Phytoremediation, a novel method for removal of heavy metals from environment: biochemical and molecular mechanisms

Shivendra V. Sahi, Ph.D.
Department of Biology
Western Kentucky University
(shiv.sahi@wku.edu)

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applied research and technology program

Outline

- Phytoremediation
- *Sesbania drummondii*

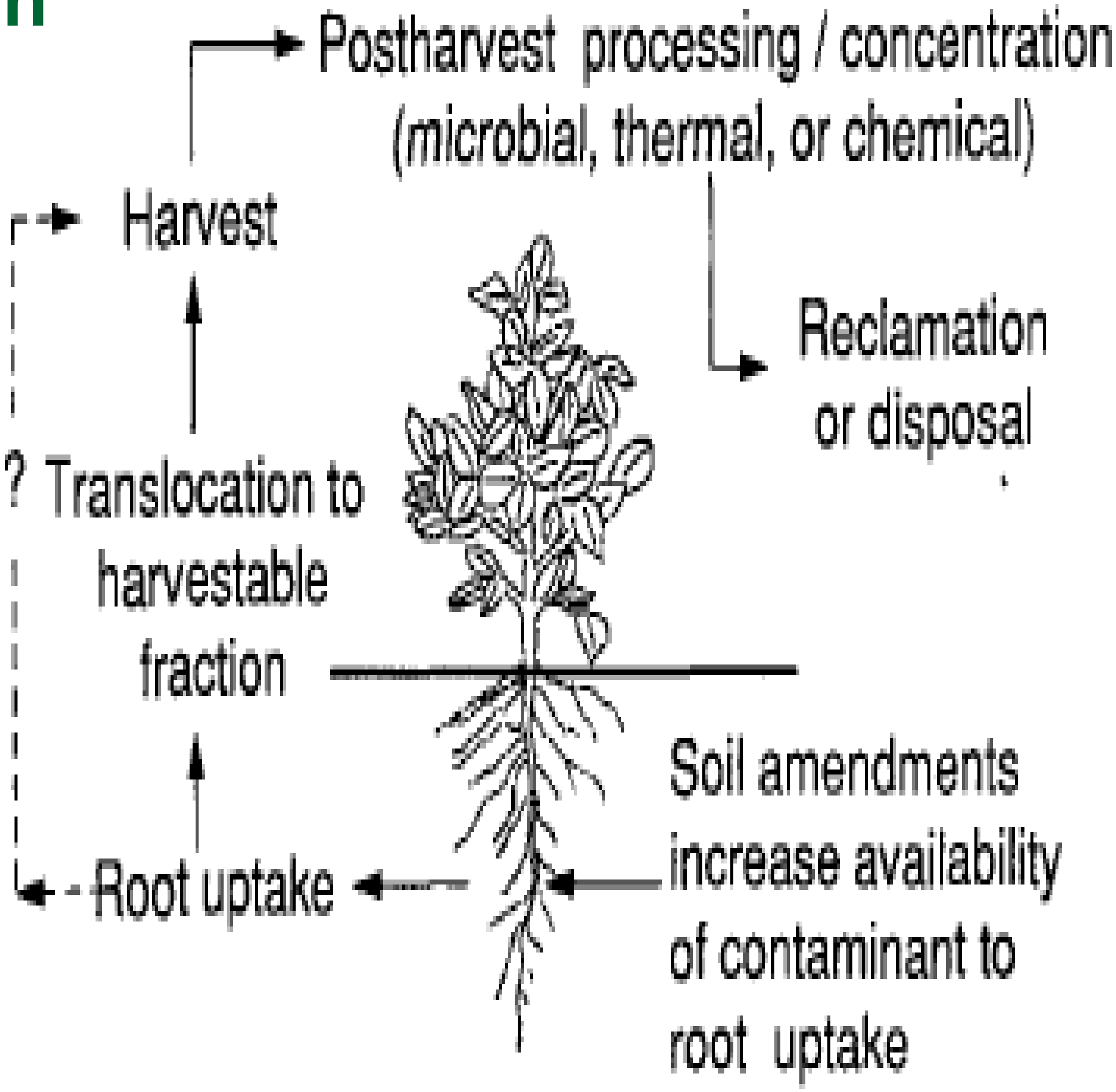
  - Metal (Pb) uptake
  - Microscopic evidence of metal transport
  - Biotransformation of toxic compounds
  - Gene identification/expression
  - Conclusion

