

Preliminary study

Investigation into participation of annexins in the response of *Arabidopsis thaliana* roots to lead (Pb) exposure.

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Introduction of Annexins

- Diverse
- Multigene family of Ca²⁺-regulated phospholipids
- Membrane-binding protein
(Delmer and Potikha, 1997; Gerke and Moss, 2002)
- Soluble
- Hydrophilic proteins
(Clark and Roux, 1995)

Importance of Annexins

- Essential cellular processes
- Defense against various types of biotic and abiotic stresses

AnnAt1 in stress response

- Rescue *oxyR* mutant in *Escherichia coli*
 - exhibit peroxidase activity
(Gidrol et al., 1996)
- Catalase motif in the first repeat
(Kush and Sabapathy, 2005)

AnnAt1 in stress response

- Limit excessive ROS levels during oxidative burst
- Upregulated by the presence of H₂O₂
- Adaptive defense mechanism
 - protects cells from oxygen toxicity
(Gidrol et al, 1996; Gorecka et al, 2005)

Hypothesis

- Pb exposure could result in oxidative burst in *Arabidopsis thaliana*, particularly the roots.

Objective

- To assess the relative contribution of annexins in the antioxidative defence against lead (Pb) stress.

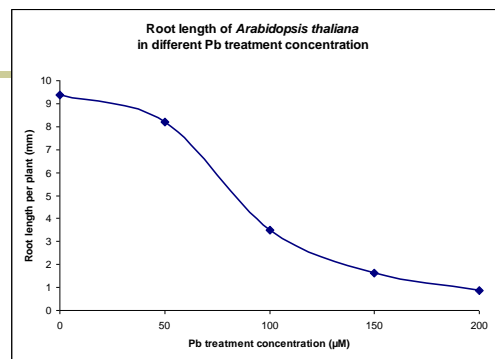
Initial Correlative Studies

- Effect of Pb on plant growth
- Determination of Pb content in plant materials
- Ultrastructural localization of Pb within plant cells
- Antioxidative enzyme assays

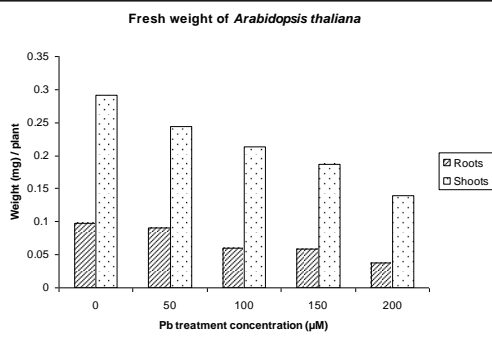
Appearance and Morphology



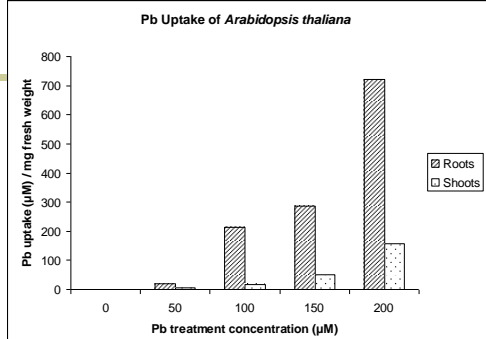
Appearance of 7-days-old *Arabidopsis thaliana* seedlings in response of different Pb concentrations.



The root length of 7-days-old seedlings grown vertically in different Pb concentrations.

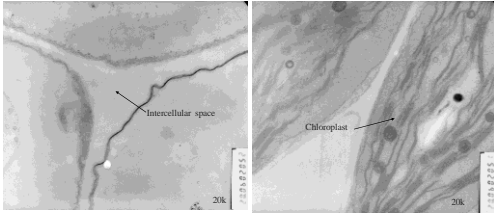


Fresh weight gain from a pool of 500 randomly selected 7-days-old *Arabidopsis thaliana* seedlings grown horizontally on modified Huang and Cunningham agar containing varying concentrations of Pb.



