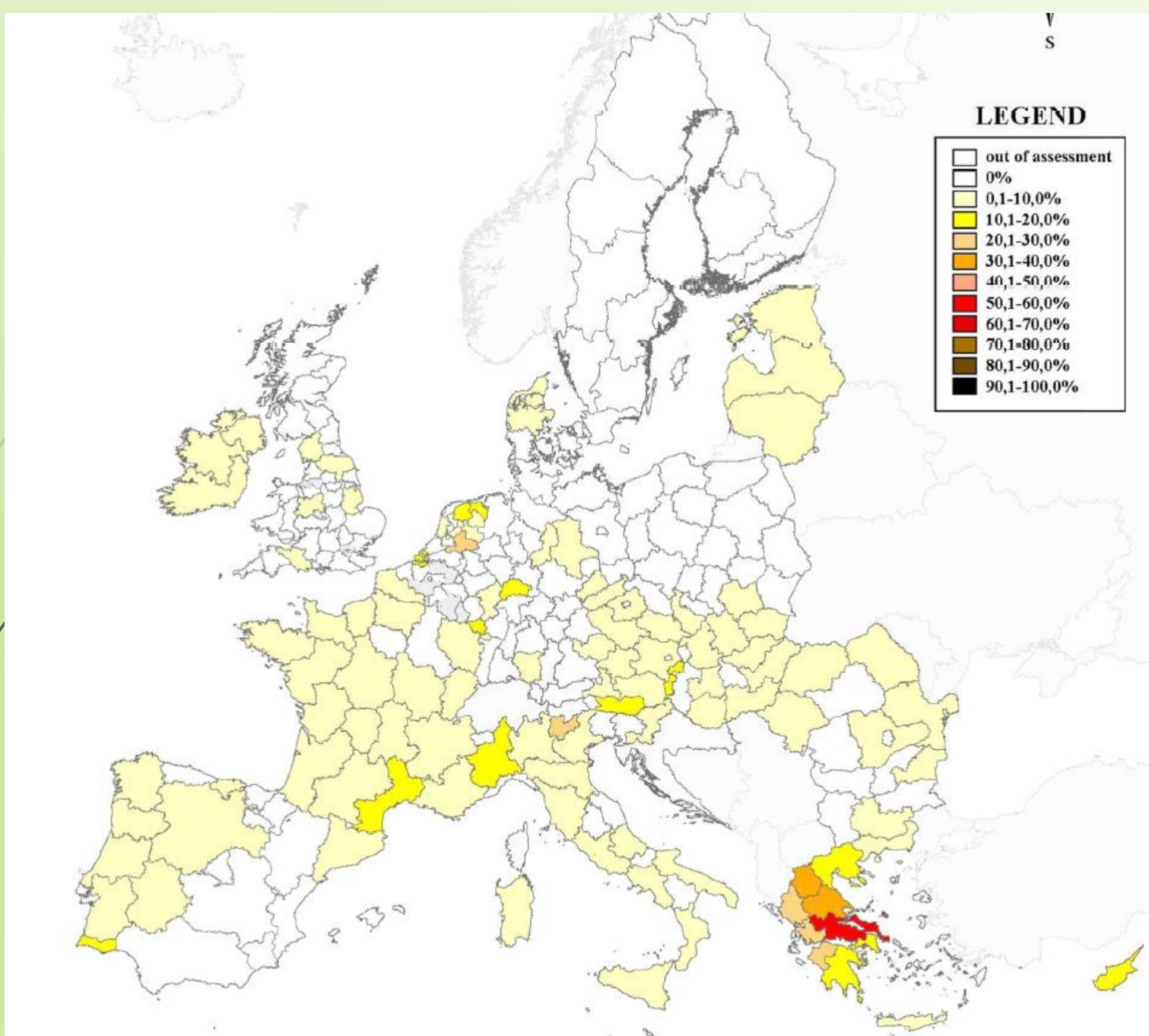


The LUCAS Topsoil Survey, with its 1 site/200 km² sampling density opened new prospect in this regard. The survey represents the first effort to build a consistent spatial database of soil properties for environmental assessments ranging from regional to continental scale on all major land use types across Europe (Tóth et al., 2013). As the inputs of

SUMMARY

In 2009, the European Commission extended the periodic Land Use/Land Cover Area Frame Survey (LUCAS) to sample and analyse the main properties of topsoil in 23 Member States of the European Union (EU). This topsoil survey represents the first attempt to build a consistent spatial database of the soil cover across the EU based on standard sampling and analytical procedures, with the analysis of all soil samples being carried out in a single laboratory.

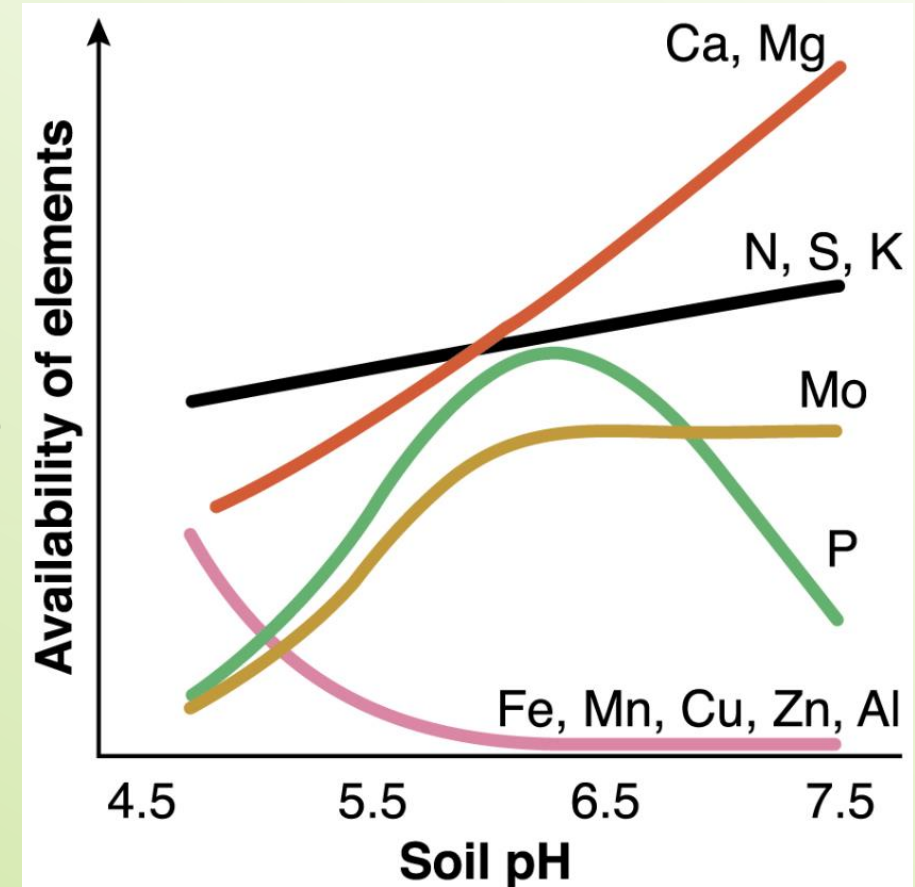
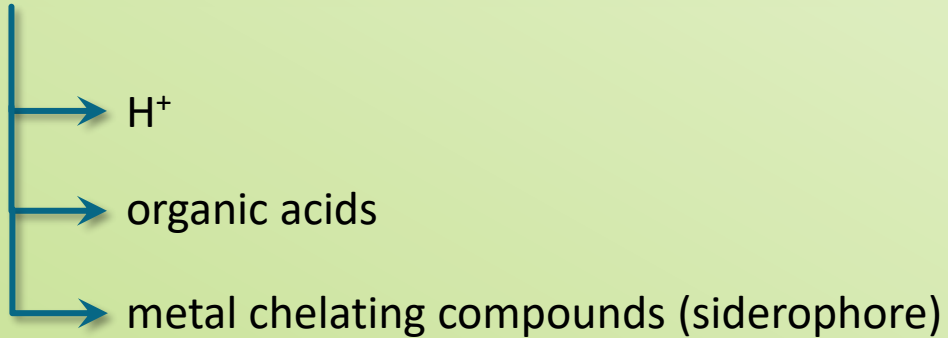
Approximately 20,000 points were selected out of the main LUCAS grid for the collection of soil samples. A standardised sampling procedure was used to collect around 0.5 kg of topsoil (0-20 cm). The samples were dispatched to a central laboratory for physical and chemical analyses.



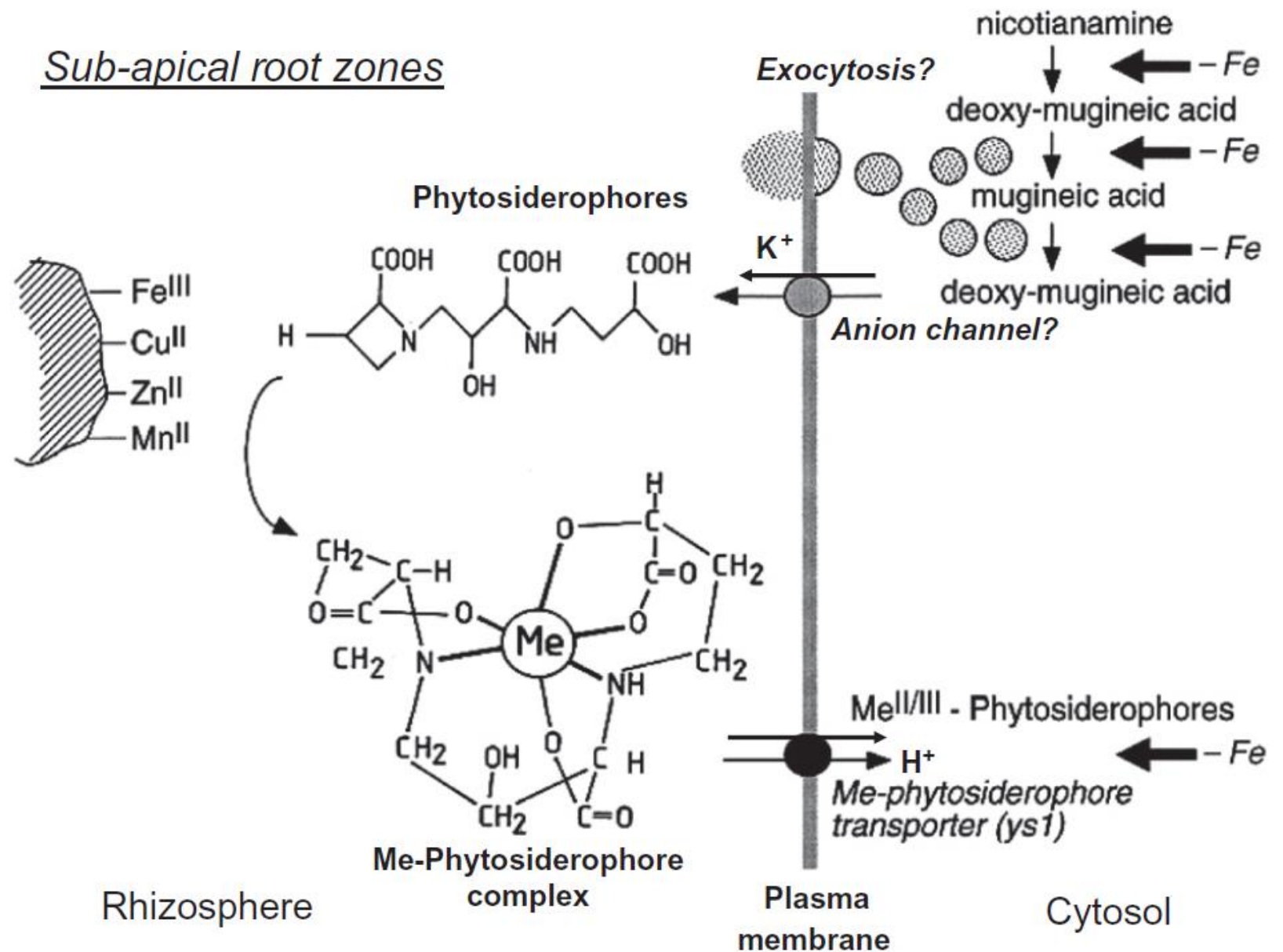
Samples from *agricultural* land that contain heavy metals in concentration which would require remediation also if these were from *industrial* or *transport* areas!!

Metal ion availability in soils is variable

- pH and soil structure and composition
- Substances present in soil solutions may act as *extractants*
- Metabolites released by plant roots and bacteria of the rhizosphere affect metal solubility and bioavailability.

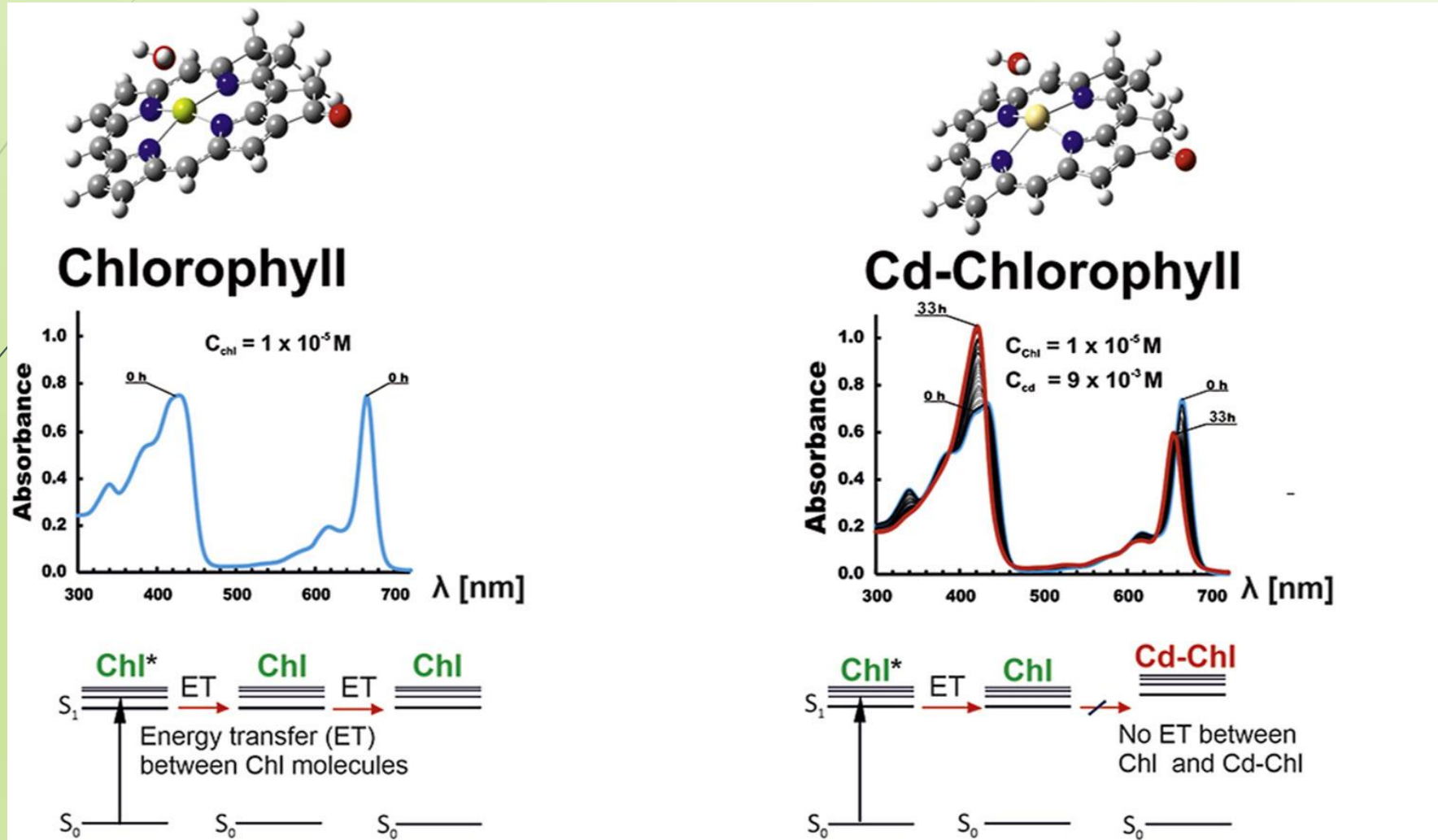


Model for root-induced mobilization of Fe and other micronutrients (Zn, Mn, Cu) in the rhizosphere of graminaceous (Strategy II) plants, mediated by release of phytosiderophores and uptake of phytosiderophores-metal complexes.