Phytoremediation

Use of vegetation for the *in situ* treatment of contaminated sites

- A fast emerging environmental clean up strategy
- Immense promise for remediation of contaminated sites (soil, ground water, waste water)
- Effective against
  - **inorganic** (toxic metals and nutrients)
  - **organic pollutants** (BTEX)
    - chlorinated solvents, ammunition wastes

Phytoextraction Process



Background

- 1980 Statute recognized over 40,000 Superfund sites endangering human health
- Mining and smelting,   municipal wastes, sewage sludge, landfill leachates, fertilizers, pesticides, nuclear accidents
- >10,000 sites remain active today (Superfund Accomplishment Figures-FY 2003)
- 40% of these sites have problems of heavy metal (Pb, Cd, Cr, As, Zn etc.) contamination

Conventional remediation strategies against metal contaminations

- Excavation and reburial of contaminated soils to another site
- Soil flushing/washing
- Solidification/stabilization
- Vitrification
- Electro-kinetics

Cost Analysis

- Conventional engineering technology v/s Phytoremediation (TIBTECH, 13, 1995)

Contaminants	Conventional Technology	Phytoremediation
Water soluble/ volatile compounds	$10-100 per m^3 soil	$ 0.02-1.00 per m^3 soil ($200-10,000 per hectare) of cropping
Compounds requiring land-filling or low temp. thermal treatments	$ 60-300 per m^3 soil	
Materials requiring special land-filling or high temp. thermal treatment	$ 200-700 per m^3 soil	
Incineration	$ 100 per m^3 soil	
Radionucleides	$ 1000-3000 per m^3 soil	

Benefits

- Economically feasible
- Socially desirable
- Environment friendly
- Improves soil health
- Effective

Terrestrial Hyperaccumulators (Brooks, 1998)

Plant	**Metal**	**% metal in shoot (DW)**
Thlaspi caerulescens	Zn, Cd	>2% Zn, >0.1% Cd,
Thlaspi spp.	Zn	>2%
Cardaminopsis hallerii	Zn	>1%
Brassica spp.	Se	
Astragalus spp.	Se	0.1-1%
Atriplex spp.	Se	
Thlaspi rotundifolium	Pb	<1% (~0.8%)
Aelloanthus subacaulis	Cu	1.3%
Haemaniastrum spp.	Co	Up to 1 %
Brake fern	As	>1.5%

Sesbania drummondii

- **A high biomass plant**
- **Common name: Rattlebox**
- **Native to Southeastern U.S.**

