The effectiveness of *Chara australis* in the phytoremediation of Cd-contaminated soils

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Outline:

1. Introduction
2. Effects does Cd have on *Chara* growth
3. Cd localization
4. Cd accumulation
5. Effects of EDTA on Cd accumulation
6. Possible tolerance mechanisms
7. Conclusions

Introduction: Cadmium

- Typical uncontaminated soil concentrations are <1 ppm Cd
- This level is increased by
  - Industrial processes (smelting, mining…)
  - Weathering of parent materials containing high Cd
  - Agricultural practices
    - Fertilizers
    - Compost
    - Pesticides

Introduction: Cadmium

- Cd is a toxic contaminant
- Accumulates within plants and animals
- Biomagnification throughout food chain
- Entry into humans through
  - Crops
  - Fish
- Residence time in human body of OVER 30 YEARS

Introduction: Cadmium

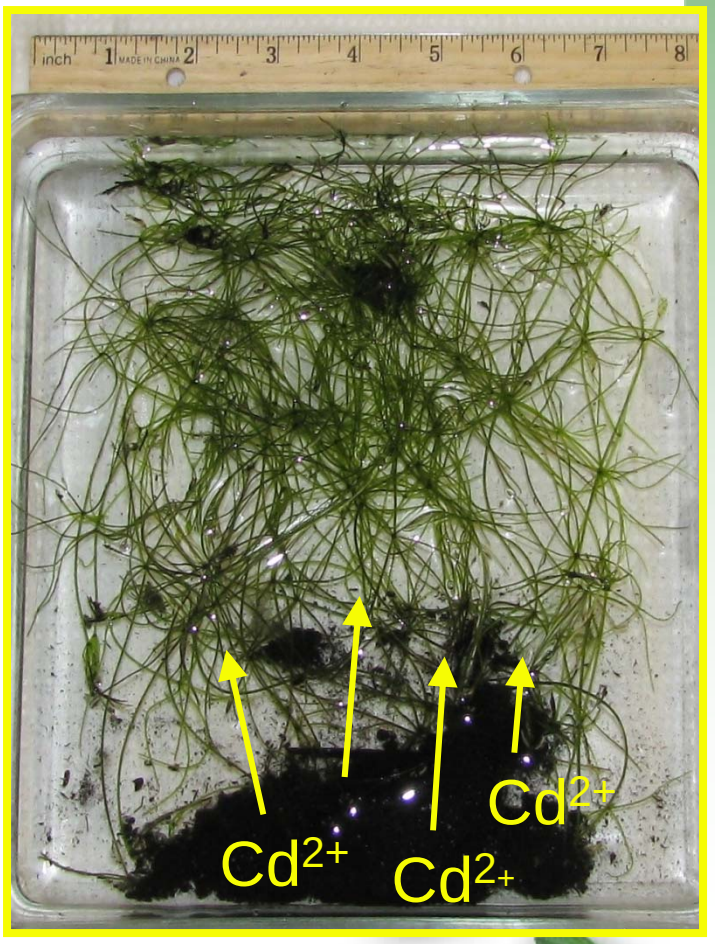
- Targets the renal cortex of the kidney
- Replaces Ca^{2+} in bones
  - Causes soft, brittle bones
- If inhaled it causes
  - Pulmonary edema
  - Lung damage
- Carcinogen

What is Phytoremediation?

- Definition:
  - A process whereby plants are employed to clean up contaminated soils
    - Some examples of contaminants include:
      - Heavy metals (cadmium, lead, mercury…)
      - Organic pollutants
      - Petroleum hydrocarbons
      - Polychlorinated biphenyls…

How do plants do this?

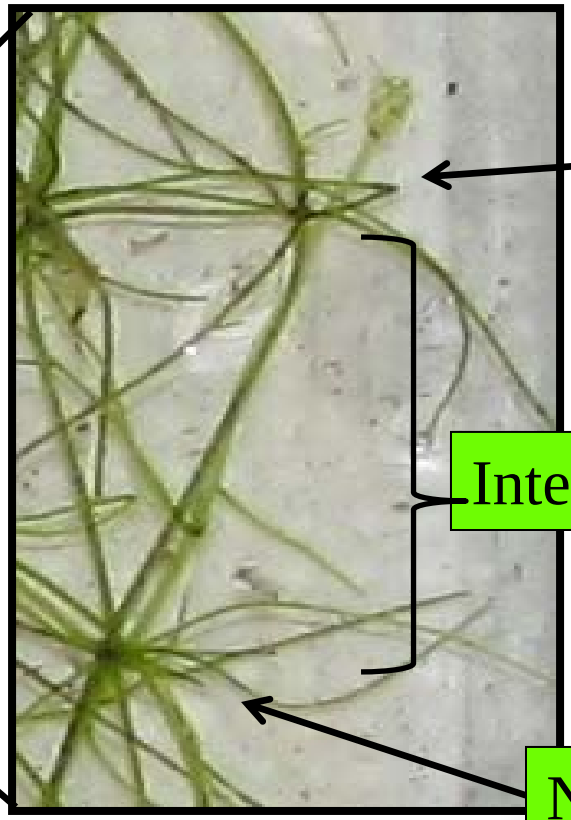
- PHYTOEXTRACTION
  1. Removal and accumulation of contaminants from soil
     - Roots/root-like structures (rhizoids)
  2. Translocation of contaminants to other tissues
     - Shoots, stems, leaves, or fruits
- Benefits:
  - Inexpensive
  - Natural method



What are Charophytes?

- Submerged aquatic plants
- Also known as stoneworts
  - Encrusted with calcium carbonate
- Wetlands, rivers, streams, lakes
- Closest living relatives to land plants

