Mongolian Academy of Sciences

SuMoCoS

Sustainability and Mobility in the Context of Smart Cities

Use of Environmental Biotechnology approaches in remediation of soil pollution in Ulaanbaatar city

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Content

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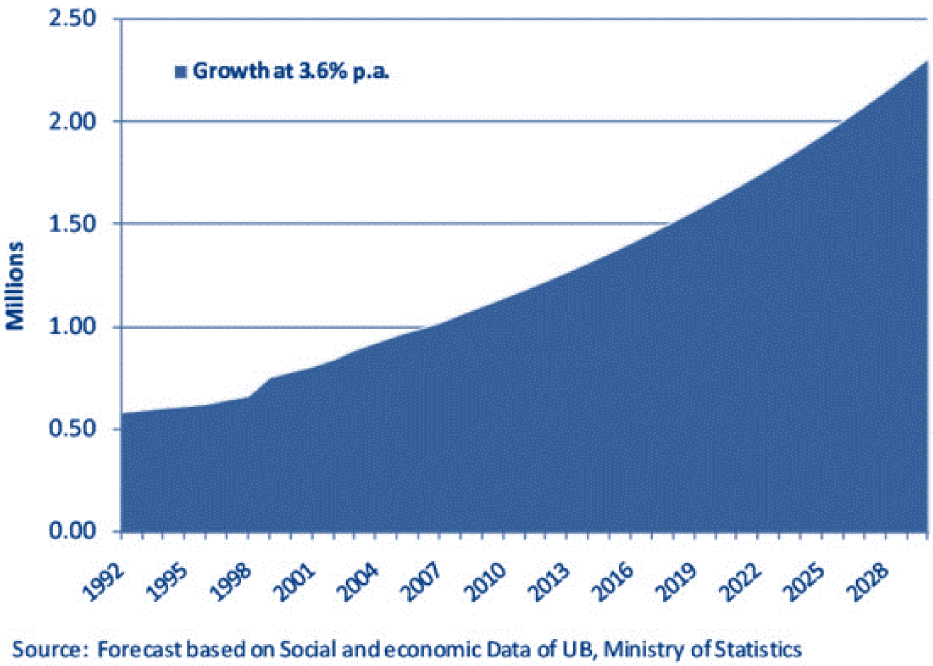
2. Studies on Zn hyper-accumulation in plants

3. Studies on Mn hyper-accumulation in green algae

1. Background

Estimation of population growth of Ulaanbaatar city



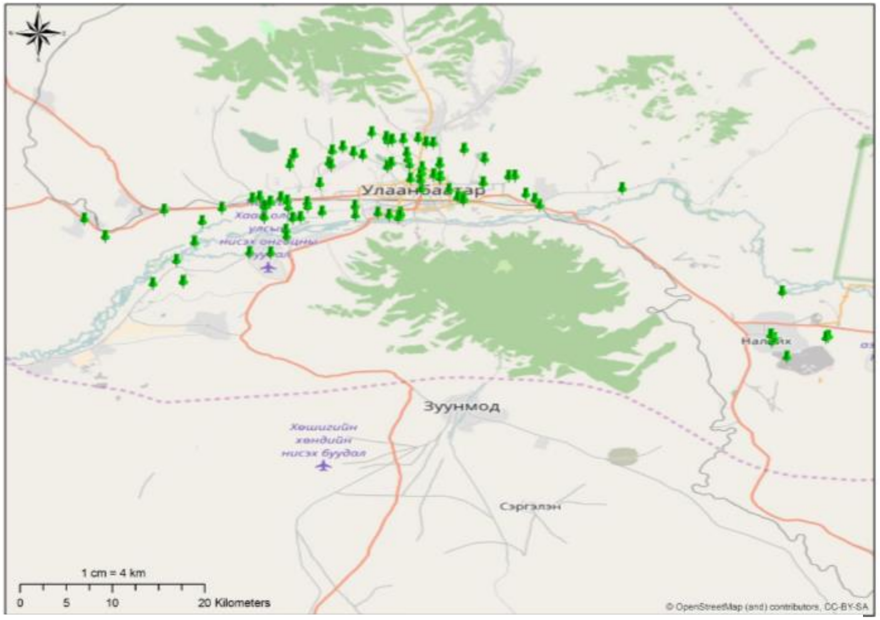
Liyer et al., 2016

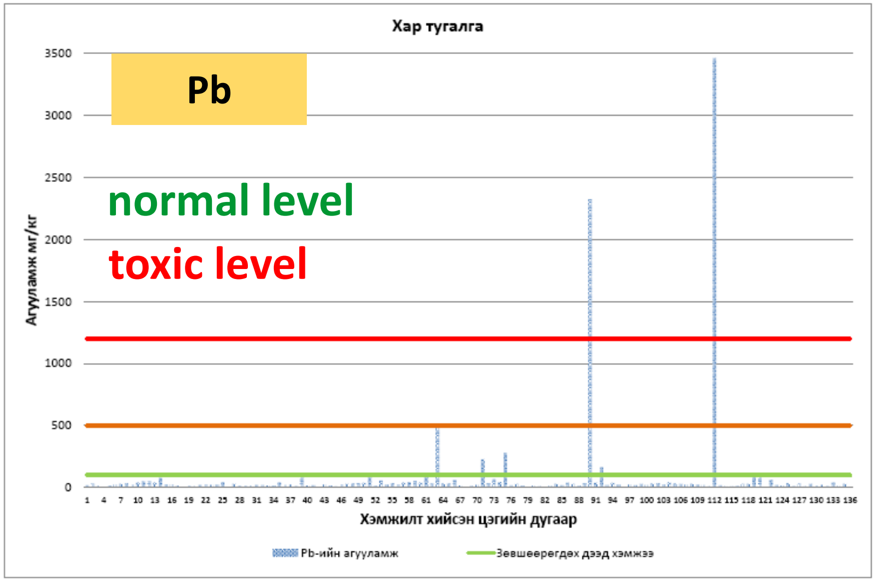
Concomitant increase of Environmental Pollution

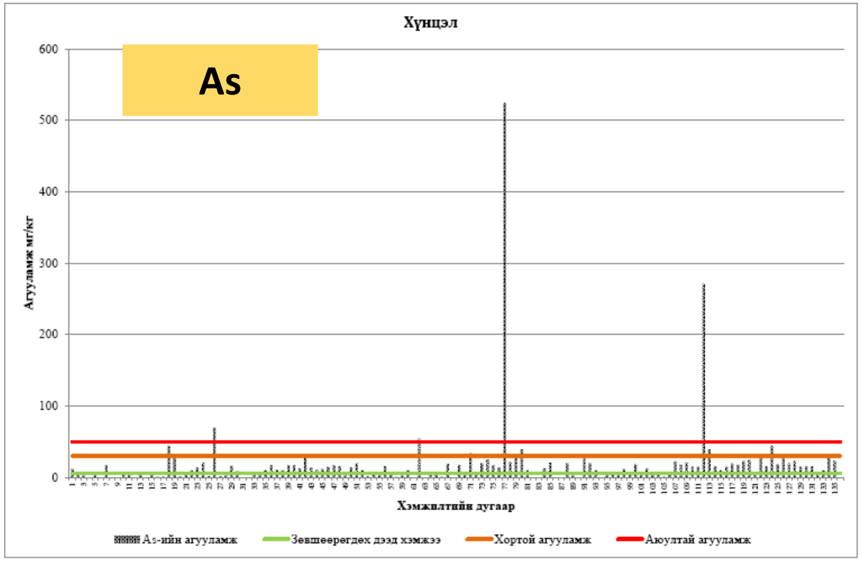
- **Soil heavy metal pollution** (especially in Khan-Uul district area)
- **Toxic bacterial contamination** (in Ger-horoolol area)

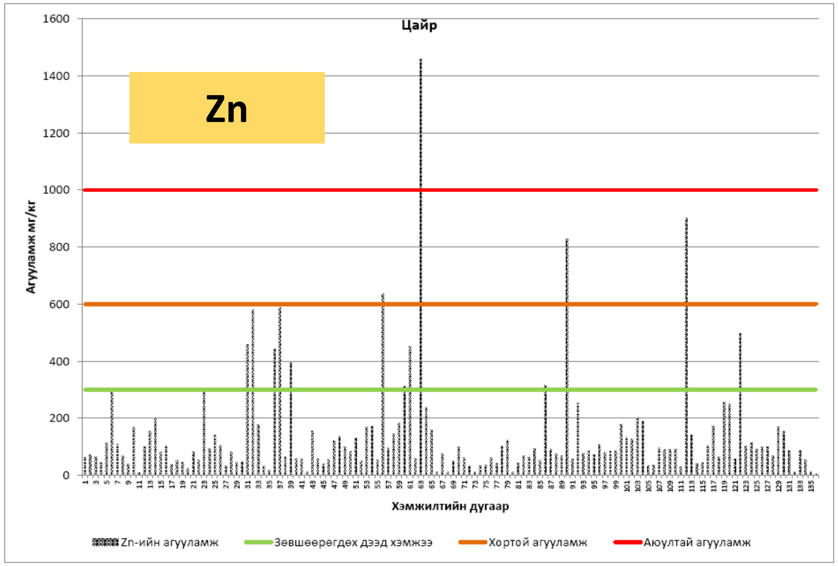
Soil heavy metal contents in UB city areas

