Cellular distribution of Zn, Cd and Pb in hyperaccumulator *Arabis paniculata*

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Outline

1 **Background**

→ *Arabis paniculata*

2 **Materials and Methods**

3 **Results and Conclusions**

- Zn, Cd and Pb distribution in roots
- Zn, Cd and Pb distribution in leaves
- Zn, Cd and Pb distribution in trichomes

4 **Related work and perspectives**

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Multi-metal hyperaccumulator

- **Multi-metal hyperaccumulators are still rare (14 species)**
- **Most work focus on *Thlaspi caerulescens*, *Arabidopsis halleri*, and *Sedum alfredii***

Thlaspi caerulescens
Zn/Cd/Ni

Arabidopsis halleri
Zn/Cd

***Sedum alfredii* (Zn/Cd)**
(Yang et al., 2002,2004)

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Multi-metal hyperaccumulators
- *Arabis paniculata*

Natural habitat---
Pb/Zn mine land

Arabis paniculata Franch., is a biennial plant belonging to *Arabis* genus, *Brassicaceae* family.

A Zn/Cd/Pb multi-metal hyperaccumulator found in China
(Tang et al., 2009, Environ.Exp.Bot)

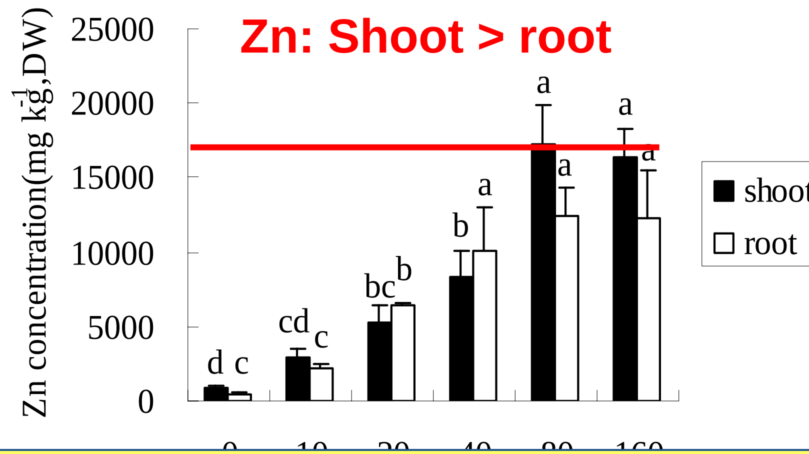
Q1: Why could this plant tolerate and accumulate such high levels of metals?

Pb,Zn,Cd concentrations in *A.paniculata* collected on P
mine land, Yunnan,China (mg kg^{-1}, n=25)

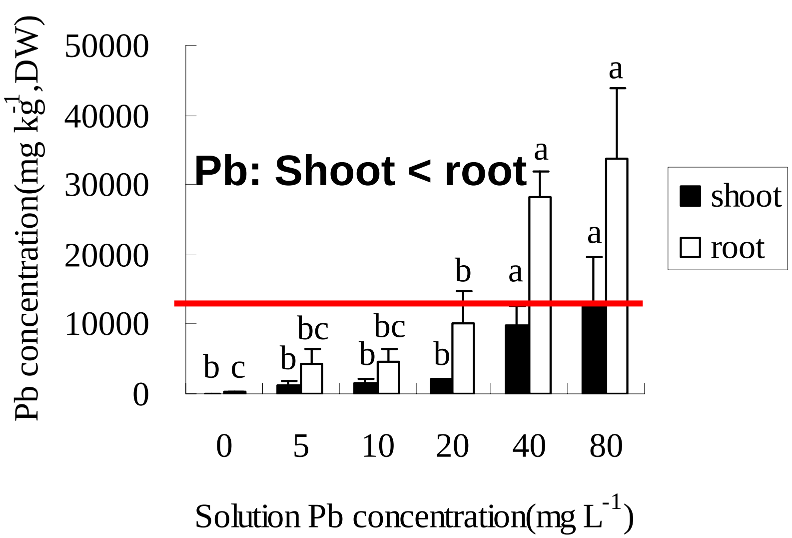
Metal	Soil	Shoot	Root	S/R
Pb	27800	2308	1465	1.93
Zn	179000	20828	16374	2.40
Cd	4240	434	415	1.49

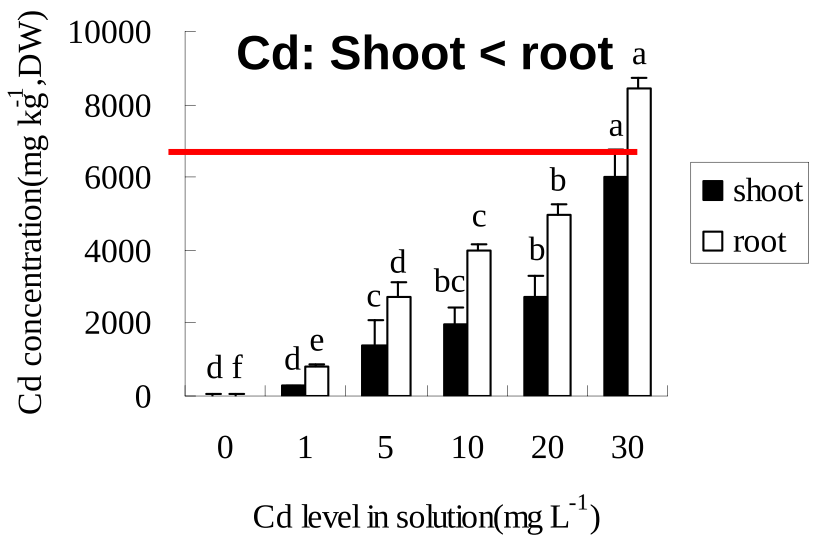
Multi-metal hyperaccumulators - *A. paniculata*

Hydroponic Study



Q2: Why there is big difference among the translocation of Zn\Cd\Pb in this plant?





