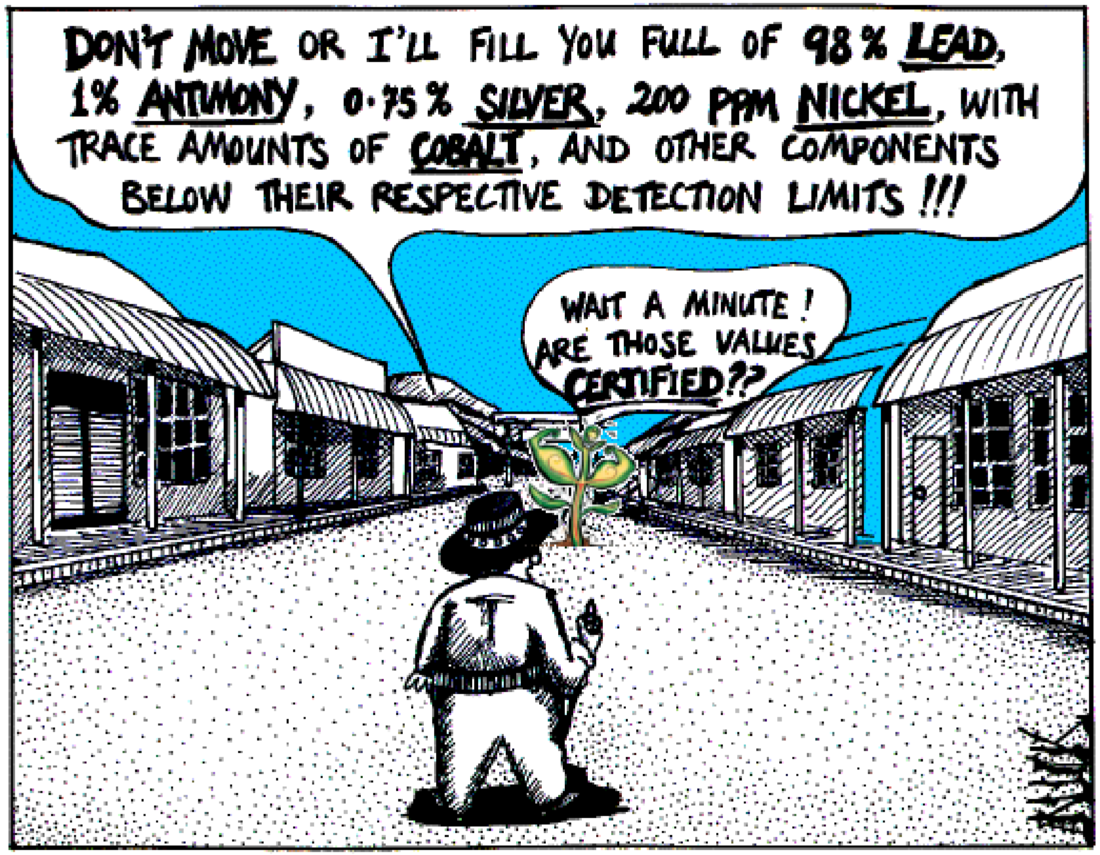
Heavy Metals and Plants - a complicated relationship

→ Copper toxicity (and deficiency) stress



Heavy metal-hyperaccumulation in the Wild West

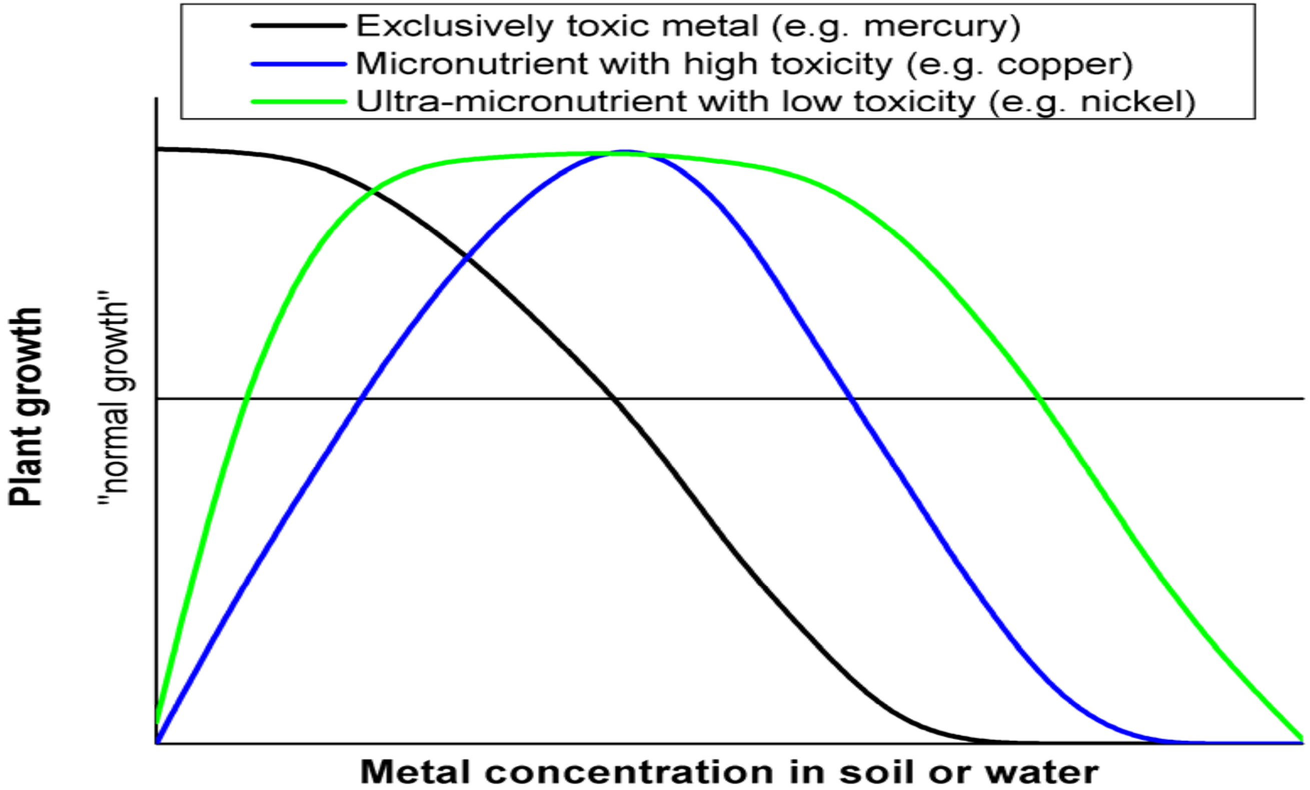
modified from: http://strangematter.sci.waikato.ac.nz/

Copper and its properties

- Redox active transition metal
- Oxidation states : Cu^{1+} / Cu^{2+}

- Soft metal and binds to soft ligands
- Cu [1] : usually bound to N in His side chains
- Cu [2] : usually bound to S in Cys/ Met
- When bound to protein – used in electron transfer reactions

- No free Cu inside the cell



- Narrow beneficial range
- Easily reach toxic effects above certain threshold

Küpper H, Kroneck PMH, 2005, Metal ions Life Sci 2, 31-62

The concentration of Cu in natural waters:

< **30 nM** in natural fresh waters, lower in oceans
> **30 nM** in polluted conditions

Yamamoto et al., 1985; Zhang et al., 2003

(Bodensee) the Cu concentration was recorded as **17.3 nM**

(Zweckverband Bodensee-Wasserversorgung)

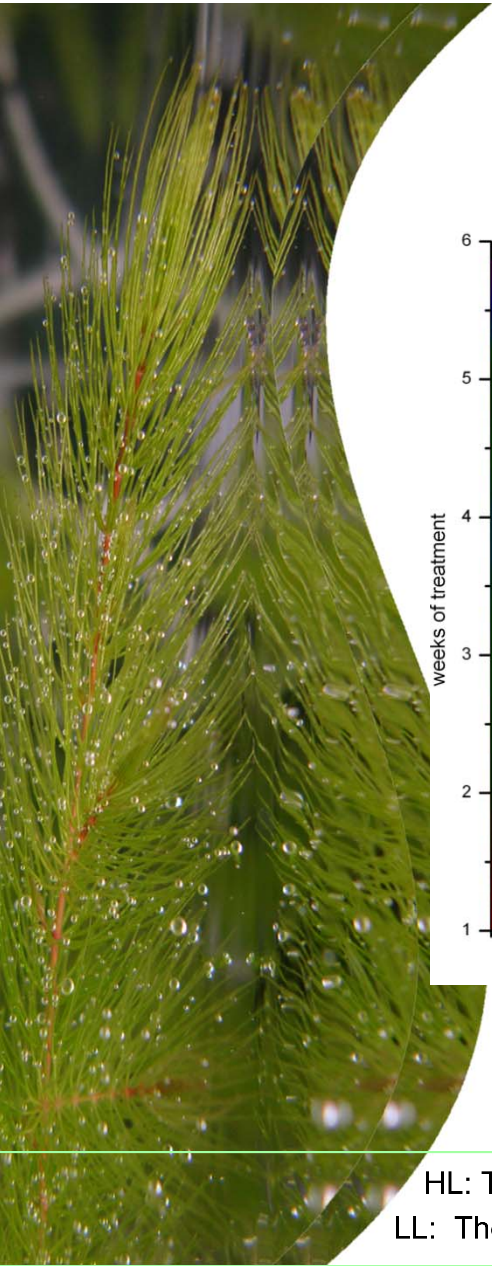
Cu toxicity in the environment

result of anthropogenic activities :