Sampling points around UB city



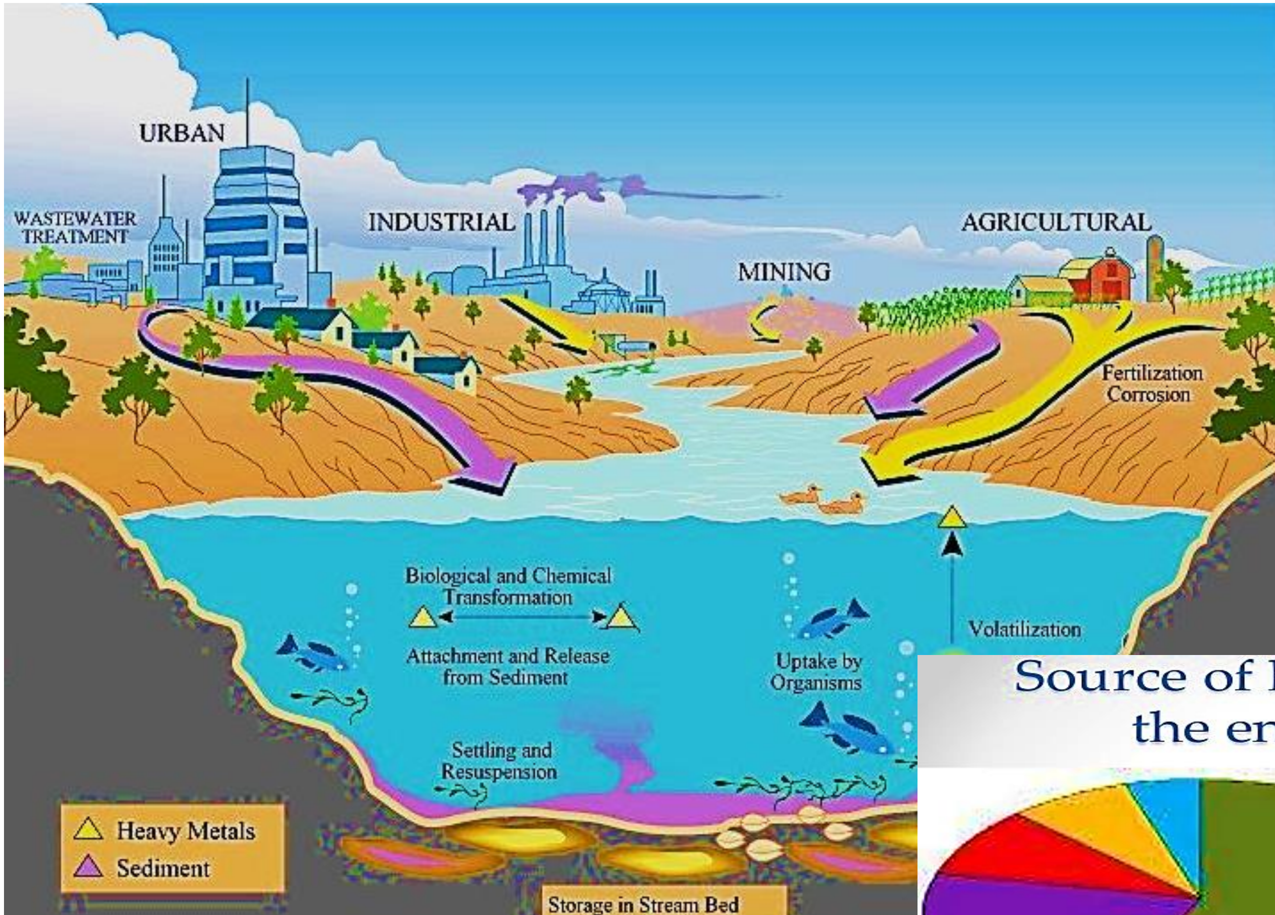


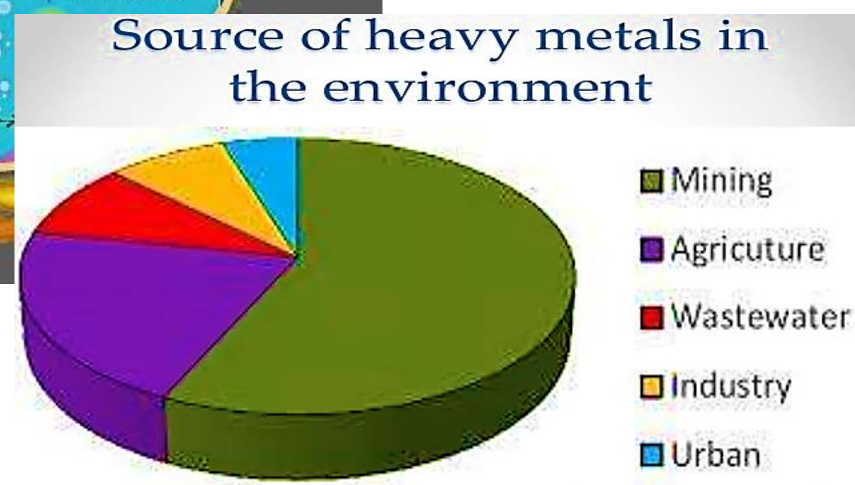




Soil heavy metal report of Ulaanbaatar city, 2017

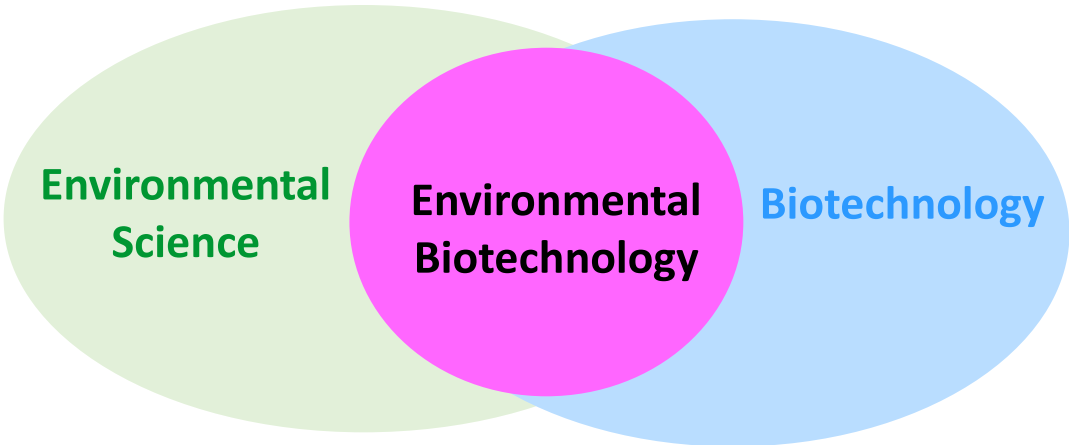
Soil heavy metal pollution





http://wwwbrr.cr.usgs.gov/

Aimed approach: Environmental Biotechnology



The development, use and regulation of biological systems

- for remediation of contaminated environments (land, air, water);
- for environment-friendly processes (green manufacturing technologies and sustainable development)" *International Soc. for Environm. Biotech*