Chara Anatomy



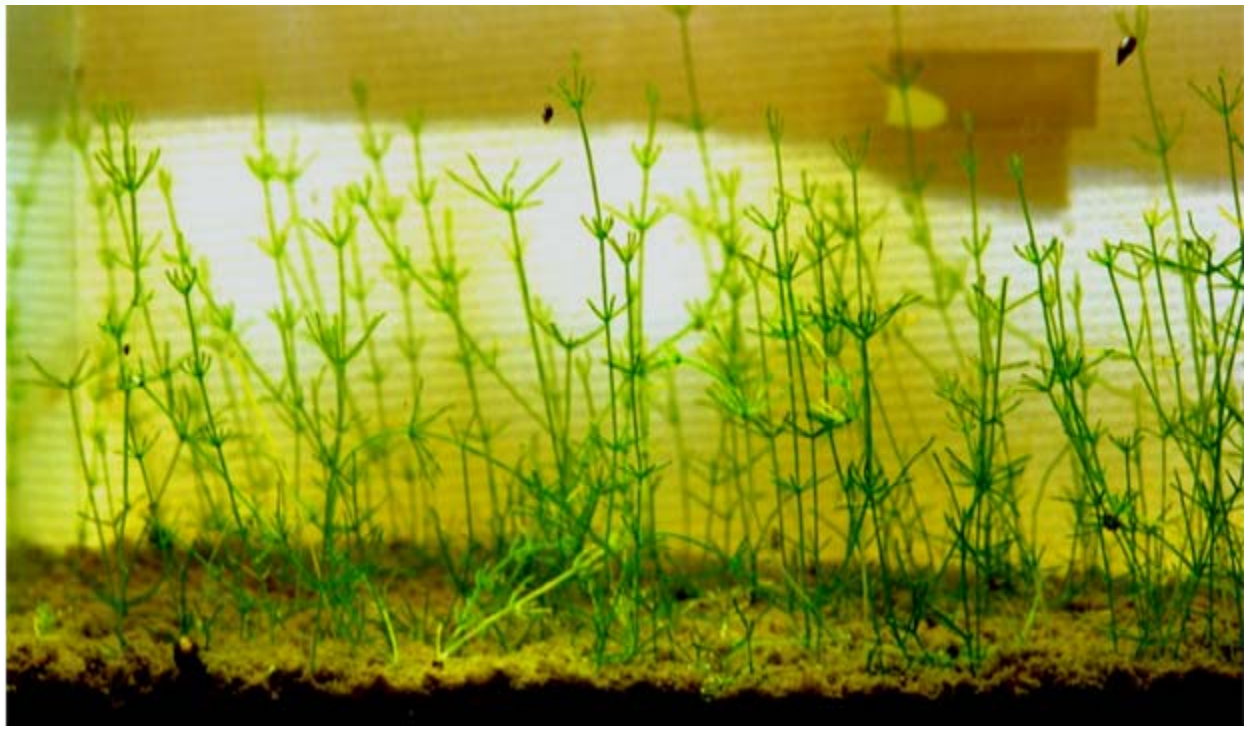
Node

Internode

Node

Why *Chara*?

- *Chara* produces dense meadows
  - Large areas can be remediated at once



Why *Chara*?

- *Chara* binds sediment and can over-winter under ice preventing…
  - Resuspension of sediment
  - Redistribution of toxins back into water column
  - (Kufel 2002)

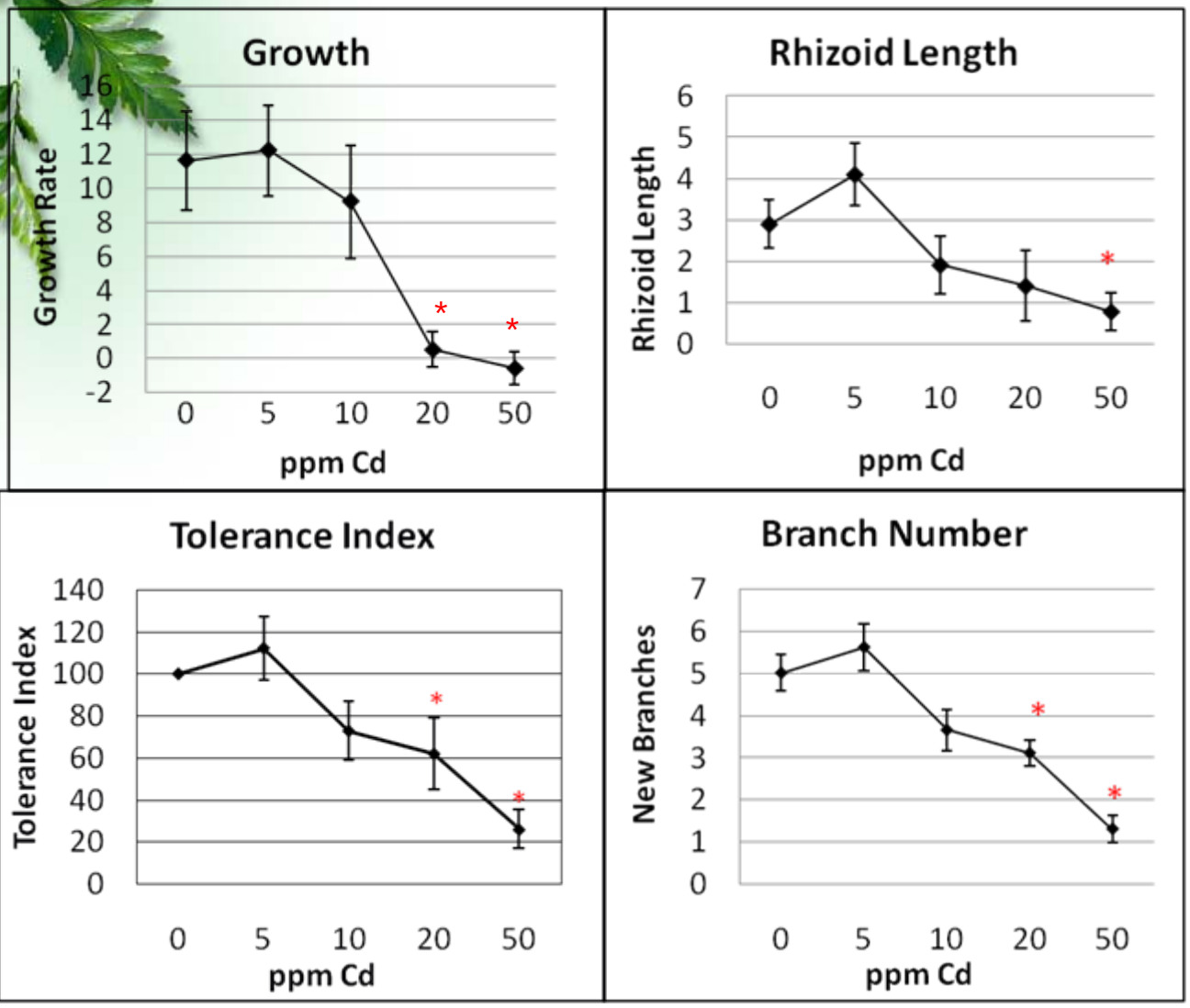
Question 1

Can *Chara* grow in soils spiked with Cd?