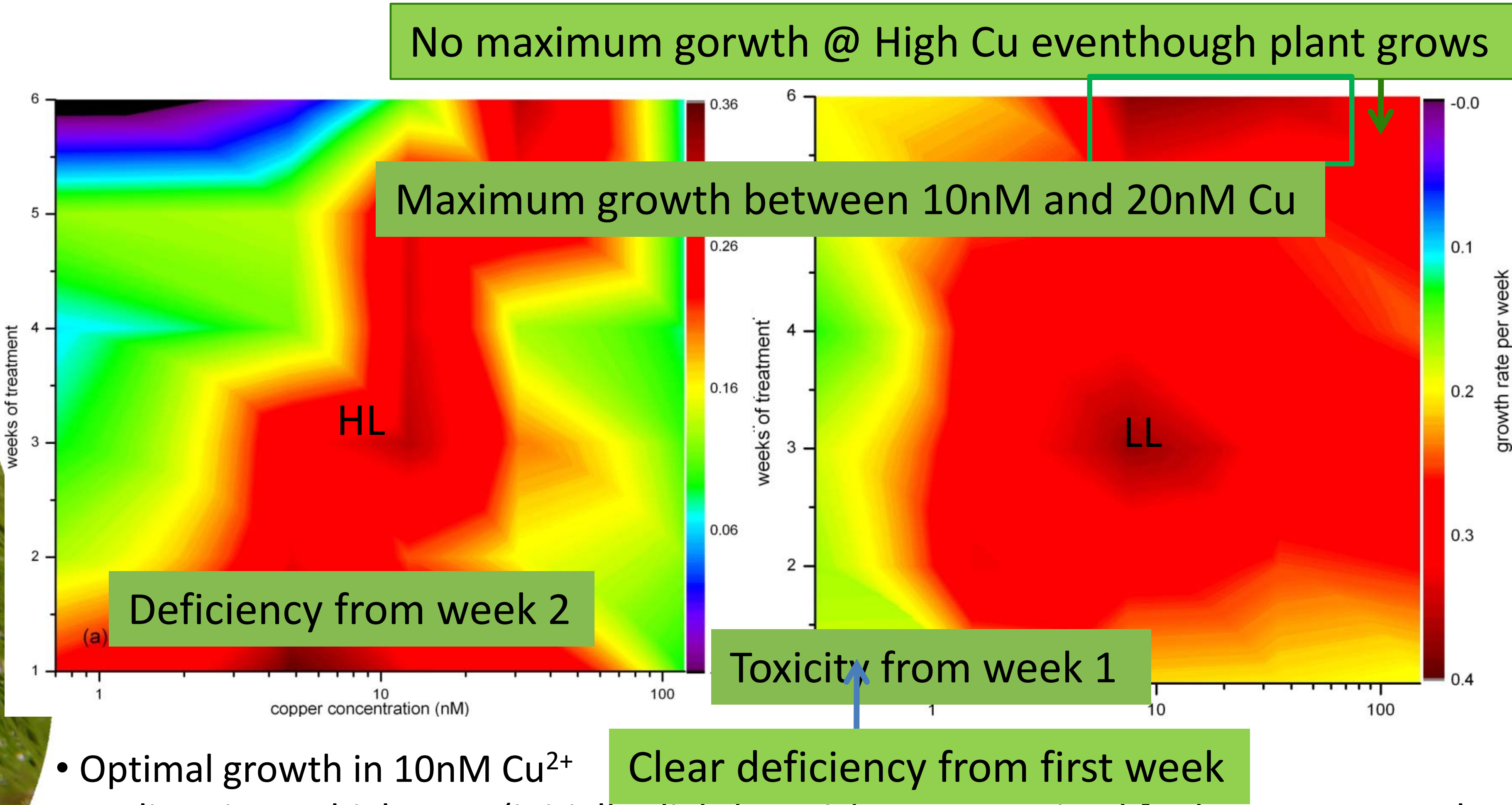
1. Application of pesticides incl. algicides and fungicides
2. Application of pig and poultry slurries (which are rich in Cu) and sewage sludge as fertilisers,
3. Mining
4. Other industrial use (metal plating, steelworks, refineries)
5. Domestic waste emissions

(Yamamoto, Nakayama & Chichester 1985, Lopez & Lee 1977)

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 Ceratophyllum.



Growth Measurement

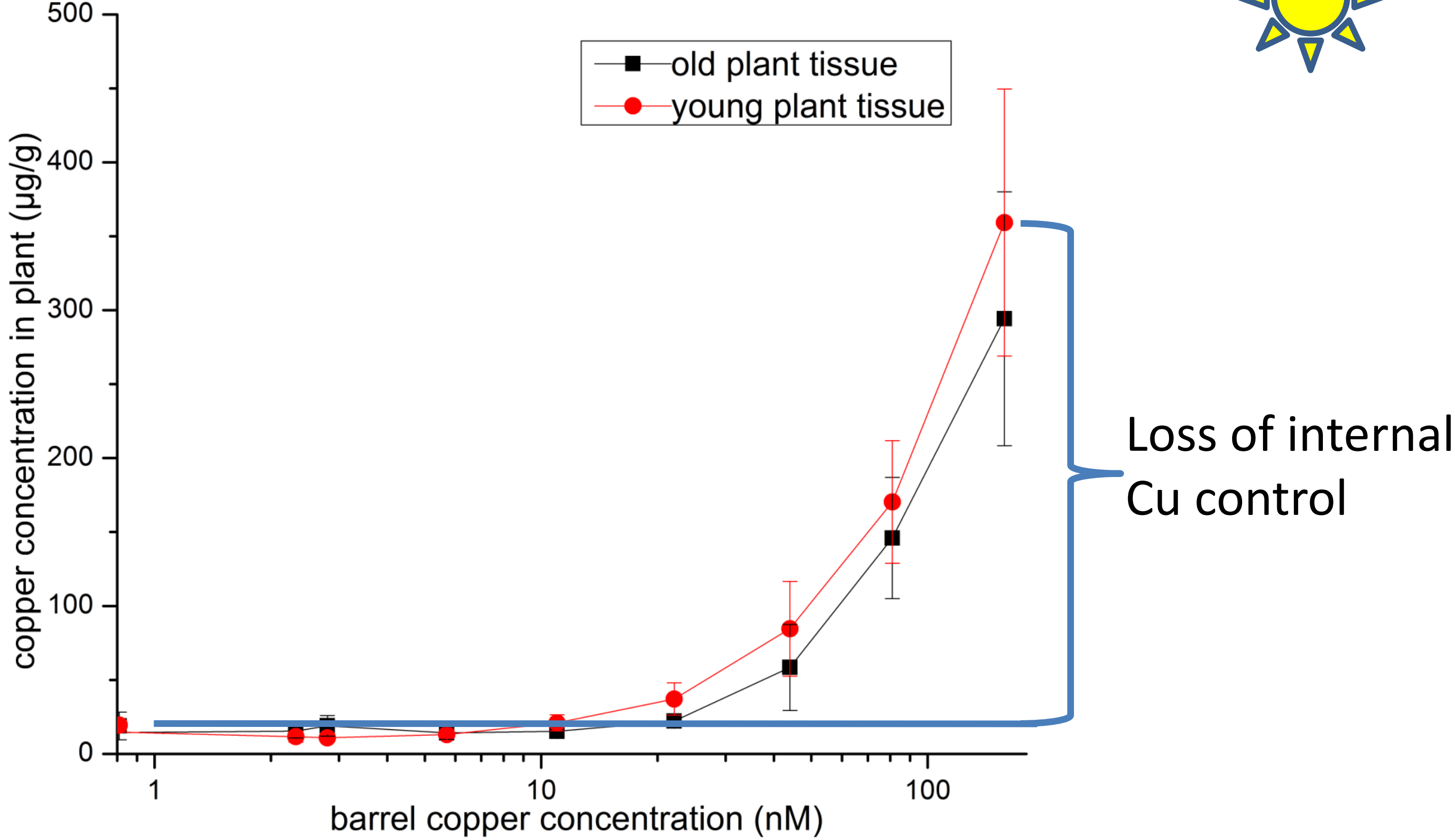


- Optimal growth in 10nM Cu^{2+}
- Acclimation to higher Cu (initially slightly toxic) : act as optimal for longterm growth

HL: Thomas G, Stärk H-J, Wellenreuther G, Dickinson BC (2013) Aquatic toxicology 140-141, 27-36

LL: Thomas G, Andresen E, Mattusch J, Hubáček T, Küpper H (2016) Aquatic Toxicology 177, 226–236

Metal Accumulation



Increase in Cu accumulation after 10nM Cu^{2+} in HL

Young tissues have higher Cu accumulation

Thomas G, Stärk H-J, Wellenreuther G, Dickinson BC (2013) Aquatic toxicology 140-141, 27-36

Irradiance-dependant physiological diversity: differences in the mechanism of heavy metal toxicity

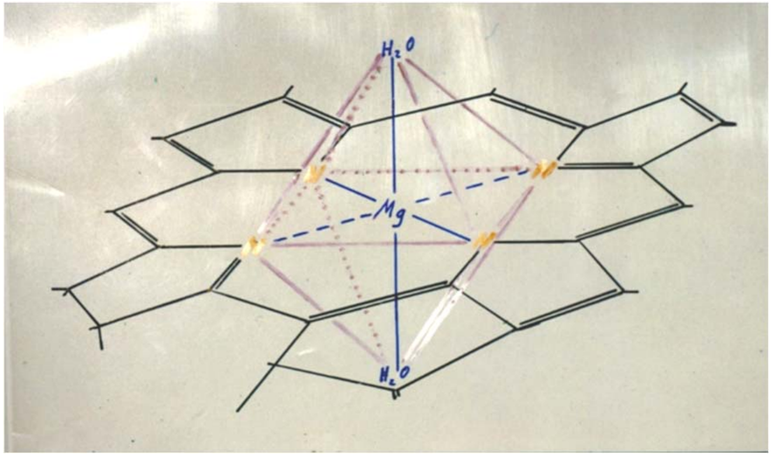
Shade Reaction
Formation of metallochlorophylls (i.e. with centre other than Mg^{2+}) in antenna (LHC II)

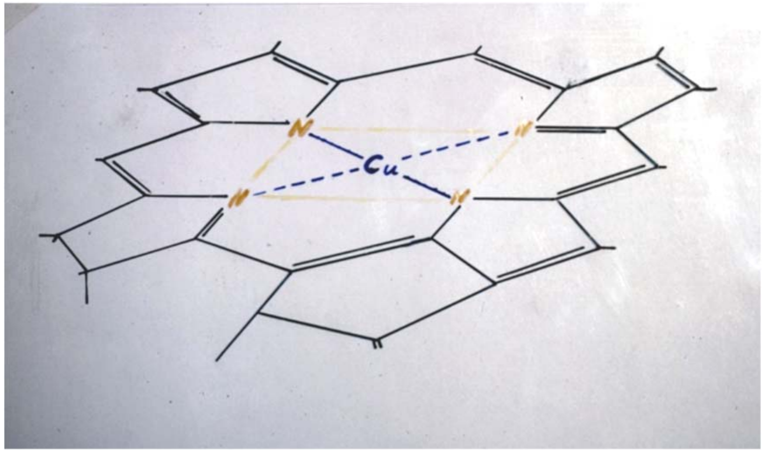
Metallochlorophylls are unsuitable for photosynthesis!