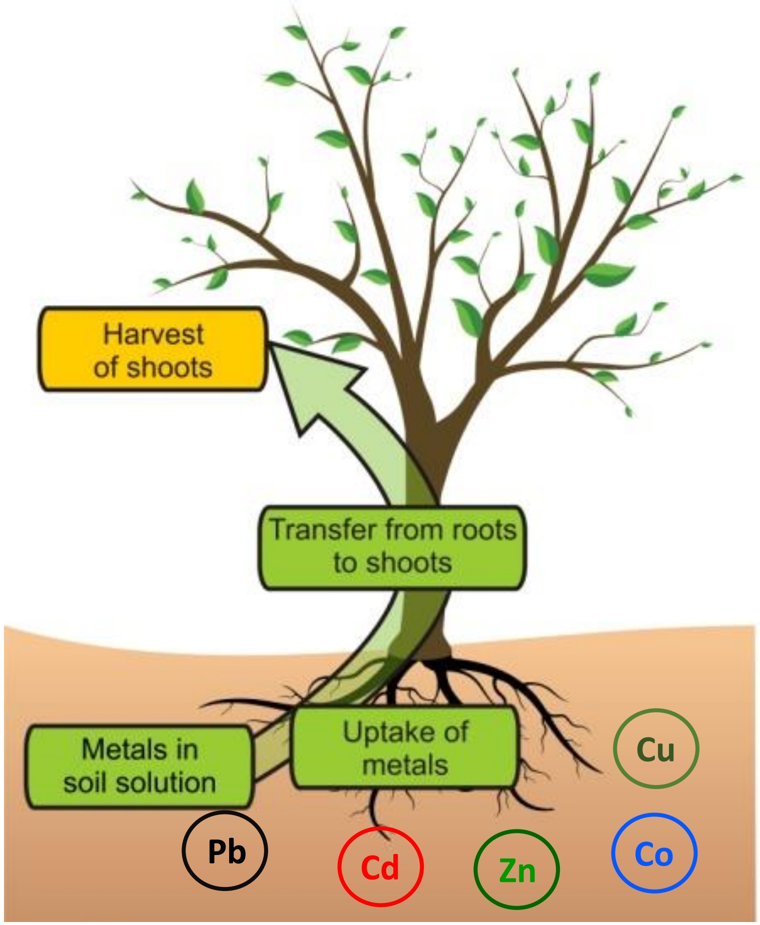
For soil remediation of heavy metals:

- Phytoremediation (plant)
- Bioremediation (microorganism)

Advantages:

- Cost-effective
- Eco-friendly
- Innovative

Phytoremediation – Green cleaning



modified from Paulo et al., 2014

In Canada: http://www.landsaga.com/phytoremediation.php

However, not every plant species can be used for phytoremediation! (most plant species die on the polluted soil)

Research topics

**To grow plants for phytoremediation,
we need to learn from metal hyper-accumulation mechanisms**

2. Studies on Zn hyper-accumulation in Zn-hyper-accumulating plants

Metal hyper-accumulating plants

> 400 different plant species (>1 % metals in plant dry weight)

Ni hyper-accumulator *Phyllanthus palawanensis* (Philippines)
~ 10% Ni in plant dry weight
~dimethyl-glyoxime-Ni complex

Zn hyper-accumulator *Thlaspi goesingense* (Austria) near Pb mine
~30% Zn in plant dry weight

Ni hyper-accumulator *Euphorbia helenae*, (Cuba) 25.74% Ni in plant dry weight

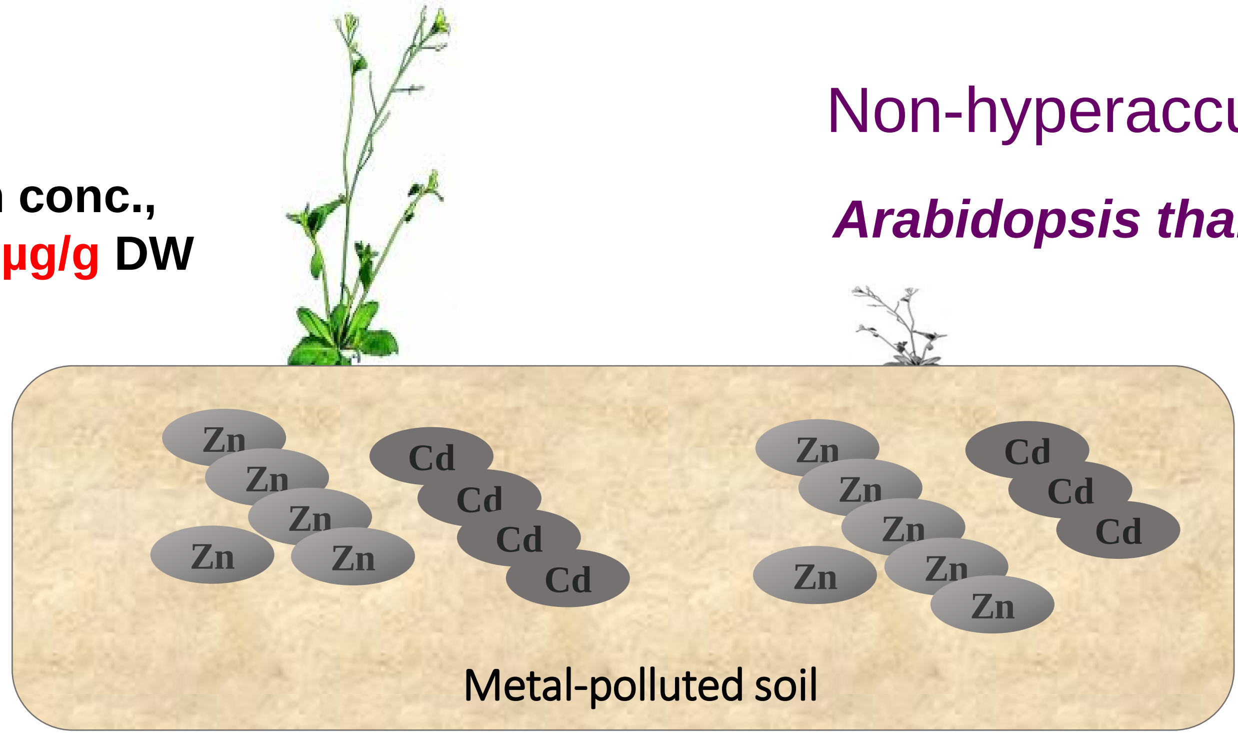
Salt 2006, Plant Physiology & Development

Our research plant: Zn/Cd hyper-accumulator *Arabidopsis halleri*

Model metal hyper-accumulator

Arabidopsis halleri

leaf Zn conc., >10 000 µg/g DW



Non-hyperaccumulator

Arabidopsis thaliana

Toxicity symptoms, leaf Zn conc., >300 µg/g DW

Q: How *A. halleri* adapted to metal-enriched soil environments?

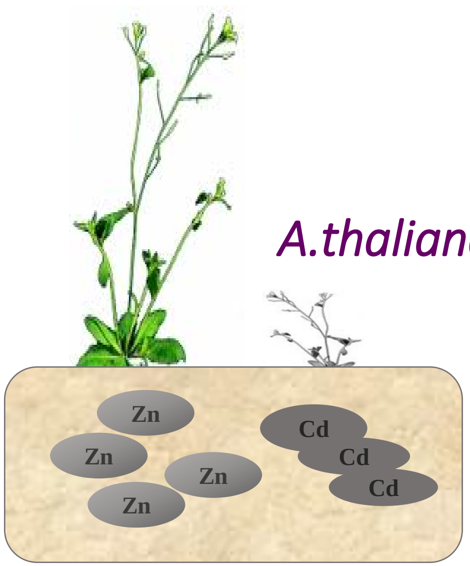
Q: What is the molecular mechanism for Zn/Cd hyper-accumulation?