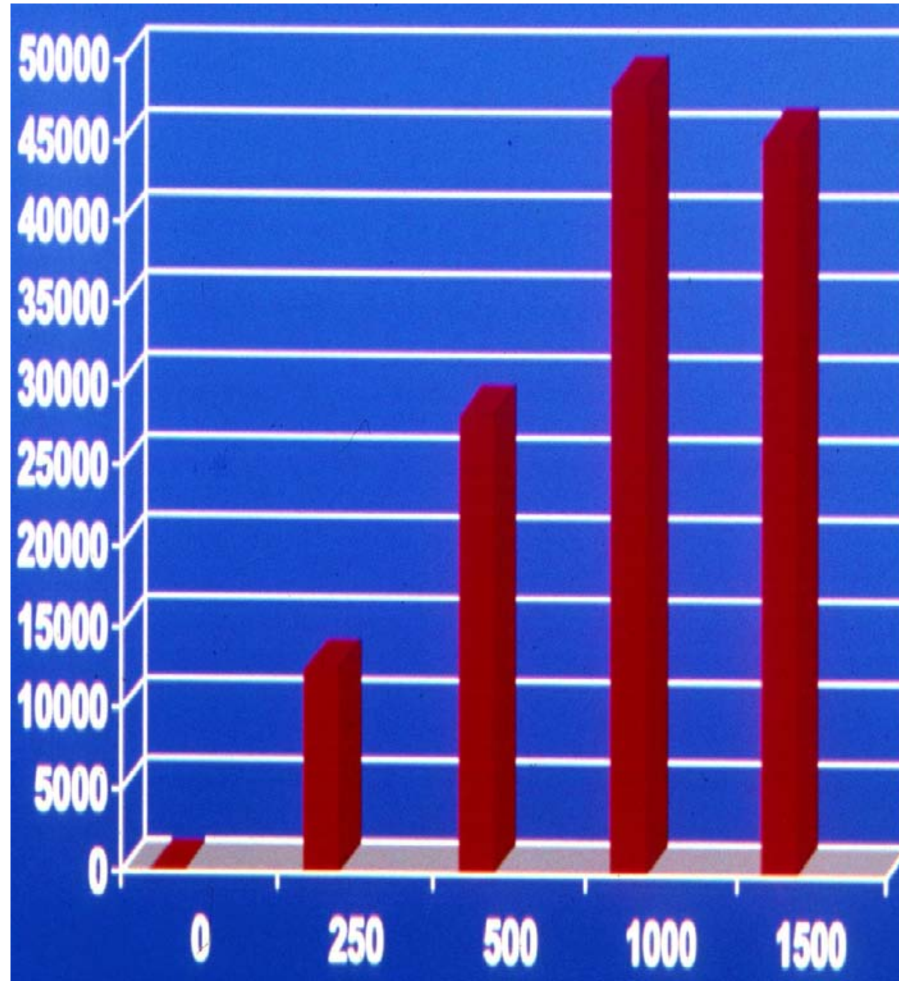
Sesbania drummondii & Lead

Demonstrated as lead hyperaccumulator

- Tolerates up to 1,000 ppm in hydroponic solution
- Accumulated >4% (DW) Pb in shoots in hydroponic conditions
- Roots showed 6% (DW) accumulation
- EDTA and low pH increased accumulation further

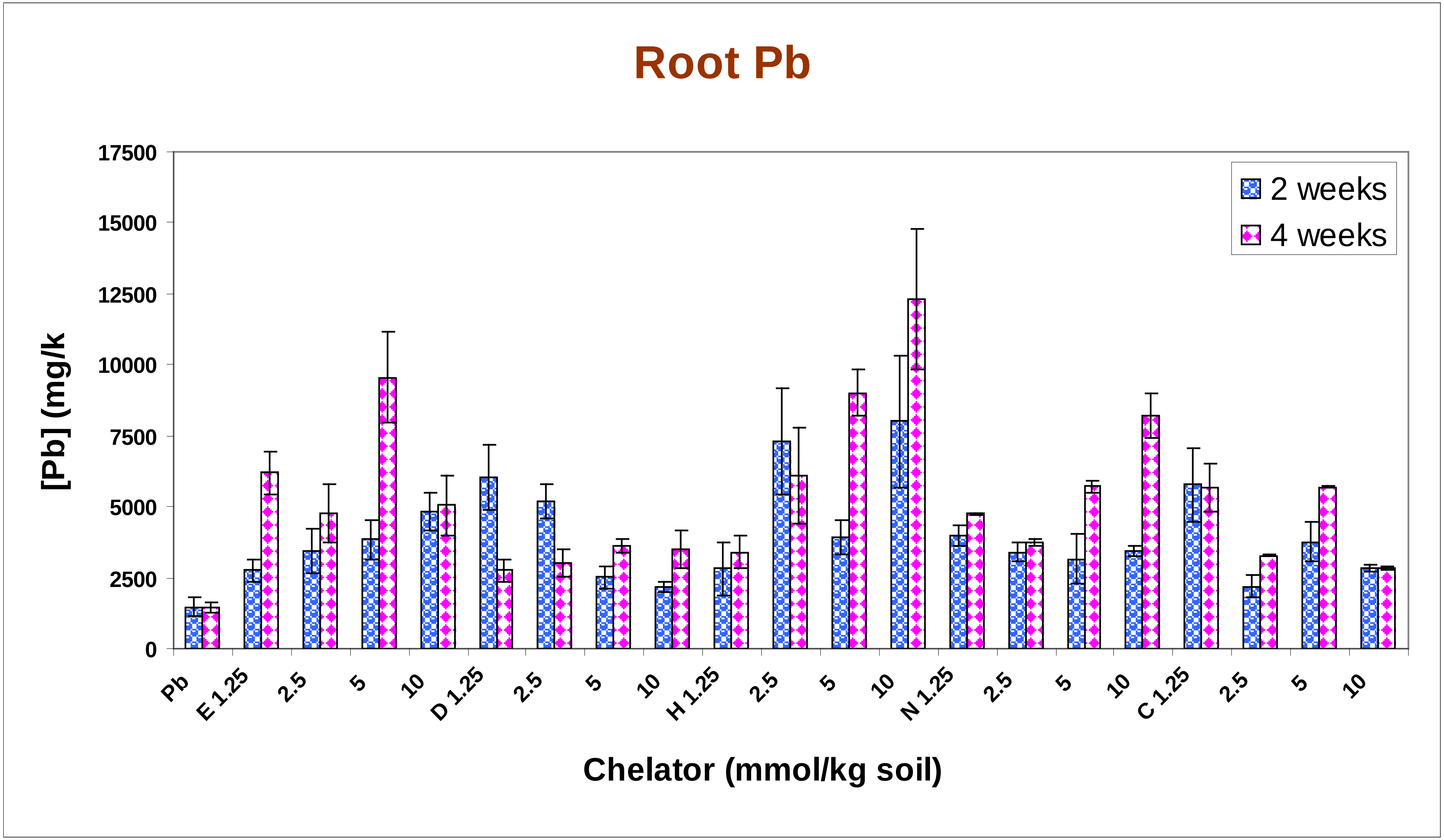
(EST 36, 4676-4680, 2002).