Sun reaction
Direct damage to the PS II core

Küpper H, Küpper F, Spiller M (1996) JExpBot 47, 259-266
Küpper H, Küpper F, Spiller M (1998) Photosynthesis Research 58, 123-133
Küpper H, Šetlík I, Spiller M, Küpper FC, Prášil O (2002) Journal of Phycology 38(3), 429-441

Why are heavy metal chlorophylls unsuitable for photosynthesis?





Main reasons

• heavy metal chlorophylls bind axial ligands only weakly (Zn-Chl) or not at all (Cu-Chl) → light harvesting proteins denature

• unstable singlet excited state → relaxation of absorbed & transferred energy as heat
→ “black holes“ for excitons

Review: Küpper H, Küpper FC, Spiller M (2006) In: Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications (B. Grimm, R. Porra, W. Rüdiger and H. Scheer, eds.), Vol. 25 of series ”Advances in Photosynthesis and Respiration” (Series editor: Govindjee). Kluwer Academic Publishers, Dordrecht; pp. 67-77.

„Sun reaction“ of copper toxicity

- Damage to the PSII by insertion into the Phe a of PSII reaction centre in high radiance. (sun reaction)

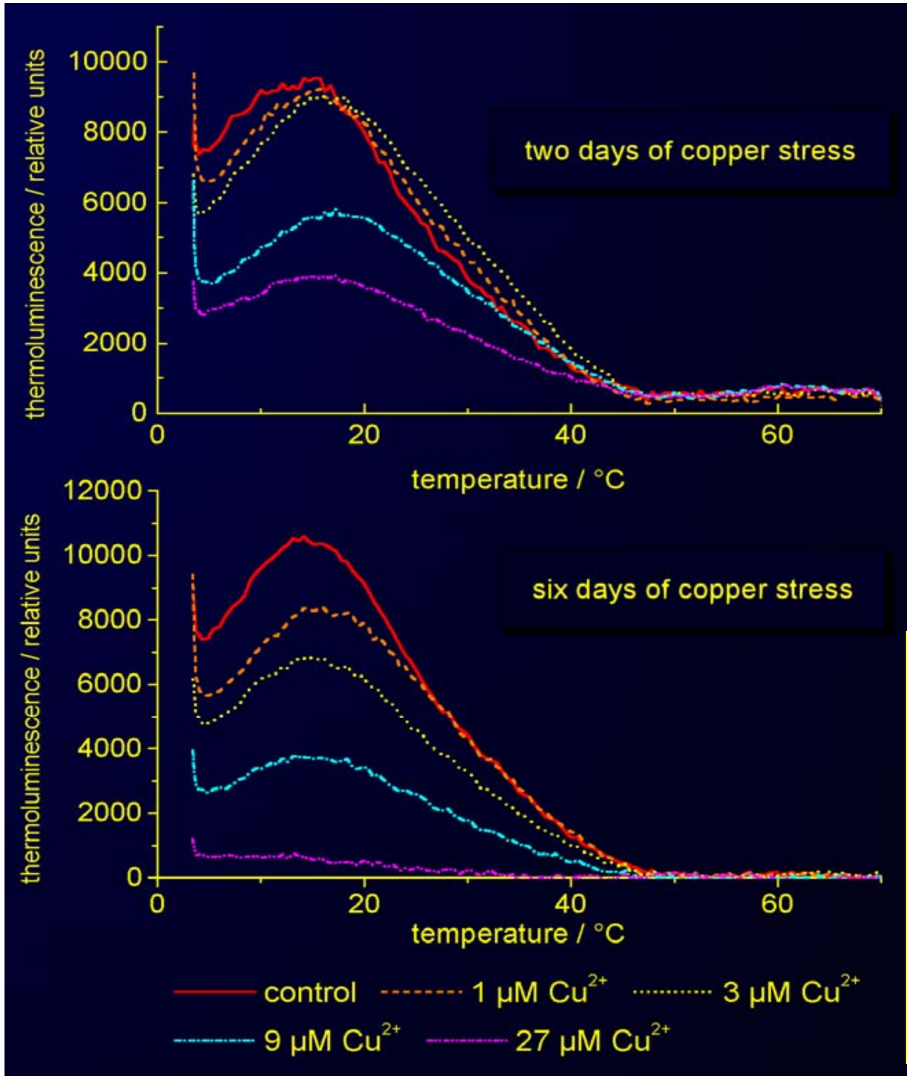
- less Chl from PS II available for substitution

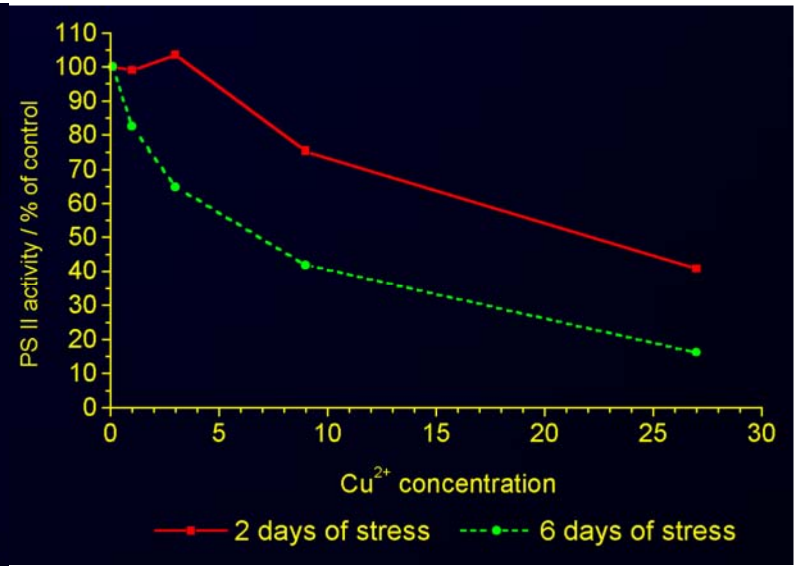
- less CuChl formation.

- bleaching of pigments

Reviews:
Küpper H, Kroneck PMH, 2005, Metal ions Life Sci 2, 31-62;
Küpper H, Andresen E (2016) Mechanisms of metal toxicity in plants. Metallomics 8, 269-285.

In vivo thermoluminescence measurements

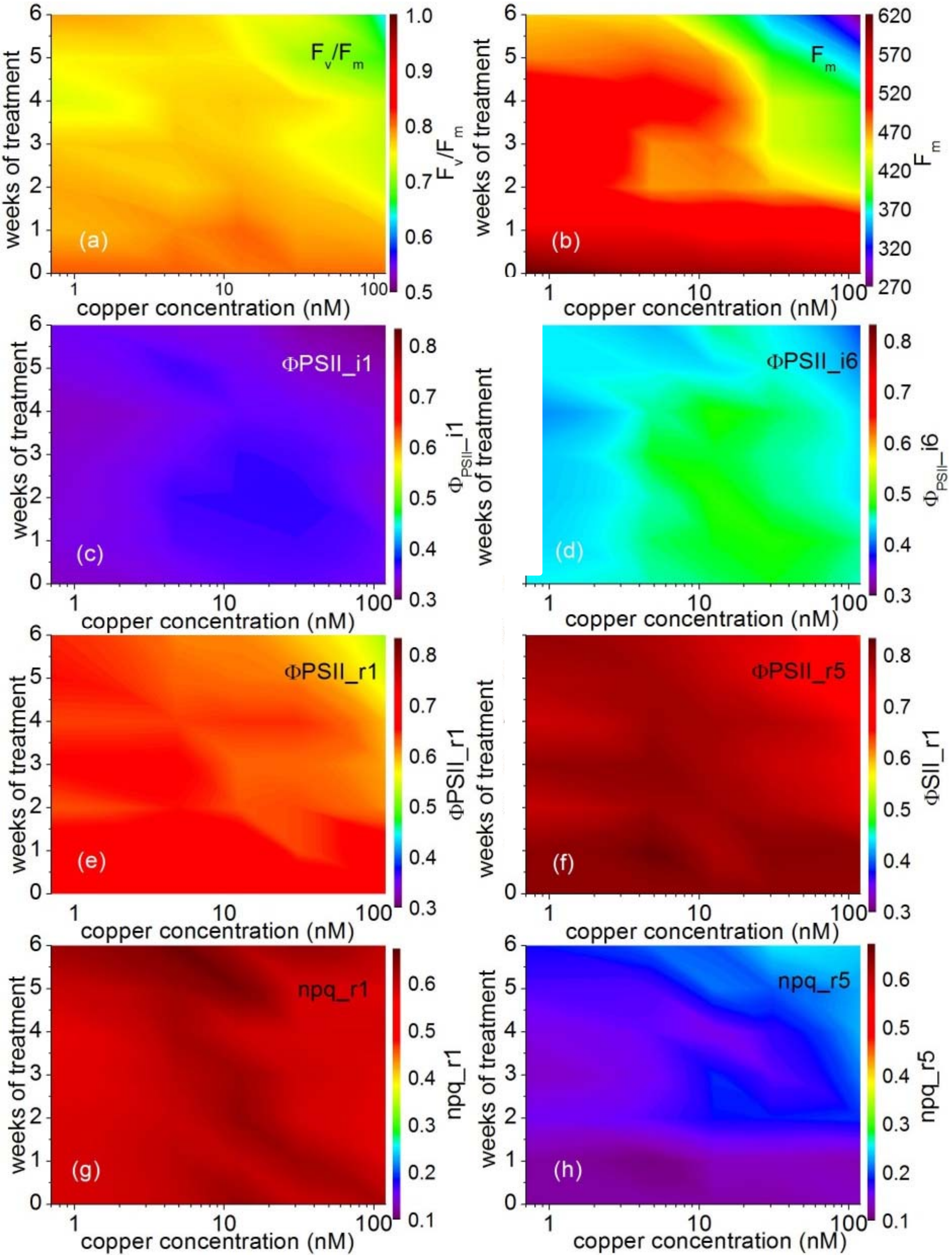




--> Heavy metal stress does not lead to a shift in the Q_A band of thermoluminescence, but only to a lowering of amplitude, i.e. a decrease of the number of active PSII