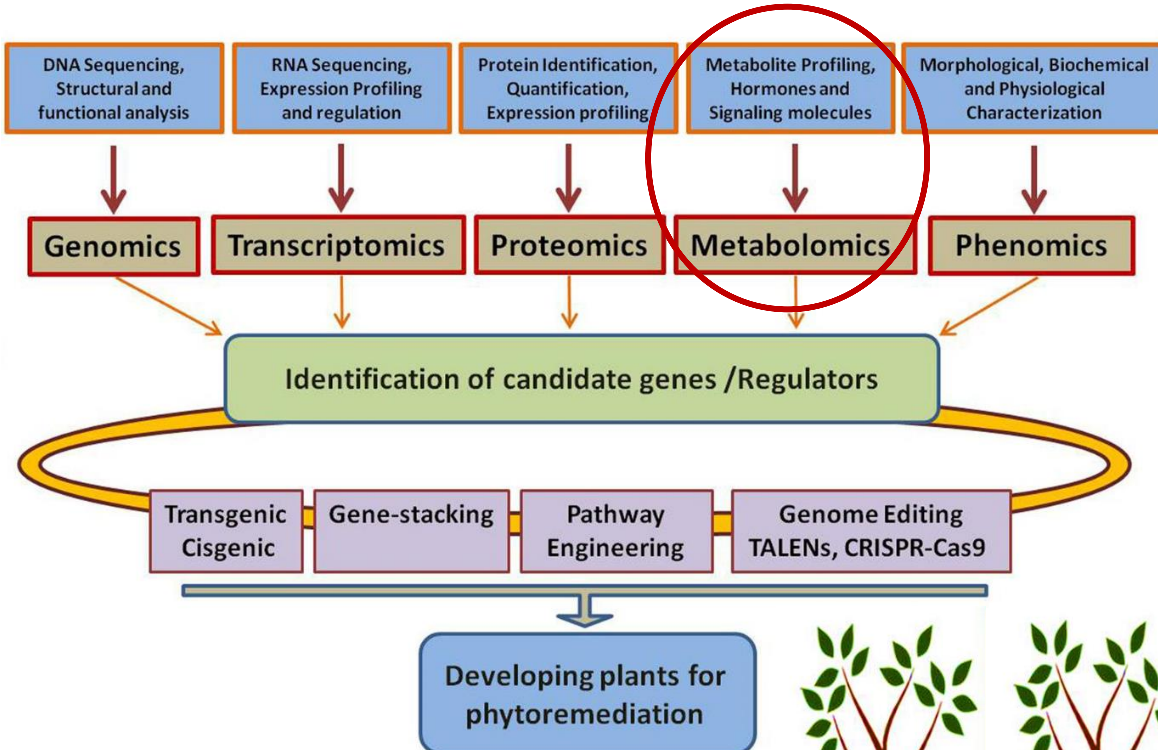
Our strategic approach

Strategy: By comparing these two plants, search the potential candidate genes from *A. halleri* for generating transgenic plants for phytoremediation

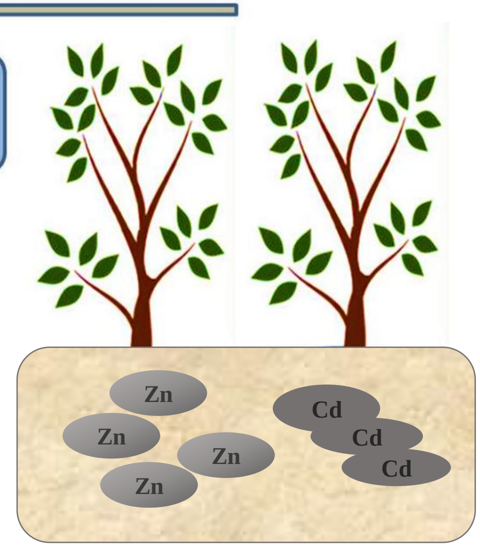
A.halleri

A.thaliana



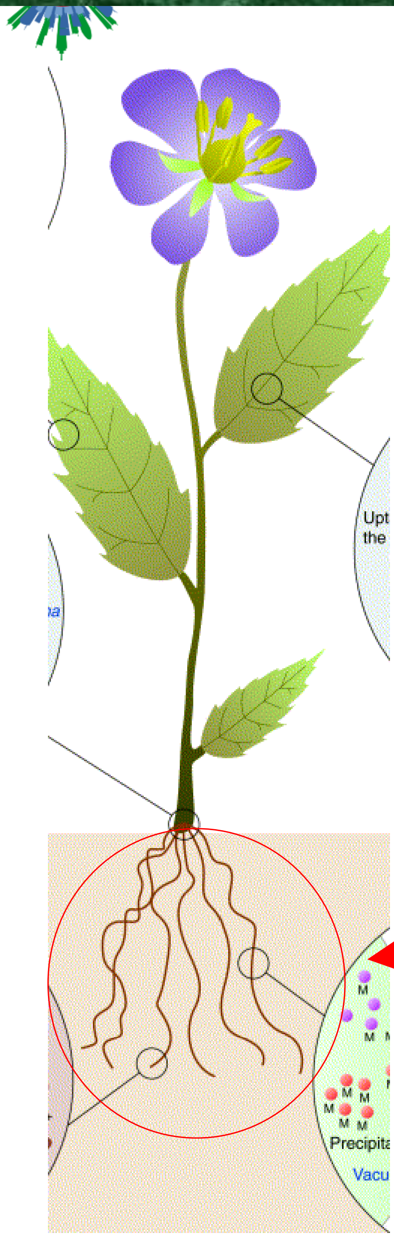


Engineered hyper-accumulator plants



Modified from Mosa et al., 2016

Specific question interested in:



Q: Whether root-secreted compounds have involved in Zn/Cd hyper-accumulation mechanism of *A. halleri* ?

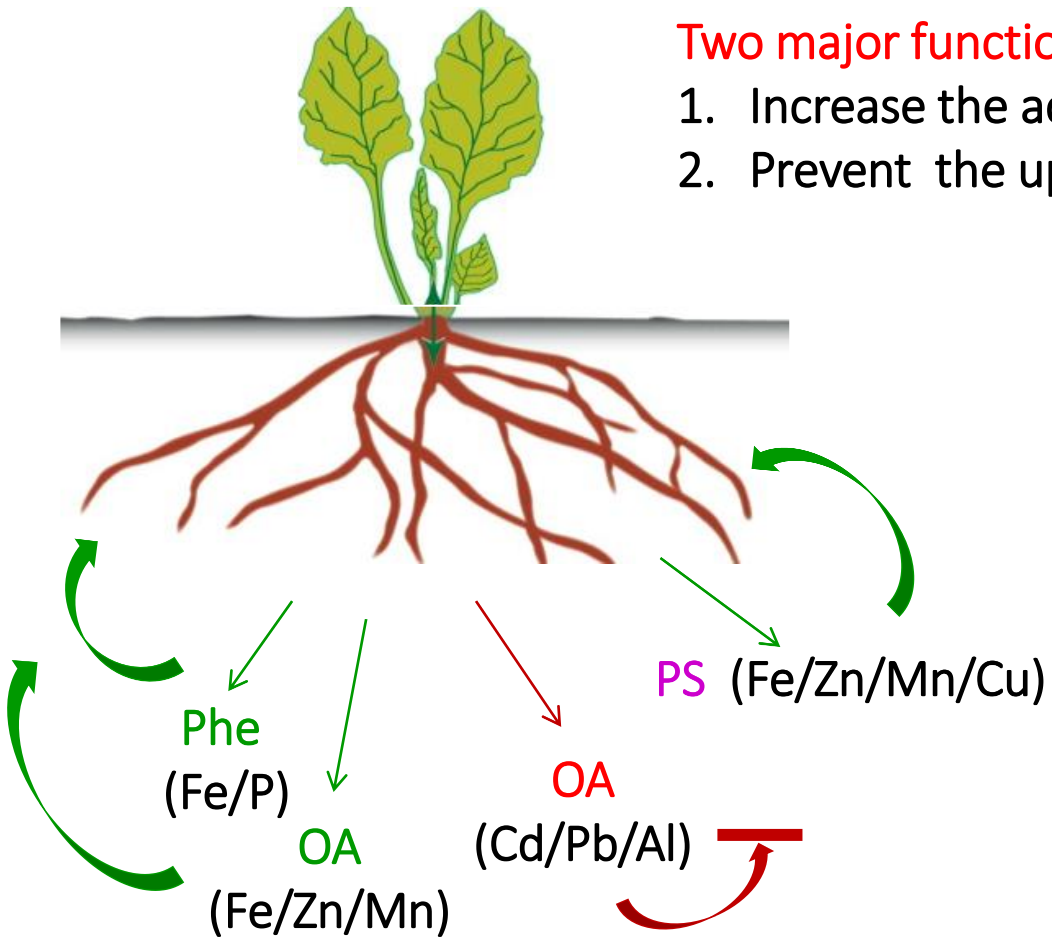
(no such studies on metal hyper-accumulators)

?