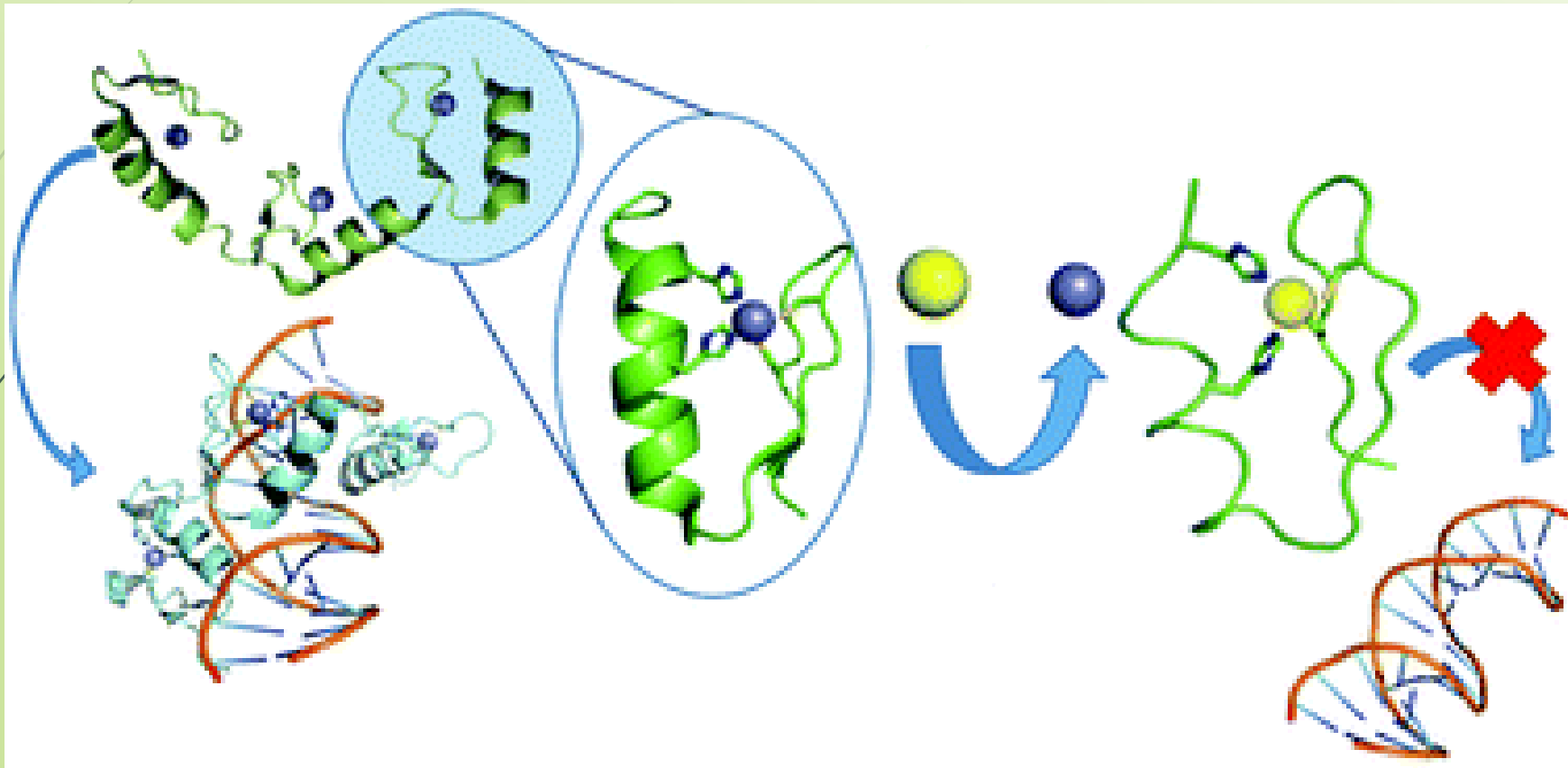
HM toxicity in plants

- 1) Displacement of essential ions in chlorophyll, enzymes and signaling proteins.



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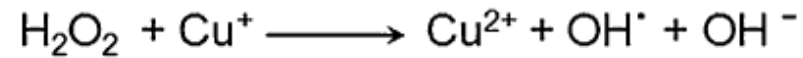
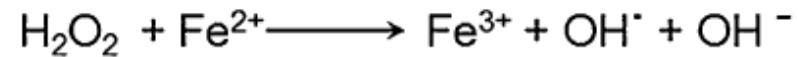
ROS: hydroxyl radical $\text{OH}^\bullet$

superoxide anion $\text{O}_2^{\bullet-}$

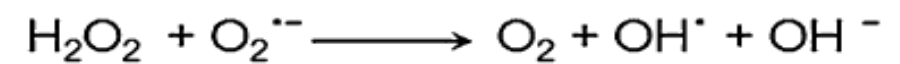
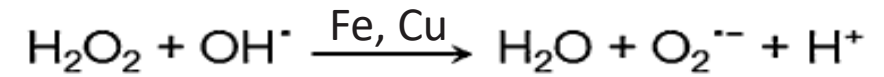
hydrogen peroxide H_2O_2

2) Generation of ROS and induction of oxidative stress

Fenton Reaction

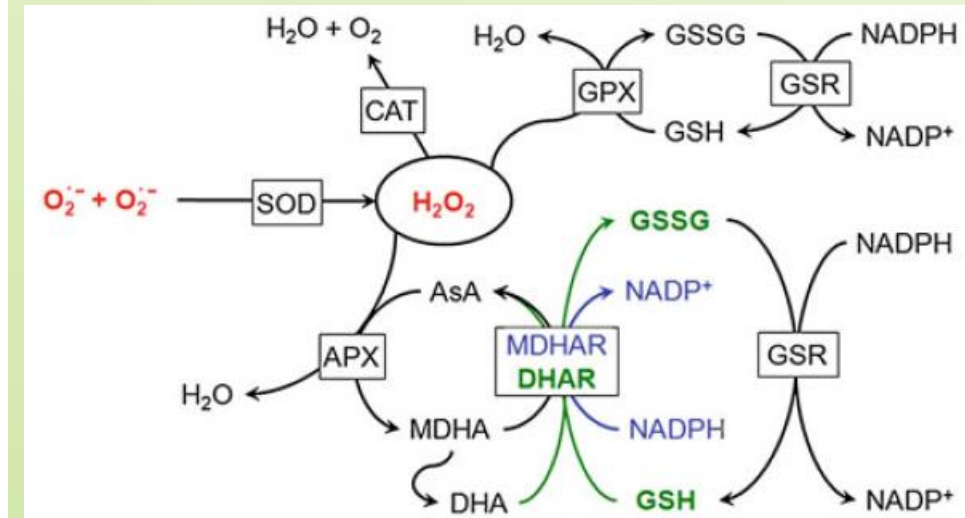
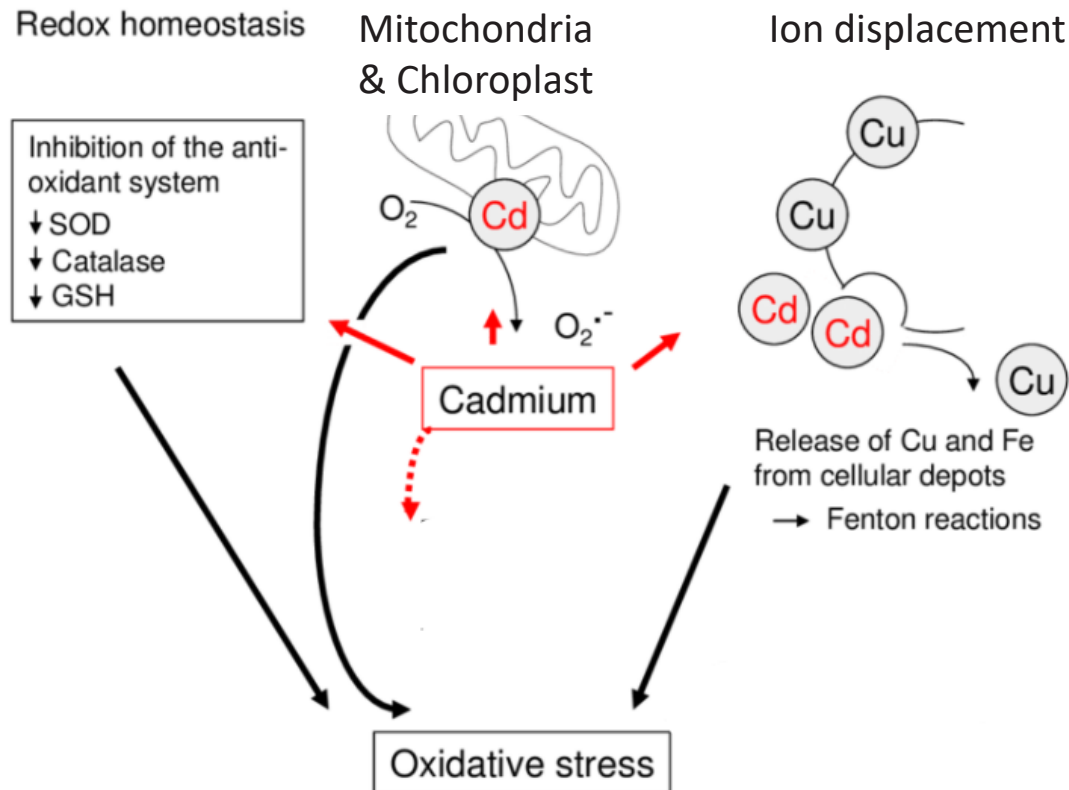


Haber-Weiss cycle



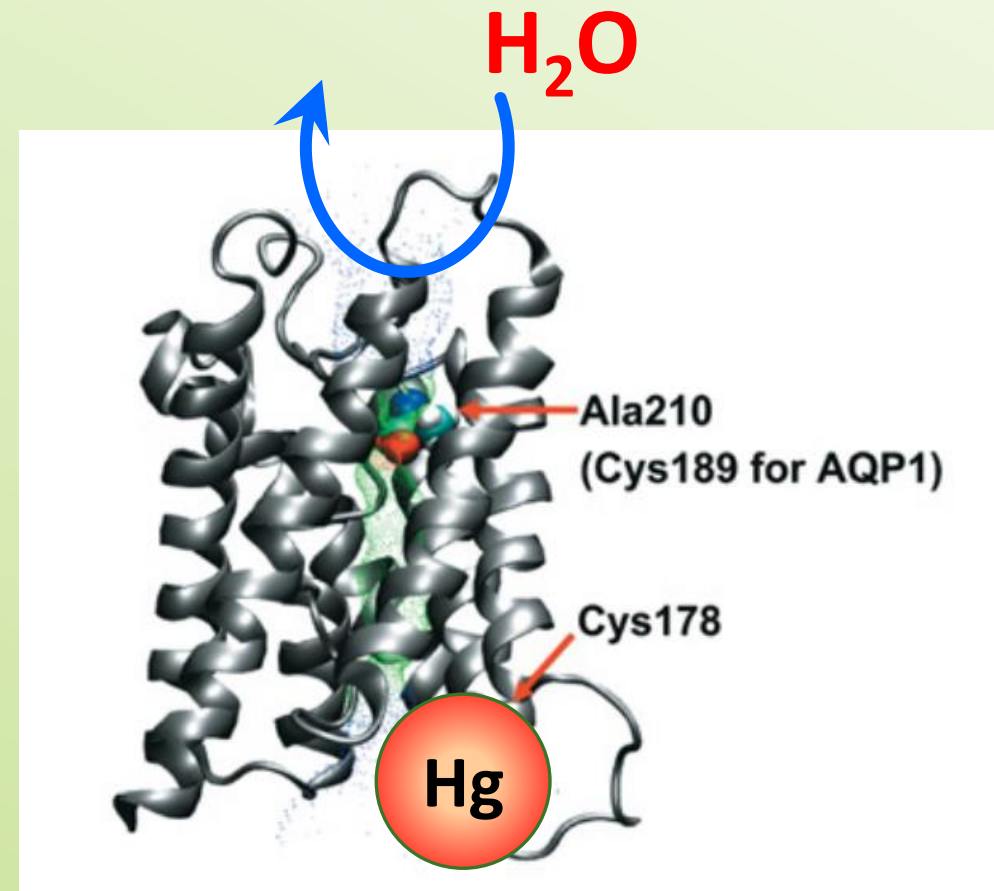
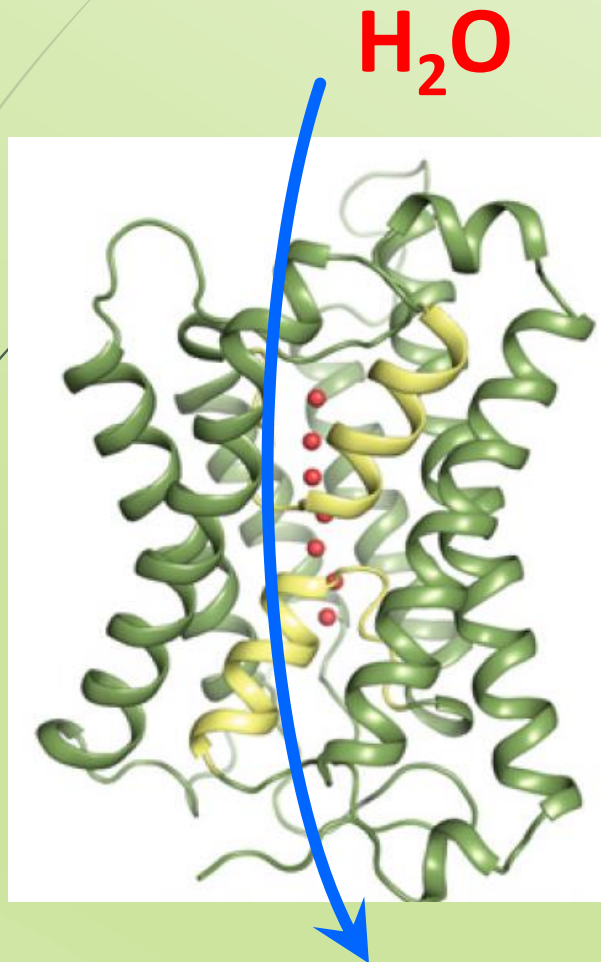
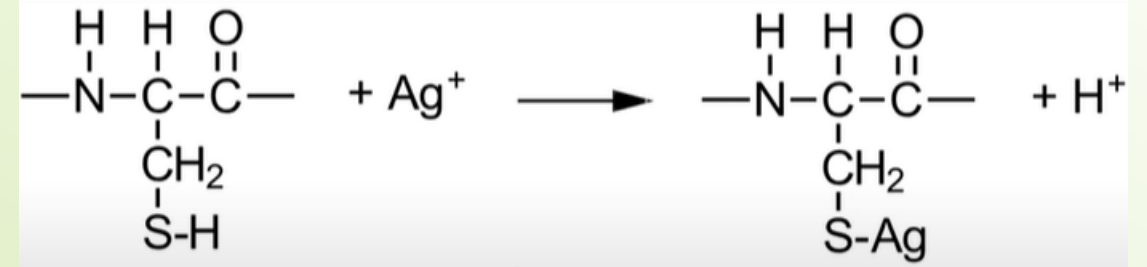
directly

indirectly



HM toxicity in plants

3) Reaction with sulfhydryl groups (–SH).