--> Q_A site is not the primary site of heavy metal action under physiological conditions

Küpper H, Šetlík I, Spiller M, Küpper FC, Prášil O (2002) Journal of Phycology 38(3), 429-441



Increased F_v/F_m @ 2 nM Cu^{2+}

↓

Cu deficiency inhibited growth and not photosynthesis

Greater F_m decrease compared to F_v/F_m

↓

Insertion of Cu into Pheo of PS II (sun reaction)

Decrease of photochemical quenching @ Cu deficiency, not in F_v/F_m

↓

Electron transport chain affected in Cu deficiency

Decrease of photochemical quenching at toxic Cu concentrations

Greater Φ_{PSII} decrease compared to F_v/F_m at highly toxic Cu

↓

Damage to the electron transport in addition to damage to PS II

Values remain less affected compared to all other parameters

↓

PSII mediated photochemistry is more vulnerable to excess Cu

NPQ increase in toxic Cu

↓

More heat relaxation

Thomas G, Stärk H-J, Wellenreuther G, Dickinson BC (2013) Aquatic toxicology 140-141, 27-36

Formation of ROS

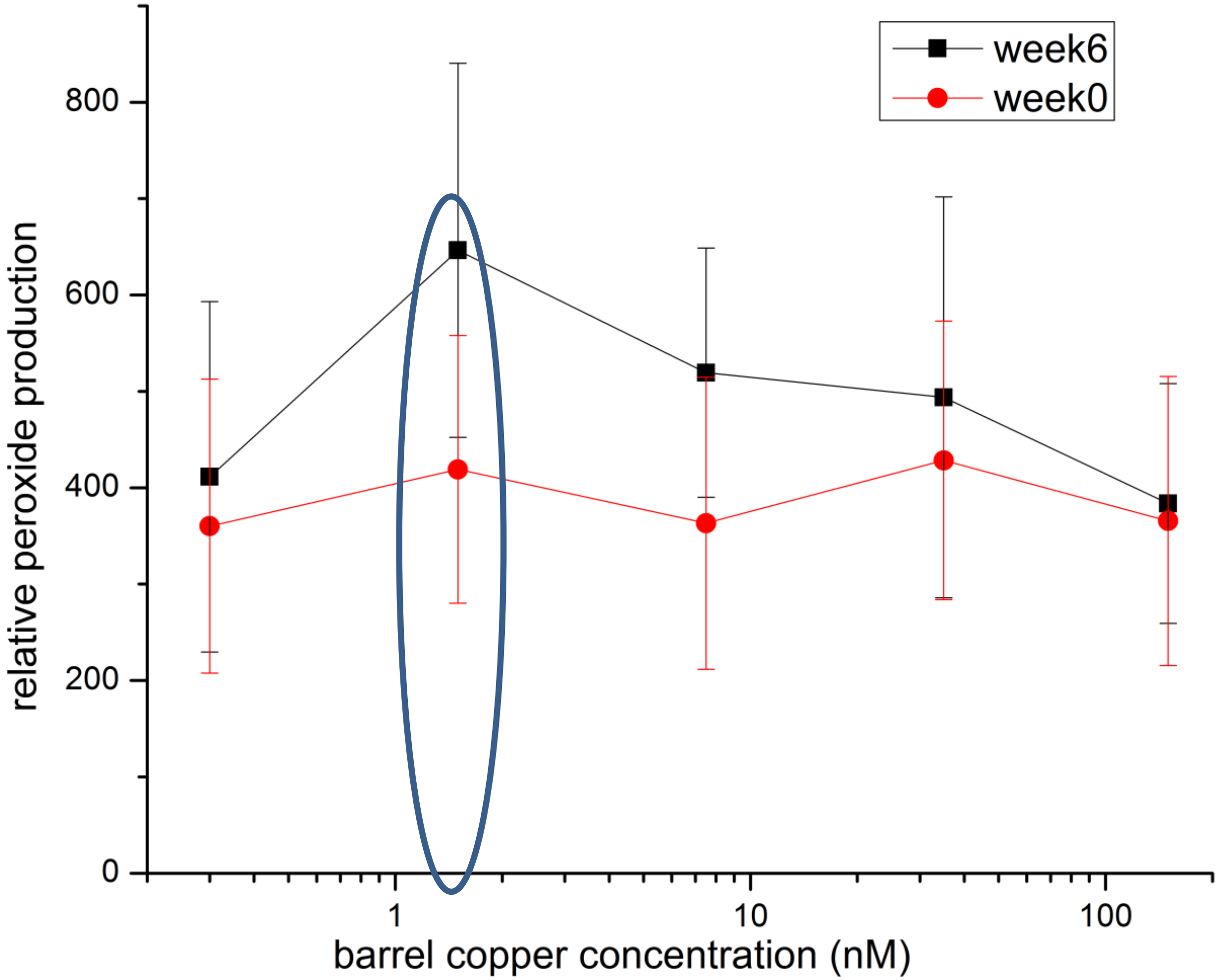
- These include various radicals like OH^-,O_2^-,etc

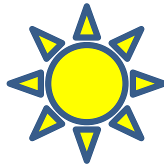
- **Direct**: Production by the Fenton reaction could be a possibility for ROS production in plants, but never proven *in vivo*

- **Indirect** *: In vivo* production may be due to the malfunctioning of the photosynthetic apparatus, e.g. by formation of [Cu]-Chl, etc.

- They cause oxidative stress which further causes oxidative damage to various biomolecules.

Reviews:
Küpper H, Kroneck PMH, 2005, Metal ions Life Sci 2, 31-62;
Küpper H, Andresen E (2016) Mechanisms of metal toxicity in plants. Metallomics 8, 269-285.





•**Higher peroxide production at around 2 nM Cu^{2+}** agreed to the high rate of photosynthesis : **peroxide is byproduct of photosynthesis**

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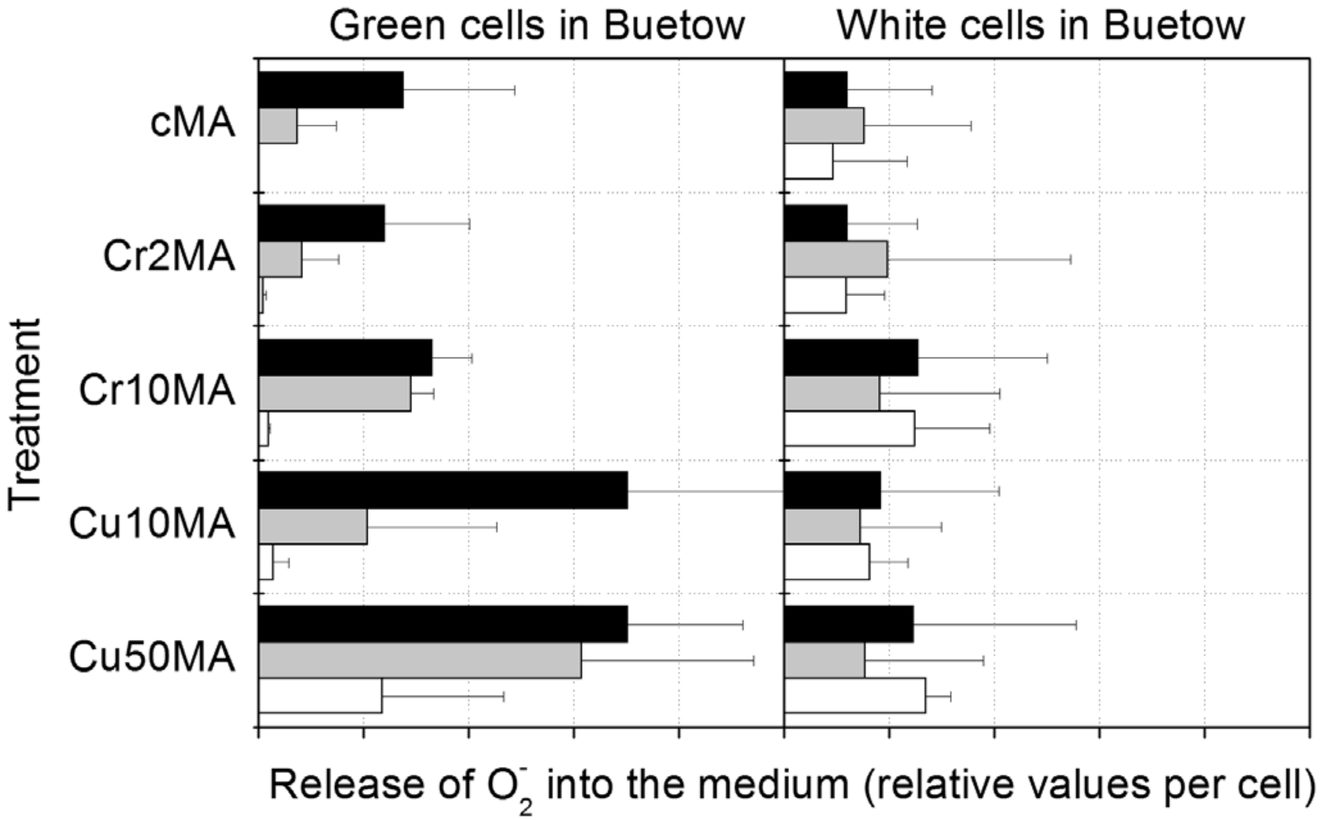
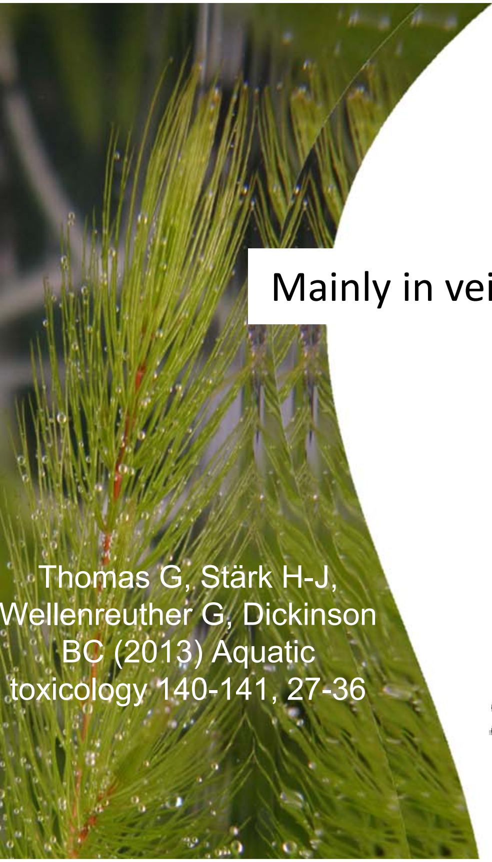
Comparison of superoxide production during Cr- and Cu-stress in *Euglena gracilis*