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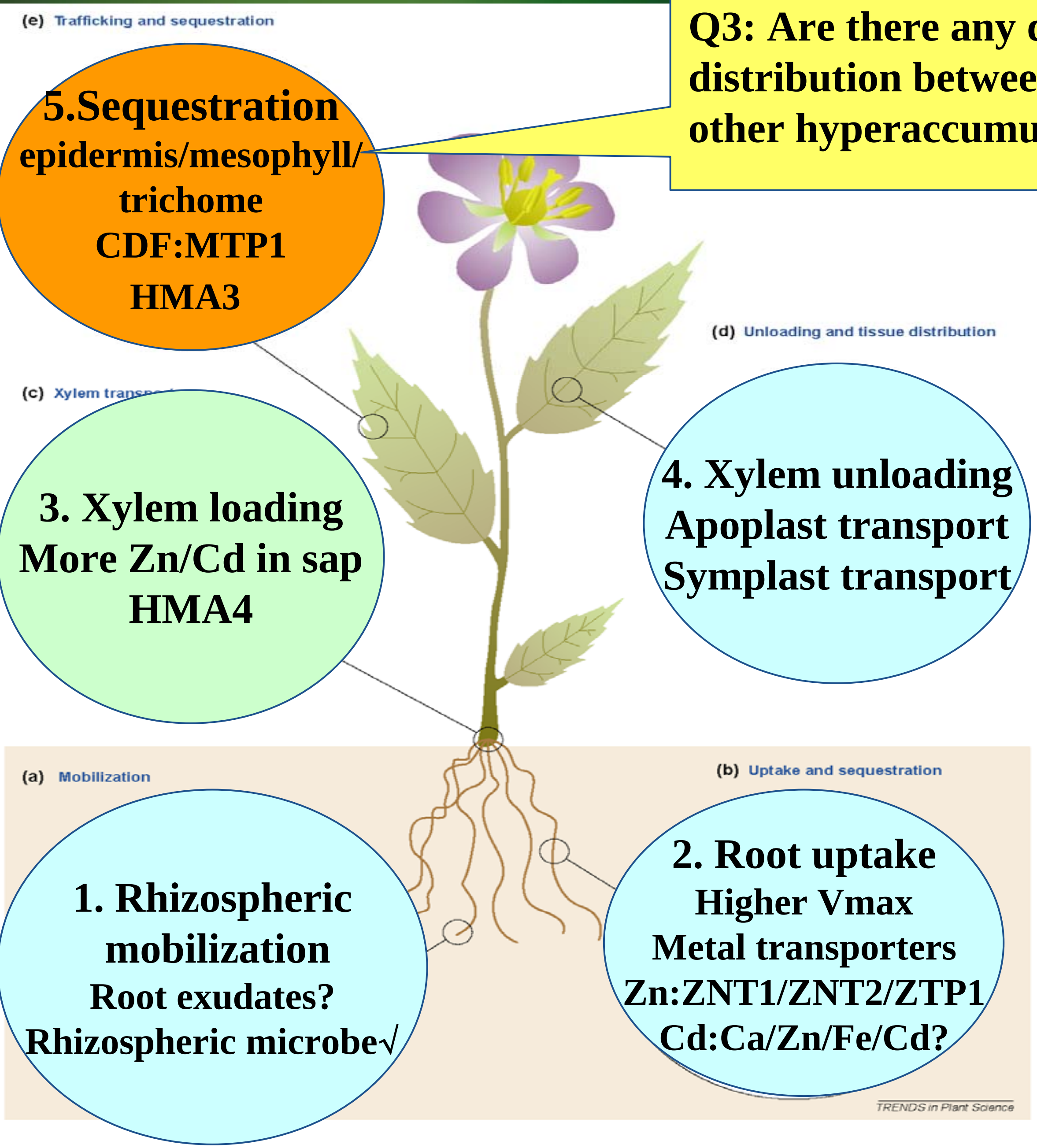
Q3: Are there any difference in metal distribution between *A. paniculata* with other hyperaccumulators at cellular level?



Uptake and accumulation mechanisms in hyperaccumulator

(Clemens e*t al*., 2002)

***Thlaspi caerulescens*,**
***Arabidopsis halleri*,**
Sedum alfredii

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Purposes of this study

- By using SEM, TEM-EDS, we studied the distribution of Zn, Cd and Pb in roots and shoots of *A. panicuata*, in order to :

1. **investigate the accumulation and distribution difference among tri-metals;**
2. **discuss their distribution characters with other hyperaccumulators;**
3. **reveal the underlying mechanisms of metal tolerance, uptake and detoxification in this plant.**

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Materials and Methods

Arabis paniculata

Hydroponic culture

Germinated in the mixture of sand and vermiculate for 3 months, cultured in 20% Hoagland solution

Four treatments: ①CK，②2000 μM Zn，③250 μM Cd，④200 μM Pb

10 days

SEM-EDS

Fixed in 1%Na_2S—in 2.5% glutaraldehyde—alcohol dehydrated—exchanged with tertiary butyl alcohol—fractured transversely in liquid N_2—freeze-dried—coated with carbon—SEM/EDS

TEM-EDS

Fixed in 5% glutaraldehyde and paraformaldehyde—post fixed with 2% OsO4—alcohol dehydrated—embedded in Spurrs resin—untramicrotomed and stained—TEM/ EDS

SEM-EDS: scanning electron microscopy — energy dispersive spectrometer

TEM-EDS: transmission electron microscopy — energy dispersive spectrometer

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Zn, Cd and Pb concentrations in *A. paniculata*

Metal	Treatment (μM)	Shoot (mg kg^{-1}, DW)		Root (mg kg^{-1}, DW)	
		Mean	SD	Mean	SD
Zn	CK	86	9	431	53
	2000	14720	3680	11600	1808
Cd	CK	Nd	-	Nd	-
	250	2380	69	7140	770
Pb	CK	Nd	-	Nd	-
	200	5960	634	54620	15320

Zn: shoot>root, TF=1.27
Cd, Pb: shoot<root, TF=0.33(Cd), 0.11(Pb)

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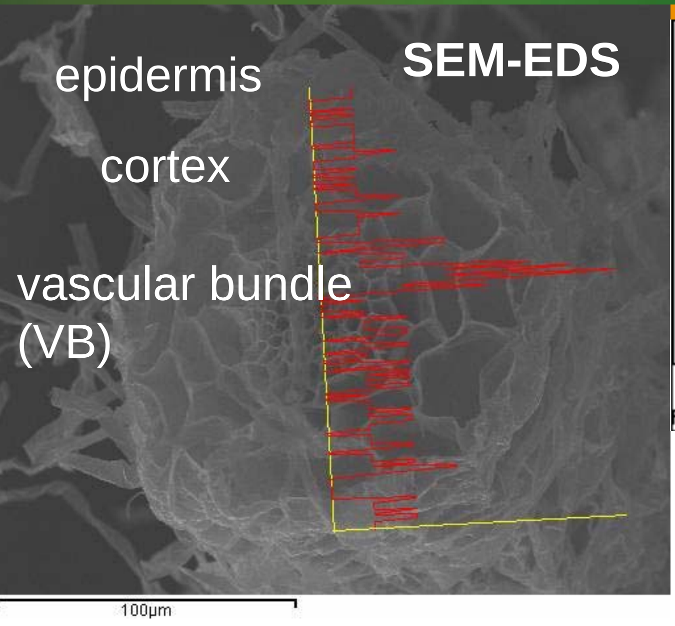
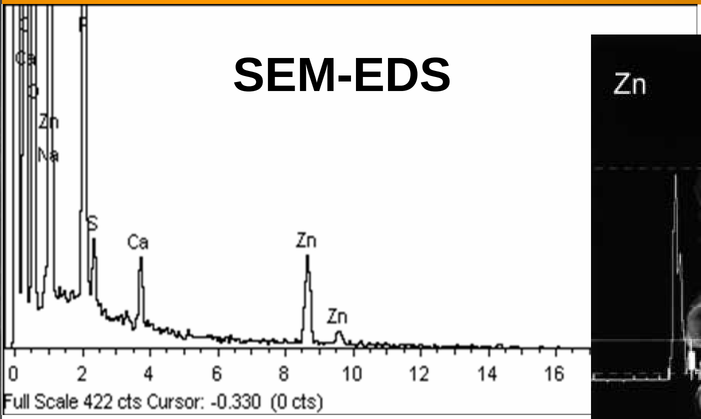
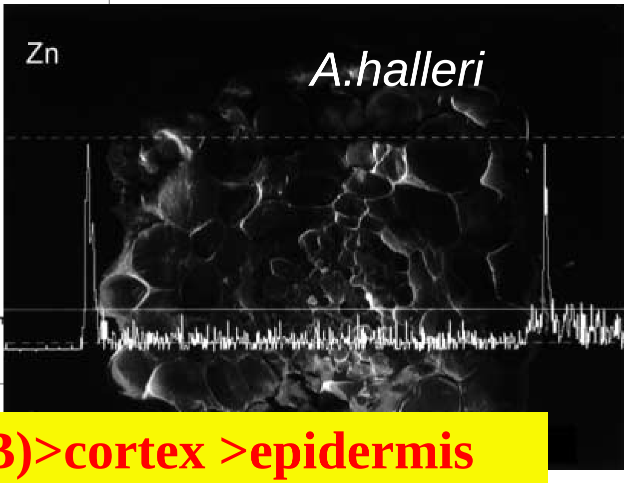
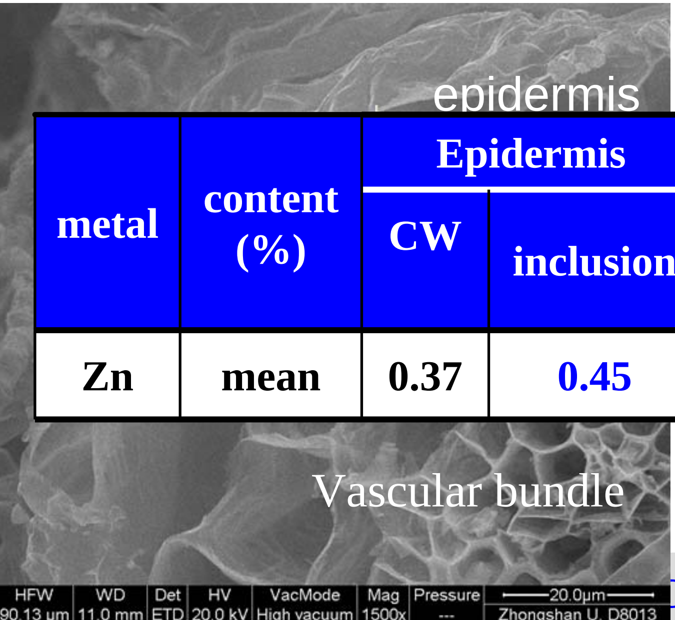
Zn distribution in roots of *A. paniculata*