Methods

- Soil spiked with 0, 5, 10, 20 or 50 ppm Cd
- Plants were grown for 29 days
- Growth parameters were recorded
  - Branch number
  - Rhizoid length
  - Growth rate
  - Tolerance index =
    - 100* rhz length *Chara* Cd-exposed /rhz length *Chara* control
- Student's t-tests were performed

Cd Effects on *Chara* Growth



Results:

- Net positive growth up to 20 ppm Cd
- 50 ppm Cd sig. decreased rhizoid length
- 20 and 50 ppm had sig. lower T.I. and branch numbers

Conclusions:

- *Chara* can tolerate levels up to 20 ppm
- 50 ppm Cd is toxic

Note:

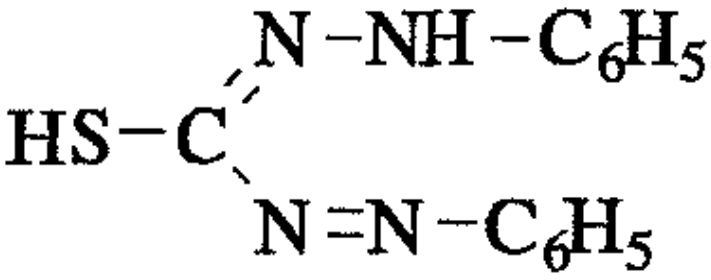
- Values are avg ± SE
- N=10; $p < 0.05$
- TI= $\text{rhizoids}_{\text{metal}}/\text{rhizoid}_{\text{control}} * 100$
- growth period 29 days.

Question 2

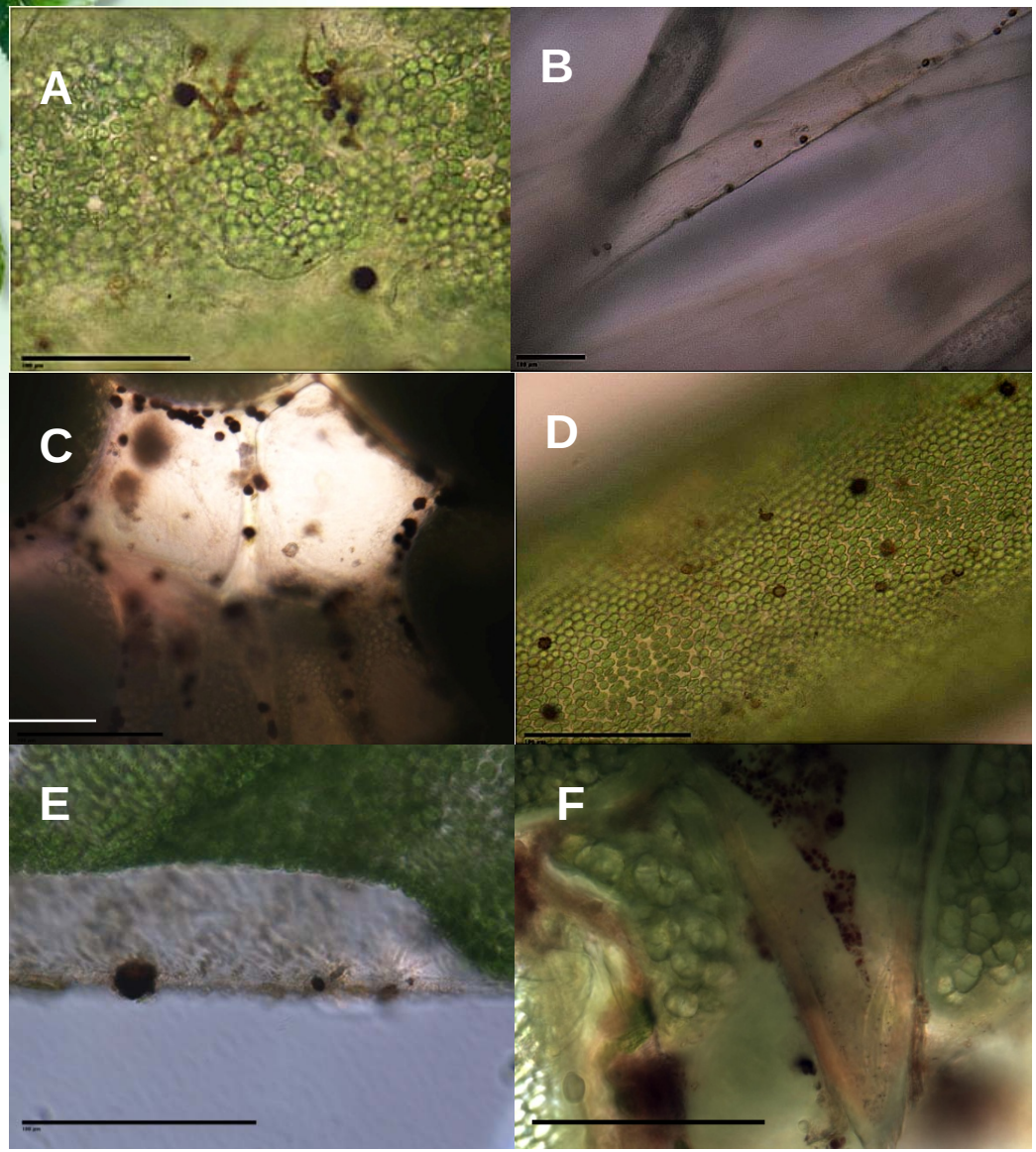
Is *Chara* actually accumulating Cd? Where is it localized?

Methods

- Histochemical method to visualize Cd
  - According to Seregin and Ivanov (1997)
  - Used **dithizone** (diphenylthiocarbazone)
    - Highly sensitive histochemical reagent
    - Forms red-black salt precipitates (dithizonates) with Cd
    - Easily viewed with light micrsoscope