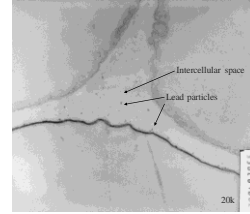
Pb uptake of each seedling of *Arabidopsis thaliana* after 7 days grown horizontally in agar plate containing $\text{Pb}(\text{NO}_3)_2$. Values are mean calibration in diluted acid digest.

Ultrastructural localization of Pb in Shoots



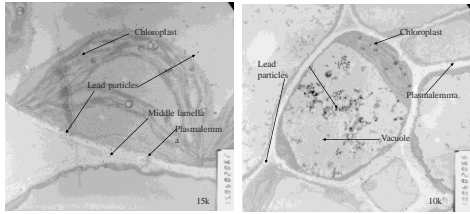
Transmission electron micrograph of *Arabidopsis thaliana* cotyledon tissue, without any $\text{Pb}(\text{NO}_3)_2$ treatment, placed vertically for 7 days in agar plates.

Ultrastructural localization of Pb in Shoots



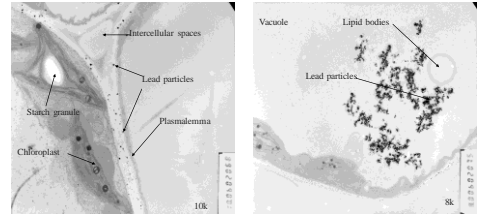
Transmission electron micrograph of *Arabidopsis thaliana* cotyledon tissue, treated with $50 \mu\text{M Pb}(\text{NO}_3)_2$, placed vertically for 7 days in agar plates.

Ultrastructural localization of Pb in Shoots



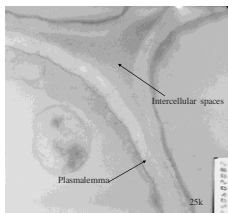
Transmission electron micrographs of *Arabidopsis thaliana* cotyledon tissue, treated with $100 \mu\text{M Pb}(\text{NO}_3)_2$, placed vertically for 7 days in agar plates.

Ultrastructural localization of Pb in Shoots



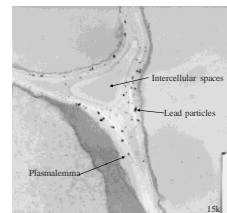
Transmission electron micrographs of *Arabidopsis thaliana* cotyledon tissue, treated with $200 \mu\text{M Pb}(\text{NO}_3)_2$, placed vertically for 7 days in agar plates.

Ultrastructural localization of Pb in Roots



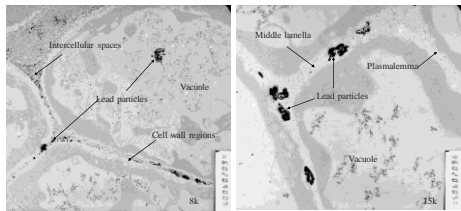
Transmission electron micrographs of *Arabidopsis thaliana* roots tissue, without any $\text{Pb}(\text{NO}_3)_2$ treatment, placed vertically for 7 days in agar plates.

Ultrastructural localization of Pb in Roots



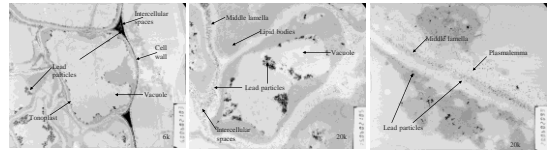
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Ultrastructural localization of Pb in Roots



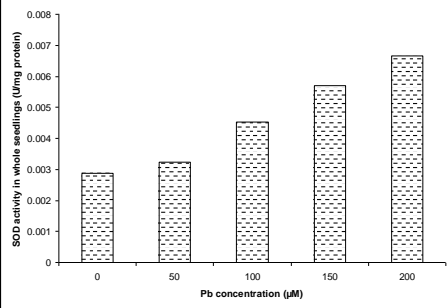
Transmission electron micrographs of *Arabidopsis thaliana* roots tissue, treated with $100 \mu\text{M Pb}(\text{NO}_3)_2$, placed vertically for 7 days in agar plates.