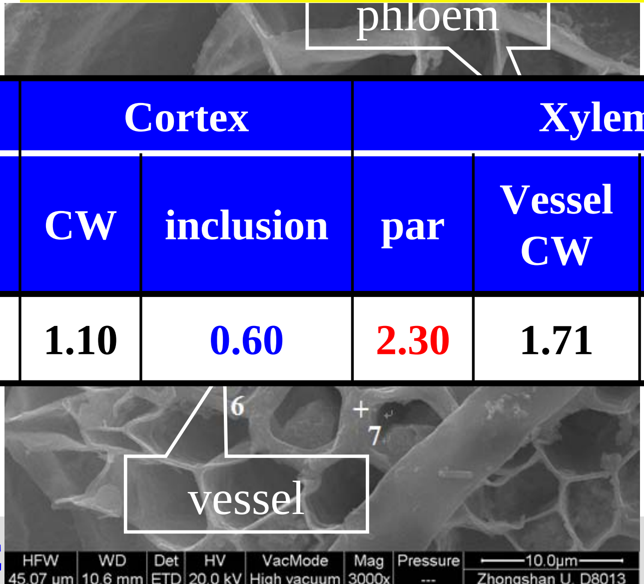






- **Vascular bundle (VB)>cortex >epidermis**
- **In VB: vessel>parenchyma>phloem, Zn could be easily translocated to shoot.**





metal	content (%)	Epidermis		Cortex		Xylem			Phloem	
		CW	inclusion	CW	inclusion	par	Vessel CW	Vessel inclusion	CW	inclusion
Zn	mean	0.37	0.45	1.10	0.60	2.30	1.71	5.03	1.07	2.31

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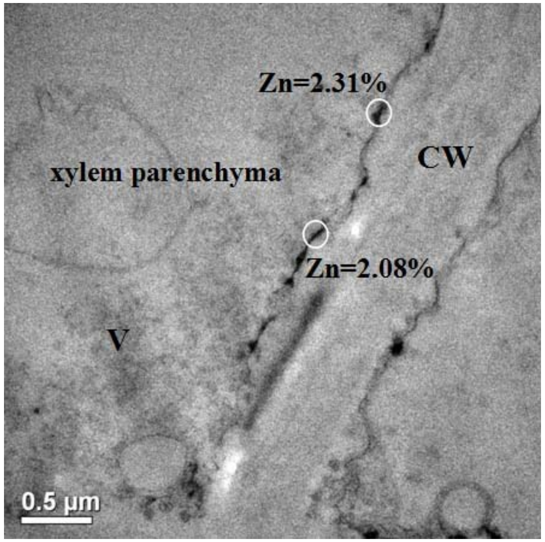
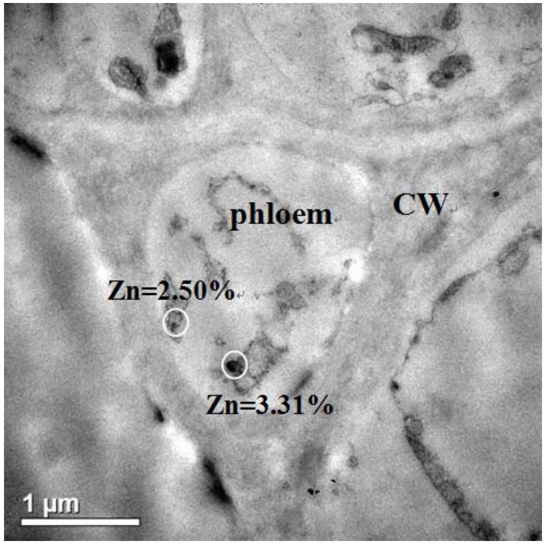
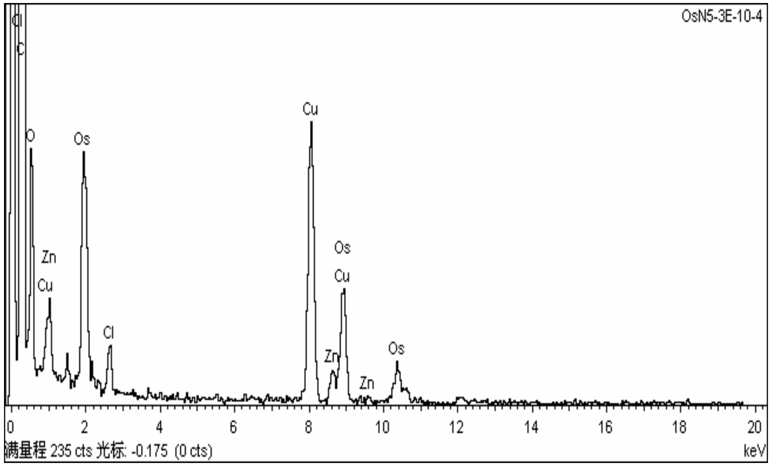
Zn distribution in roots of *A. paniculata*

TEM-EDS



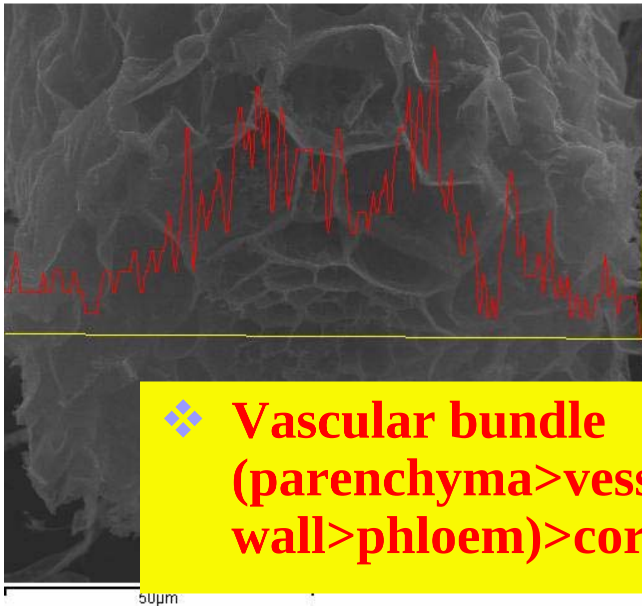


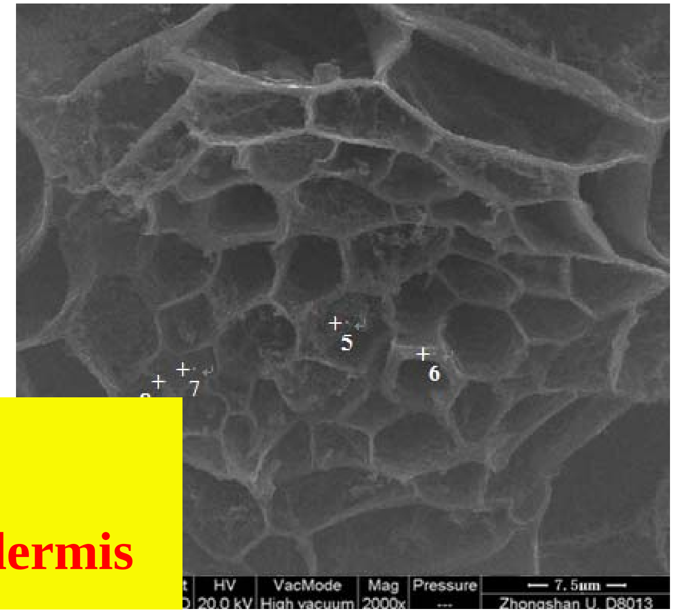


- TEM-EDS: Zn precipitates both in the centre and along the walls of xylem parenchyma and phloem in a form of black dense deposition.