Cd Localization



Histochemical detection of Cd using dithizone

A. Cd in shoot cytoplasm.

B. Cd along cell wall of rhizoid.

C. Cross-section of nodal complex. Cd in apoplast and cytoplasm of cells.

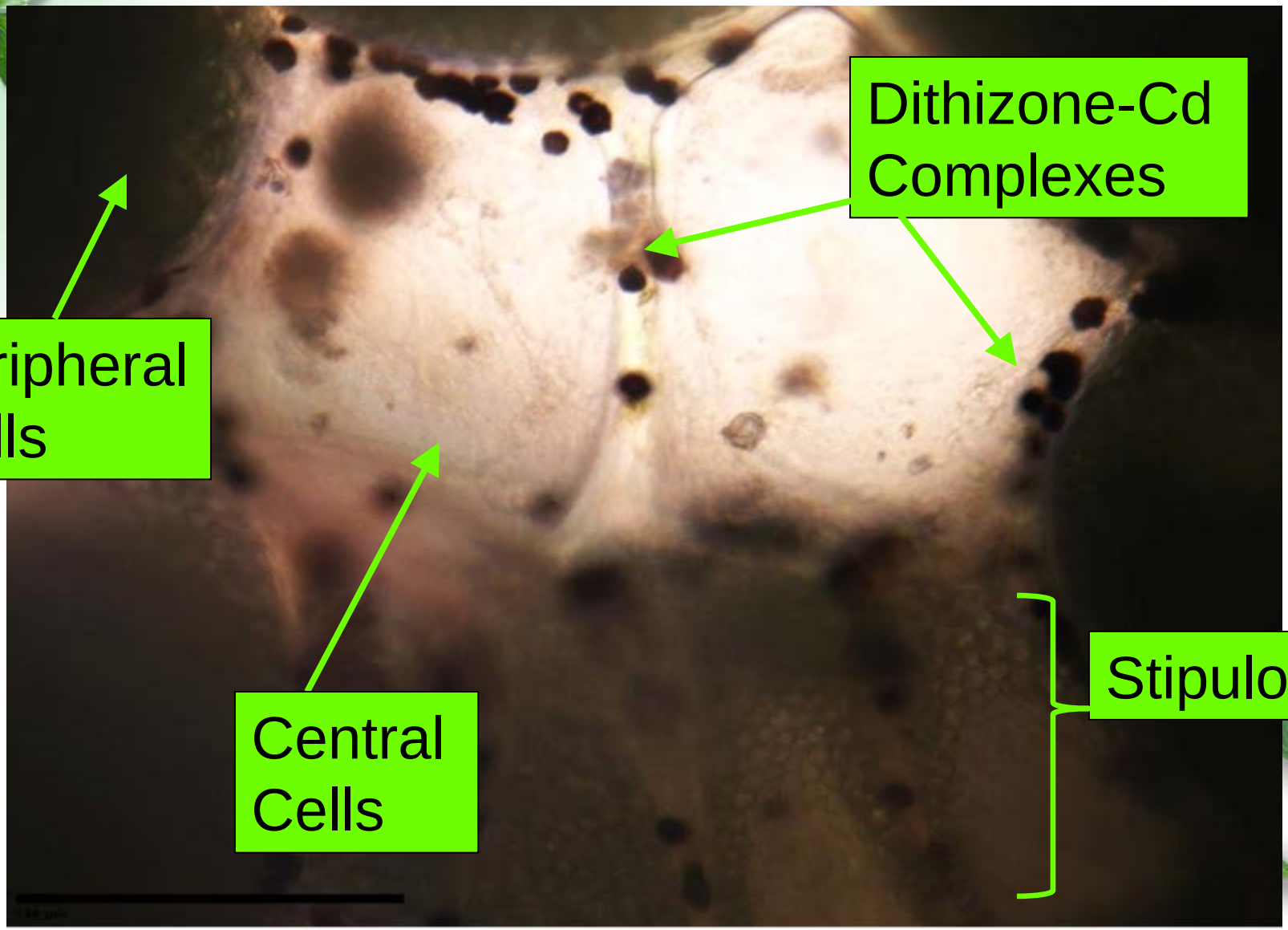
D. Cd in shoot.

E. Plasmolyzed shoot. Cd in shoot cell wall.

F. Cd in stipulode.

- Scale bars are 100 µm
- Method accdg. to Seregin and Ivanov (1997)

Cd Localization



Conclusions

- Cd is localized within Chara shoots and rhizoids
- Indicates that there is possible translocation of the metal from rhizoids to shoots

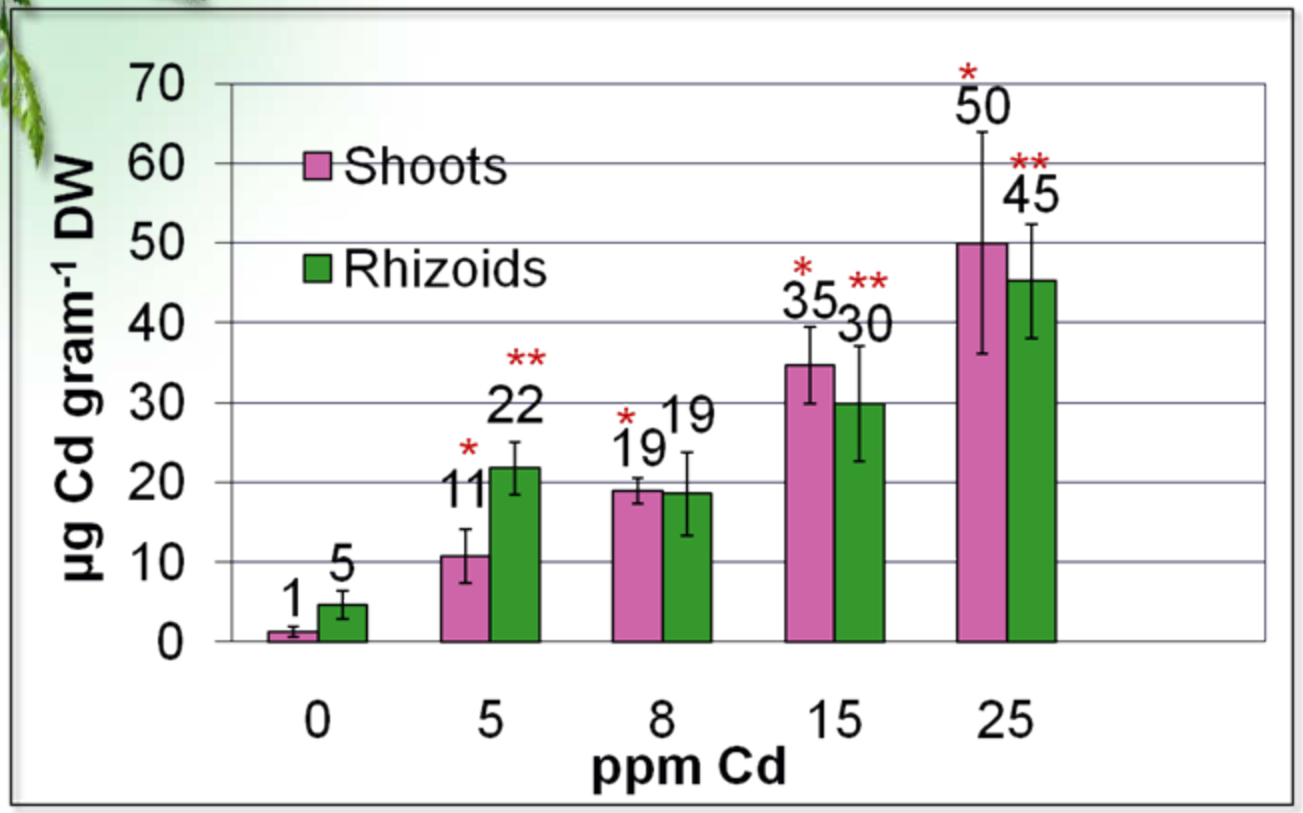
Question 3

How much Cd can *Chara* accumulate in its shoots and rhizoids?