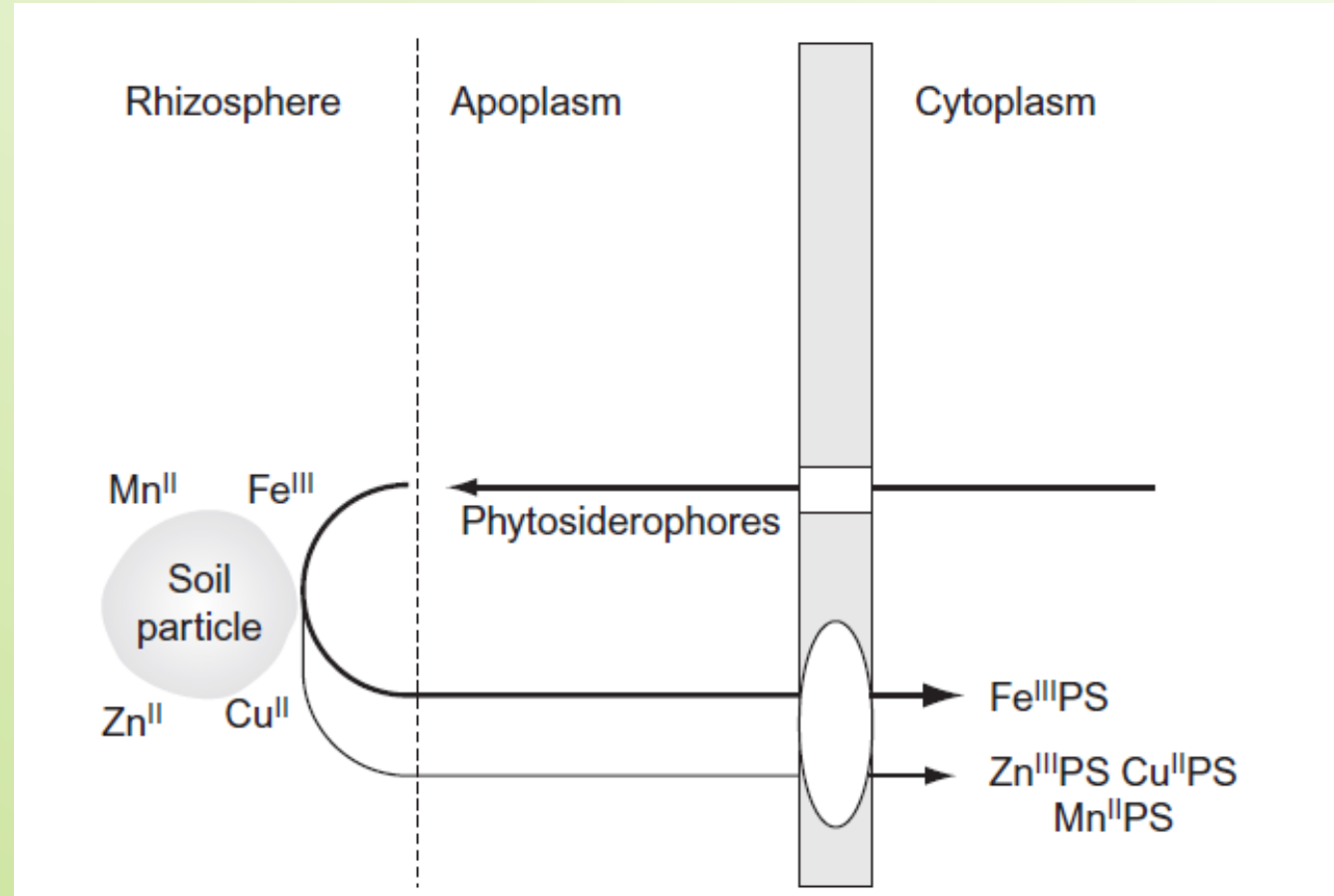


Es: decrease activity of aquaporins

HM toxicity in plants

4) Similarity with nutrient cations and oxyanions, and competition for root absorption

Elements that resemble essential minerals, such as Hg, Pb, and Cd, which are more likely to enter the cell through the existing mineral uptake machinery



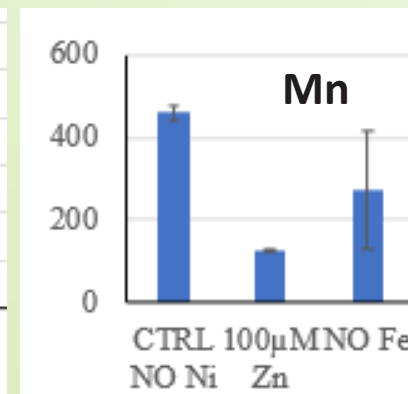
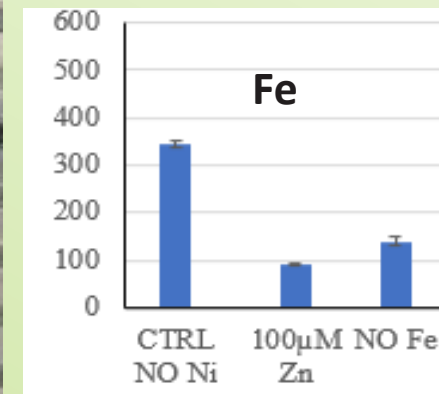
Zn toxicity leads to chlorosis in young leaves. This may be an induced deficiency of Fe, because of the similar ion radius of Zn and Fe. Induced Mn deficiency may also be of importance, as high Zn supply strongly decreases the Mn concentration of plants

Control



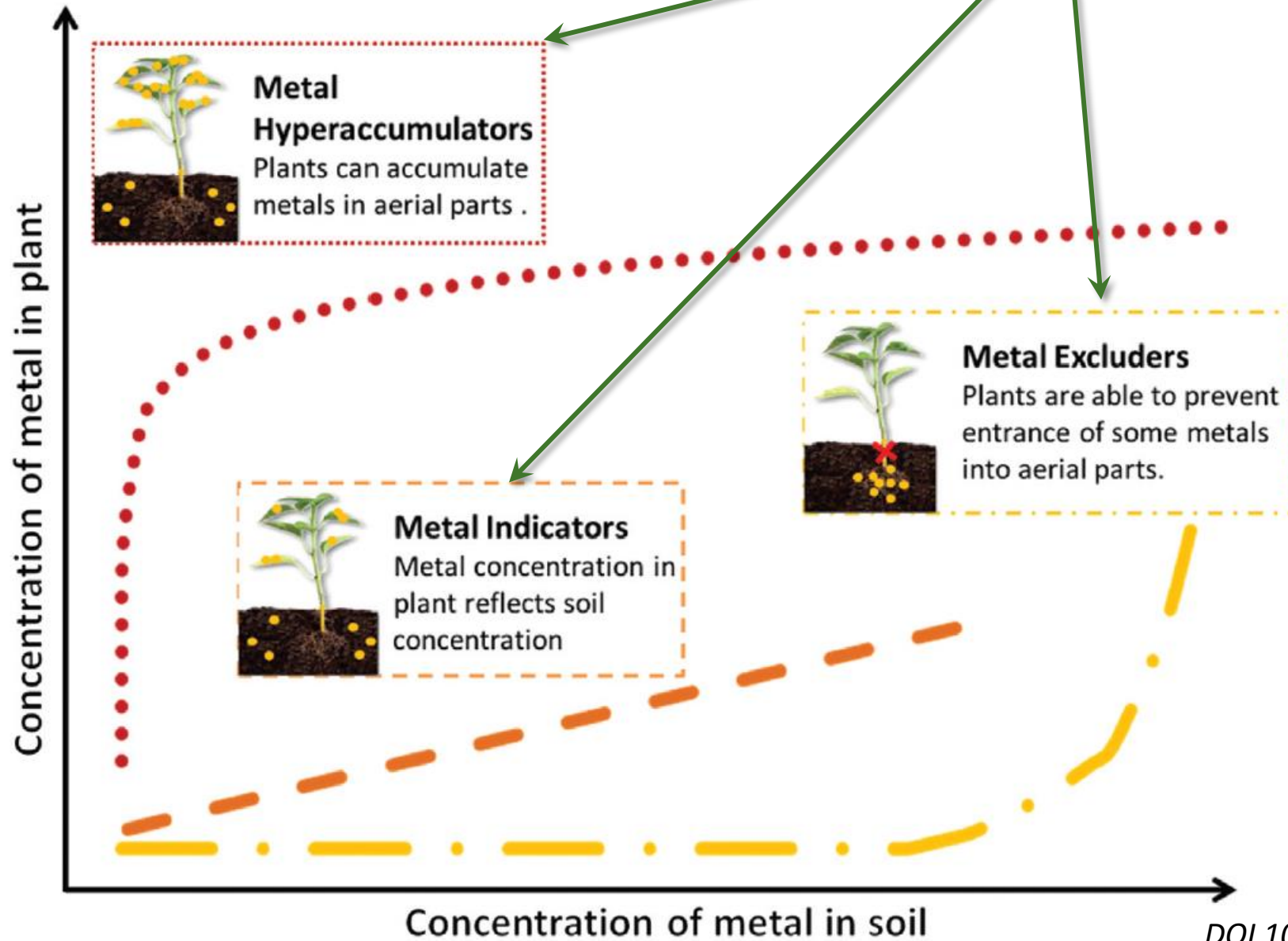
With 100 μ M Zn

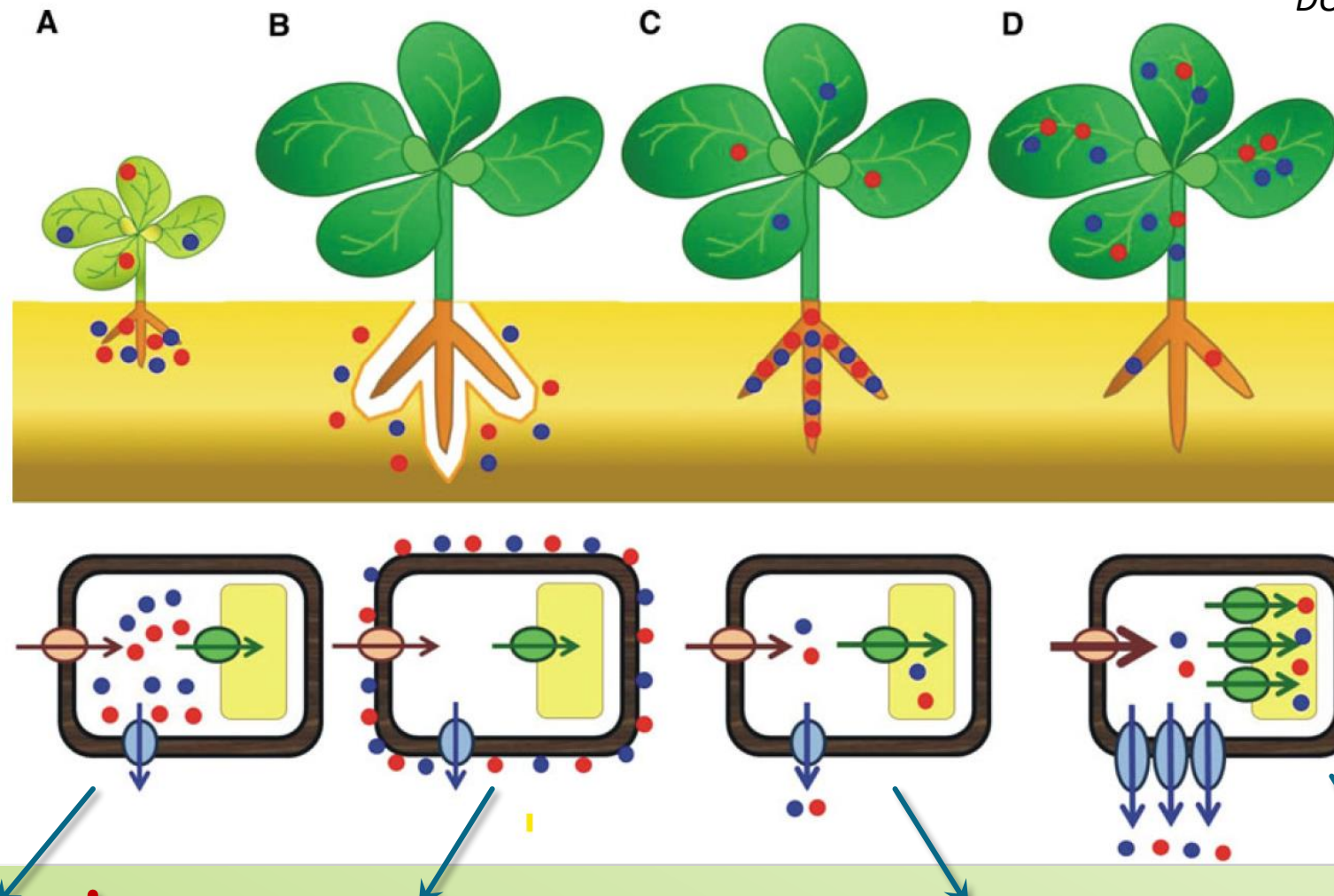
Without Fe



How do plants respond!?

Plants can be





Heavy **metal-sensitive plants** cannot keep metals out of their roots or prevent transport to the shoot

excluder plants are able to keep metals outside the roots or take care of rapid efflux in case toxic metals have entered root cells

tolerant non-hyperaccumulators sequester HMs into root vacuoles, preventing translocation to shoots

hyperaccumulator plants take up HMs through the root and load them into xylem. In the shoot, HMs are safely sequestered in vacuoles

Plant tolerance to metal stress

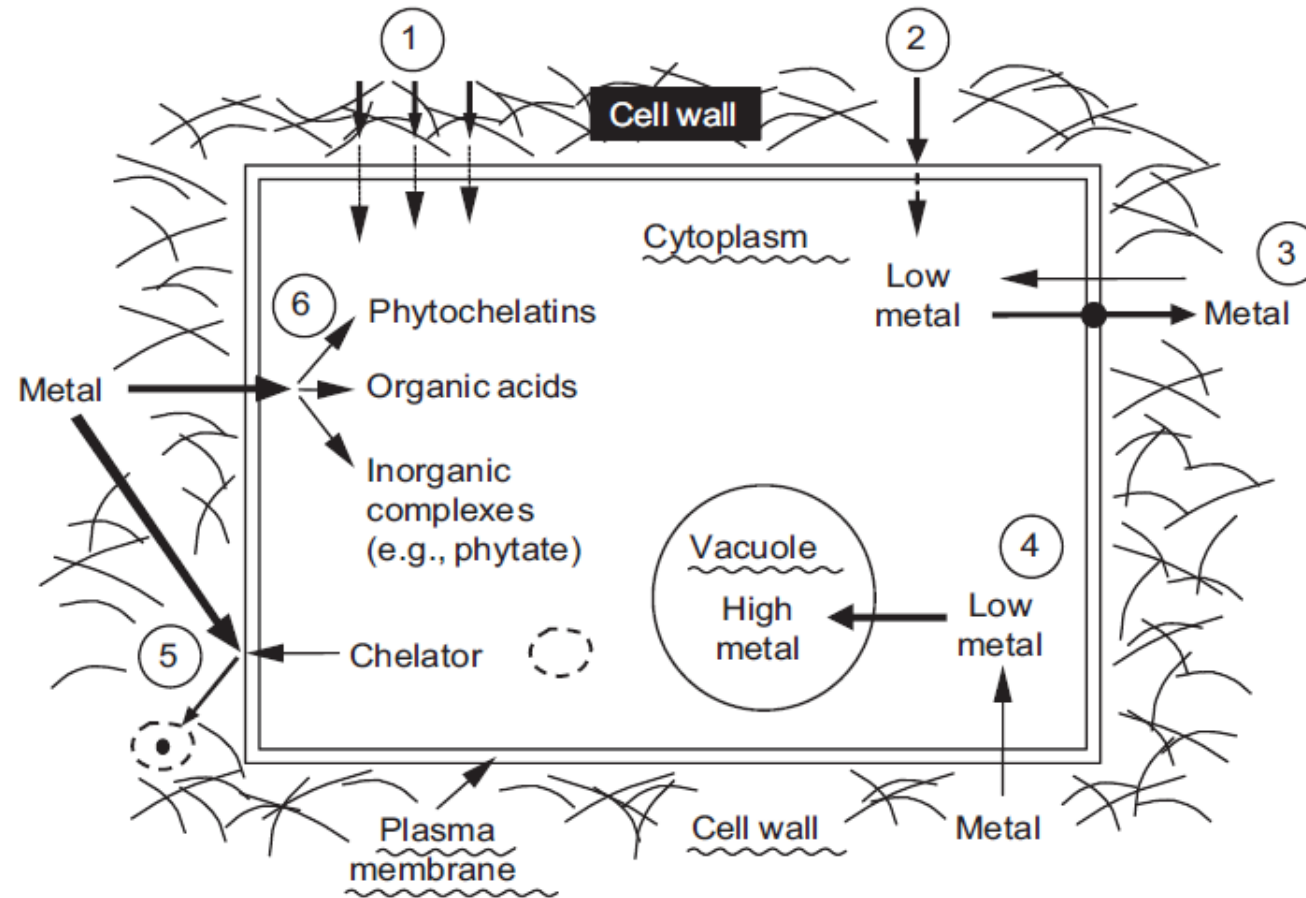


FIGURE 7.14 Possible mechanisms of heavy metal tolerance of plants. (1) Binding to cell wall; (2) restricted influx through plasma membrane; (3) active flux; (4) compartmentation in vacuole; (5) chelation at the cell wall-plasma membrane interface; (6) chelation in the cytoplasm. *Modified from Tomsett and Thurman, 1988.*