Cd distribution in roots of *A. paniculata*

SEM-EDS





- **Vascular bundle (parenchyma>vessel cell wall>phloem)>cortex >epidermis**

metal	content (%)	Epidermis		Cortex		Xylem			Phloem	
		CW	inclusion	CW	inclusion	Par	Vessel CW	vessel	CW	Inclusion
Cd	mean	0.33	0.34	0.94	1.02	2.19	2.03	-	1.64	1.48

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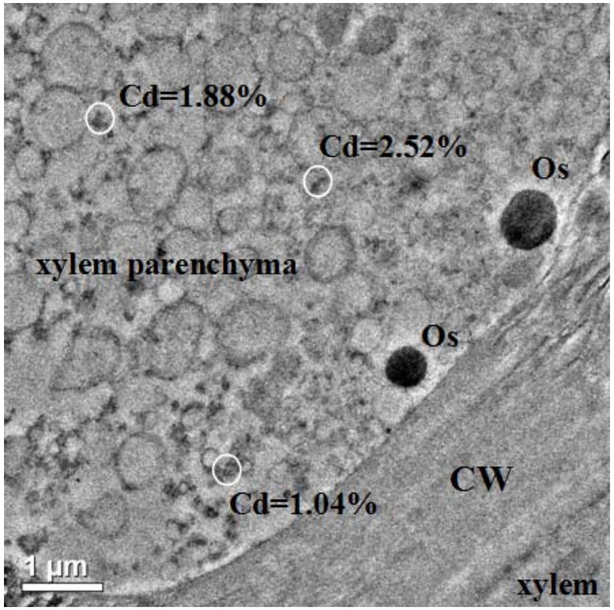
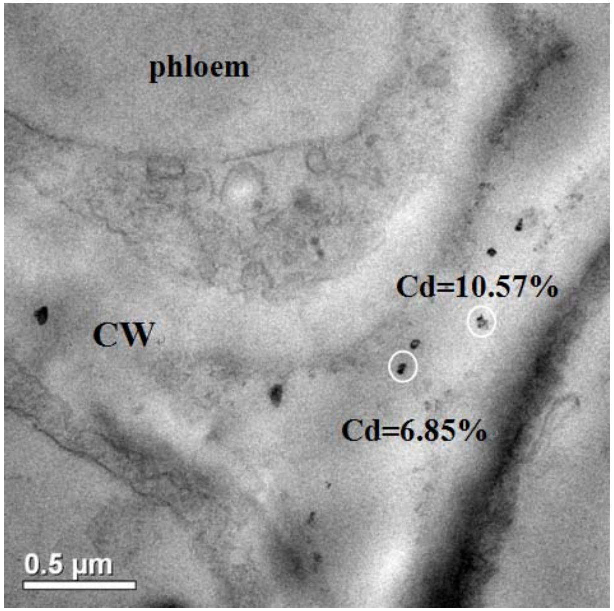
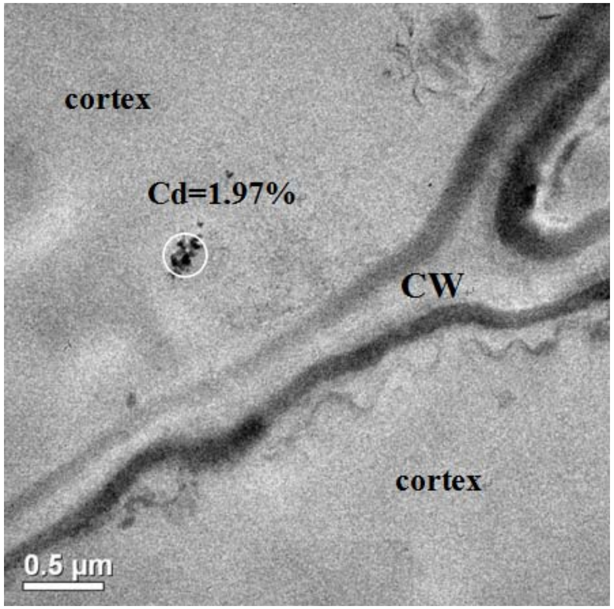
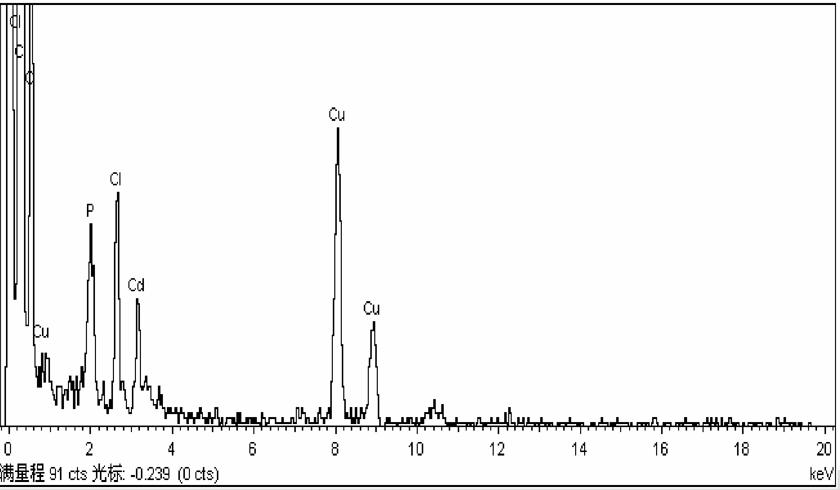
Cd distribution in roots of *A. paniculata*

TEM-EDS









- TEM-EDS: Cd mainly distributed in xylem parenchyma, cortex and the cell wall of phloem in a form of black dense electron deposition.