Methods

- 30 g soil was spiked with 0, 5, 8, 15, or 25 ppm Cd
- Plants were grown for 50 days
- Shoots and rhizoids were separated and oven-dried
- Digested with 1 mL of nitric acid
- Centrifuged
- Bulked to 50 mL with ddH2O
- 0, 1, 2, 3, and 4 ppb Cd standards were prepared in 2 % nitric (background)
- Samples read in duplicate by graphite furnace atomic absorption spectroscopy

Cd Accumulation



Results:

- As Cd in the soil increases, so does the amount in *Chara* shoots and rhz
- *Chara* accumulates Cd beyond the conc. added to soil

Note:

- Student's t test $p<0.05$;
- * = shoots sig. different from controls
- **= rhz sig. different from controls

Question 4

Does EDTA effect Cd accumulation in *Chara*?

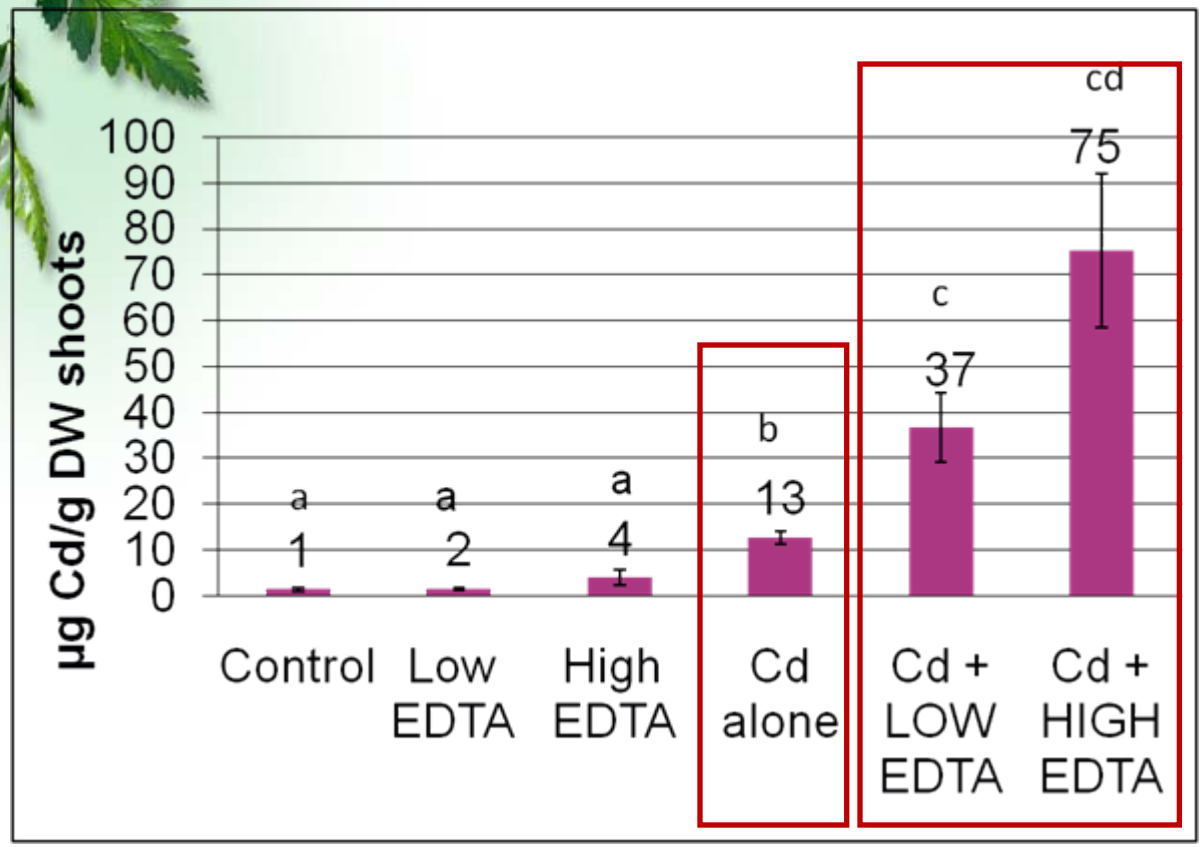
EDTA

- EDTA=Ethylethylenediaminetriacetic acid
- EDTA is a chelator that increases solubility of contaminants in soil
- Makes contaminants more available for plant uptake

Methods

- 30 g soil was spiked with 0, 5, 8, 15, or 25 ppm Cd
- Plants were grown for 50 days
- Shoots and rhizoids were separated and oven-dried
- Digested with 1 mL of concentrated nitric acid
- Centrifuged 3x 3,000 rpm 5 minutes each
- Bulked to 50 mL with ddH2O
- 0, 1, 2, 3, and 4 ppb Cd standards were prepared in 2 % nitric (background)
- Samples read in duplicate by graphite furnace atomic absorption spectroscopy

Enhanced Phytoextraction



Results:

- *Chara* shoots exposed to 8 ppm Cd + low or high EDTA had a sig. greater conc. of Cd than 8 ppm Cd alone ($p<0.005$, $n=7-9$)

Conclusion:

- EDTA may enhance the translocation of Cd to *Chara* shoots

- **Note:** Data for 2 experiments was pooled
- Values are avg ±SE
- Different letters indicate significant differences

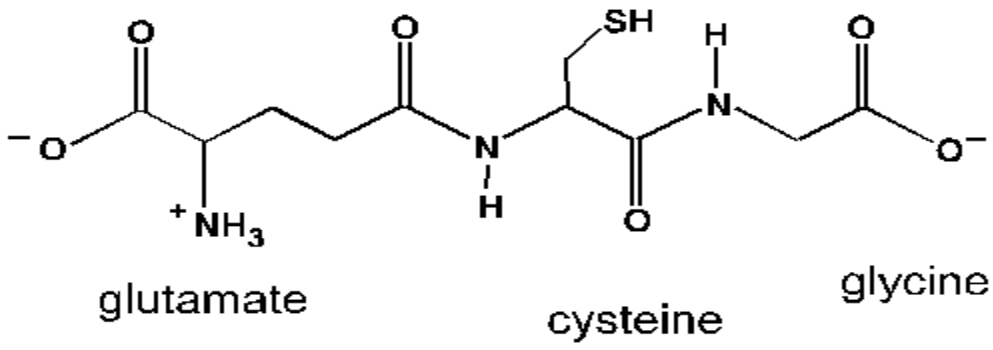
Question 5

Is there an effect of Cd on glutathione levels in *Chara*?