mg/kgdw Pb

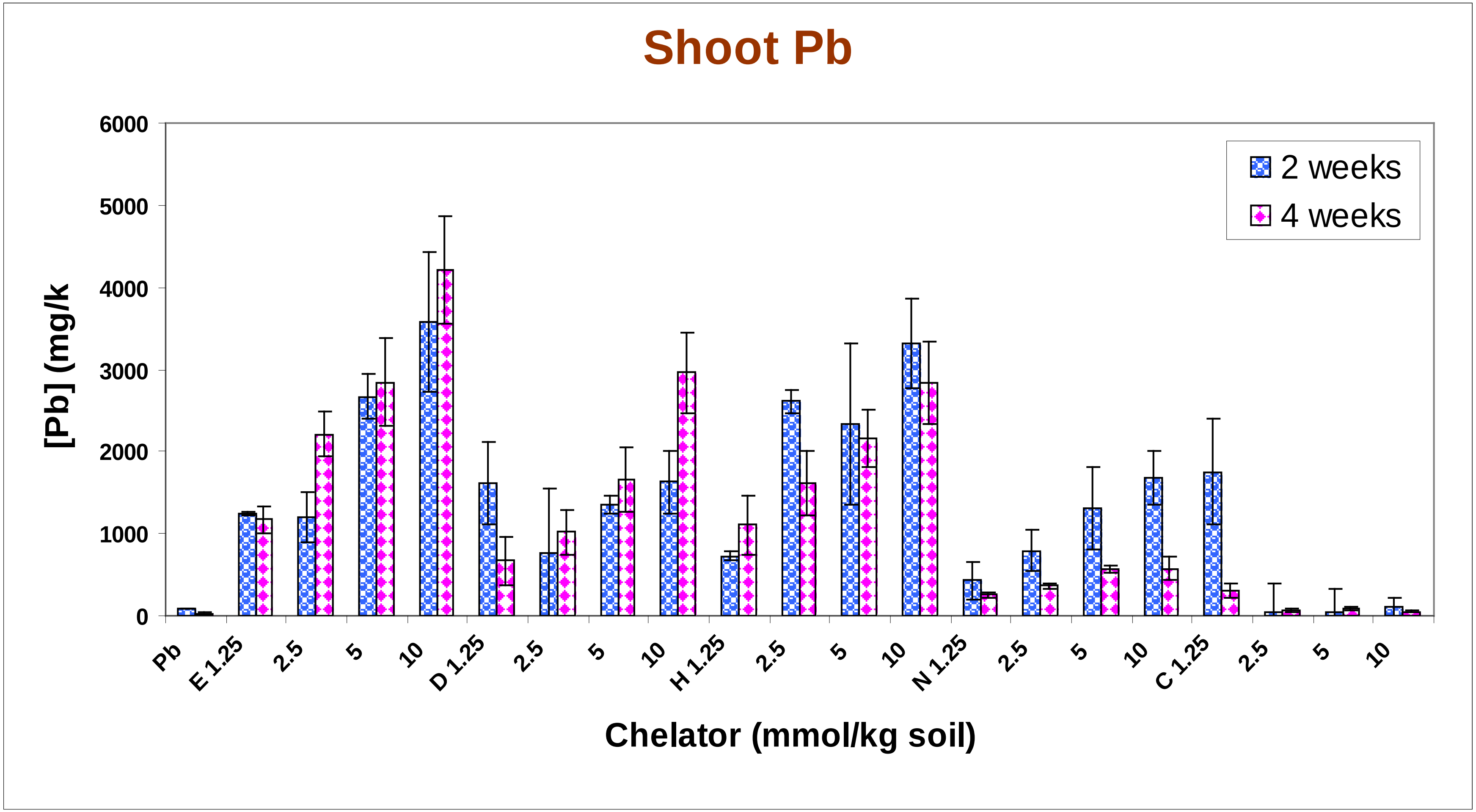


mg/L Pb

Sesbania in soil supplemented with Pb



Sesbania in soil supplemented with Pb