Functions of root secreted compounds in mineral availabilities

Two major functions:

1. Increase the acquisition of essential nutrients
2. Prevent the uptake of toxic metals

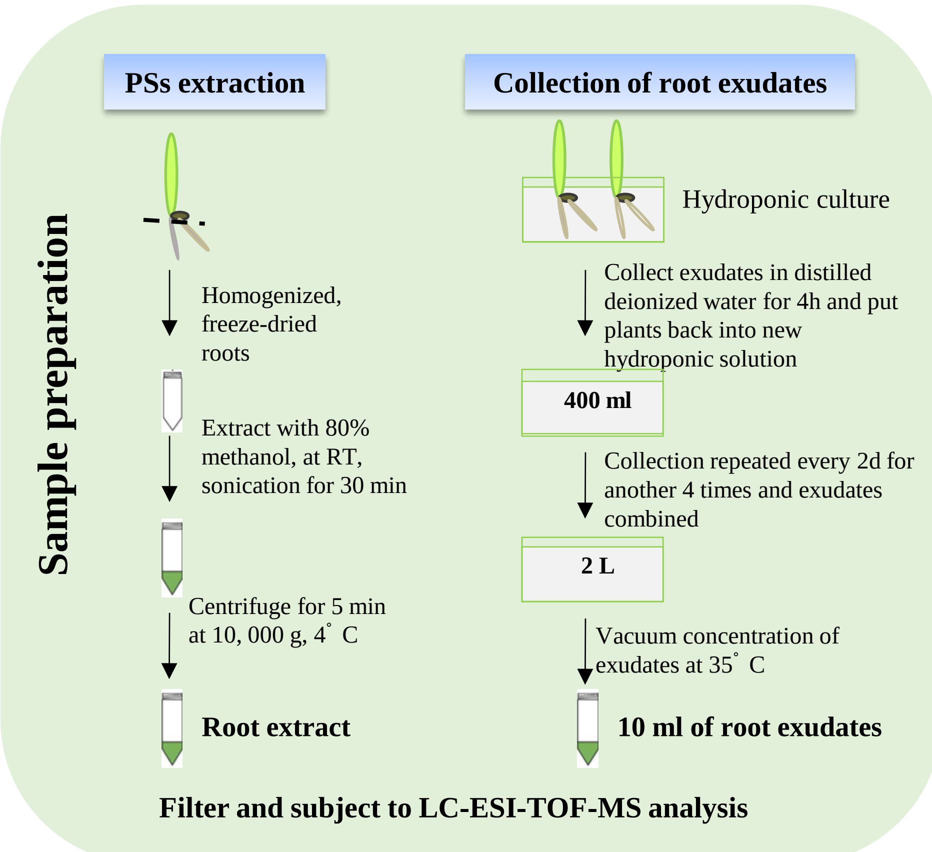


Phe- phenolics
OA- organic acids
PS- phytosiderophores

Dakora & Philipps, 2002
Martinez & Motto, 2000
Kochian et al., 2004
Kobayashi et al., 2010

We developed a mass spectrometry method for root secretion analysis.

Workflow of LC-ESI-Q-TOF-MS (without chemical derivatization)



Sample Anal.

UPLC-ESI-TOF-MS Analysis

ESI+ mode (for free PSs) **ESI- mode (for PS-Fe complexes)**

Experimental conditions

- ESI mode: ESI+ and ESI-
- solvent temperature : 4 C
- ESI needle voltage: 3KV (ESI+), 2.5KV(ESI-)
- detection mass range: 50- 990 m/z
- column: T3 column (1.7 µm, 2.1 mm x 150 mm)
- column temperature: 40° C
- mobile phase: Acetonitrile (ACN), Formic acid (FA)

  ESI+: A buffer- 0.1% FA in 2% ACN, B buffer- 0.1% FA in 100% ACN

  ESI- : A buffer- 2% ACN, B buffer- 100% ACN

Time (min)	**Flow Rate** (ml/min)	**%A**	**%B**
0	0.300	99.5	0.5
4.00	0.300	5.0	95.0
4.50	0.300	5.0	95.0
4.80	0.300	99.5	0.5
6.30	0.300	99.5	0.5

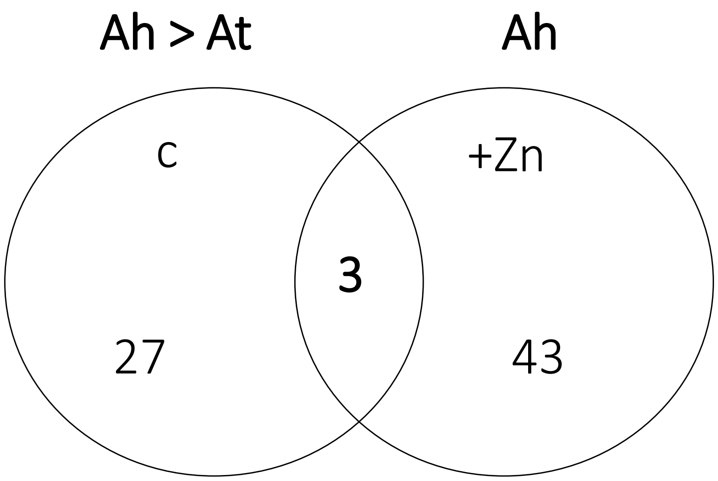
Data Anal.

- MS signal processing with Markerlynx 4.1
- PSs identification with MS/MS using MassFragment
- MS data generation with retention time-m/z pairs
- MS filter of peaks with >30 S/N ratio

Tsednee et al., 2012

Comparative root secretion analysis identified an interesting compound “nicotianamine”.

Metabolite profiling in LC-ESI-Q-TOF-MS (Hilic column, ESI+)



3 metabolites:

1. 144.983 m/z
2. 304.150 m/z
3. 286.140 m/z

* 304.150 m/z

- predicted elemental composition: $C_{12}H_{22}H_3O_8$

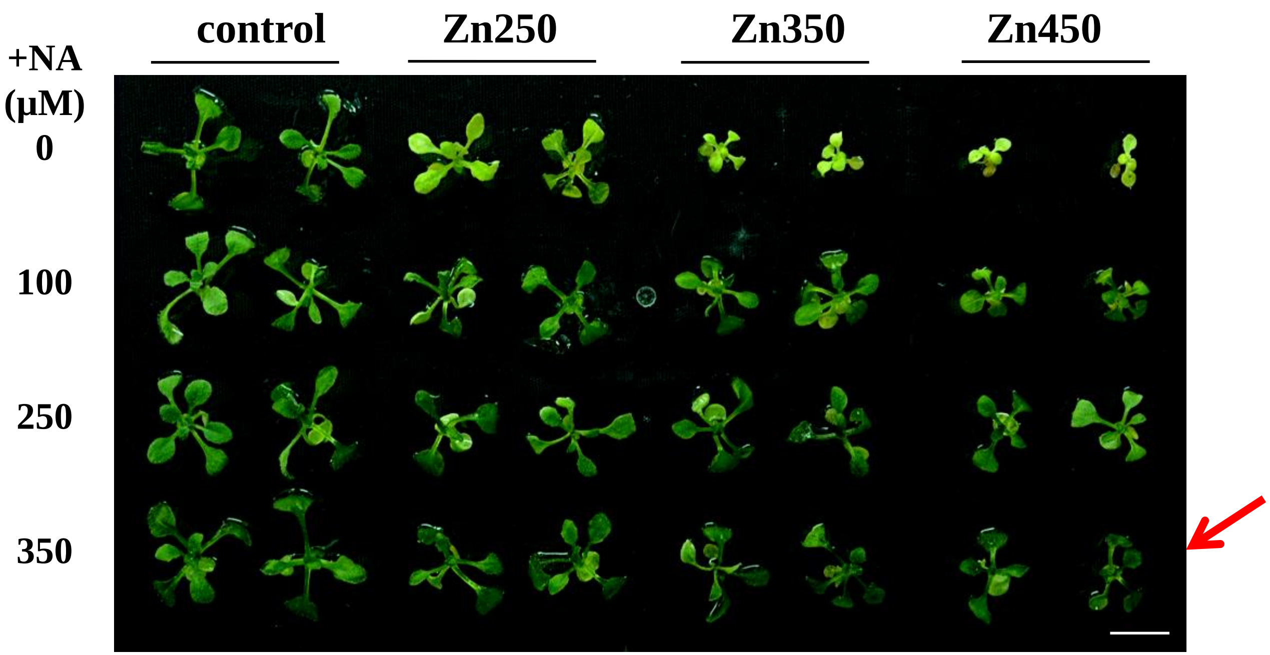
as similar to NA (nicotianamine, a marker compound of *A. halleri*)