Plant tolerance to metal stress

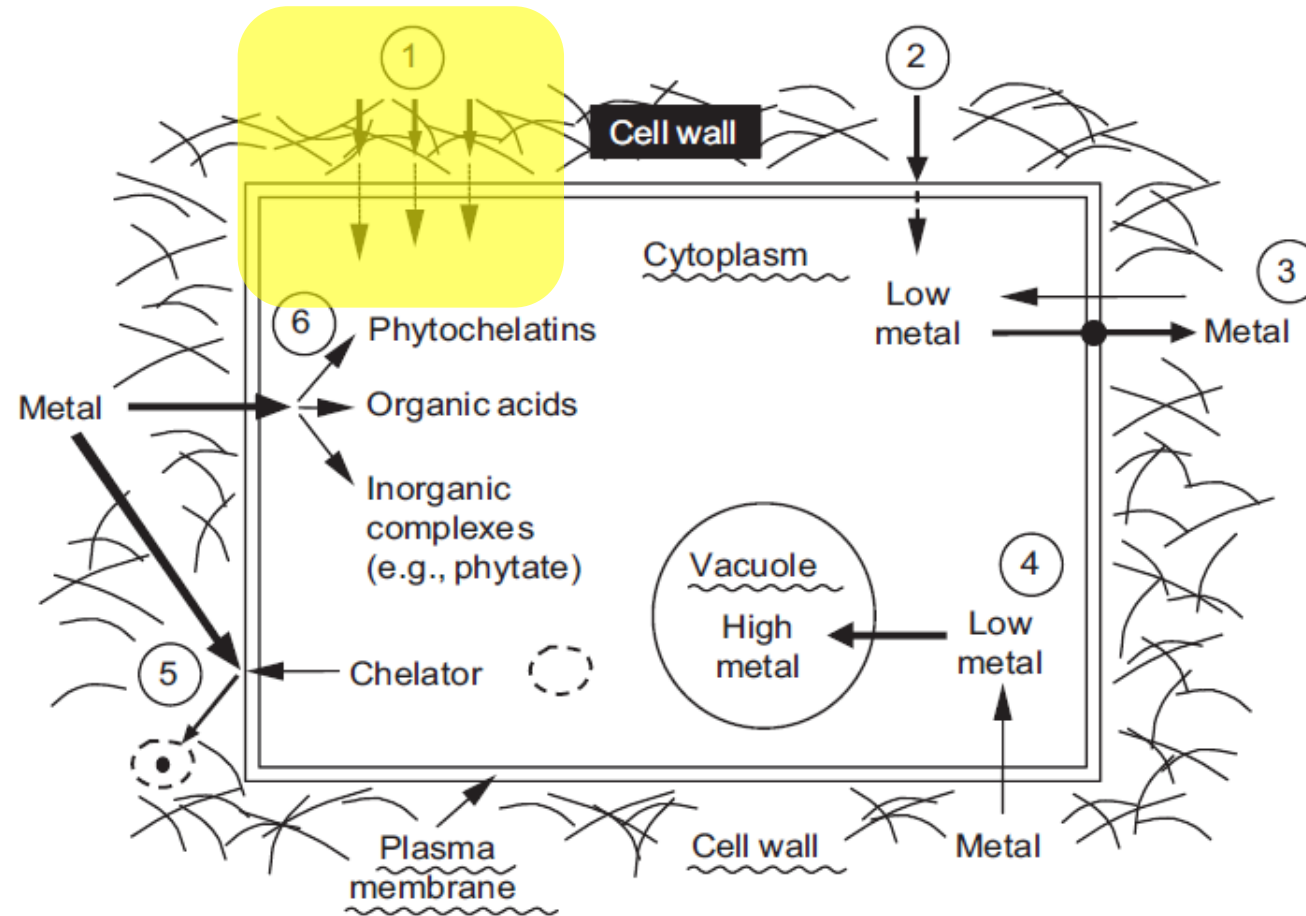
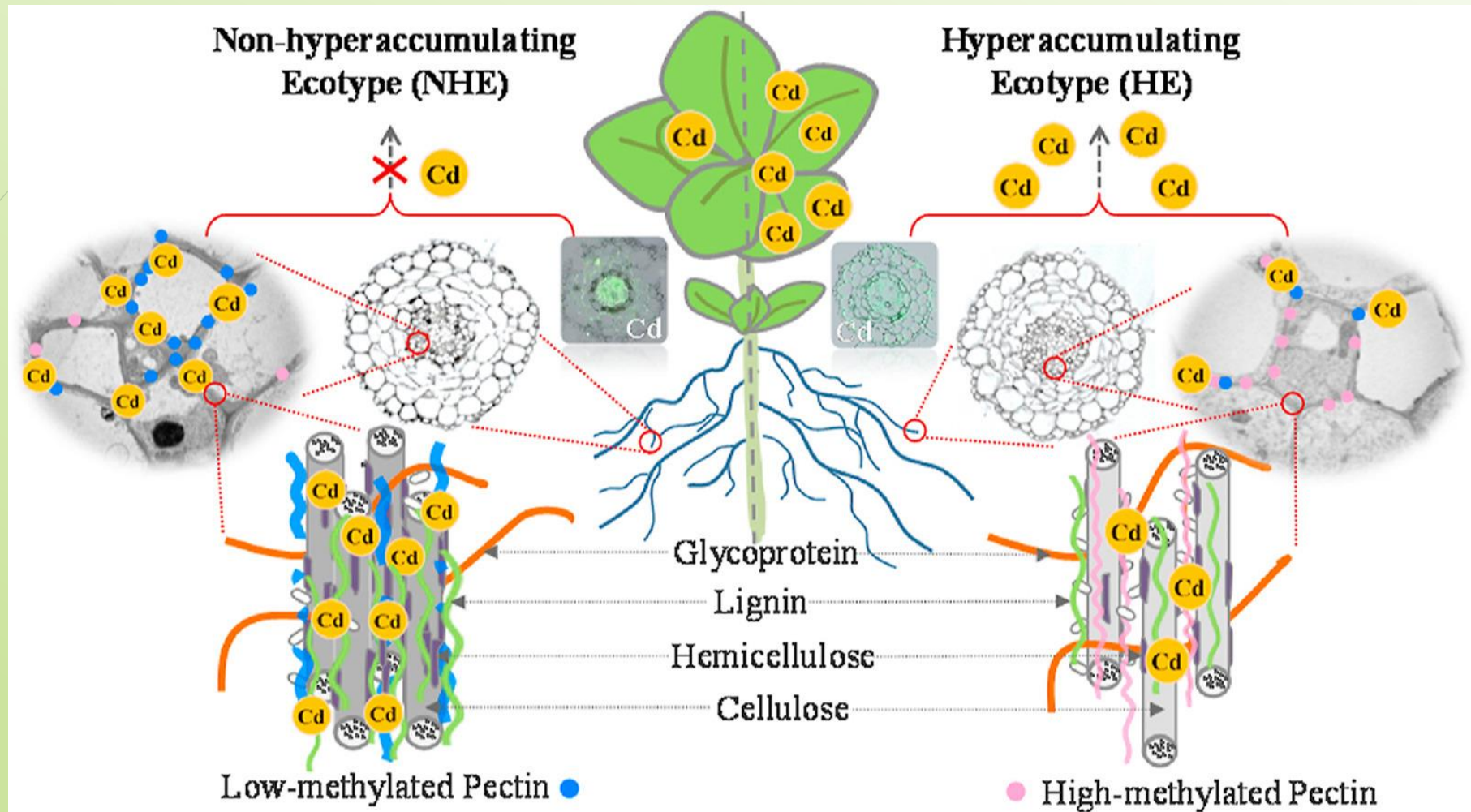


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1) Binding to the cell wall

*Sedum alfredii*

- a) Increased cellulose and lignin gave rise to cell wall thickenings (trapping more Cd in the root).
- b) thickened cell wall was enriched in low-methylated pectin (more COO^- sites for binding to Cd^{2+})

- a) no significant change in root cell wall thickness
- b) high methylated cell wall caused less and looser Cd bound in the root

Plant tolerance to metal stress

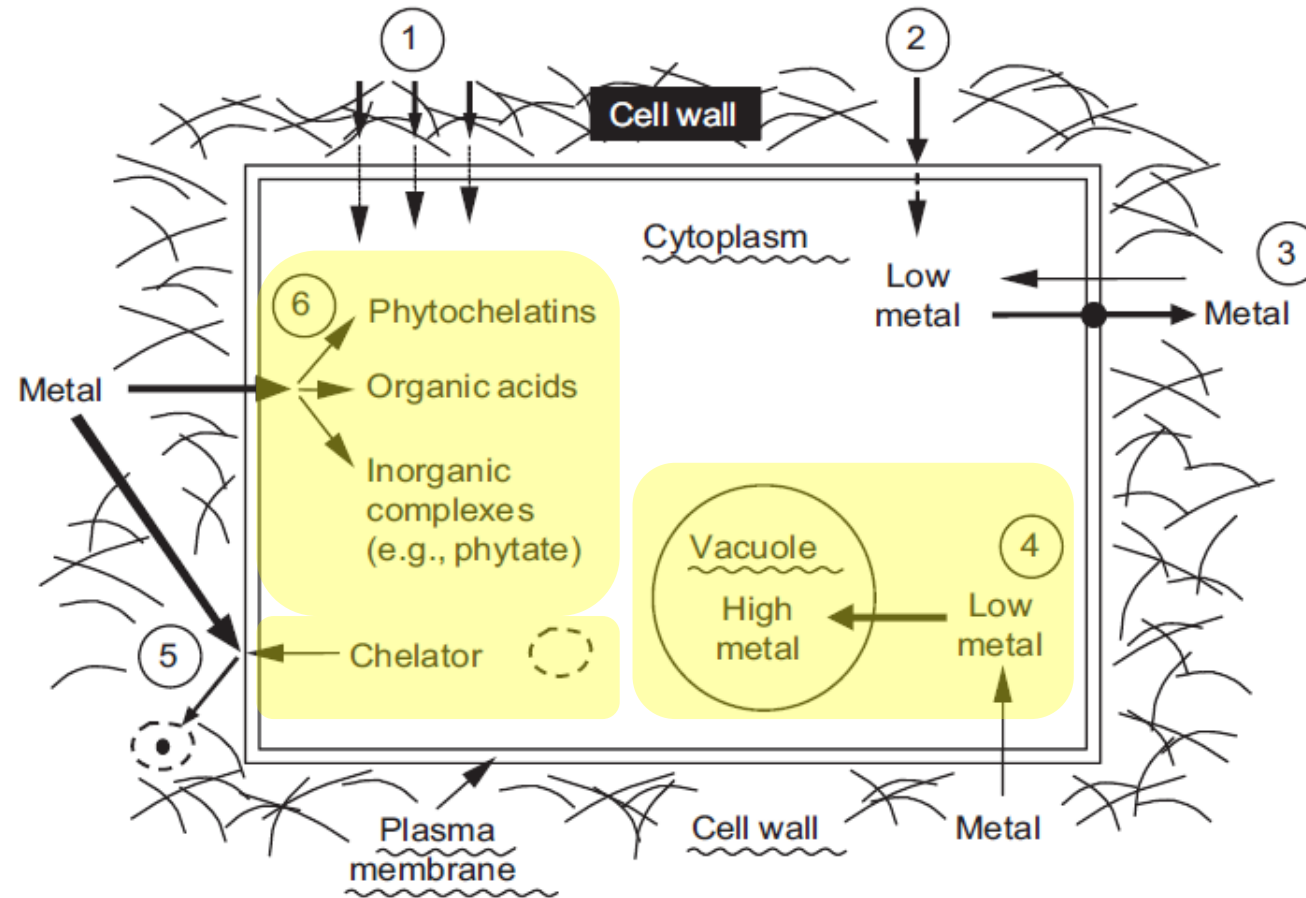


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5-6) Metal chelation

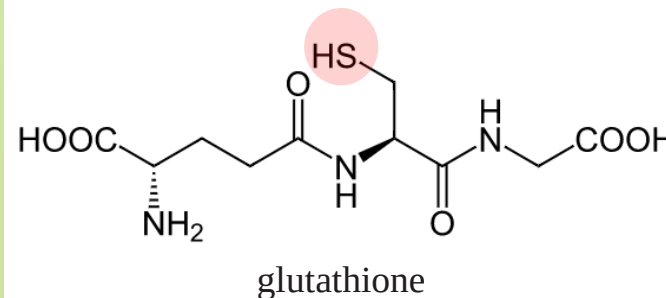
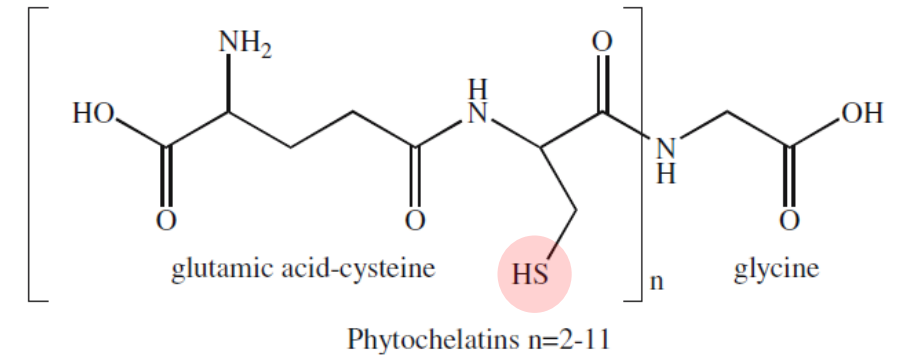
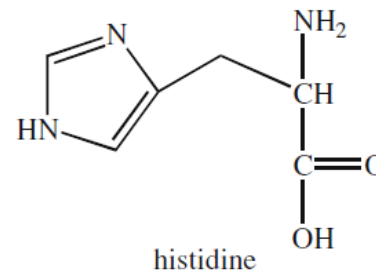
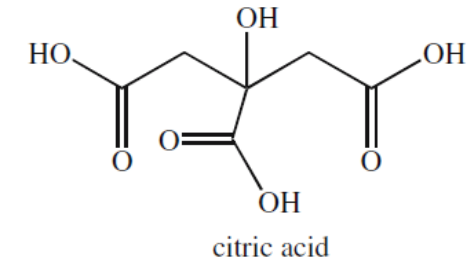
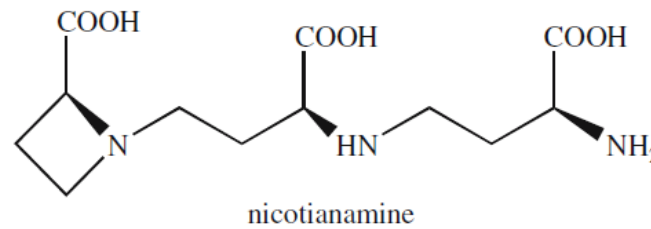
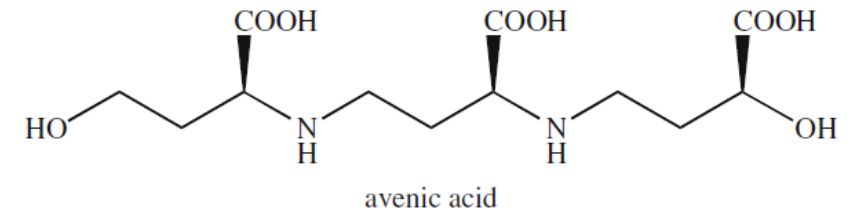
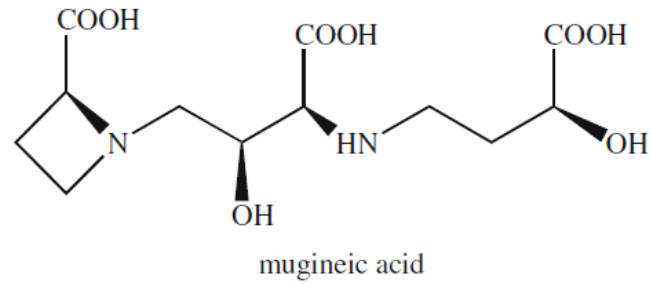
Binding of metals by ligands is the main detoxification strategy

Compounds containing thiols (-SH):

- glutathione
- phytochelatins
- metallothioneins (proteins!)

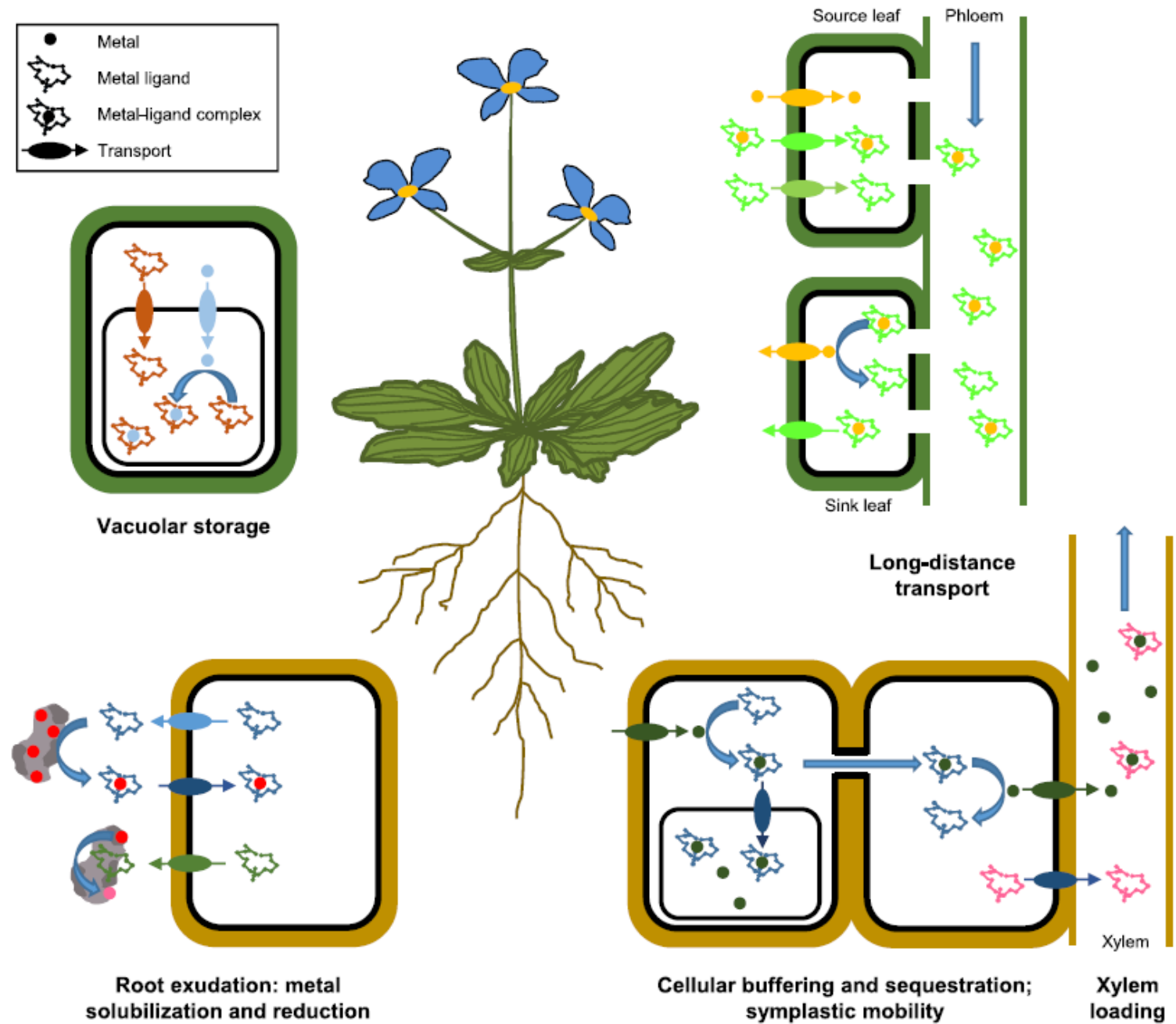
Compounds that do not contain thiols:

- free aminoacids (histidine)
- nicotianamine
- organic acids (citrate, malate)



ligands are usually involved
in metal nutrient
homeostasis...
... and excess HM tolerance

- 1) secreted into the extracellular space and influence the phytoavailability of metal ions
- 2) buffering of metals in the cytosol and other cellular compartments (no free-metal ions in the cytosol)
- 3) storage of metals, predominantly in vacuoles



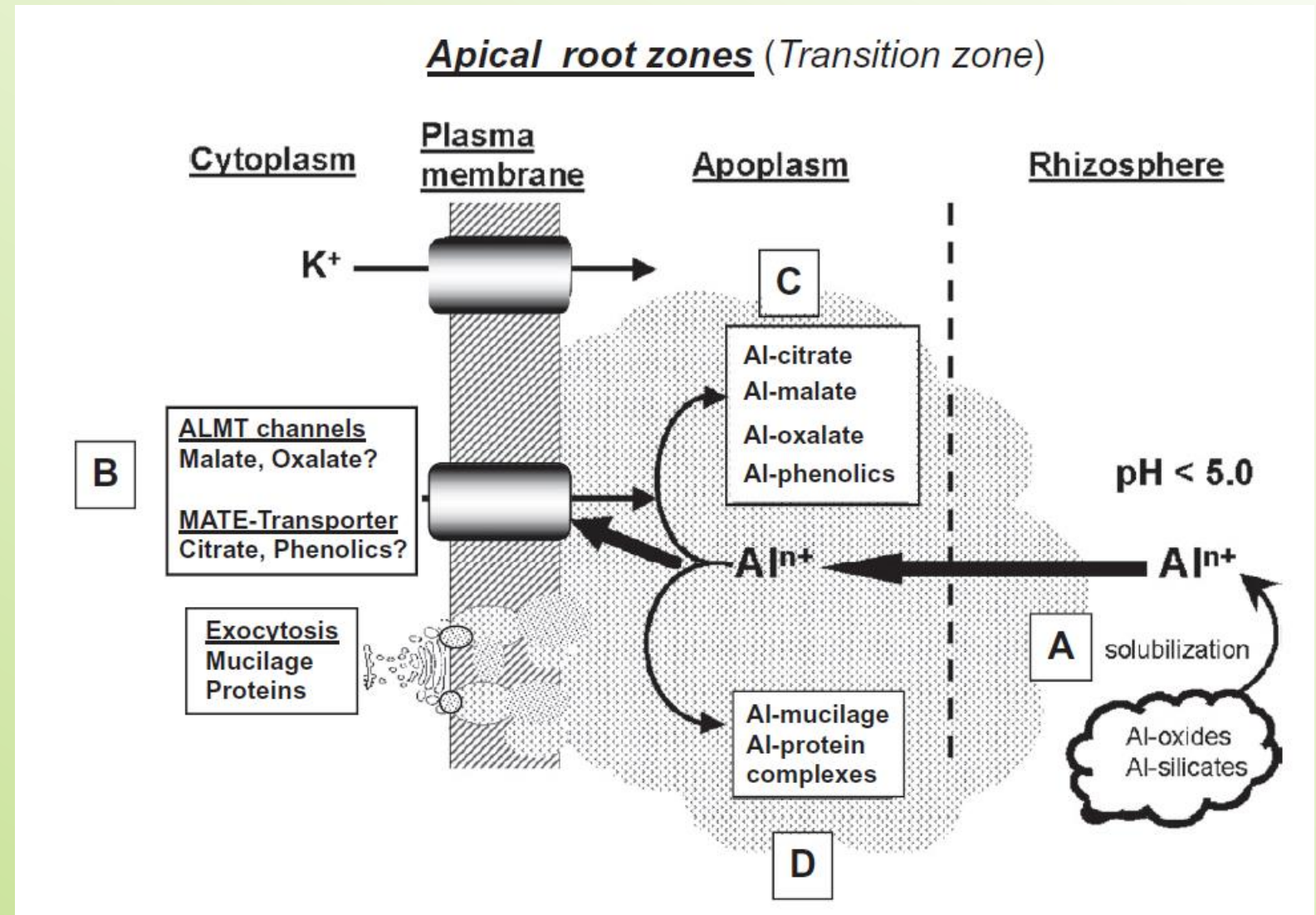
They can help
detoxification, preventing
metal absorption

Model for root-induced Al
detoxification by secretion of organic
Al chelators.

A. Solubilization of toxic Al species in
acid mineral soils at $\text{pH} < 5.0$

B. Al-induced activation of anion
channels in the Al-sensitive apical root
zones and release of organic Al
chelators

C. detoxification of Al by complexation
with low-molecular-weight (LMW)
chelators with proteins and mucilage

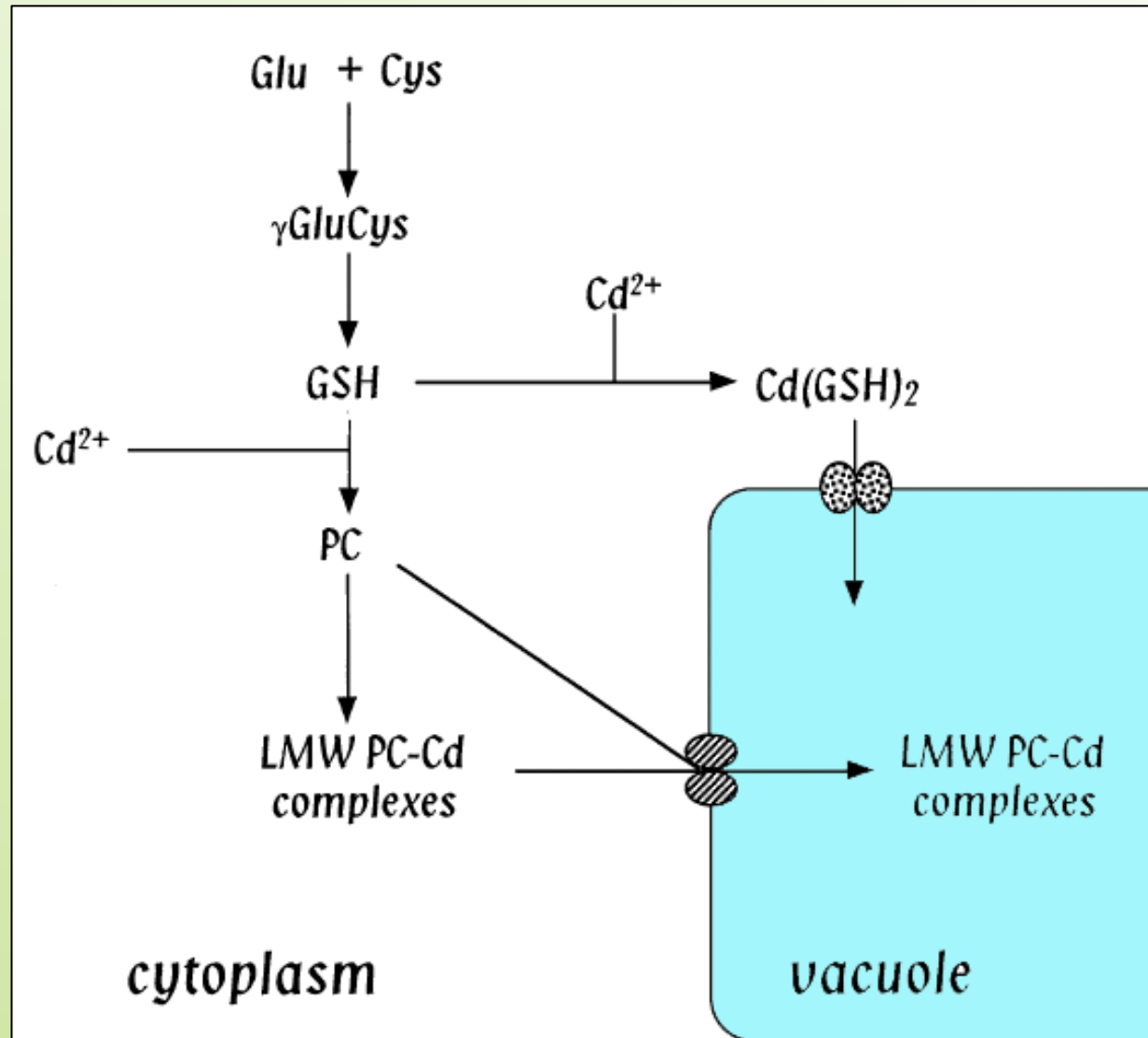


Thiol containing compounds/ proteins

The metal-binding activity of thiols has been mostly implicated in *cytosolic buffering*, *vacuolar sequestration*, and long-distance transport in the *phloem*

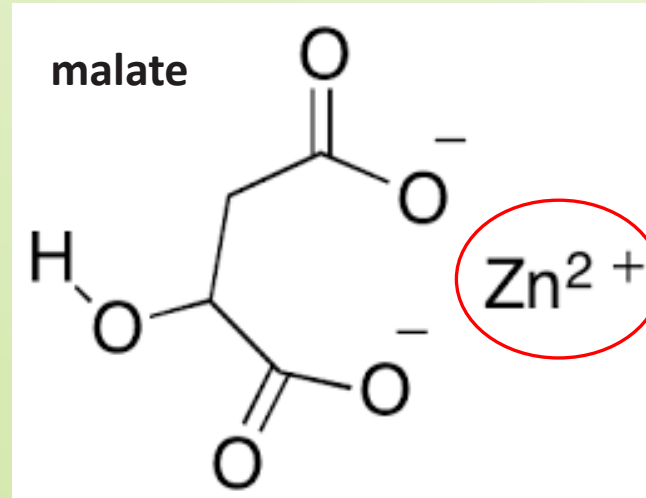
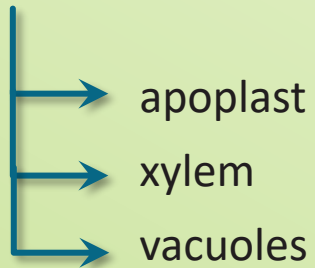
- **Glutathione** γ -GluCysGly
- GHS derived **Phytochelatins** $(\gamma$ -GluCys) $_n$ -Gly
- **Metallothioneins** (MTs)

- The complex GHS/PCs – HM (Cd, As, Cu) are usually store into the vacuole: detoxification system.
- PCs do not seem to play a significant role in hyperaccumulators, even if they are associated to HM toxicity stress
- MTs, mainly involved in HM physiology (es, reallocation of Cu upon senescence) → maybe involved in Cu tolerance?



Organic acids

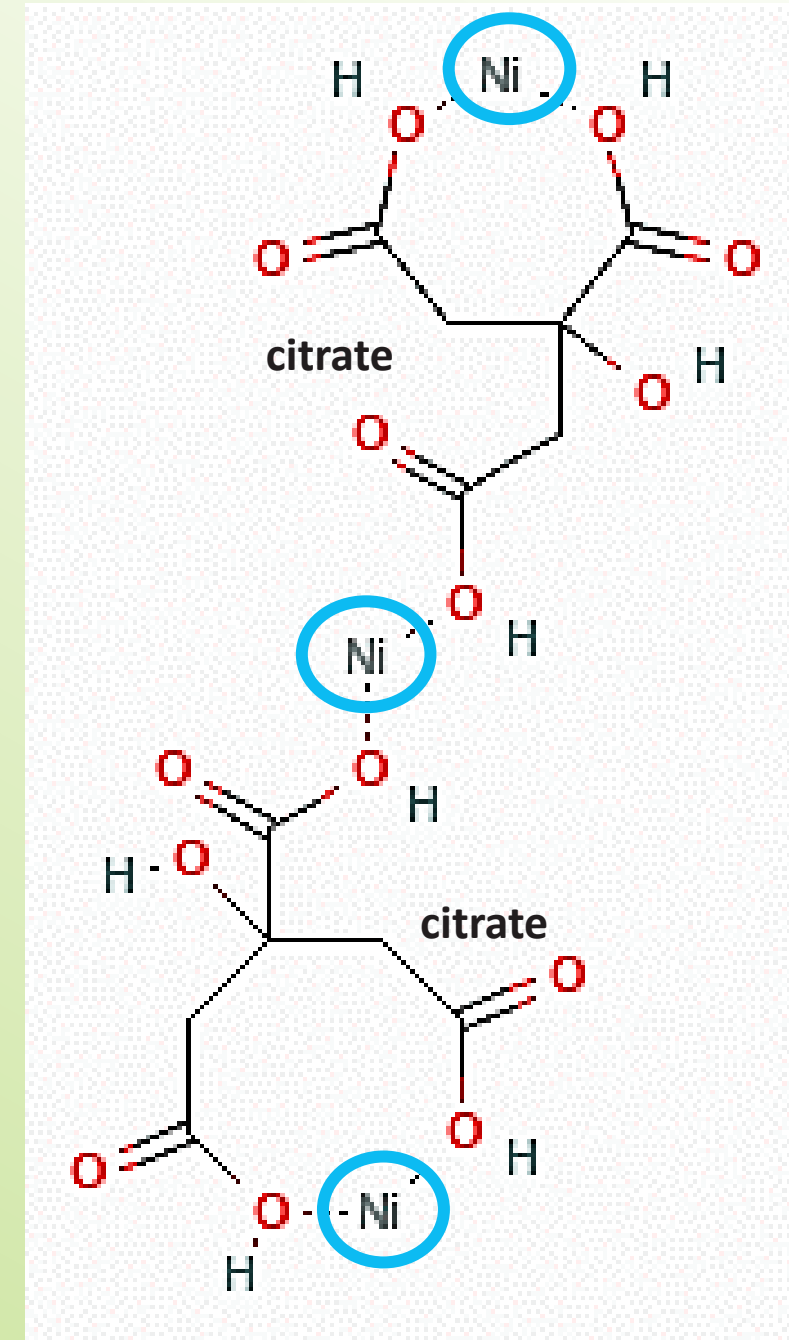
- They form a stable complex in acidic environments



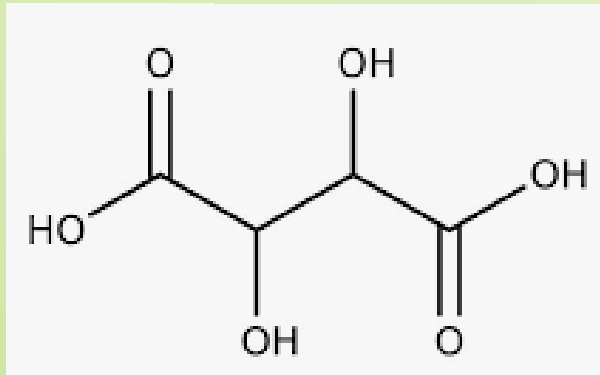
* Fe - **citrate** and **malate** → in pea xylem