→ Increase in superoxide production under heavy metal stress is mainly caused by malfunctioning photosynthesis!

Rocchetta I, Küpper H (2009) New Phytologist 182, 405-420



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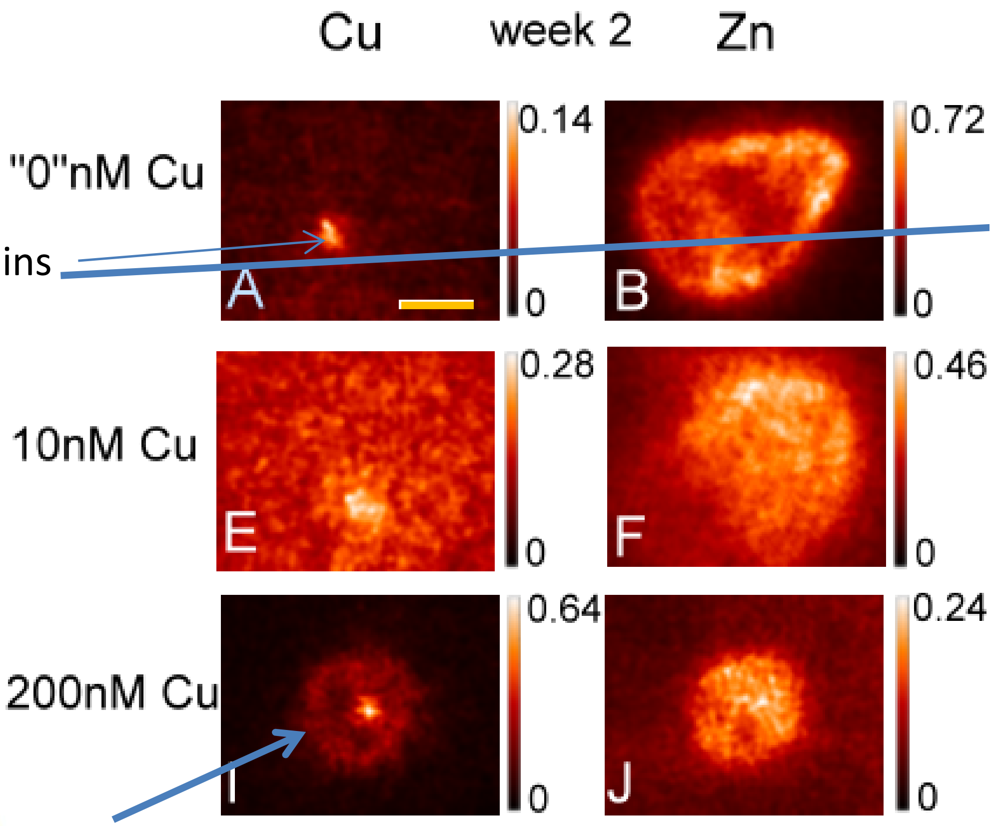


Mainly in veins

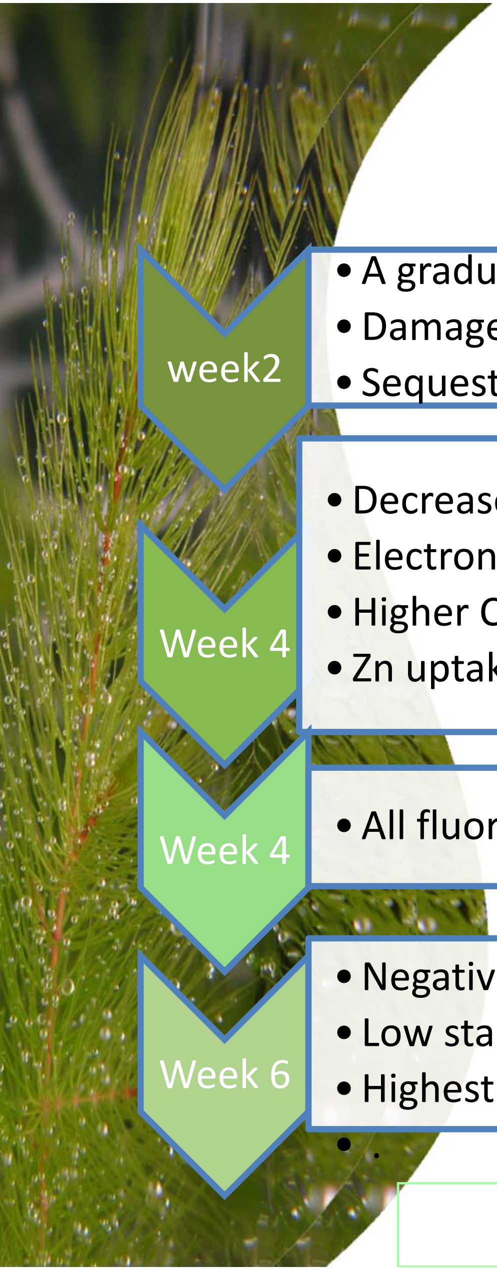
Sequestration of Cu from veins to mesophyll and epidermis

2 phase response to Cu toxicity

1. Initially sequestered from veins to mesophyll and epidermis
2. Further accumulation in veins contributing to the damage of the plant

Furthe

- Zn compartmentation is damaged in Cu deficiency (optimal Cu homogenous distribution)
- Additional Zn is sequestered to epidermis
- Zn uptake inhibit at toxic Cu



Copper Toxicity in HL

week2

- A gradual decrease in growth is observed
- Damage to the PSII reaction centre becomes significant
- Sequestration of Cu from vein to mesophyll affects photosynthesis

Week 4

- Decrease in Chlorophyll pigments
- Electron transport inhibited apart from PSII damage
- Higher Cu accumulation in veins : transport inhibited
- Zn uptake inhibited

Week 4

- All fluorescence parameters decrease including (NPQ) at 200 nM Cu^{2+} especially towards the end

Week 6

- Negative growth and unhealthy symptoms visible
- Low starch in older tissue : no photosynthesis
- Highest Cu accumulation.
- .