OPLS-DA: S-Plot analysis
Metabolite peaks with normalized intensity:
>2-fold in Ah > At (c)
>2-fold in Ah (+Zn) > Ah (c)

c - control condition
+Zn - excess Zn treatment

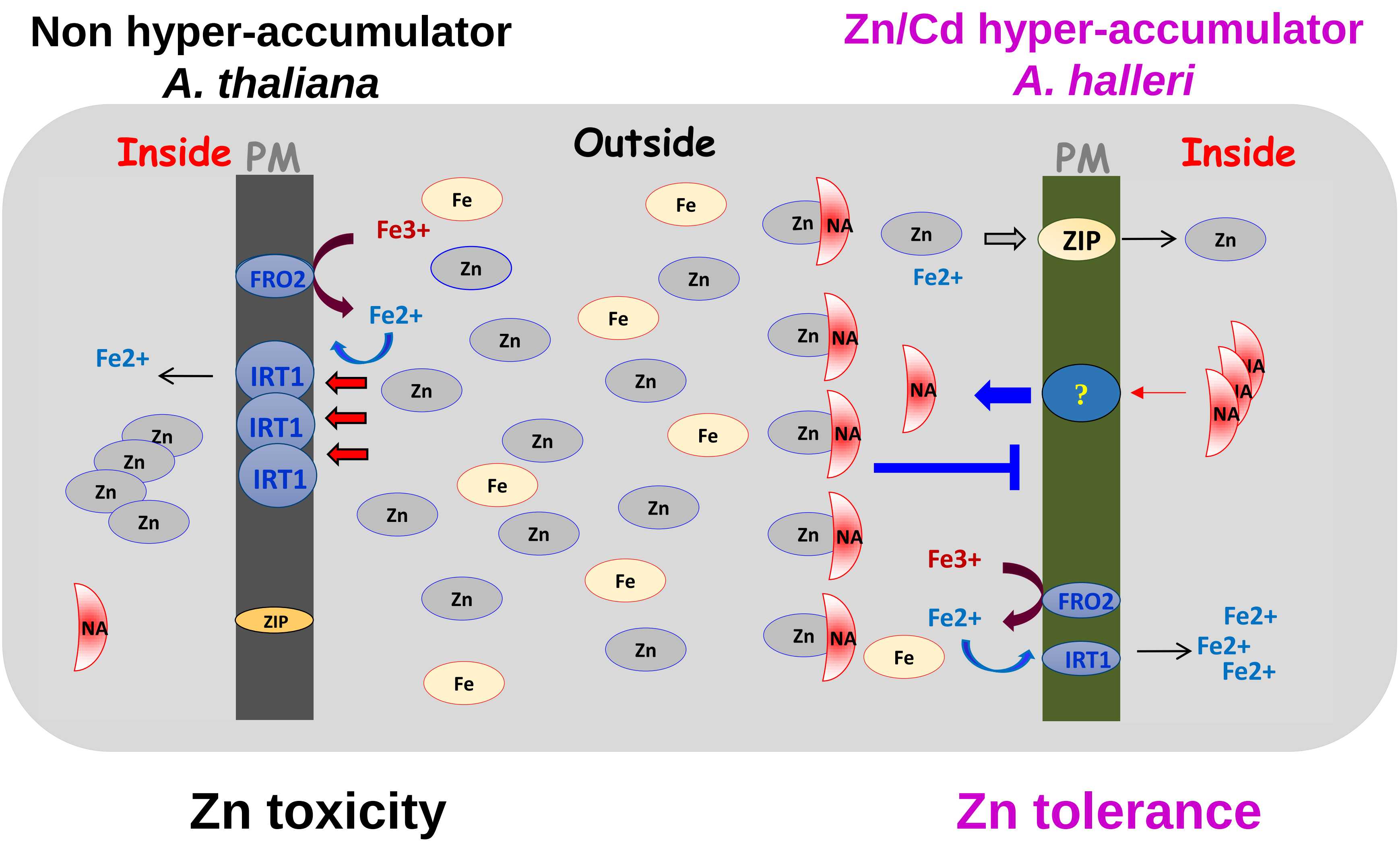
Tsednee et al., 2014

Exogenous NA application makes *A. thaliana* tolerant to excess Zn.



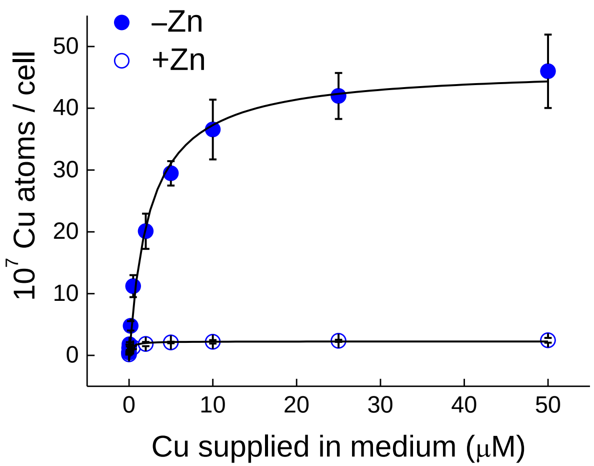
Overexpression of NA synthesis genes in Arabidopsis, tomato, tobacco, and crops increase tolerance to excess heavy metals (Zn, Cd, Cu and Ni).
Clemens et al., 2014; Chen et al., 2018

Working hypothesis:
Zn tolerance by root-secreted NA

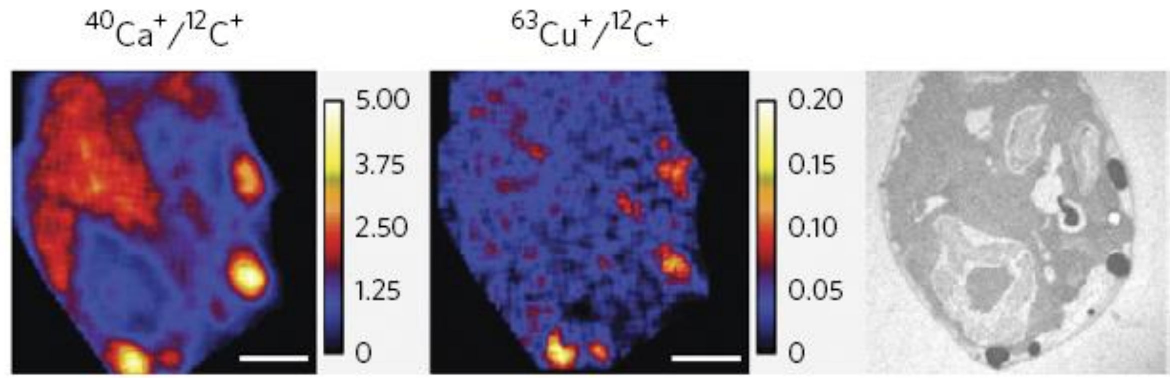


2. Mn hyper-accumulation in *Chlamydomonas*

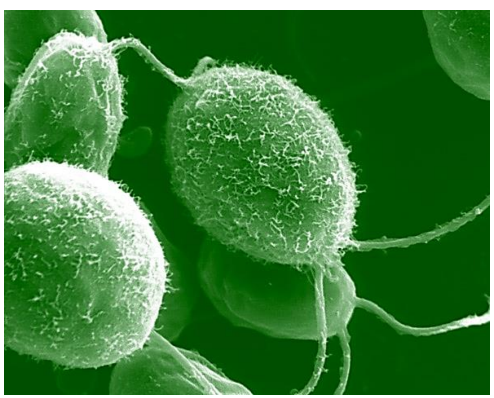
Cu hyper-accumulation



Subcellular metal imaging identifies dynamic sites of Cu accumulation in *Chlamydomonas*



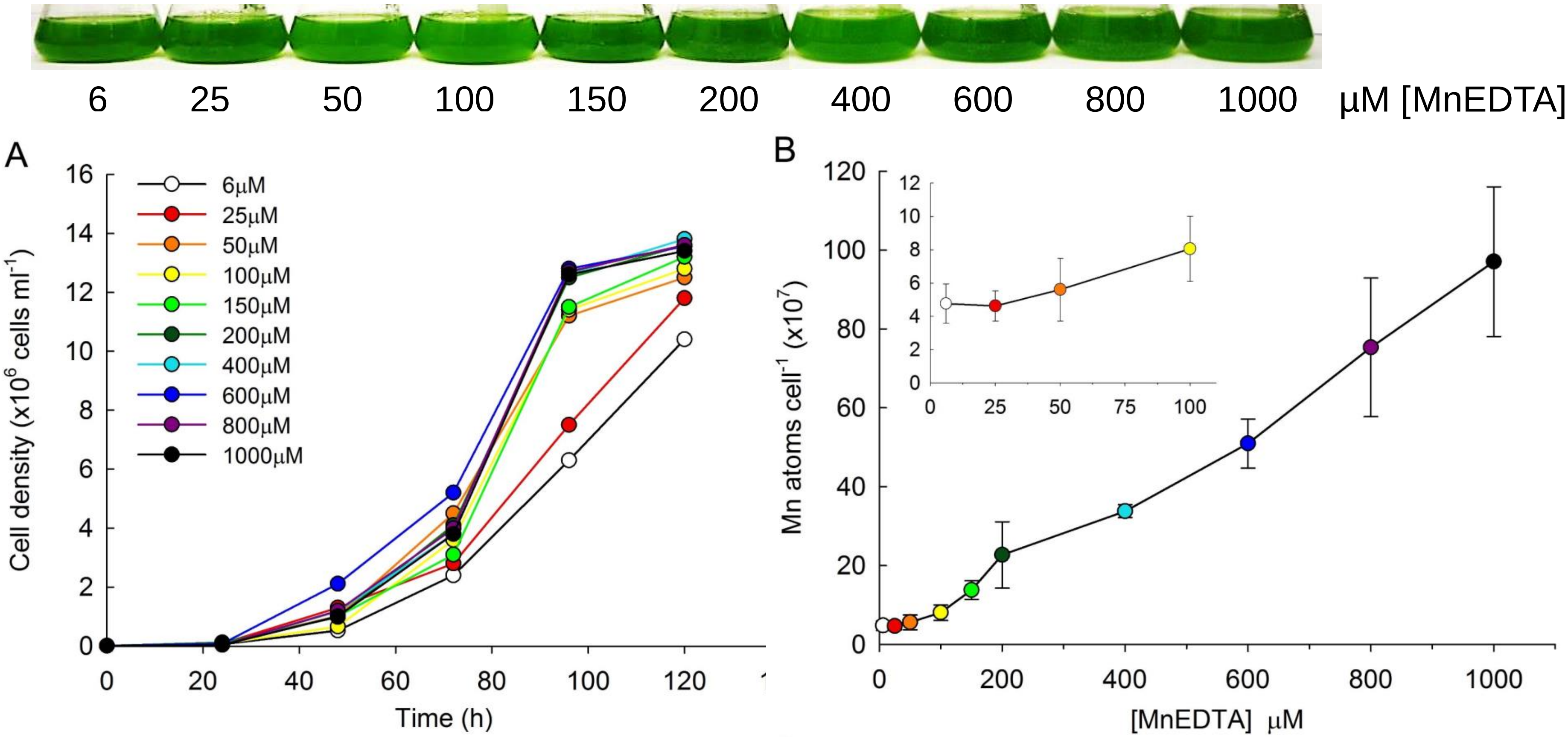
Anne HH *et al., Nat. Chem. Biol.* 2014 (Sabeeha lab, UCLA)