What is Glutathione?

- Tripeptide synthesized from the amino acids:
  - L-cysteine
  - γ-glutamate
  - Glycine
- Found in animals and plants

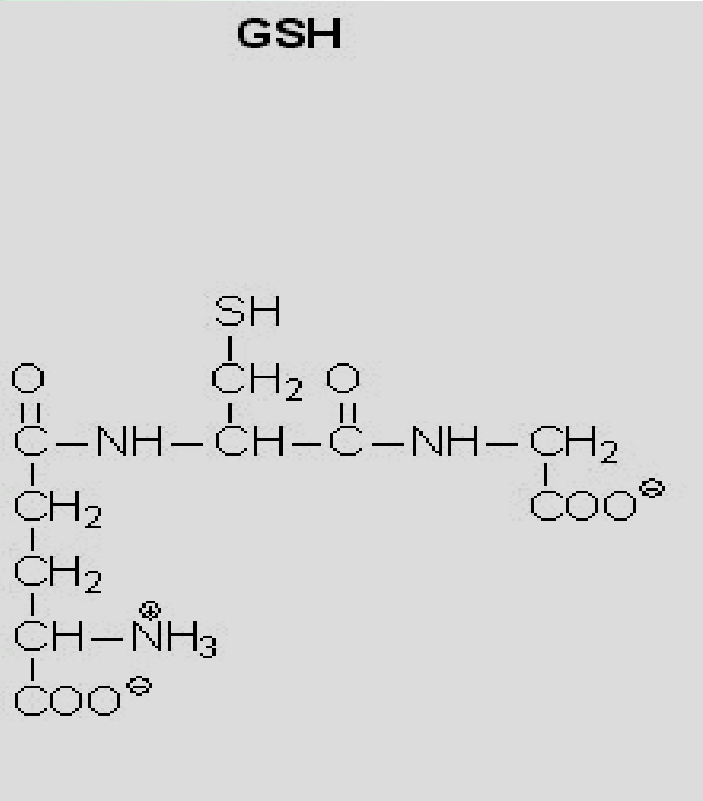
glutathione (GSH)



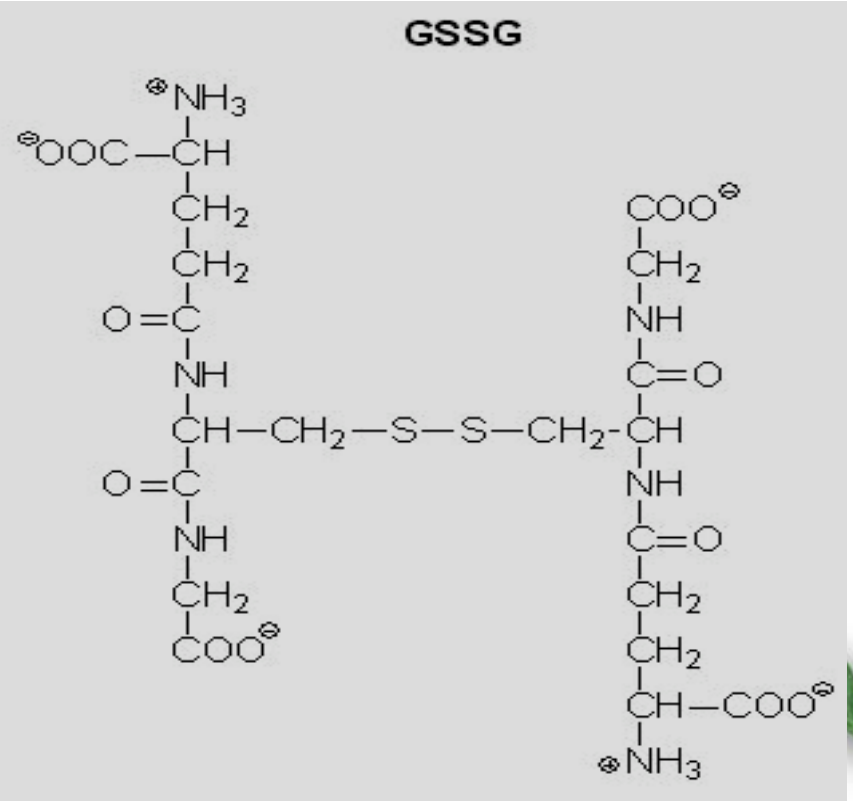
www.bb.iastate.edu/%7Ejat/glutchp.html

Forms of Glutathione

Reduced (GSH)



Oxidized (GSSG)



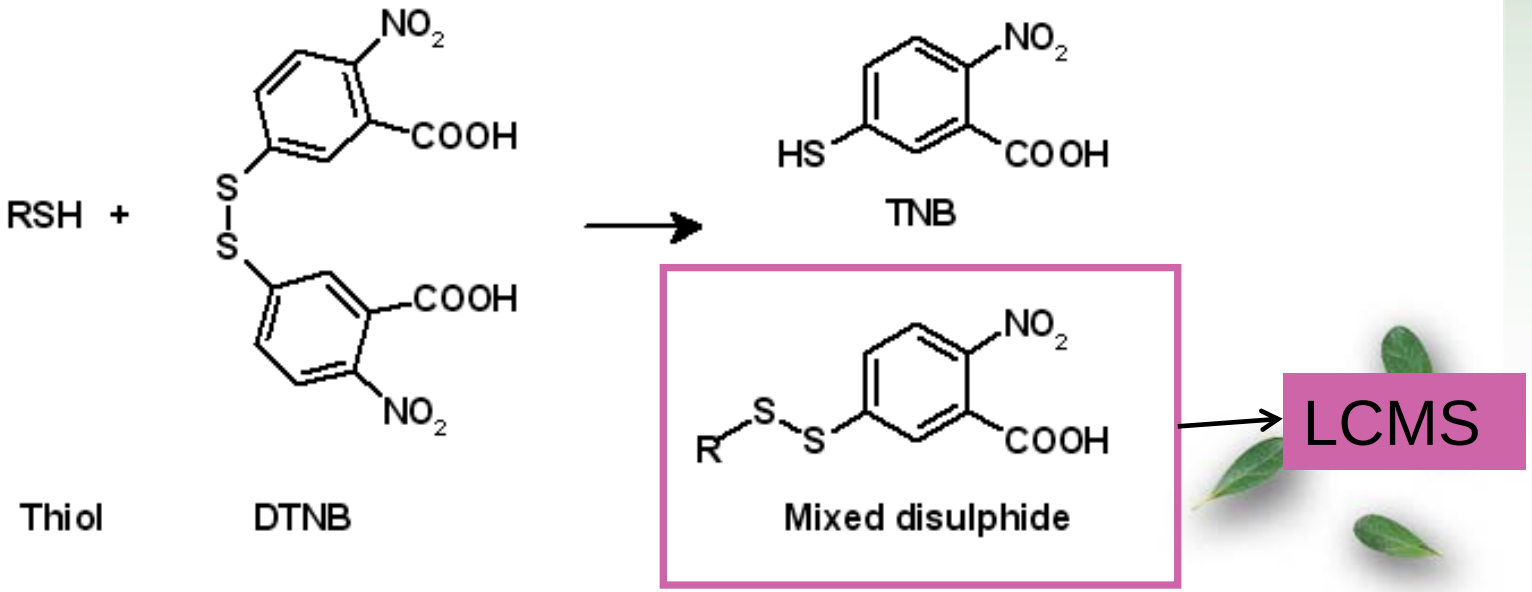
http://themedicalbiochemistrypage.org/images/gssgfigure.jpg