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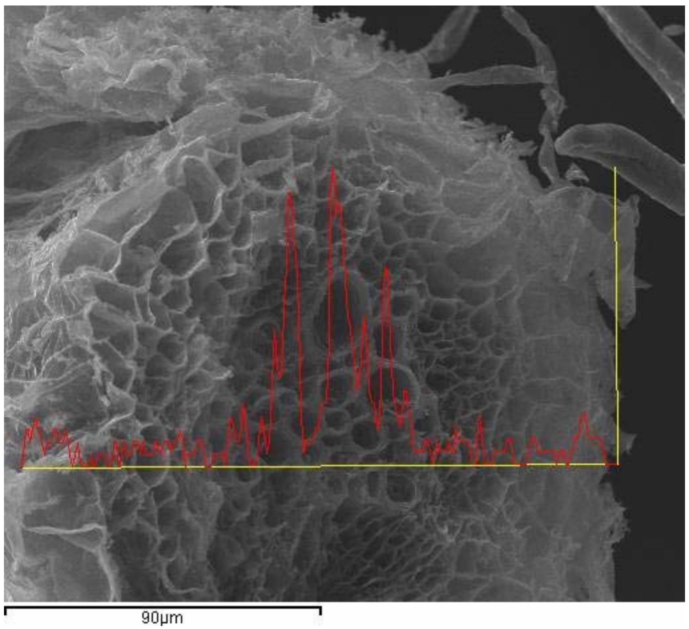
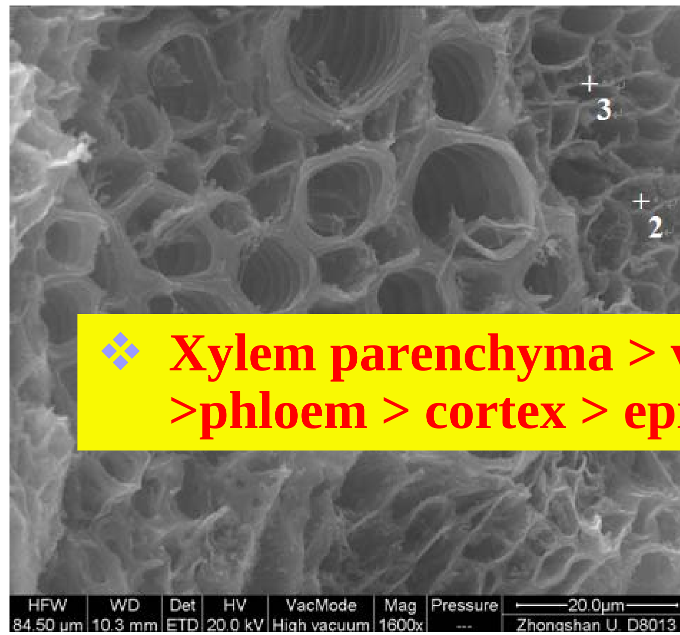
Pb distribution in roots of *A. paniculata*

SEM-EDS



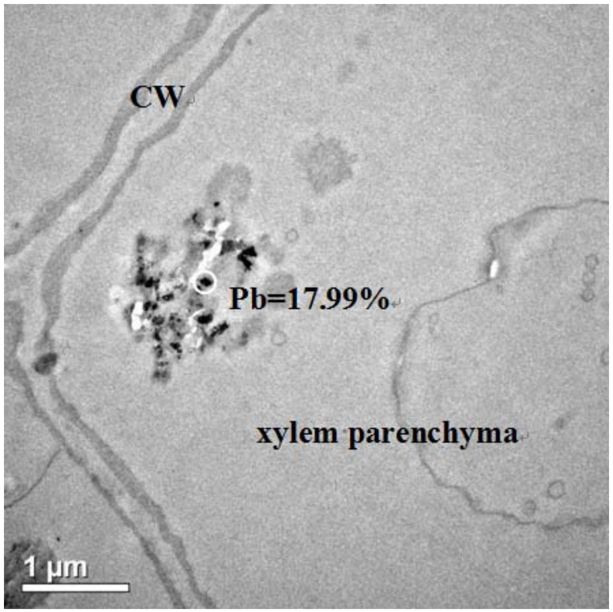


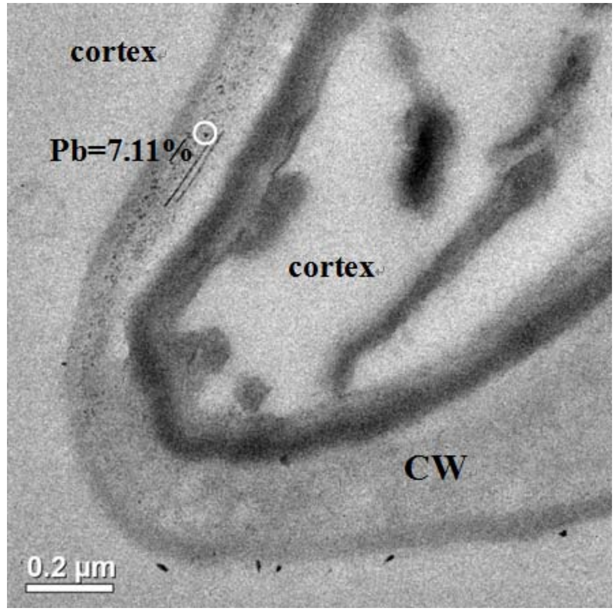
- **Xylem parenchyma > vessel cell wall >phloem > cortex > epidermis**

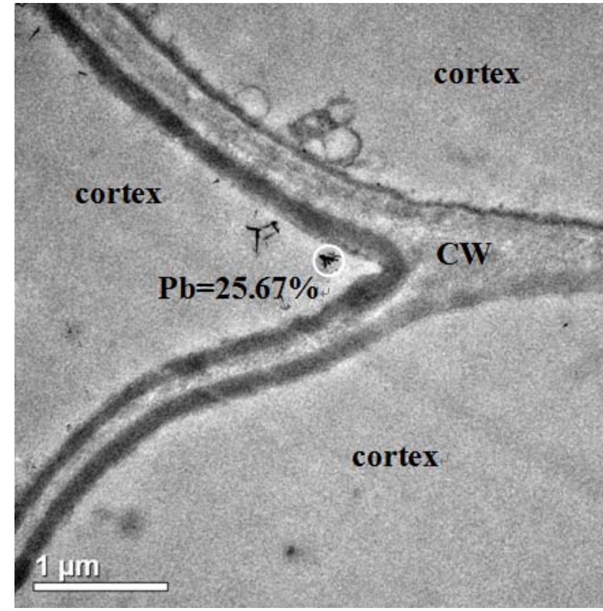
metal	content (%)	Epidermis		Cortex		Xylem			Phloem	
		CW	inclusion	CW	inclusion	Par	Vessel CW	Vessel	CW	inclusion
Pb	mean	0.82	1.06	1.76	7.28	12.63	1.16	-	1.39	2.37

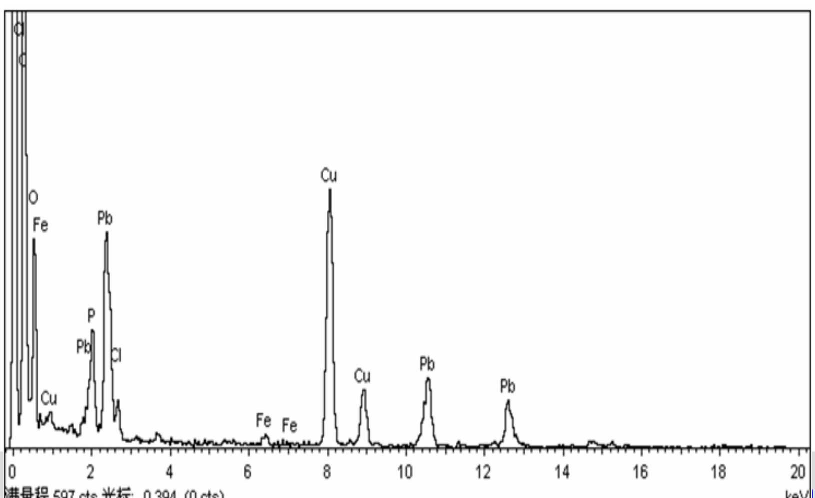
Pb distribution in roots of *A. paniculata*

TEM-EDS









- TEM-EDS: very high content of Pb was found in xylem parenchyma and cortex. Pb particles were small and evenly distributed in cell wall, while were much larger in cytosols.