Chlamydomonas reinhardtii

Q: Whether chlamy cells also hyper-accumulate other metals?

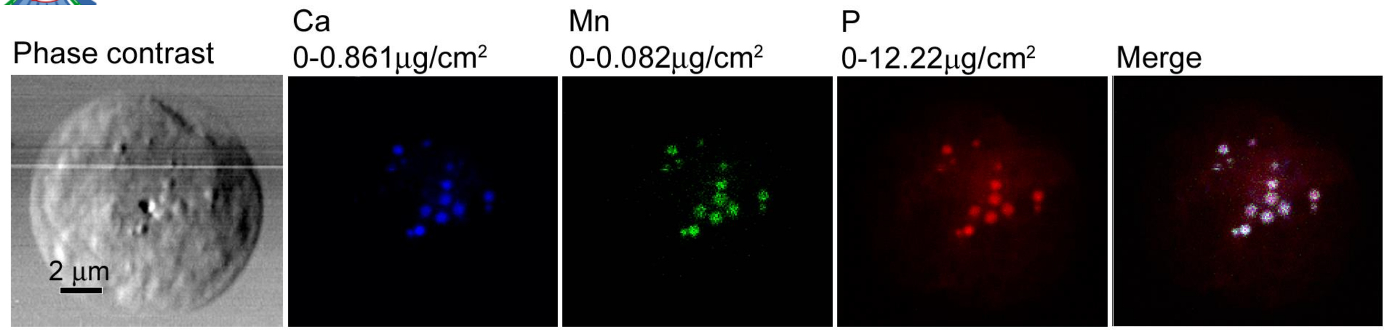
Mn hyper-accumulation in chlamy



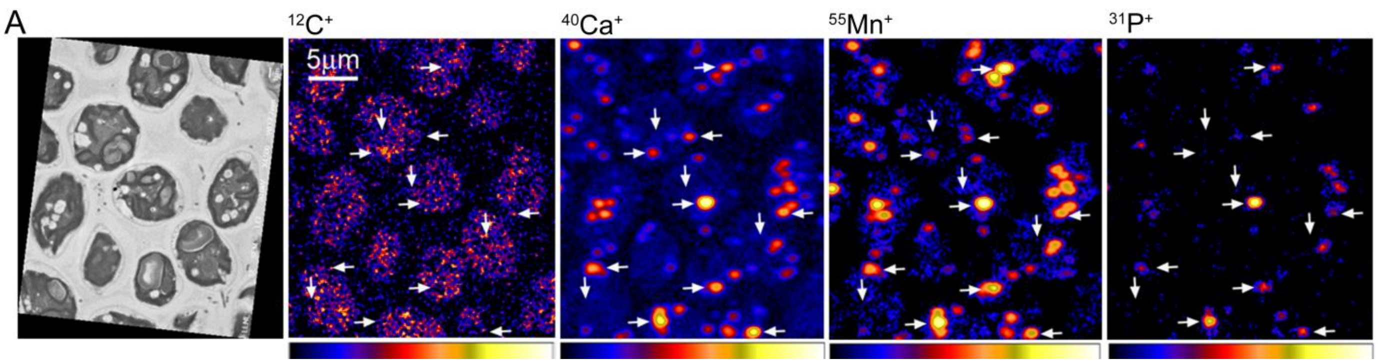
Q: Where this hyper-accumulated Mn goes?

Tsednee et al., 2019, in press

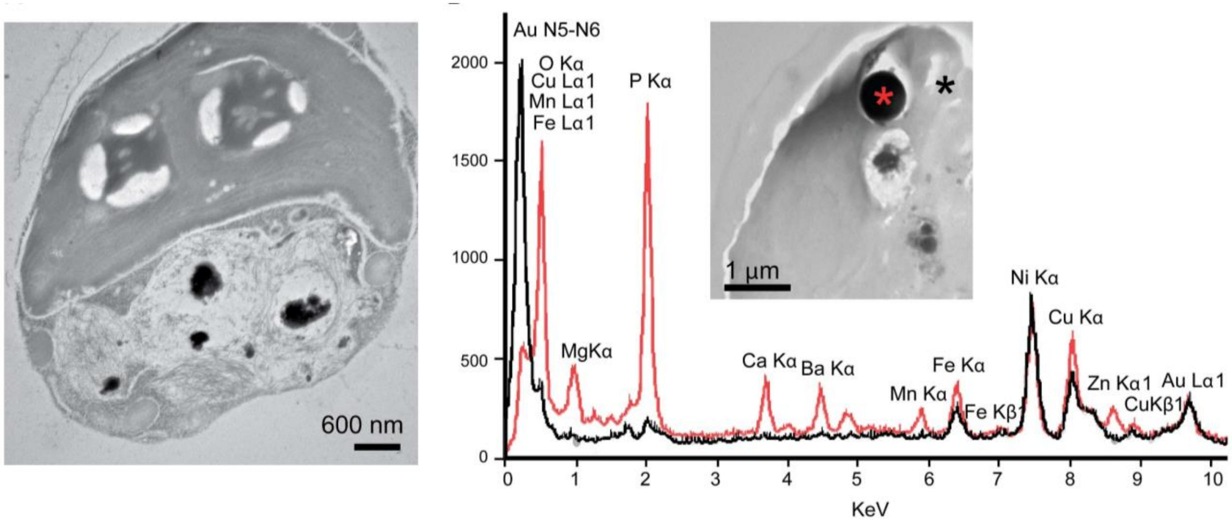
Mn hyper-accumulation site - acidic vacuole