* Zn - citrate and malate → *Noccaea caerulea* epidermal cell vacuoles

* Ni - malate *Alyssum*

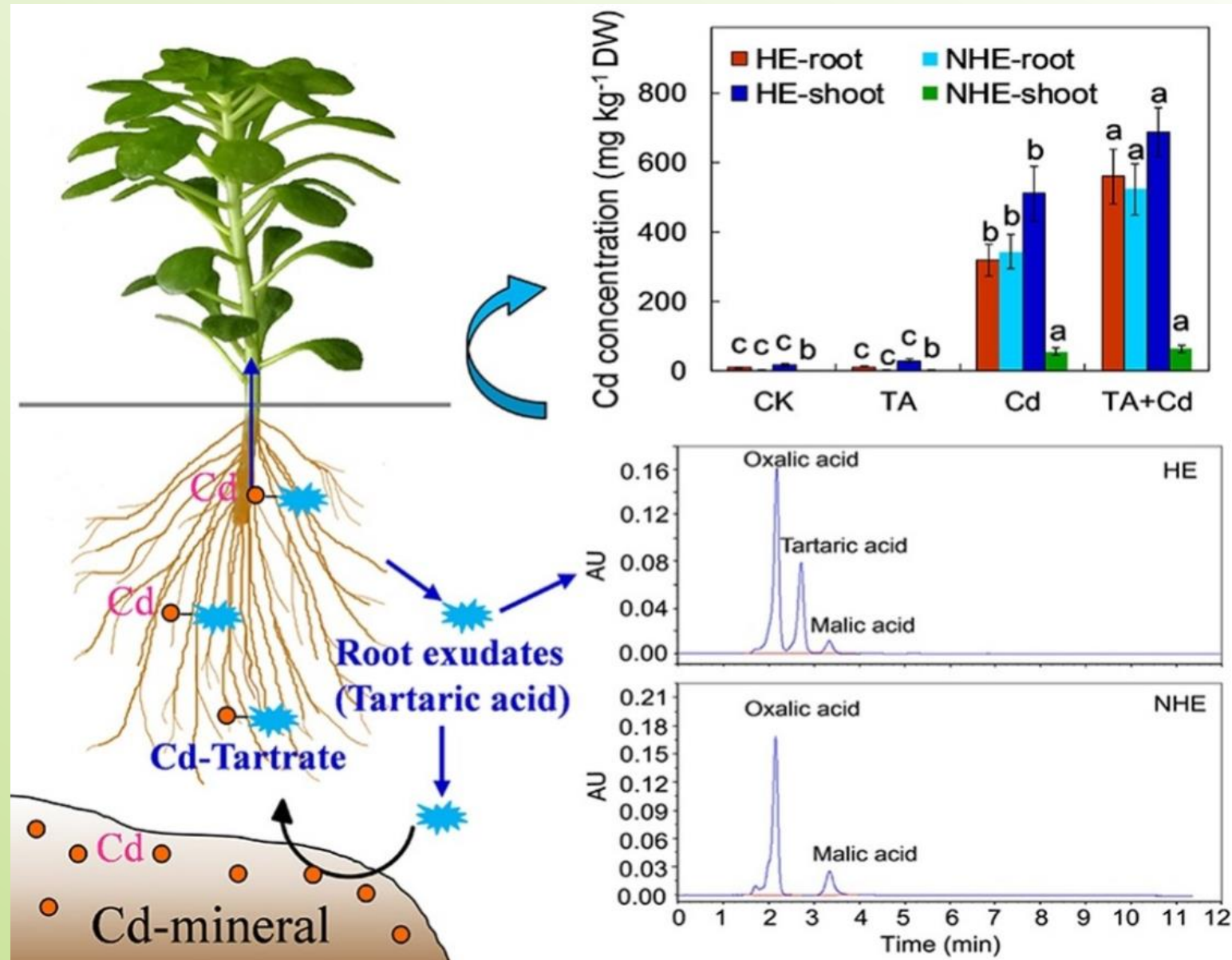


Tartaric acid



Example:

The hyperaccumulating ecotype (HE) of *S. alfredii* was collected from a Pb/Zn mine area in Quzhou, China, and the non-hyperaccumulating ecotype (NHE) of *S. alfredii* was obtained from a tea plantation in Hangzhou, China

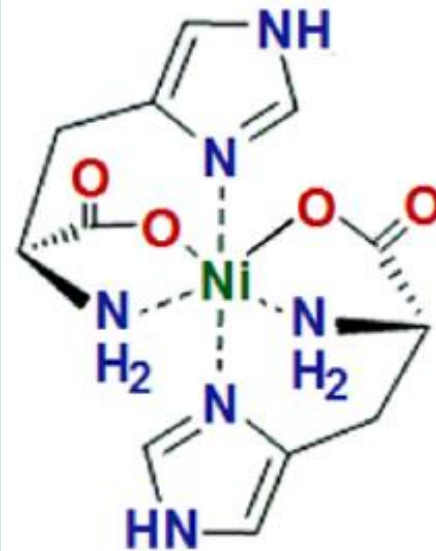


Aminoacids

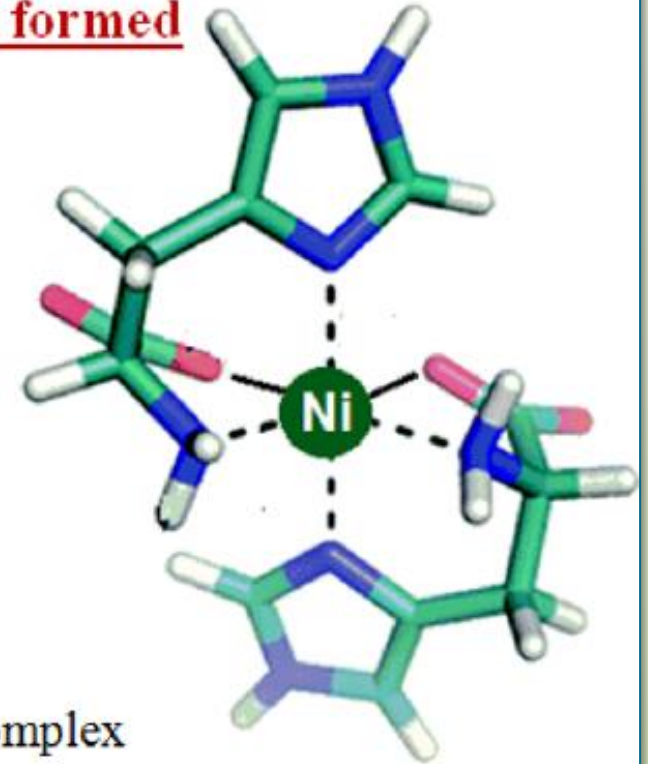
- **Histidine** is the main aa involved in metal chelation (in environment with neutral pH):
 - stable complex Ni – HIS (symplastic mobility and xylem loading) in *Alyssum lesbiacum*
 - Complex Cu – HIS (phloem sap)
 - Zn – HIS in *N. caerulescens*

Cytosolic pH 7.2-7.5

A stable complex of histidine with metals
is formed

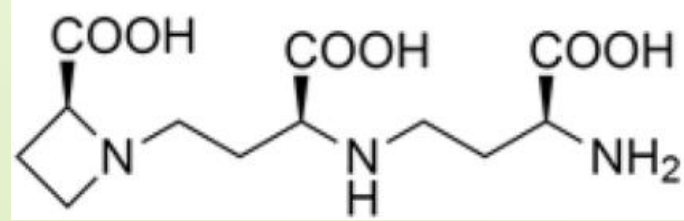


Ni(His)₂ complex



Stability of the metal-histidine complexes:
Cu > **Ni** > Co > **Zn**

Nicotianamine - NA



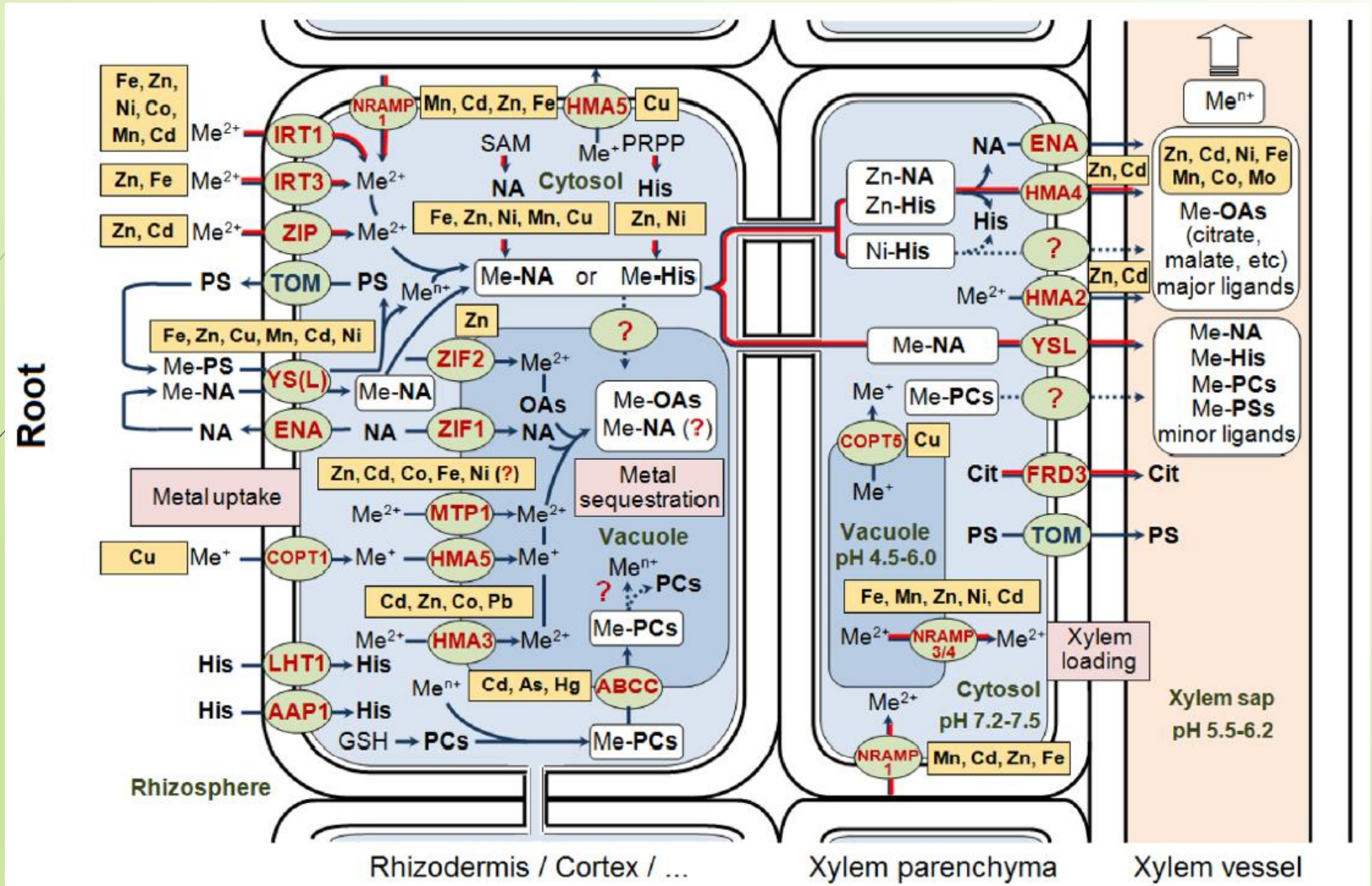
Good chelating agents under neutral – acidic conditions:

- Zn–NA in xylem of *A. halleri*;
- Zn–NA in phloem of rice;
- Cu–NA and Ni–NA in xylem and phloem of *Noccaea caerulescens*;



Involved in root-to-shoot translocation?

All these molecules as well as trace metal ions need to be transported across membranes.



Filters

Higher Classification:

Order:

Family:

Genus:

Species:

Species Id:

Obligate / Facultative:

Element:

Distribution:

Catalog #:

PDF's:

Arsenic
Cadmium
Chromium
Copper
Cobalt
Lead
Manganese
Nickel
REE
Selenium
Thallium
Zinc

Species Id

Family ▲

Genus

Species

Element Name

Threshold Range

Element	Threshold for hyperaccumulators (mg/kg dw)	Genera (No.)	Species (No.)
Arsenic (As)	>1,000	2	5
Cadmium (Cd)	>100	9	43
Cobalt (Co)	>300	33	43
Copper (Cu)	>300	44	53
Chromium (Cr)	>1,000	1	1
Lead (Pb)	>1,000	9	9
Manganese (Mn)	>10,000	24	42
Nickel (Ni)	>1,000	131	532
Selenium (Se)	>100	15	41
Thallium (Tl)	>100	2	2
Zinc (Zn)	>3,000	12	21

<http://hyperaccumulators.smi.uq.edu.au/collection/>

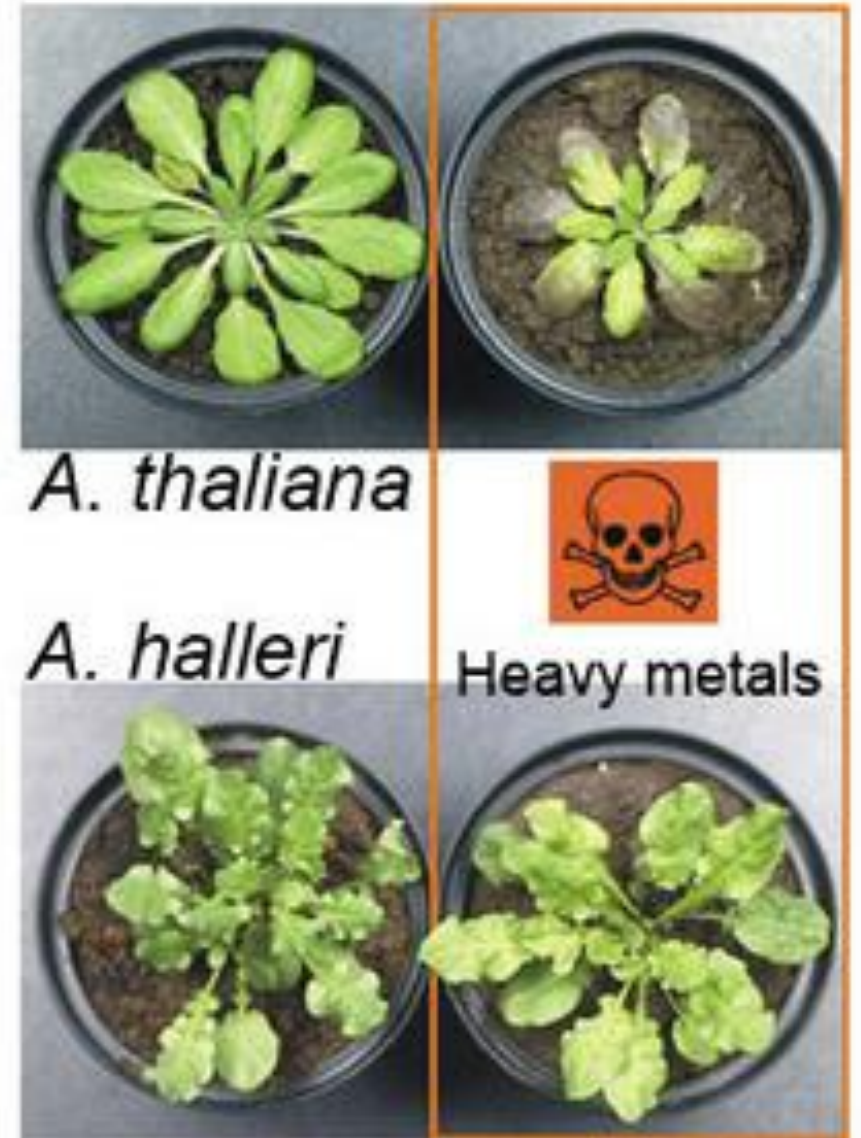
Over 600 plant species (0.2% of angiosperms) have been identified as hyperaccumulators of trace metals (Zn, Ni, Mn, Cu, Co and Cd), metalloids (As) and nonmetals (Se), the majority of them being Ni hyperaccumulators (75%)

Hyperaccumulators occur in more than 80 different families. The **Brassicaceae** family is relatively rich in them, in particular the genera *Alyssum* and *Noccaea*

http://www.pflanzenphysiologie.uni-bayreuth.de/pflanzenphysiologie/en/forschung/gru/html.php?id_obj=75803