Roles of Glutathione

- Detoxification of
  - Heavy metals (Cd)
  - Reactive oxygen species (ROS)
- Activates defense mechanisms
- Regulates growth, development, cell cycle, gene expression, protein activity
  - Glutathionylation
  - Thiol-disulfide conversion

Ellman's Reagent (DTNB)

- 5, 5'-dithiobis-(2-nitrobenzoic acid) or DTNB
- Measures the amount of thiol present

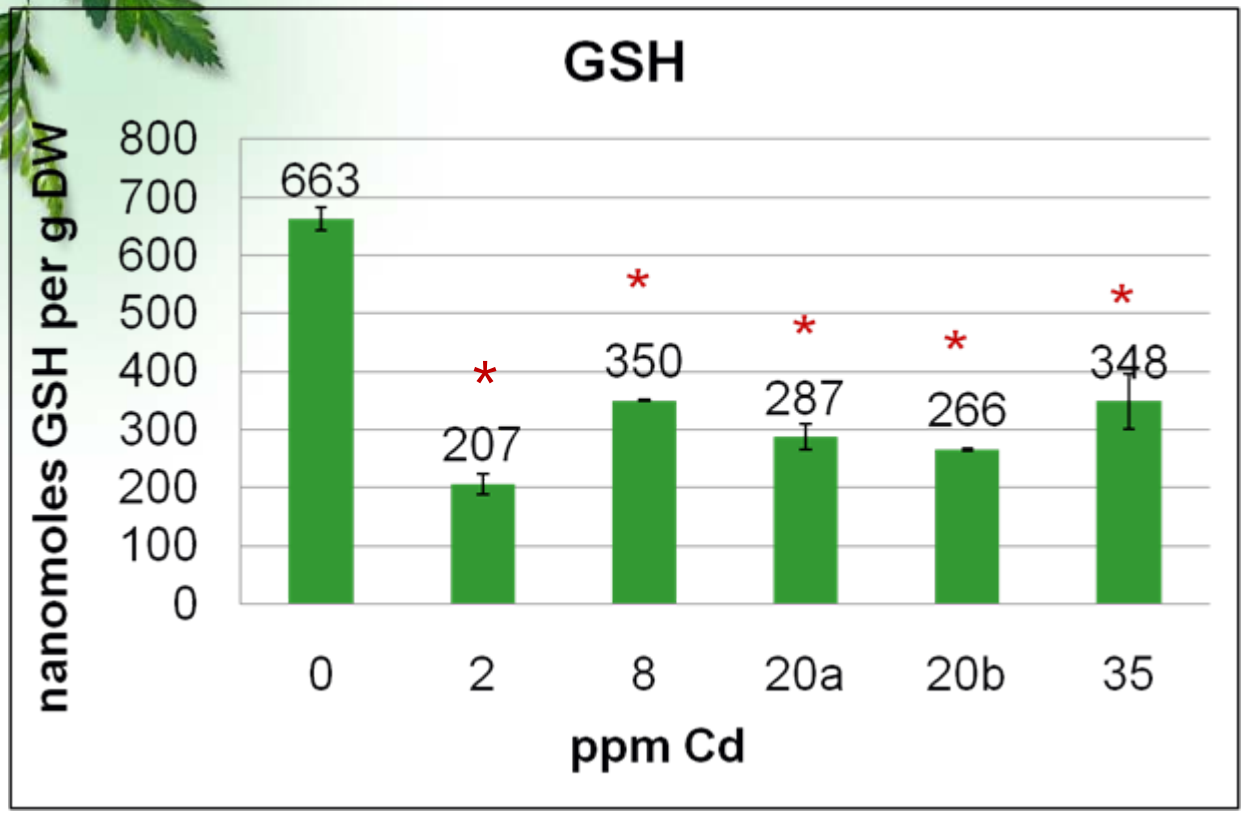


http://en.wikipedia.org/wiki/File:DTNB_reaction.png

Methods: Sample Prep

- *Chara* explants were exposed to 0 to 35 ppm Cd for 97 days
- Individual *Chara* internodes with intact apex were cut weighed and hand homogenized in cold nanopure water
  - Method was followed according to Guan et al. 2003
  - Ellman's Reagent

Results: GSH



Results:

- Nanomoles of GSH/ g DW in *Chara* shoots was significantly lower in plants exposed to all Cd treatments compared to control ($p<0.005$)

Conclusions:

- GSH may be playing a role in the tolerance of Cd-exposed *Chara* by forming larger proteins (PCs).

Overall Conclusions

- *Chara* can tolerate up to 20 ppm Cd.
- Cd is localized to *Chara* shoot cell walls and cytoplasm, rhizoids, stipulodes, and apoplast of the nodal complex.
- As Cd in the soil increases, so does the amount in *Chara* shoots and rhizoids.
- *Chara* accumulates Cd beyond the concentration added to soil.
- EDTA enhances the amount of Cd accumulated by *Chara* shoots
- Glutathione levels are significantly reduced in Cd-exposed *Chara* shoots.