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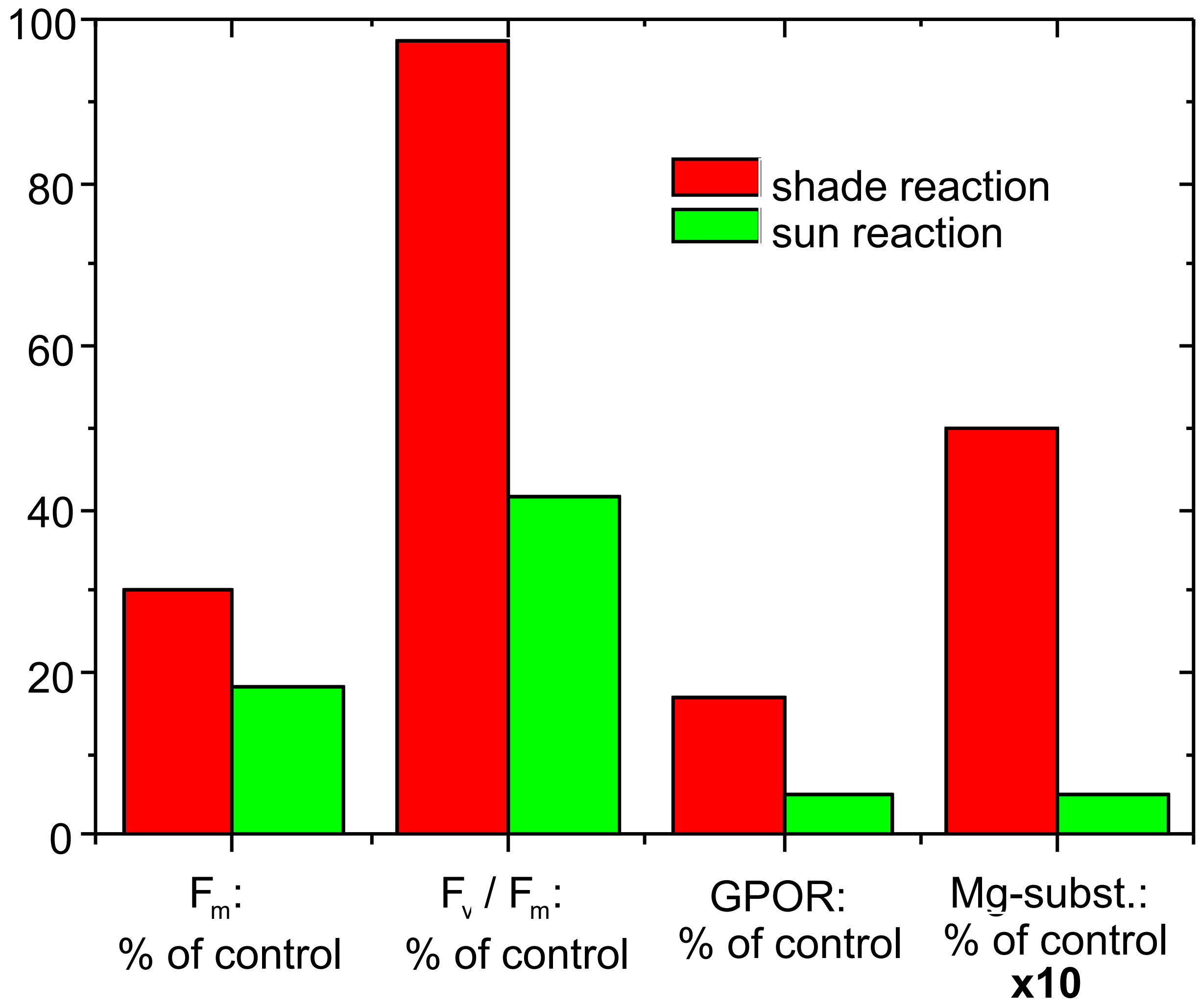
Photosynthesis activity: Sun- vs. Shade-reaction



Küpper H, Šetlík I, Spiller M, Küpper FC, Prášil O (2002) Journal of Phycology 38(3), 429-441

„Shade reaction“ of copper toxicity

- [Cu]-Chl formation in LHC II in low irradiance.

  - Substitution of Mg^{2+} in the Chl forming heavy metal substituted Chl / (hms)-Chl (LHC antenna Chl which are more accessible)

  - Pheophytinization : Cu-Chl conformational change exposing MgChl to acidic thylakoid lumen.

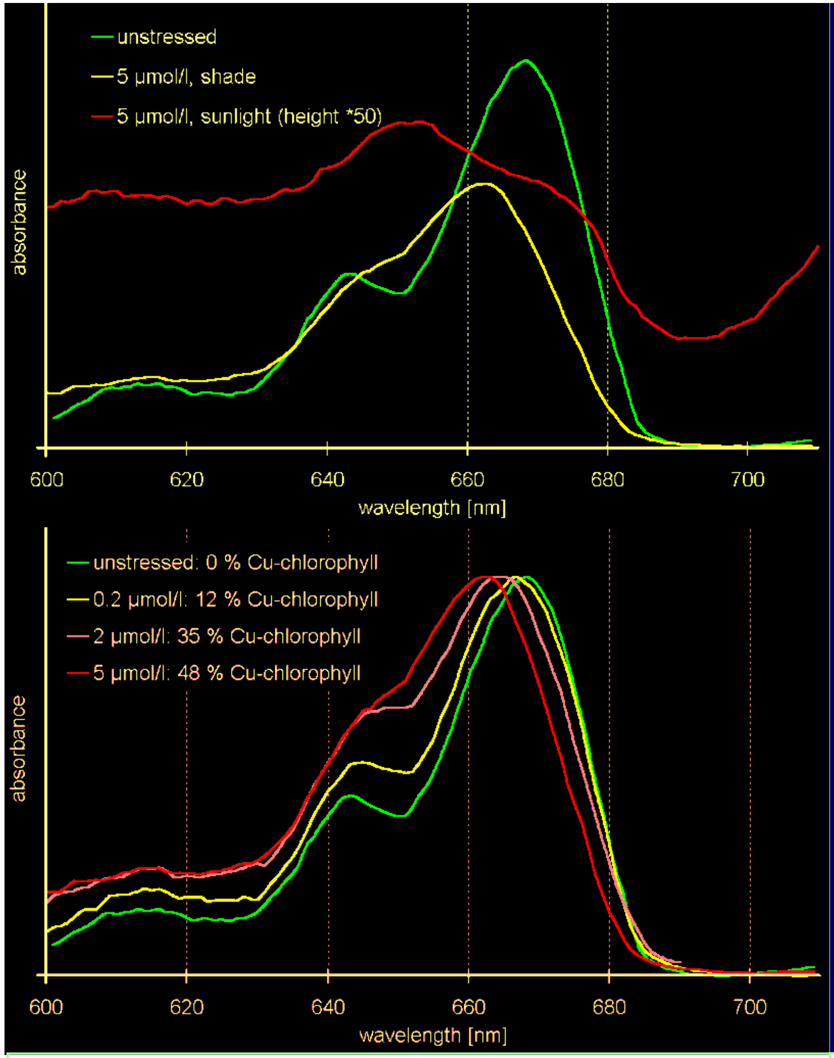
  - photochemical quenching less affected

Reviews:
Küpper H, Kroneck PMH, 2005, Metal ions Life Sci 2, 31-62;
Küpper H, Andresen E (2016) Mechanisms of metal toxicity in plants. Metallomics 8, 269-285.

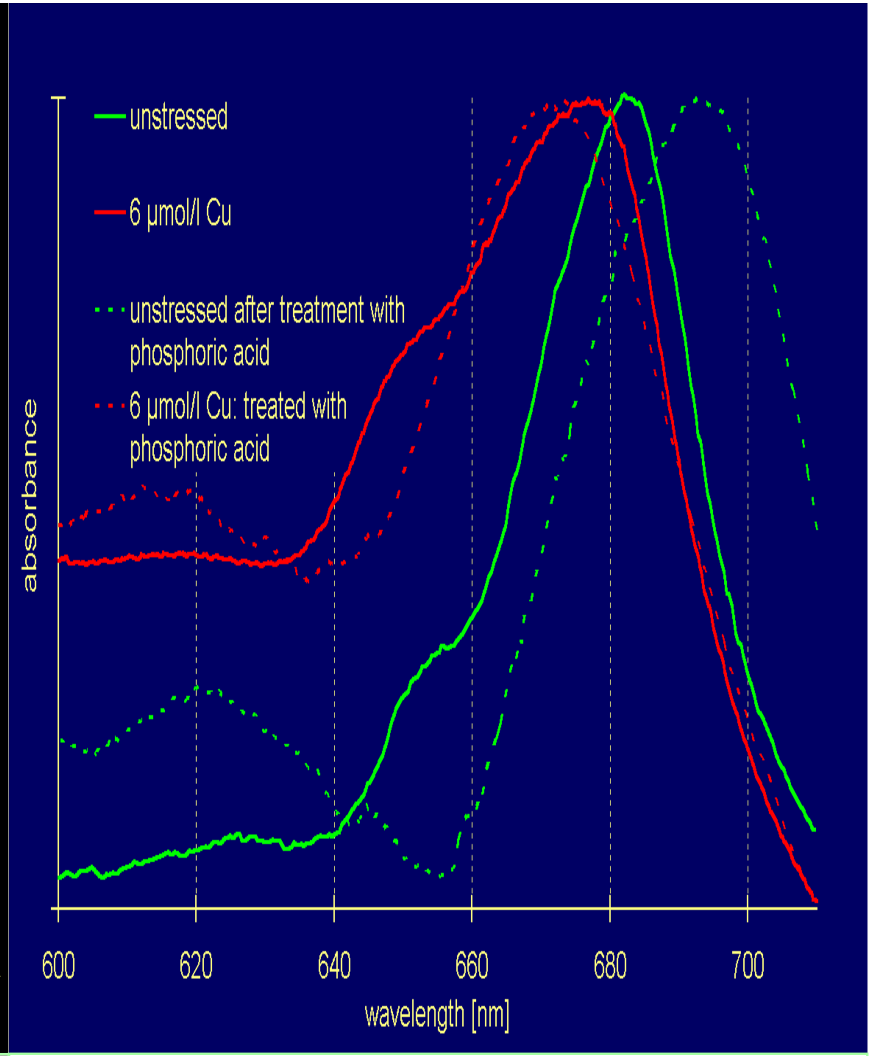
UV/VIS absorbance spectroscopy

Spectra of *Elodea canadensis* extracts

In vivo UV/VIS absorbance spectroscopy



Küpper H, Küpper F, Spiller M (1996) Journal of Experimental Botany 47 (295), 259-66