XRF



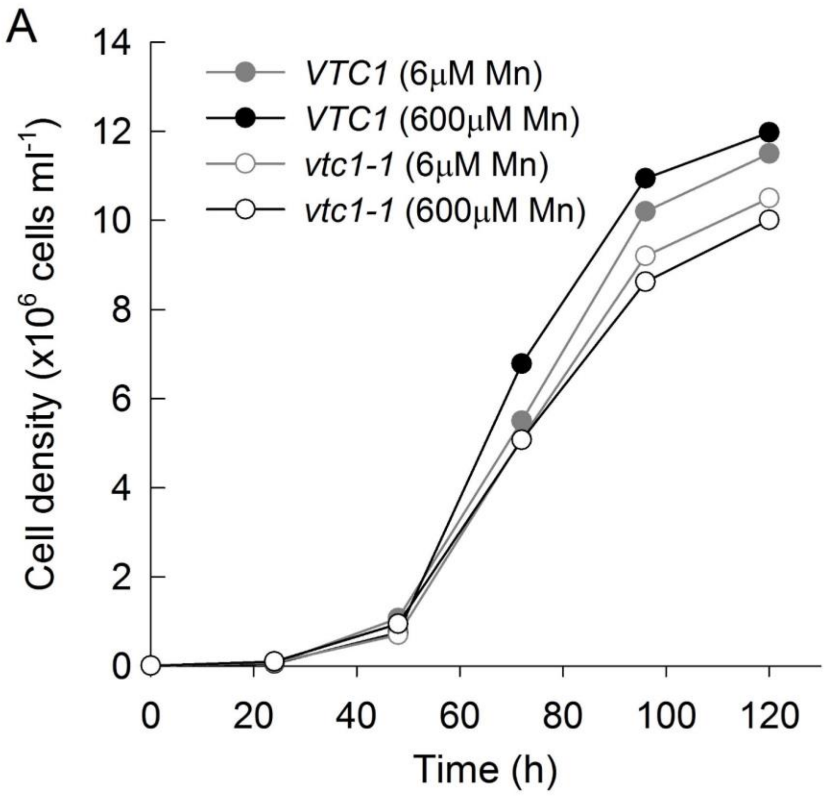
Nano-SIMS

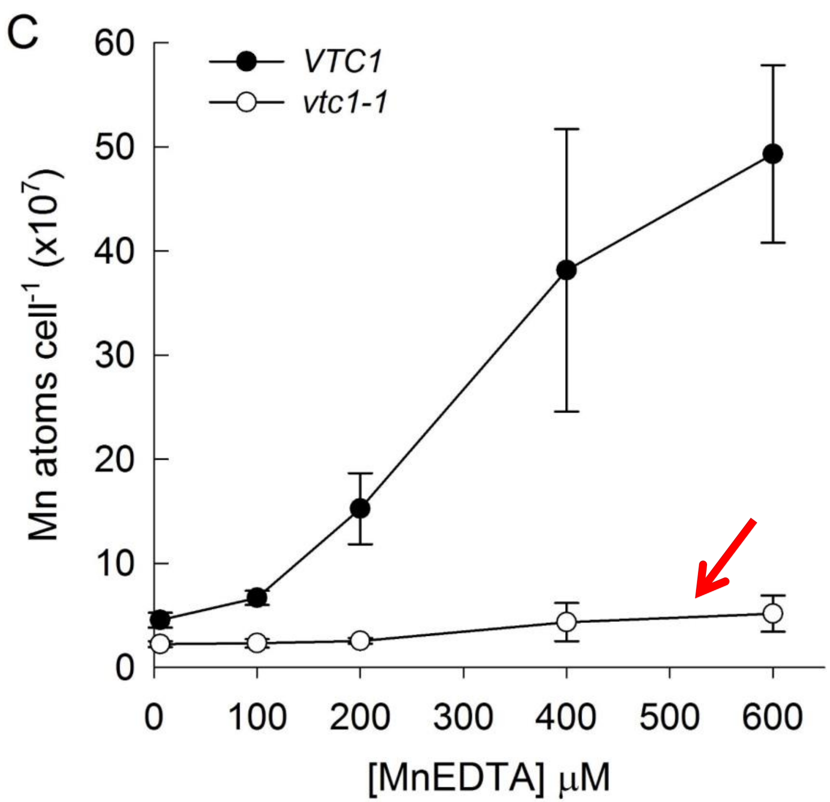


TEM-EDXS

Tsednee et al., 2019, in press

Mutant defected in biogenesis of acidic compartment can not hyper-accumulate Mn.





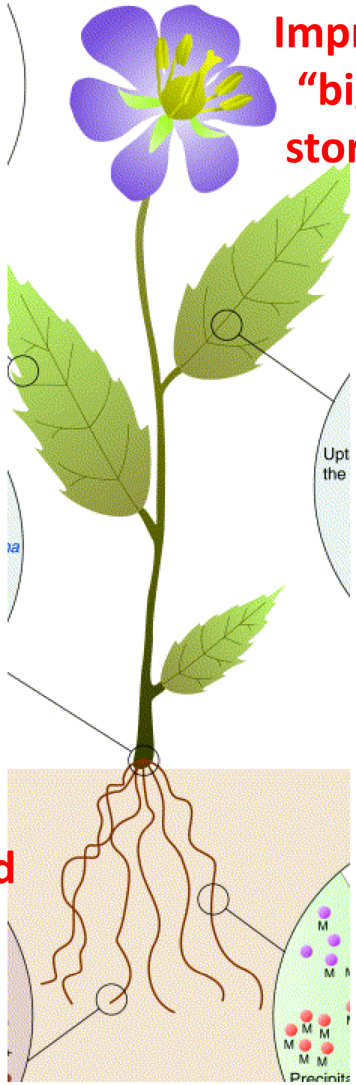
Tsednee et al., 2019, in press

Conclusion

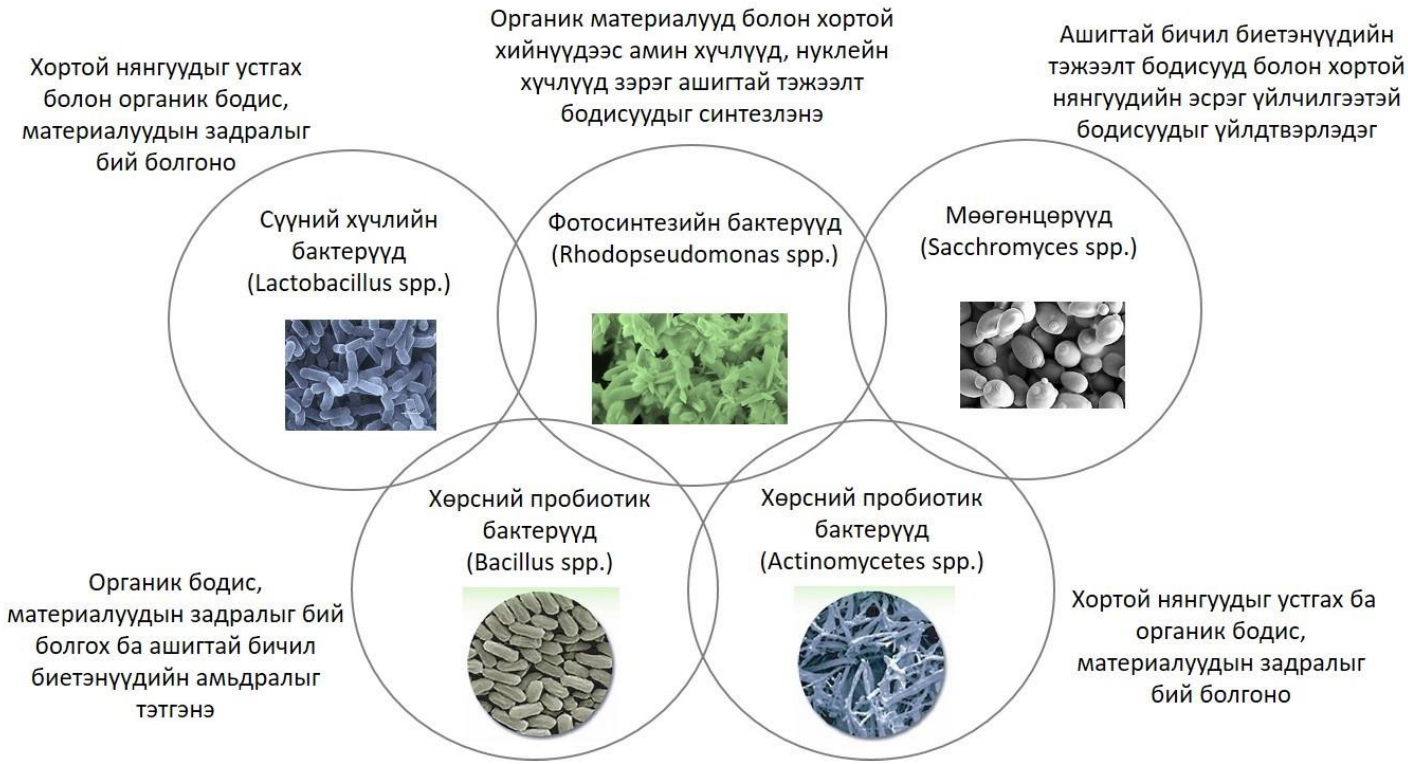
1. **We have identified root-secretion mechanism and NA exudation for Zn hyper-accumulation of *A. helleri.***

2. **We have identified the acidic vacuolar compartment for Mn hyper-accumulation of *Chlamydomonas.***

3. **Using NA-biosynthesis and efflux transporter gene, the generation of transgenic plants are currently conducting.**



Bioremediation for soil toxic bacterial contaminations



Use of community of microorganisms against toxic/ harmful bacterial contaminations (collaborative project, on-going)

Future work

1. Generation of transgenic plants (using NA- and acidocalisome-related genes) for soil heavy metal remediation in Ulaanbaatar city

2. Application of Environmental Biotechnology in soil toxic bacterial contamination

Acknowledgement

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Thank you!

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