Acknowledgements

- NSF ERIE IGERT
- Alan Siegel
- Lab members
- Husband and Family

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Ecosystem Restoration through Interdisciplinary Exchange

References

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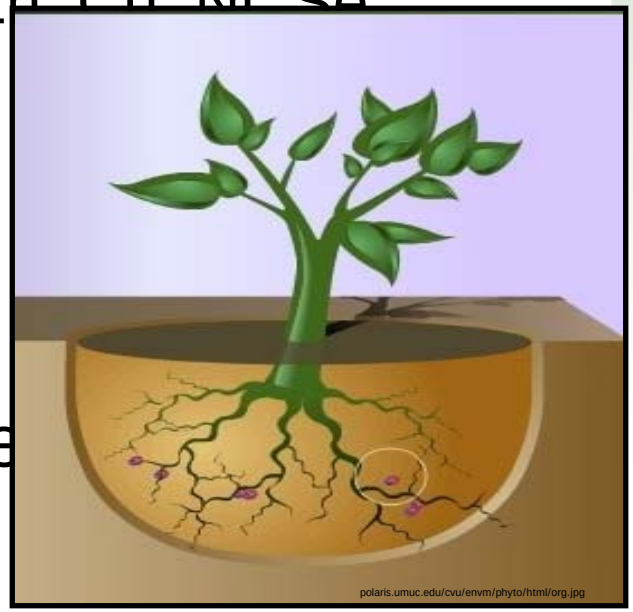
- Seregin, I.V. and Ivanov, V.B. 1997. Histochemcial Investigation of Cadmium and Lead Distribution in Plants. *Russian Journal of Plant Physiology*. 44:6 . 791-796.

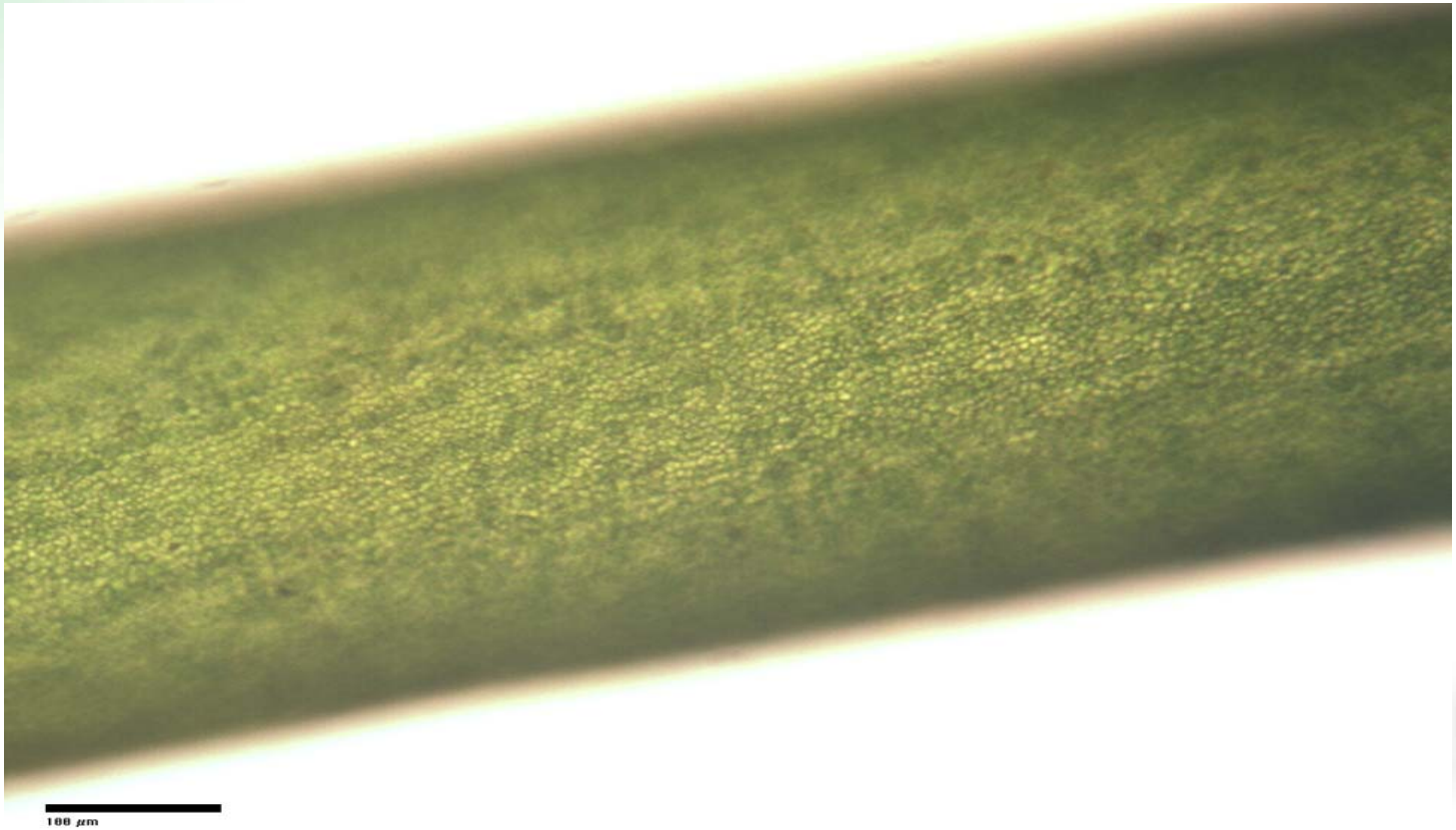
Thank you for your attention!

Phytoextraction

- ***Definition***:
  - A specific process whereby plants take up and concentrate substances, such as toxic materials, into their plant biomass
    - Ex. heavy metals such as Cd, Zn, Cu, Ni, Se
- ***Benefits***:
  - Natural method
  - Efficient
  - Not environmentally disruptive
  - Inexpensive



Control *Chara* Shoot



Sample and standard preparation

- The standards were prepared using 10 ppm gsh and gssg solution in water
  - Glutathione ethyl ester (GEE) served as internal standard for the GSH thiol
  - L-cystine-1 served as internal standard for disulfide (GSSG)
  - Standards ranged from 50 ppb to 500 ppb of GSH and GSSG, with 100 ppb GEE and 1000 ppb cystine
- Thiols in water were derivatized with 2mM ER after which, 5% sulfosalicylic acid was added
- The same derivatization method applied to the samples was used for the standard preparation
- Liquid chromatography followed by mass spectrometry was performed in Dr. Diana Aga's lab with the help of Divina Navarro
- Analysis of peak areas was performed using Qual Browser