Ultrastructural localization of Pb in Roots



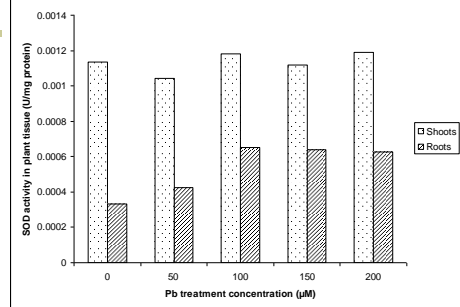
Transmission electron micrographs of *Arabidopsis thaliana* roots tissue, treated with $200 \mu\text{M Pb}(\text{NO}_3)_2$, placed vertically for 7 days in agar plates.

Superoxide dismutase activity in whole seedlings



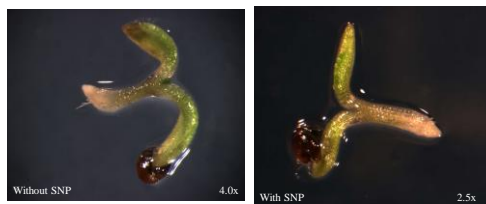
Effect of Pb treatment on SOD activity in extracts of whole seedlings of 7-days-old *Arabidopsis thaliana* grown in agar plates placed horizontally (n = 100 randomly selected plants).

Superoxide dismutase activity in shoots and roots



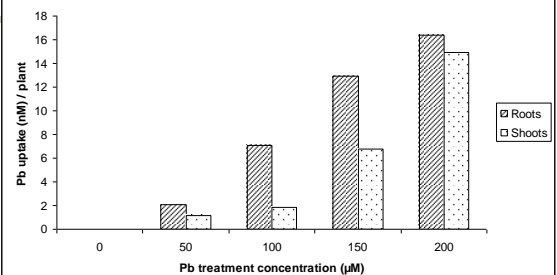
Effect of Pb treatment on SOD activity in extracts of shoots and roots tissues of 7-days-old *Arabidopsis thaliana* grown in agar plates placed horizontally (n = 200 randomly selected plants).

Manipulation studies : Effect of nitric oxide (NO)

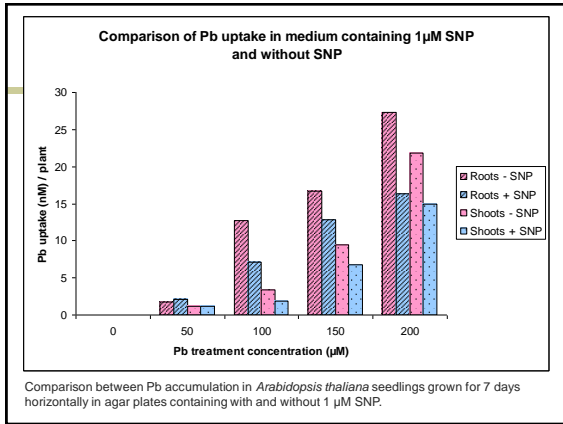


Interaction of $1 \mu\text{M SNP}$ and $200 \mu\text{M Pb}$. The 7-days-old seedlings were grown vertically in agar plates.

The effect of NO on Pb uptake in Arabidopsis thaliana



Pb accumulation in *Arabidopsis thaliana* seedlings grown for 7 days horizontally in agar plates containing $1 \mu\text{M SNP}$ and different Pb concentrations.



Isolation of Mutants ~ Challenges

- Unsure if seedlings were mutants / adapted to the stress
- Malnutrition after > 1 month
- Modification

Works in progress

- ROS and enzymes activity determination
- Pb mutant selection
- TEM, GFS, PCR work (quantitation of annexin transcript levels)
- Effect of NO and Ca^{2+}

Acknowledgement

- Manfred Ingerfeld - Transmission Electron Microscopy
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- Matt Walters – Photography

Thank you!