Noccaea caerulescens



A. thaliana

A. halleri



Heavy metals

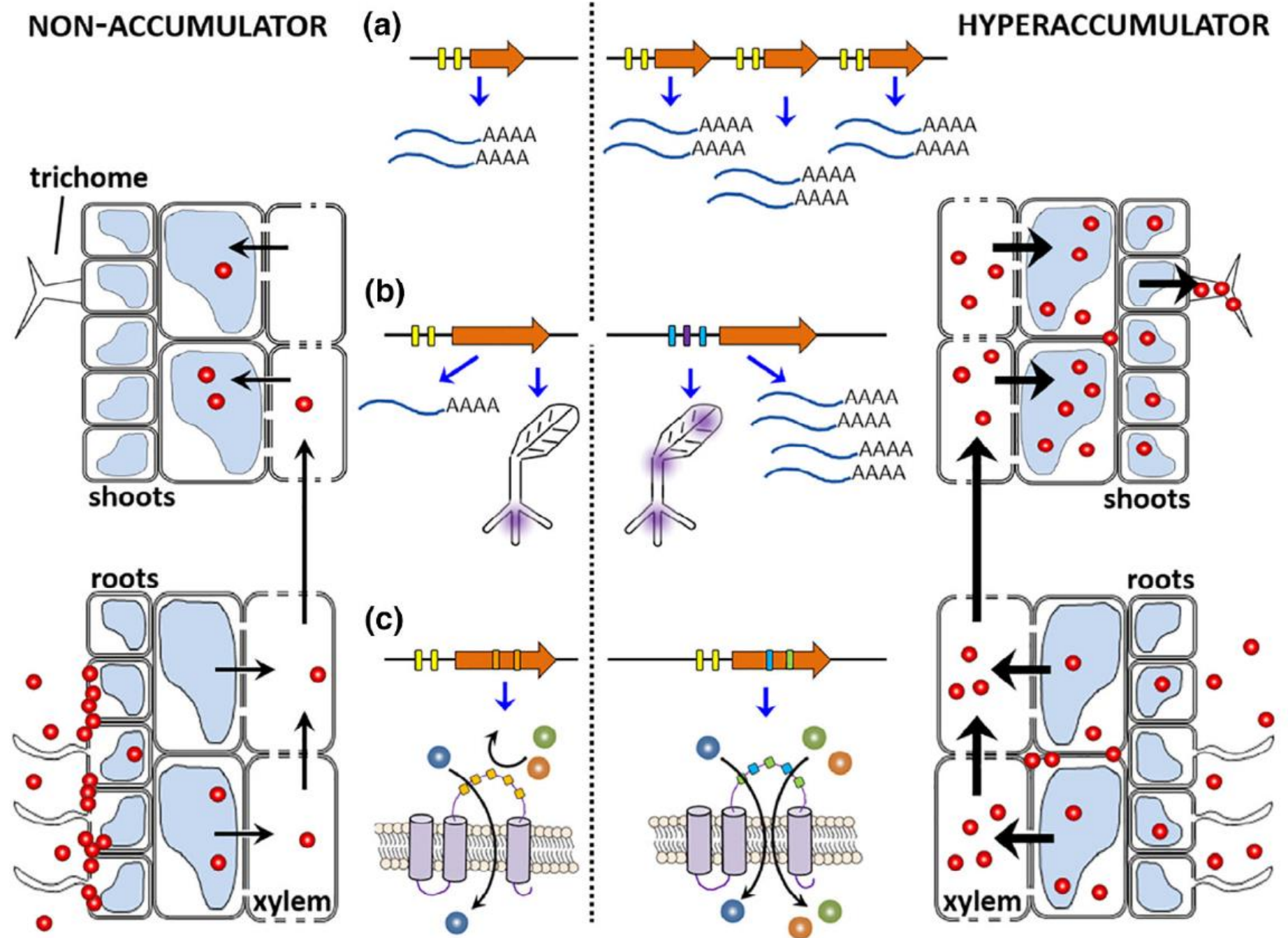
Ni-hyperaccumulating tree from New Caledonia, *Pycnandra acuminata* (ex-Sebertia)

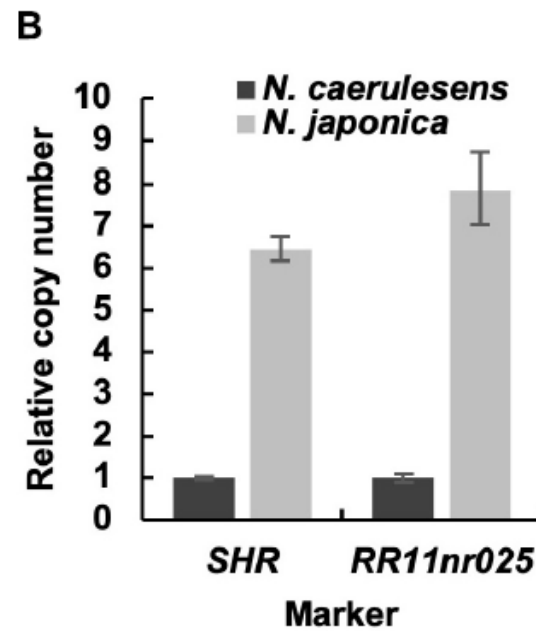
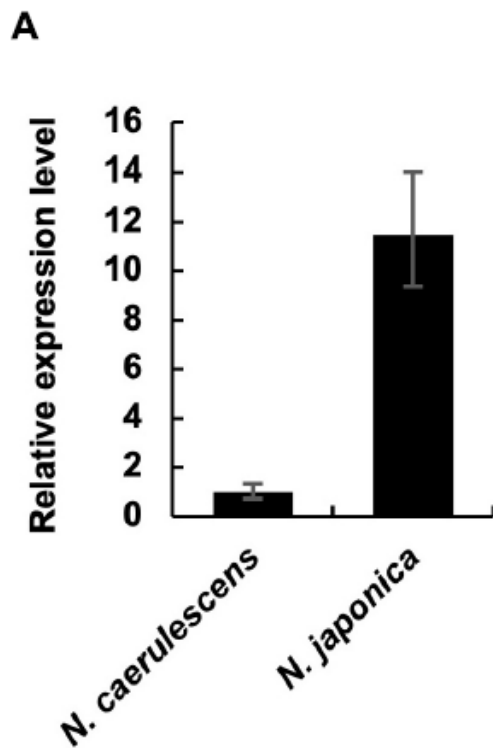
The green-light blue color in the exuded latex in *P. acuminata* is due to the high concentration of Ni



can contain up to 26% (w/w) Ni

How did it evolve
(genetically speaking)?!

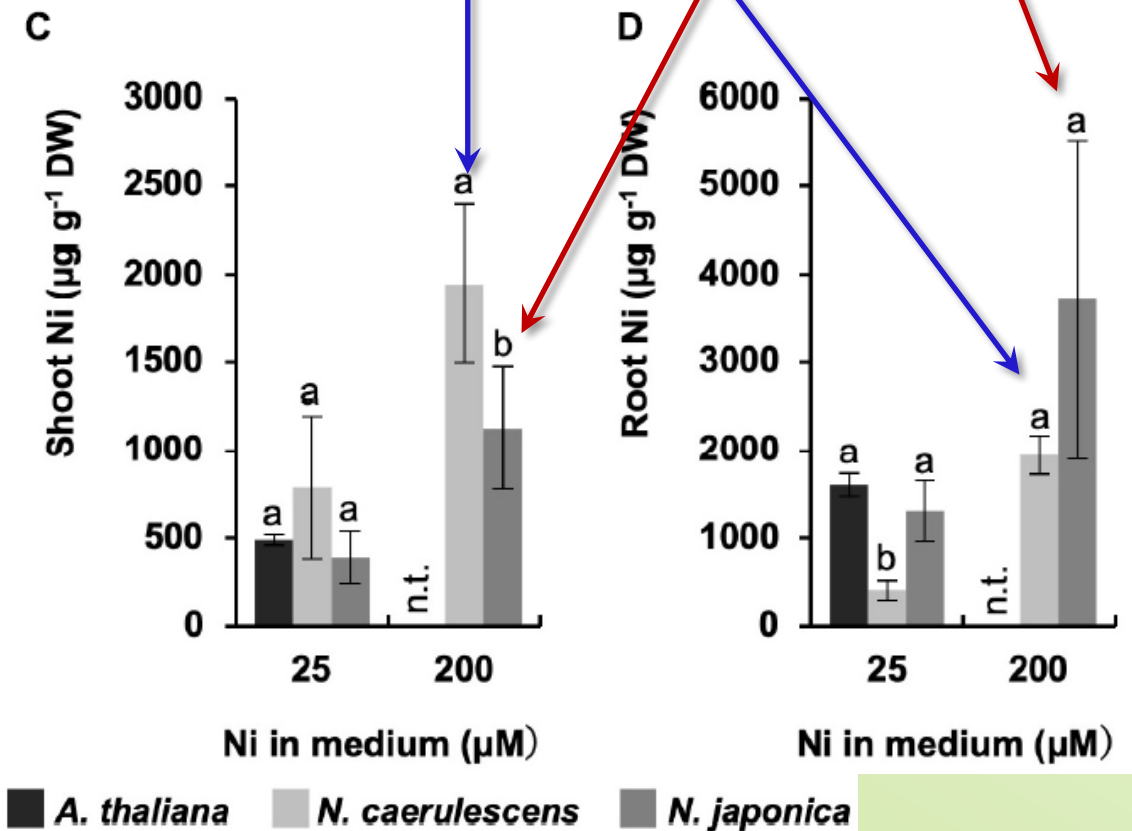




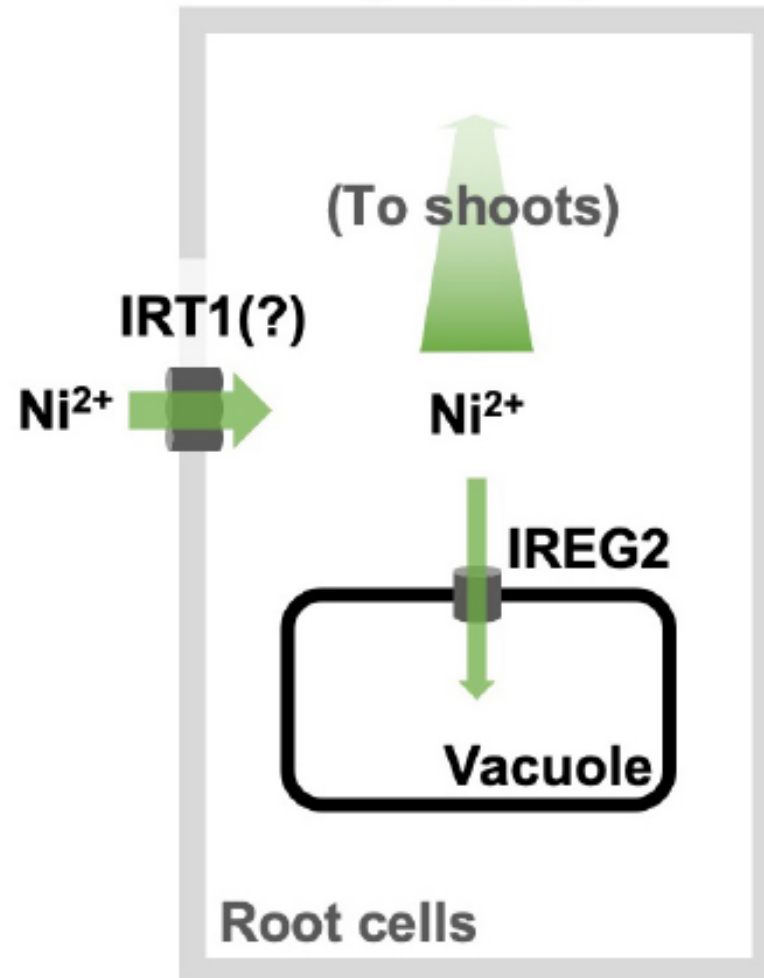
The copy number of *IREG2* per genome

Root expression level of *IREG2* - plants were exposed to 200 mM Ni for 7 days in hydroponic culture

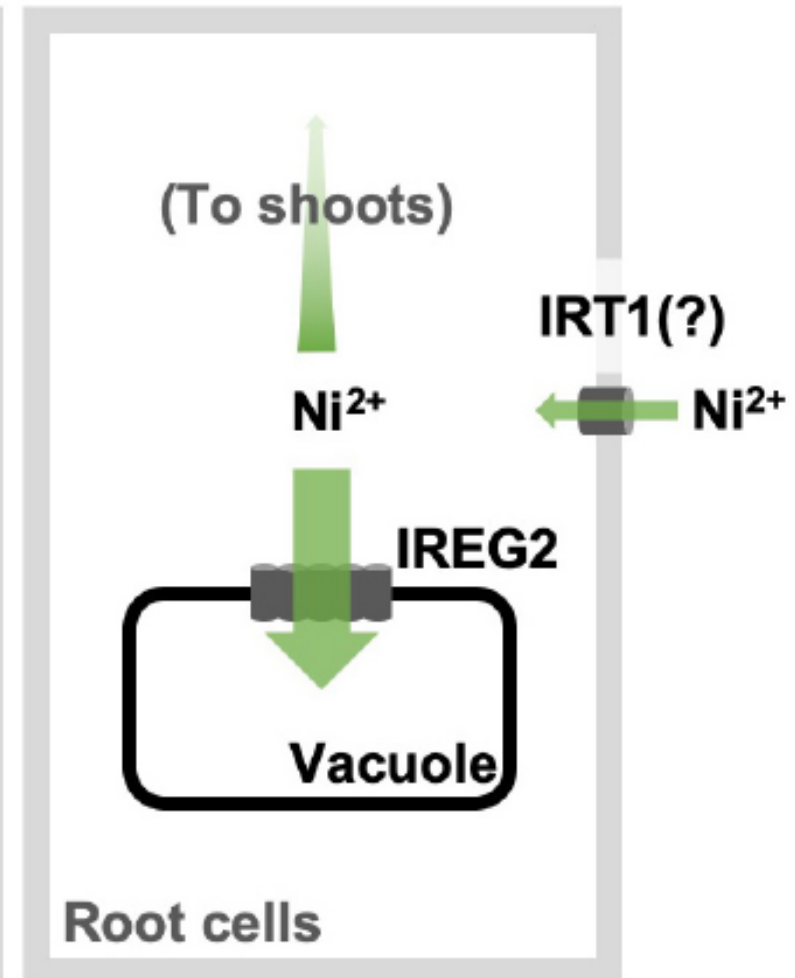
Example:
Noccaea caerulescens and *Noccaea japonica*



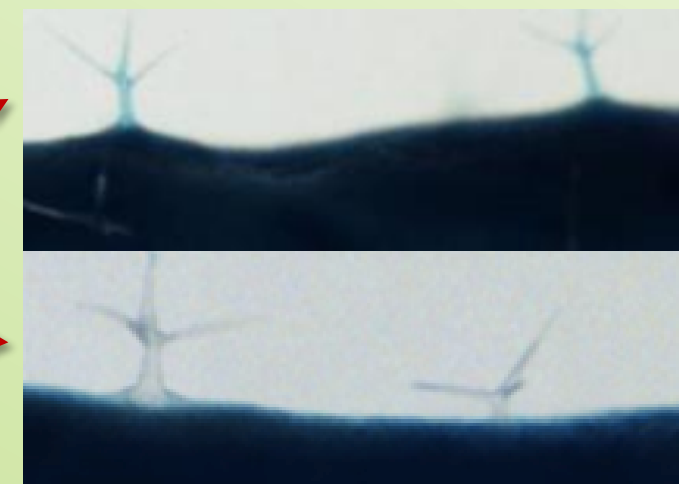
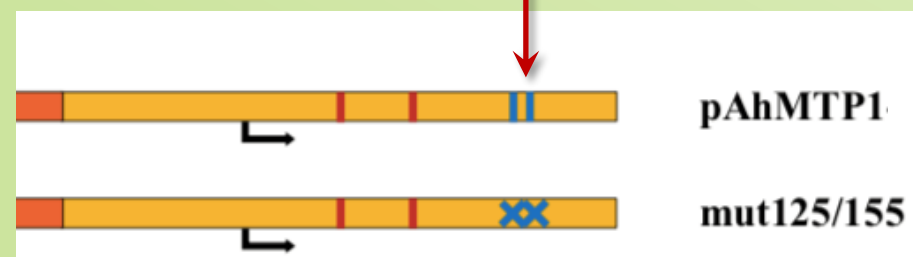
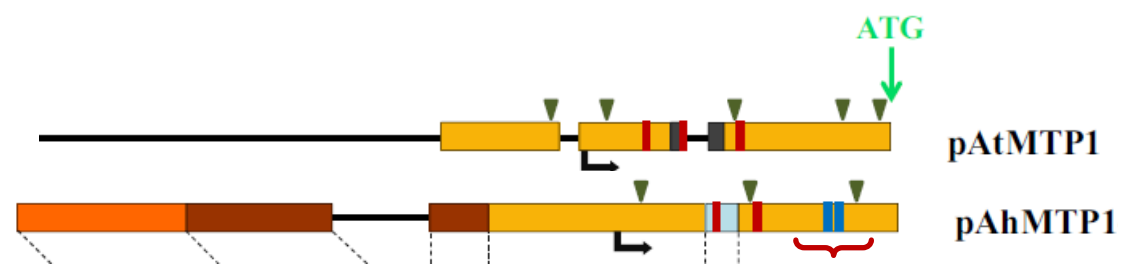
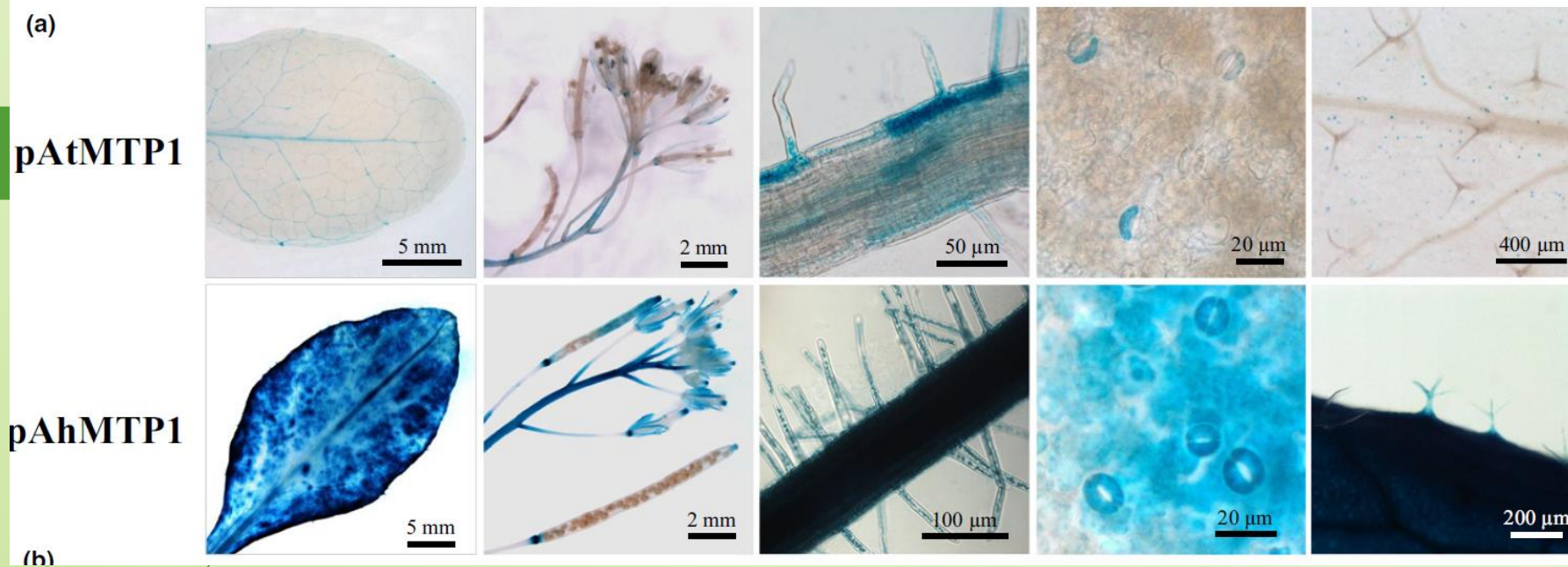
N. caerulescens
(Ganges)