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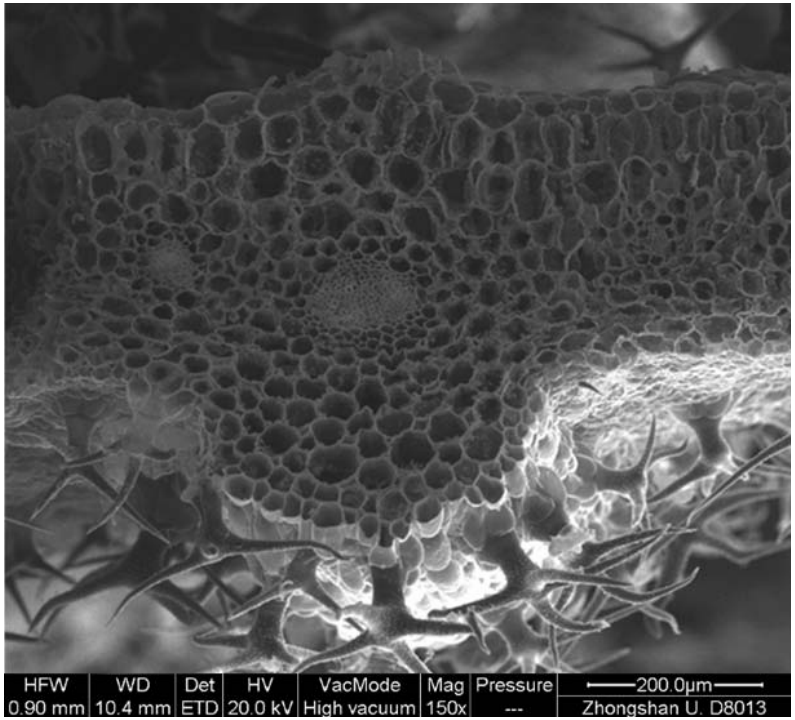
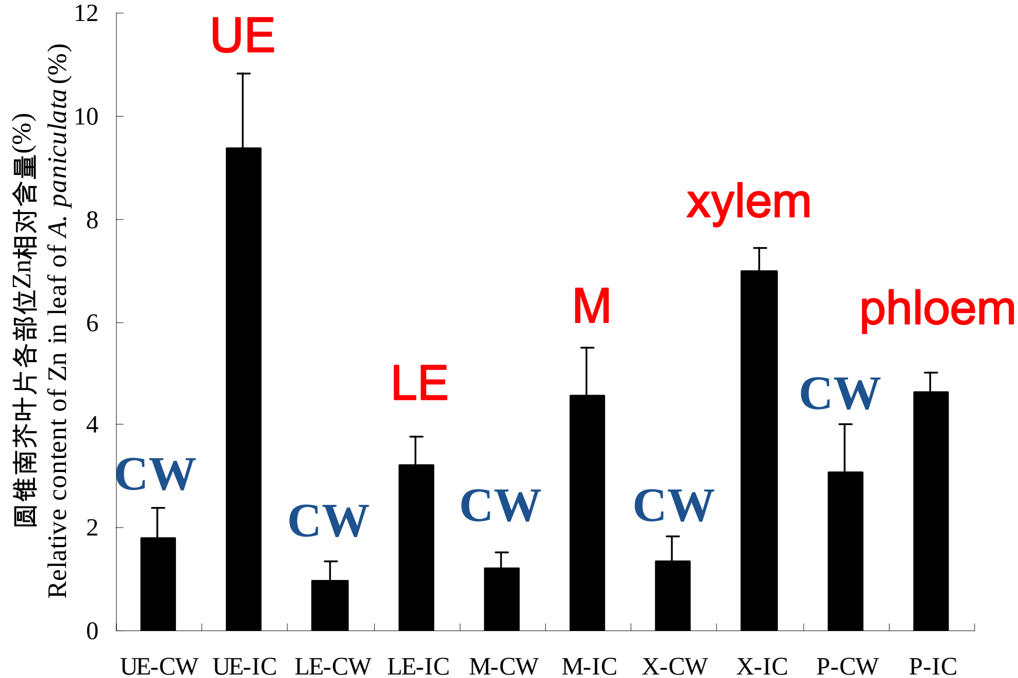
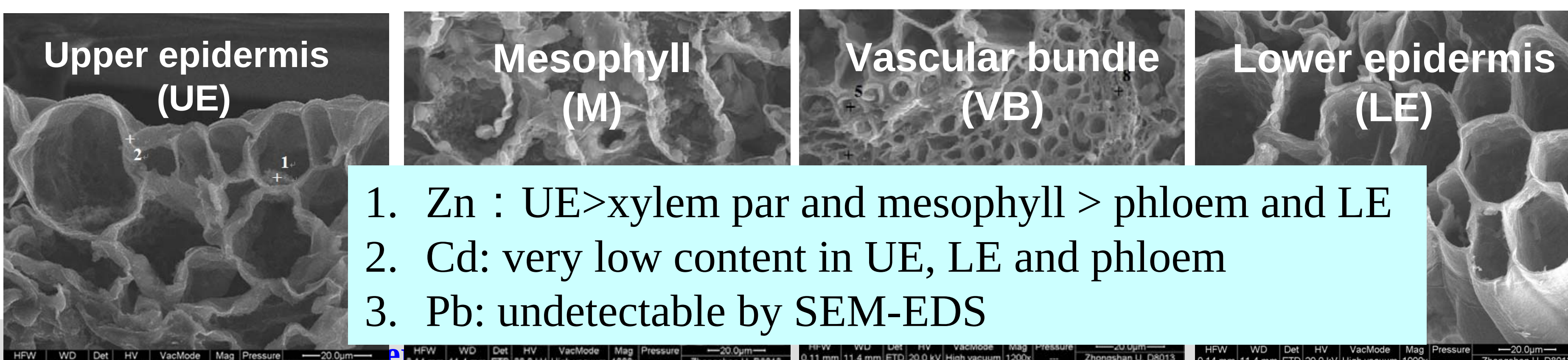
Zn distribution in leaves of *A. paniculata*