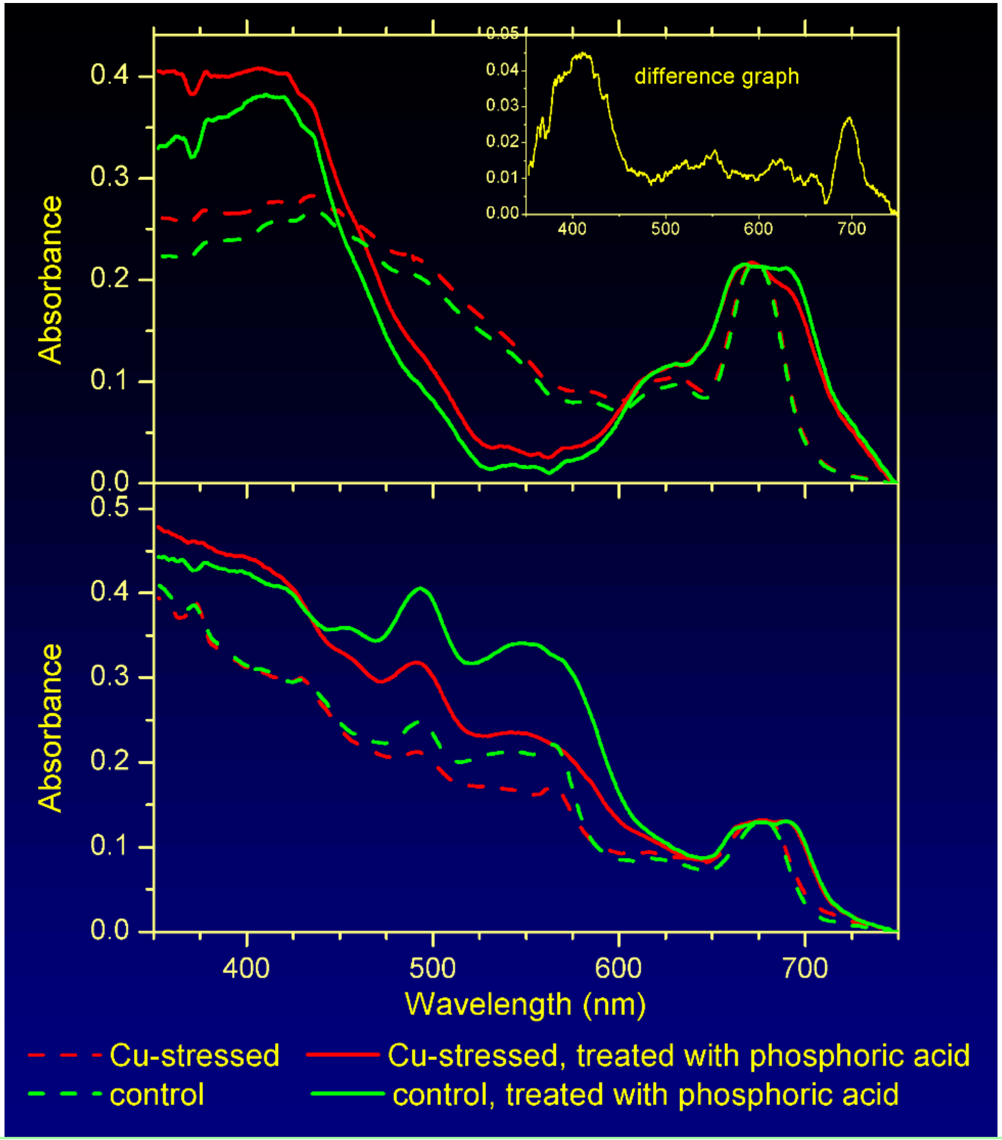


Küpper H, Küpper F, Spiller M (1998) Photosynthesis Research 58, 125-33

UV/VIS -spectroscopy: non-Chlorophyta with 10 µM Cu^{2+}

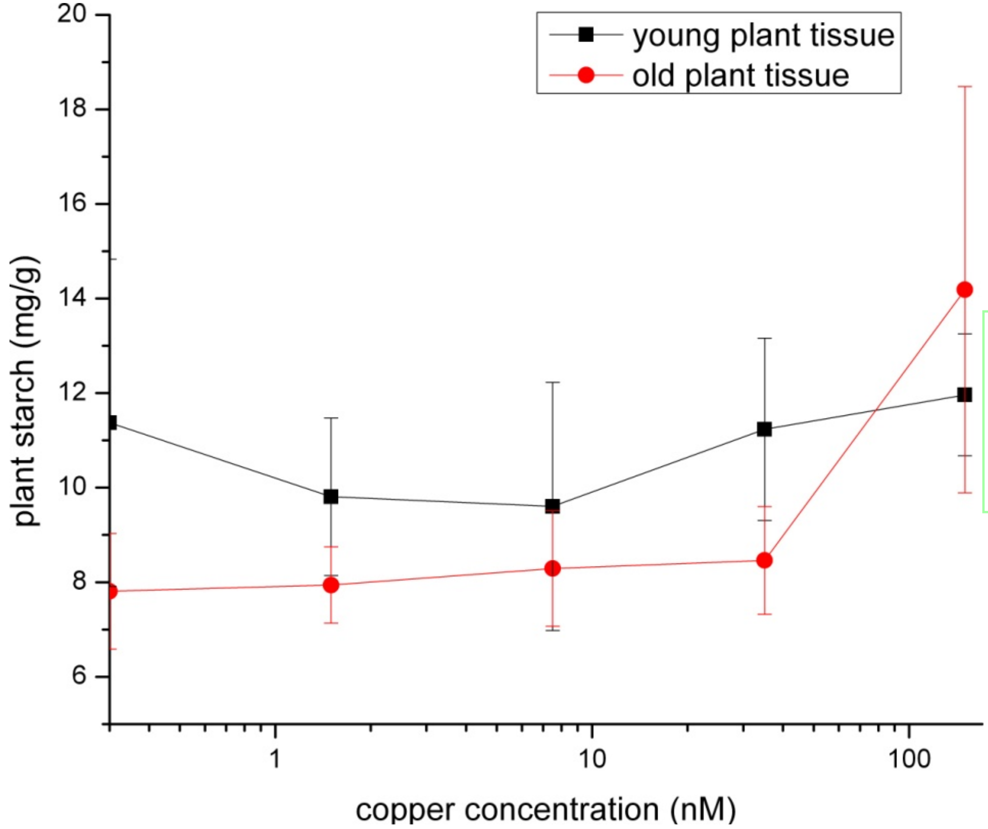
Brown alga
Ectocarpus siliculosus:
Chl a/c-LHC always accessible to Mg-substitution
--> always shade reaction

Red alga
Antithamnion plumula:
Phycobilisomes: no Chl
--> always sun reaction



Küpper H, Šetlík I, Spiller M, Küpper FC, Prášil O (2002) Journal of Phycology 38(3), 429-441

Starch accumulation



Thomas G, Andresen E, Mattusch J, Hubáček T, Küpper H (2016) Aquatic Toxicology 177, 226–236

Low light

- Less starch accumulation
- Increase in starch with increase in Copper concentration after optimal

Copper Toxicity in LL

Week 3

- LHCII damage (decrease in Fm, very little in Fv/Fm and photochemical quenching)
- More damage to LHCII

Week 6

- Decrease in Chl A (more Cu Chl formation + pheophytination)
- Further damage to LHC
- Highest starch accumulation .
- Highest Cu accumulation.
- Zn uptake inhibited
- Reduction in oxygen production

Thomas G, Andresen E, Mattusch J, Hubáček T, Küpper H (2016) Aquatic Toxicology 177, 226–236

Copper deficiency

- Decrease in the PSII activity , damages the thylakoid membrane, decrease in the chlorophyll pigments.
- The damage to physiological functions causes the formation of various symptoms. These may cause the up-regulation or down-regulation of genes.
- Affect the various proteins including Plastocyanin, SOD, etc
- Affect the various protein activity including Cu-SOD, AO, etc

Review: Andresen E, Peiter E, Küpper H (2018) Trace metal metabolism in plants. Journal of Experimental Botany 69, 909-954

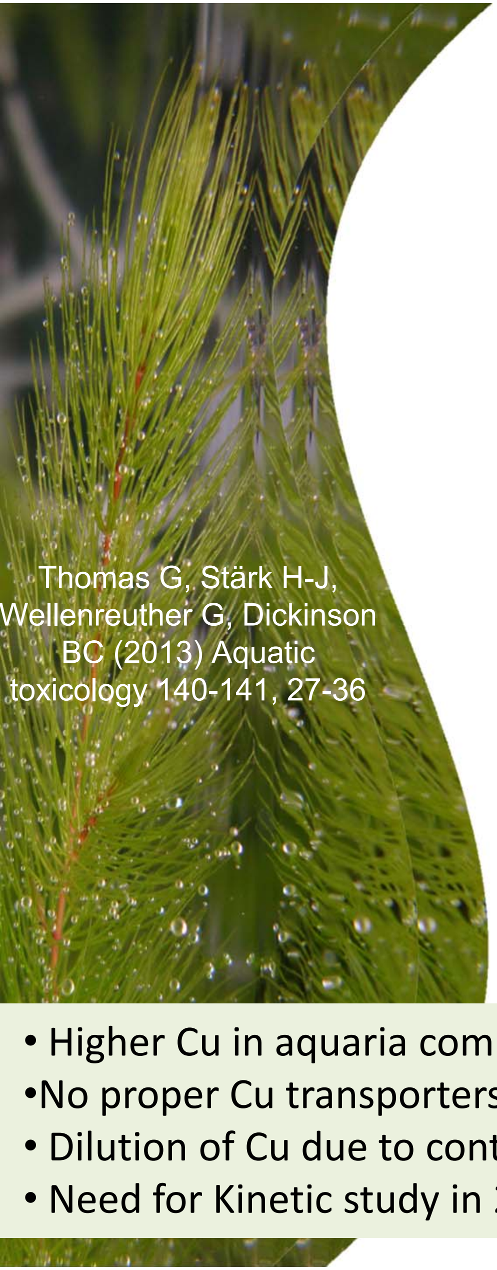
During Cu deficiency

- metal chelate reductase activity increase ($Cu^{2+} \rightarrow Cu^{+}$)
- MT transcripts shows increased expression

Fordham-Skelton et al . 1997 Acta Physiologiae Plantarum

Higher peroxide formation (ROS) : release of metals from MTs (also seen in Fungi)