N. japonica

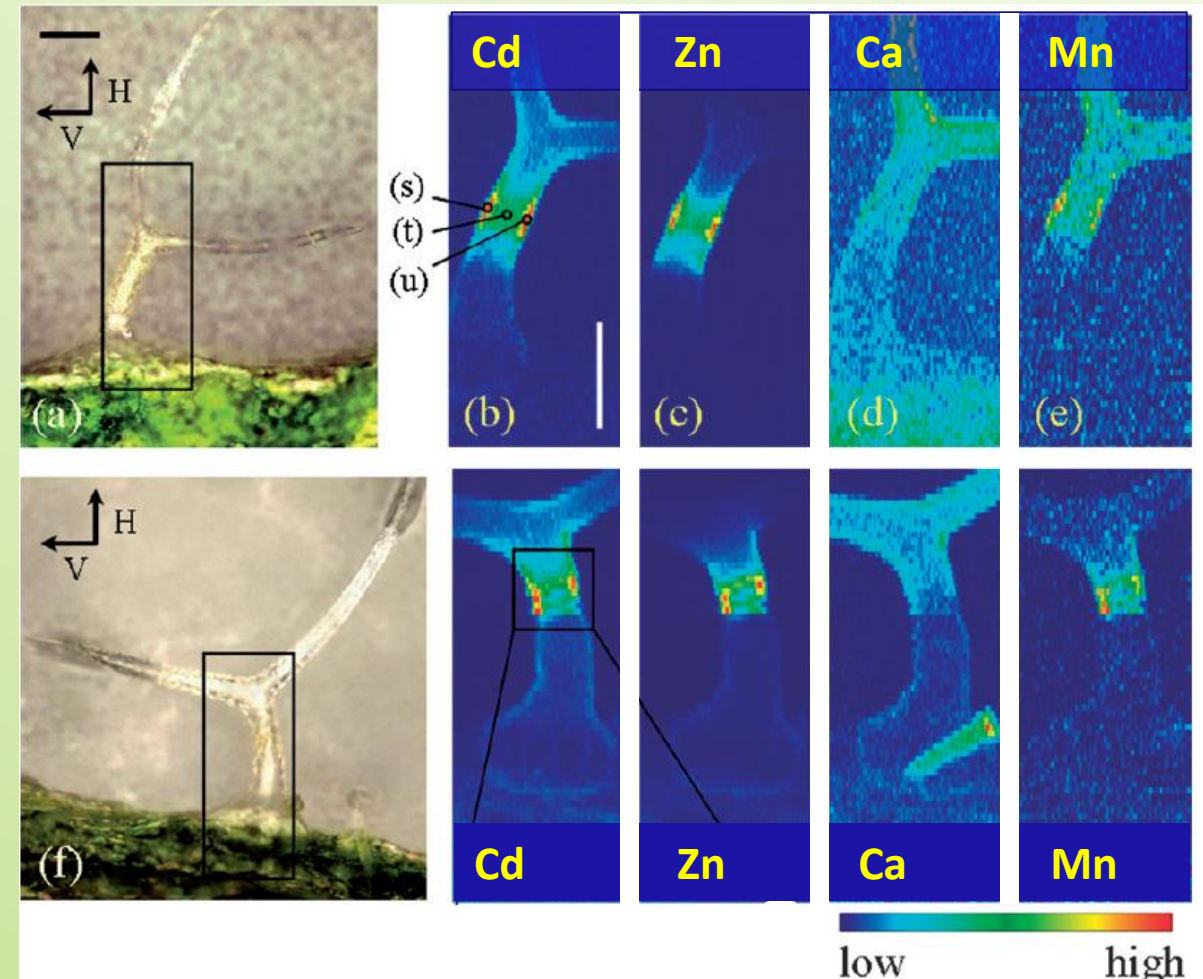
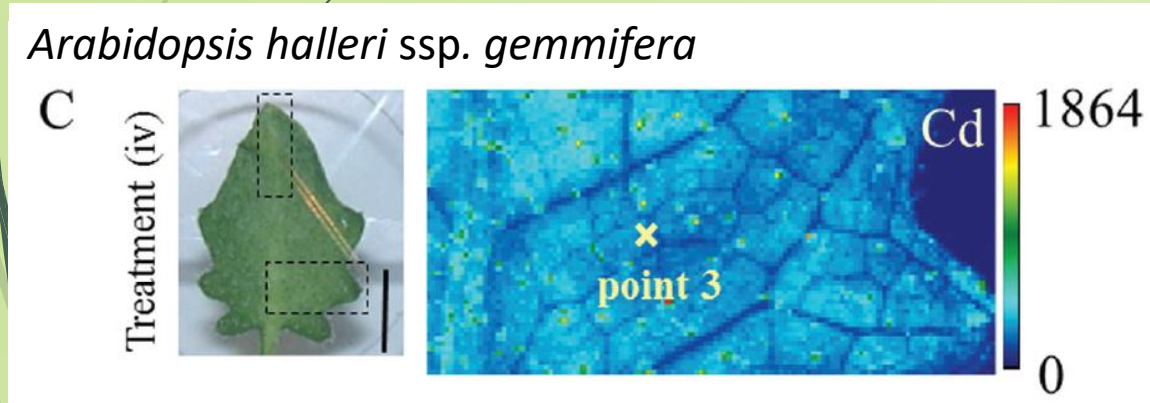
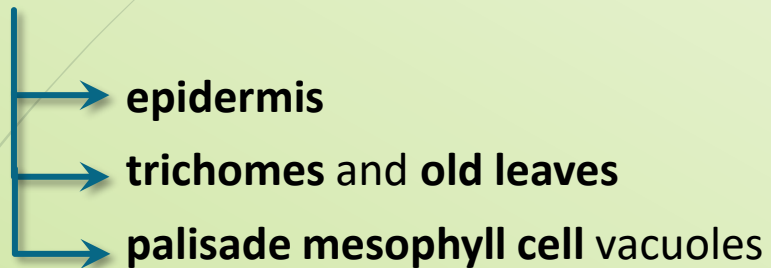


Example:
Arabidopsis halleri (Zn)

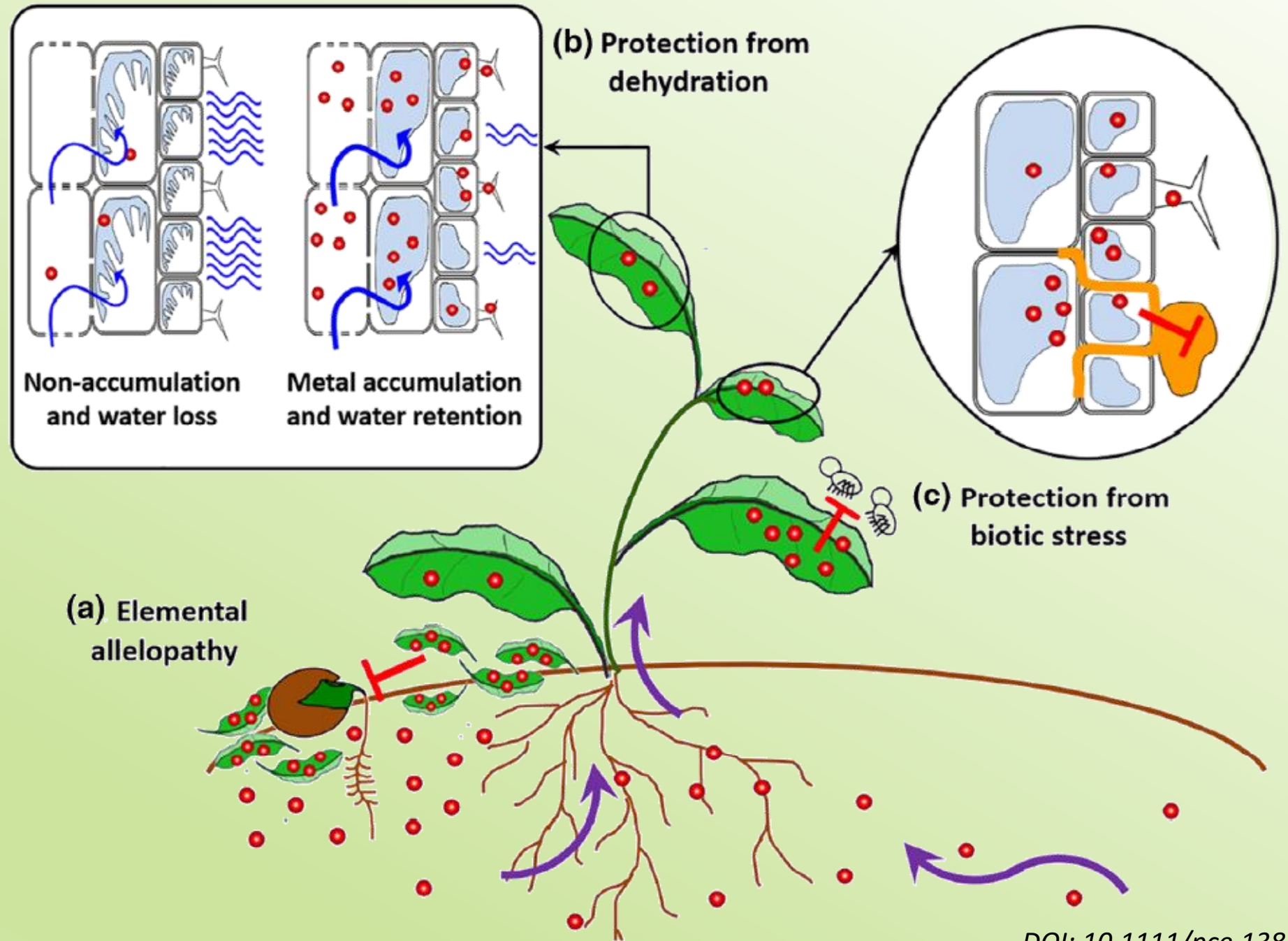


Main component of hyperaccumulation: metals are translocated from roots to above-ground tissues, via xylem, and distributed over aerial tissues

They need to be compartmentalized or sequestered in (sometimes) photosynthetically inactive tissues:



Why hyperaccumulation?!



The study of hyperaccumulation may contribute to a biotechnological approach to

i) *phytoremediation* and

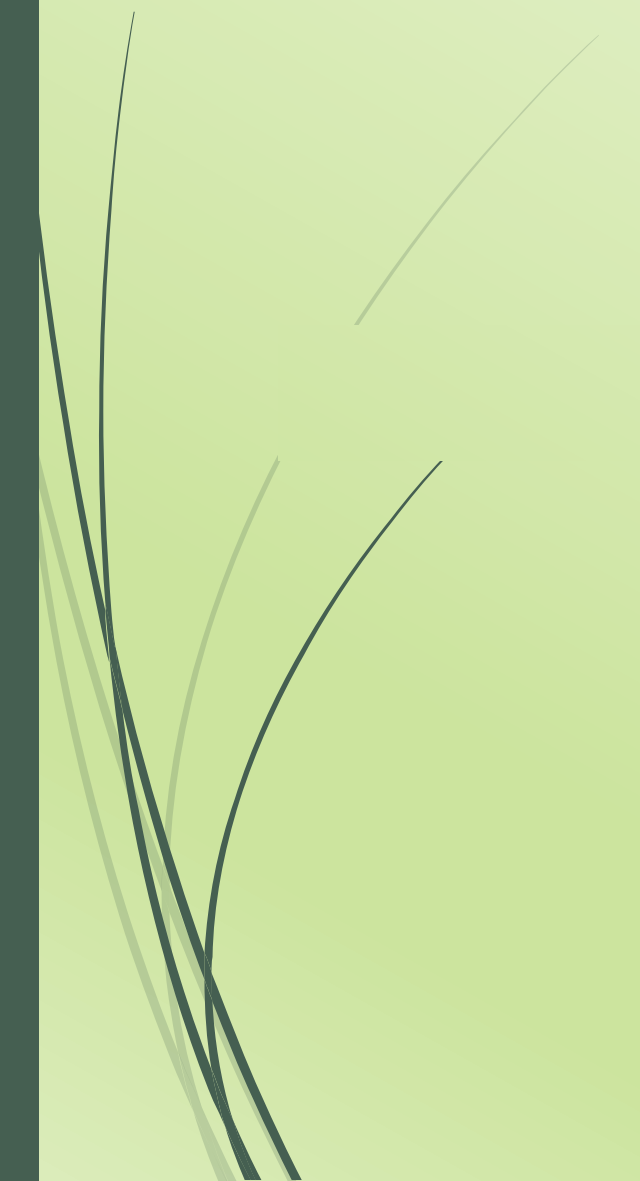
ii) *biofortification*

Table 1. Selection of Genes Correlating to Zn²⁺, Cd²⁺, or Ni²⁺ Accumulation and Tolerance^a

ID	gene description	mean expression value			P-FDR	correlation	trait
		LC	GA	MP			
isotig02262	unknown <i>N. caerulea</i> transcript	3473	7874	2368	0.0031	0.9487	Cd acc
isotig08052	unknown <i>N. caerulea</i> transcript	1083	1292	663	0.0031	0.9487	Cd acc
isotig02715	unknown <i>N. caerulea</i> transcript	62	278	26	0.0031	0.9487	Cd acc
AT1G76430	phosphate transporter PHT1;9	42	56	16	0.0102	0.9487	Cd acc
AT2G30080	zinc transporter ZIP6	78	211	35	0.0102	0.9487	Cd acc
AT3G58060	metal tolerance protein MTP8	88	278	44	0.0102	0.9487	Cd acc
AT5G16570	glutamine synthetase GLN1;4	1545	2362	1303	0.0102	0.9487	Cd acc
AT5G57110	autoinhibited Ca ²⁺ -ATPase ACA8	2683	3474	1532	0.0102	0.9487	Cd acc
AT3G05920	heavy metal transport/detoxification superfamily protein	23	49	15	0.0555	0.8960	Cd acc
AT5G50160	ferric reduction oxidase FRO8	15	39	13	0.0555	0.8960	Cd acc
AT1G77760	nitrate reductase NR1	4199	5774	2360	0.0555	0.8960	Cd acc
AT3G61940	metal tolerance protein MTP2	55	82	37	0.0555	0.8960	Cd acc
isotig00200	unknown <i>N. caerulea</i> transcript	138	247	209	0.0468	0.8216	Zn acc
AT2G40300	ferritin FER4	43	121	144	0.0809	0.8216	Zn acc
AT3G11050	ferritin FER2	13	40	38	0.0809	0.8216	Zn acc
AT2G39450	metal tolerance protein MTP11	150	457	311	0.0809	0.8216	Zn acc
AT2G28160	FER-like regulator of iron uptake FIT	71	226	147	0.0809	0.8216	Zn acc
AT5G25220	knotted1-like homeobox gene KNAT3	870	711	452	0.0701	0.8216	Zn/Cd tol
AT3G43790	zinc induced facilitator-like ZIFL2	415	282	124	0.0701	0.8216	Zn/Cd tol
AT1G51460	ABC-2 type transporter family protein	128	77	23	0.0701	0.8216	Zn/Cd tol
AT3G13100	multidrug resistance-associated protein MRP7	1042	1024	519	0.0701	0.8216	Zn/Cd tol
AT4G16430	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	163	146	107	0.0701	0.8216	Zn/Cd tol
AT5G56080	nicotianamine synthase NAS2	1172	1051	477	0.0701	0.8216	Zn/Cd tol
AT3G60100	citrate synthase CSY5	189	236	109	0.0701	0.8216	Zn/Cd tol
AT2G19110	cadmium/zinc-transporting ATPase HMA4	26074	25124	6447	0.0701	0.8216	Zn/Cd tol

Gene Expression Differences between *Nocca caerulea* Ecotypes Help to Identify Candidate Genes for Metal Phytoremediation

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Thank you for your attention!