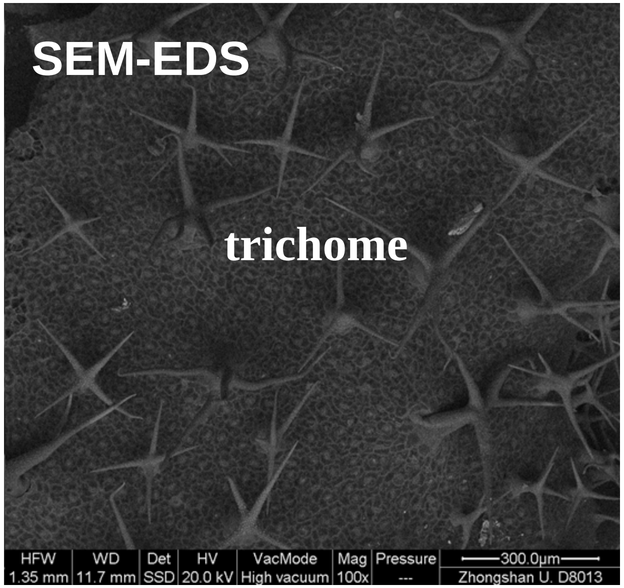


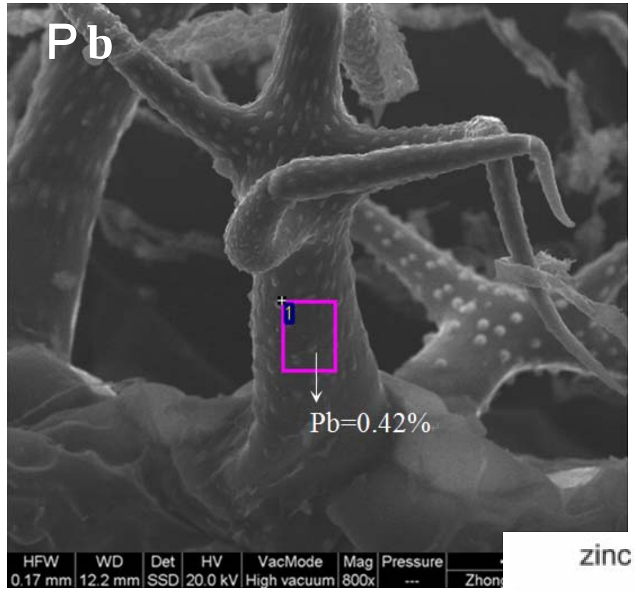




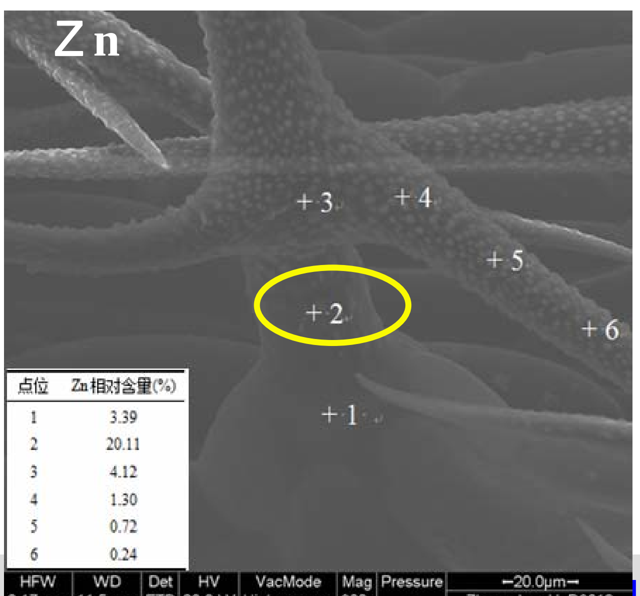
1. Zn：UE>xylem par and mesophyll > phloem and LE
2. Cd: very low content in UE, LE and phloem
3. Pb: undetectable by SEM-EDS

Zn distribution in leaf trichomes of *A. paniculata*

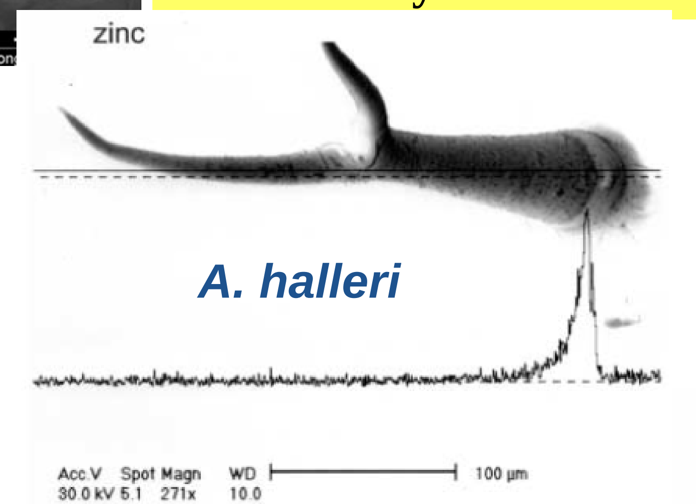




1. **Zn：up to 20% was distributed in the base of leaf trichomes, similar to *A. halleri.***
2. **Pb and Cd accumulation in trichomes are relatively weak.**



Spot No	Zn(%)
1	3.39
2	20.11
3	4.12
4	1.30
5	0.72
6	0.24



In leaf of *A. halleri*, highest Zn and Cd content was observed in a narrow ring in the base of trichome (Küpper *et al.*，2000)

Conclusions

1. In the roots of *A. paniculata*, cell-wall-precipitation may not be the main storage way since Zn, Cd and Pb can be translocated from apoplast to vascular bundle parenchyma effectively. In addition, it was easier for Zn than Cd and Pb to load into xylem for further transportation.
2. In the leaves, the dominant distribution of Zn was in upper epidermis, followed by xylem vascular, phloem, mesophyll and the least, cell wall.
3. In leaf trichomes, up to 20% of relative Zn content observed in a narrow ring in the middle of trichome base was very similar to *A. halleri*. This fact indicated that the compartmentation in the trichomes was another important detoxification mechanism for Zn.

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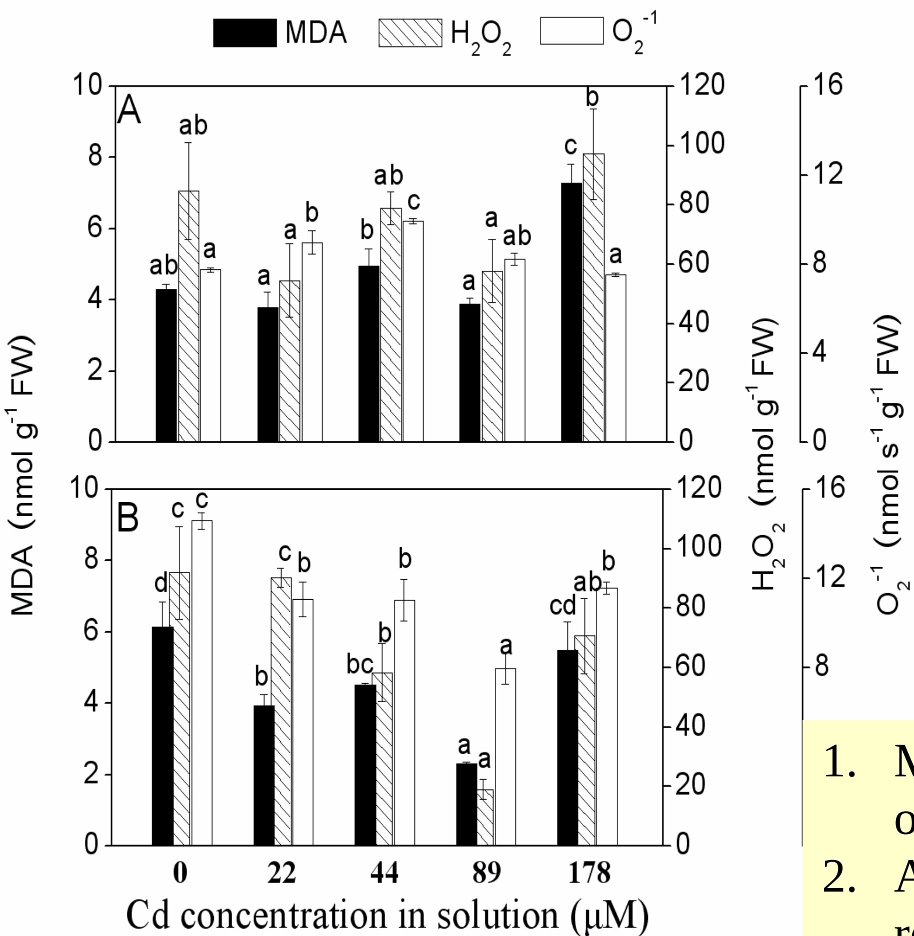
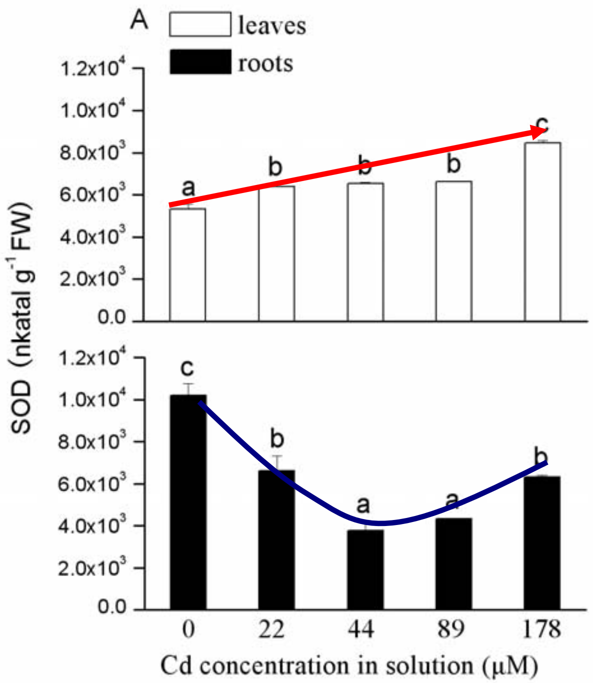
Our related work: Mechanisms of metal uptake and detoxification in *A. paniculata*