Welch et al. 1993 Planta



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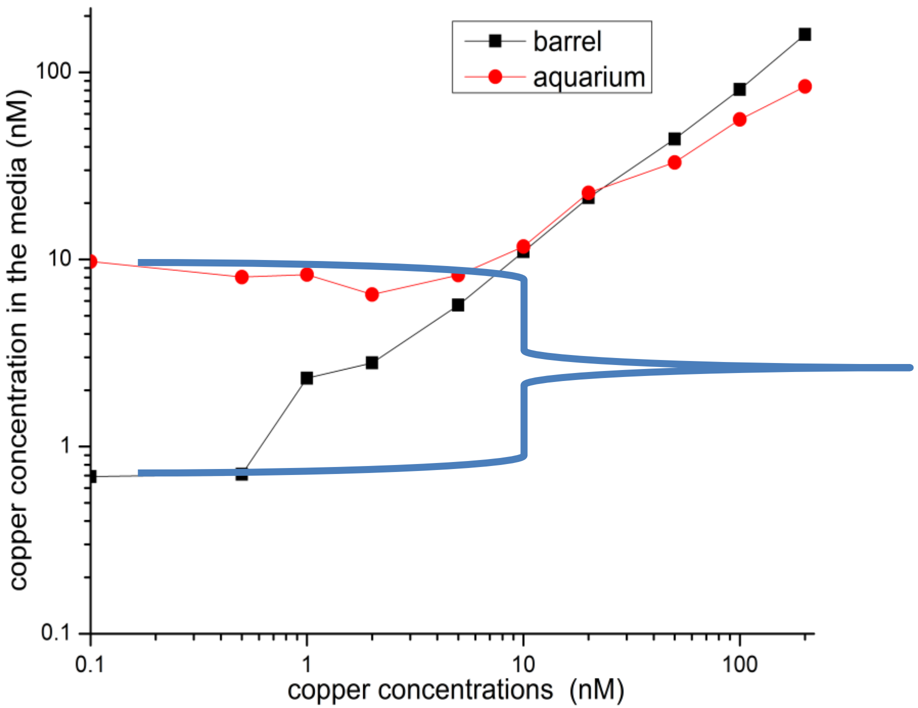
Barrels Vs Aquaria

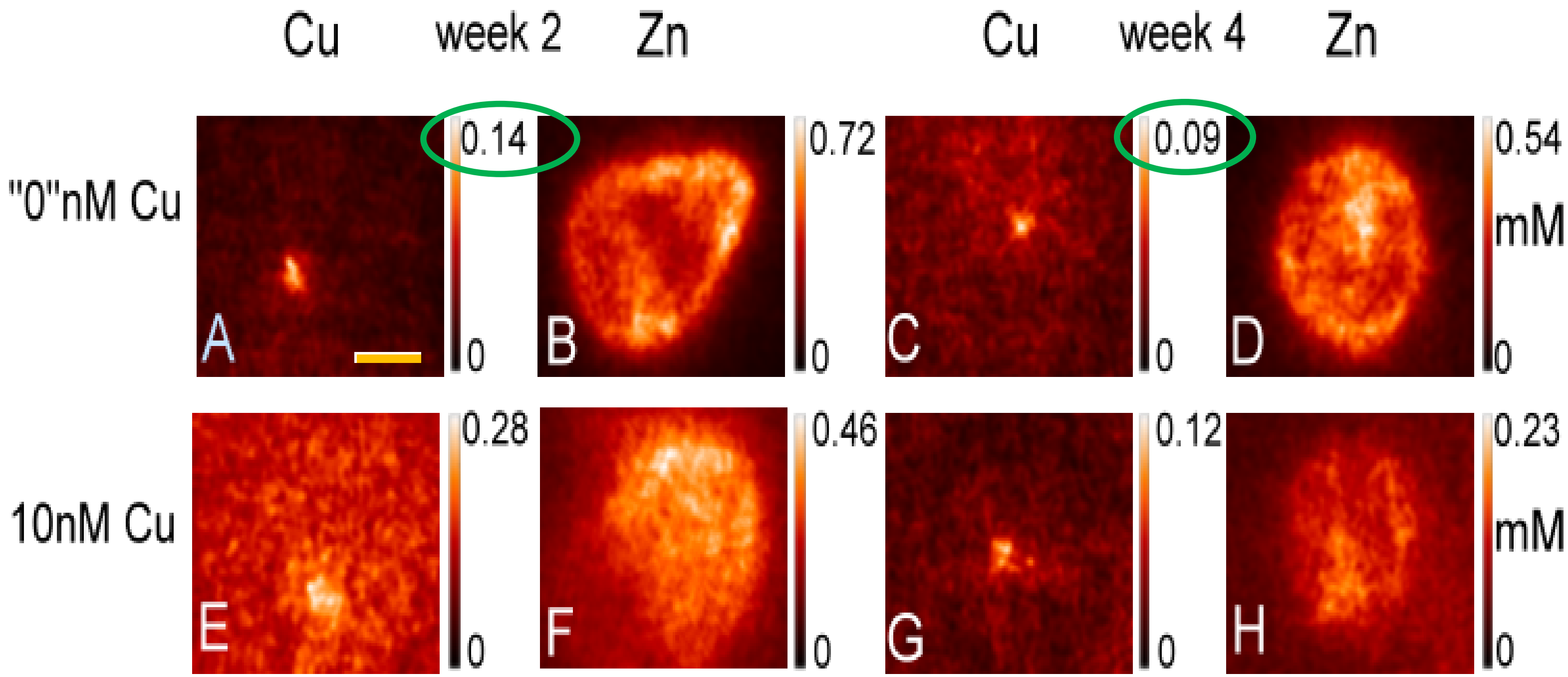
Copper in the media



So where is this Copper coming from ?

- Higher Cu in aquaria compared to barrels at Cu deficiency conditions **due to release from the plants**
- No proper Cu transporters for active uptake below 10 nM Cu^{2+}
- Dilution of Cu due to continuous circulation of media within 1 week
- Need for Kinetic study in 24 Hrs

Distribution of Cu during Cu deficiency



Deficiency: Reduction in tissue concentration

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Combating Cu deficiency

Deficiency observed in ***Arabidopsis thaliana*** from 5 nM Cu^{2+} grown for 5 weeks (Pilon et al., 2008)

↓

- Down-regulation of SOD by micro RNA activation for Cu to be available for plastocyanin
- Use of alternative proteins like FeSOD.

Deficiency observed in **pea plant** below 100nM Cu^{2+} grown for 18d (Welch et al., 1993)

↓

Metallothioneins were involved in increased Cu chelate reductase activity

Deficiency observed in **Algae** @ "0" nM Cu^{2+}(Quinn & Merchant, 1998) (< 3 nM Cu^{2+})

↓

PC function is replaced by cytochrome C6

Deficiency of Cu in *C. demersum*

- Unable to redistribute Cu to make best use of the available Cu
- Therefore Cu remains in vein in deficient Cu
- Release of Cu from plant

- HL & LL : Light acclimated electron flow inhibited but not dark adapted photochemical quantum yield of the PSII reaction centre : lack of plastocyanin

- LL : decrease of starch

- Involvement of proteins : SOD, PC ?
- Involvement of MTs ?
- Kinetic study would yield better understanding.

Copper Deficiency in HL (<10nM Cu^{2+})

week2

- Deficiency symptoms and reduction in growth rate observed at "0" nM Cu^{2+}
- Light acclimated electron transport is affected by lack of plastocyanin

week4

- Zn compartmentation is damaged
- Reserve Cu utilized and growth reduction observed from 5 nM Cu^{2+}

week5

- Reduction in pigments : light harvesting affected
- Complete stop of growth : death of the plant

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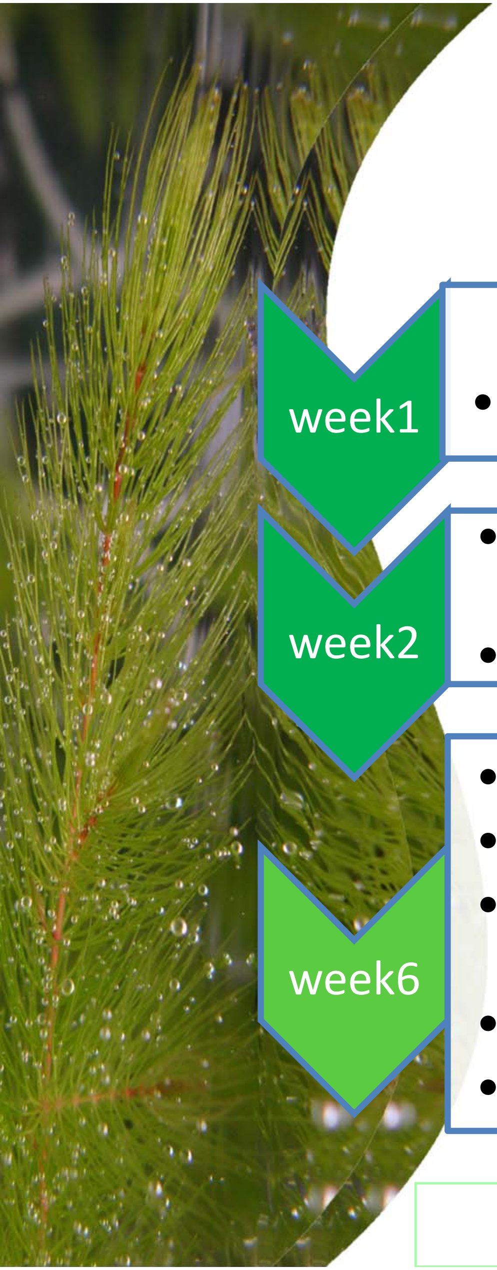

Copper Deficiency in LL (<10nM Cu^{2+})

week1

- reduction in growth rate observed at "0" nM Cu^{2+}

week2

- Light acclimated electron transfer affected by malfunctioning of plastocyanin
- Growth reduction observed from 5 nM Cu^{2+} (similar to HL)

week6

- Reduction in pigments : light harvesting affected
- Oxygen production affected.
- Further decrease in light acclimated electron transfer (no or less Plastocyanin)
- Decrease in starch
- Growth reduction

Thomas G, Andresen E, Mattusch J, Hubáček T, Küpper H (2016) Aquatic Toxicology 177, 226–236

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