(1)Antioxidant response to Cd in *Arabis paniculata*

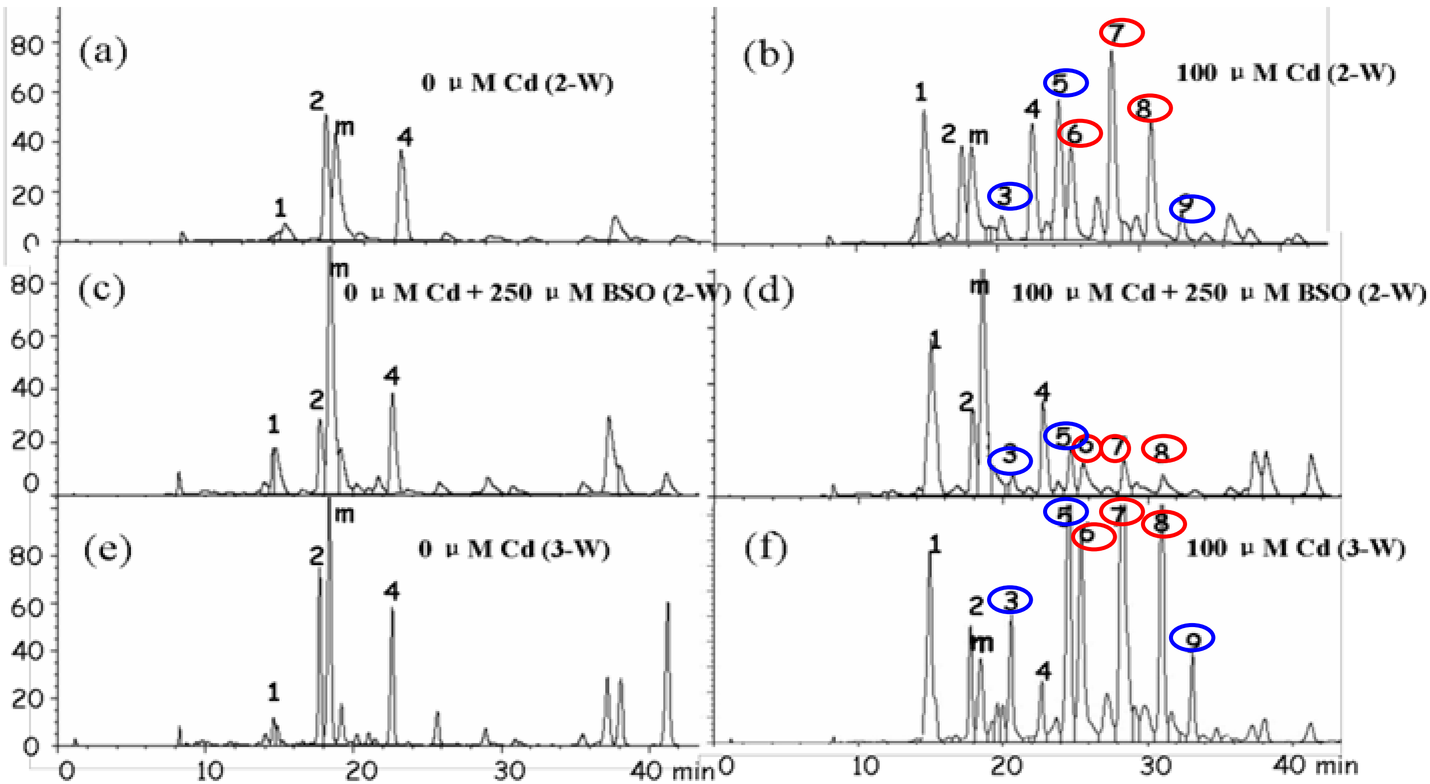




1. Moderate addition of Cd could enhance growth of *A. paniculata* and alleviate lipid peroxide.
2. Antioxidant enzymes in shoots and roots responded to Cd in totally different way.

(Qiu et al., 2008, Chemosphere)

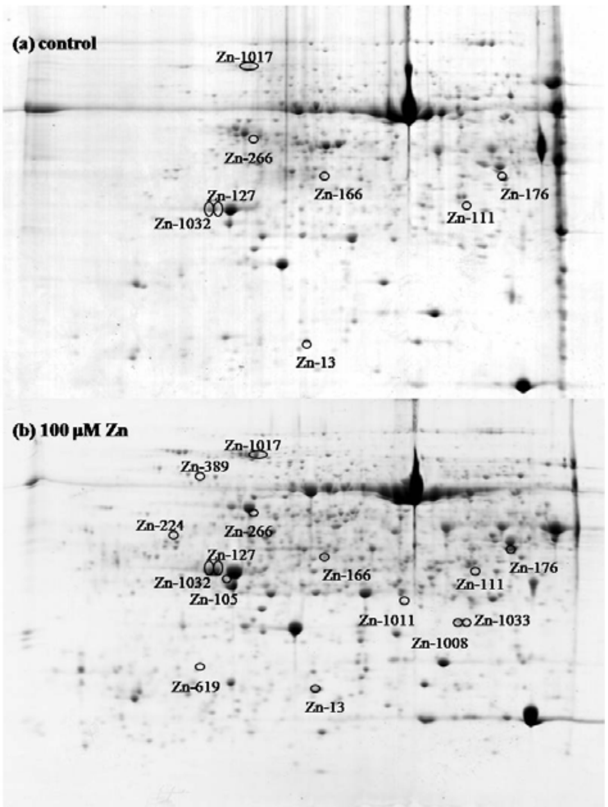
(2) PCs and non-protein thiols in *A. paniculata* under Zn/Cd exposure---HPLC

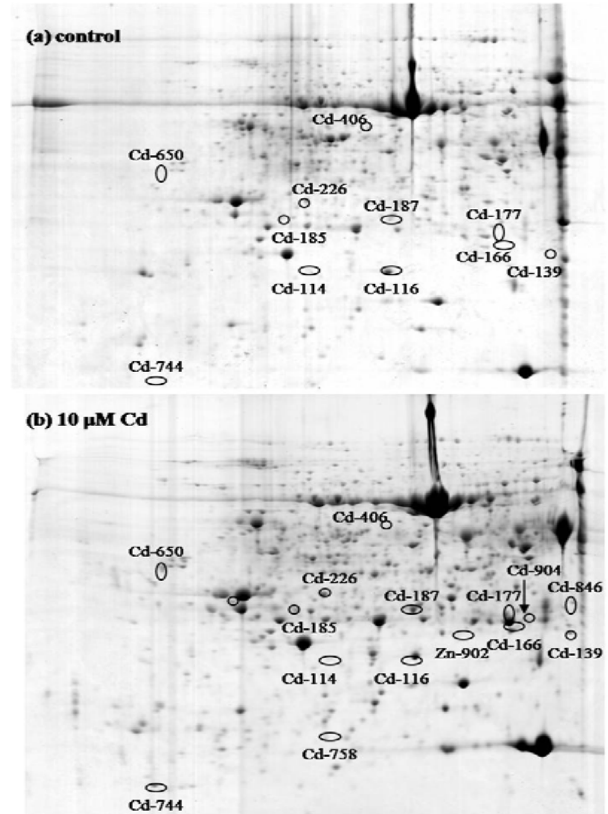


(1) Cys; (2) GSH; (3) X20.5; (4) NAC; (5) X25.5; (6) PC2; (7) PC3; (8) PC4

1. PCs could only be induced by Cd in root of *A. paniculata*, but not induced by Zn.
2. Both PCs and GSH did not play primary role in Cd tolerance but may be important for Cd uptake by *A. paniculata* (Zeng et al., 2009, Environ.Exp.Bot)

This work is still on-going...





- We are now applying proteomic approaches to investigate differentiated expressed proteins responded to Zn and Cd in *A. paniculata*.

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Thanks for your attention!