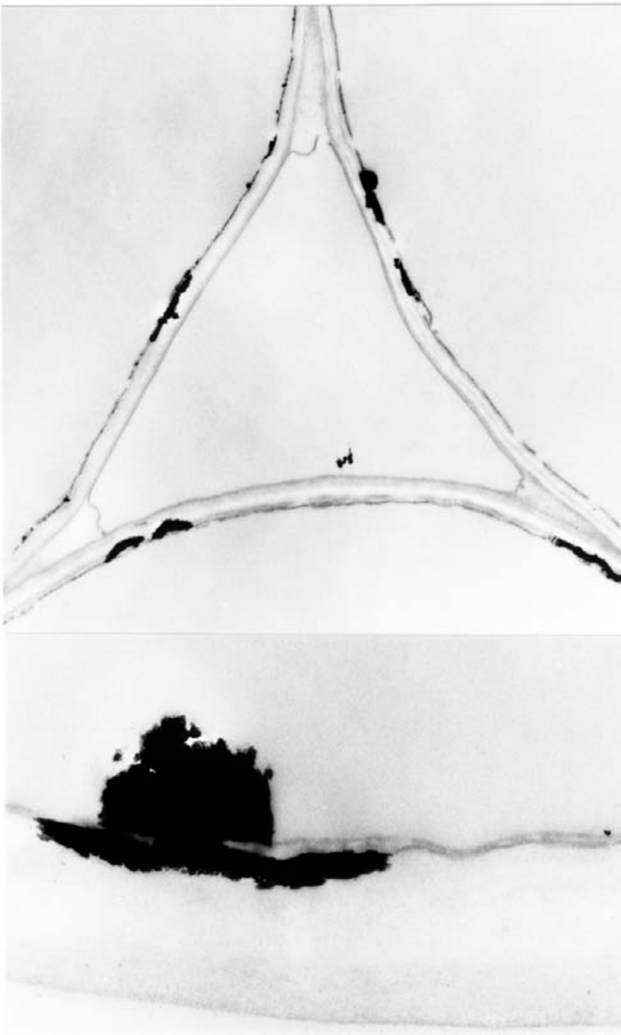
Estimated total Pb removed from soil by several plants

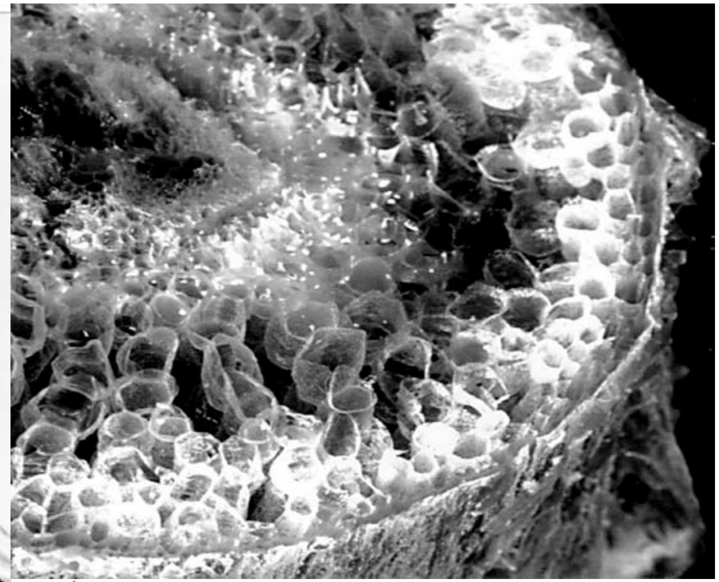
(Ruley 2004)

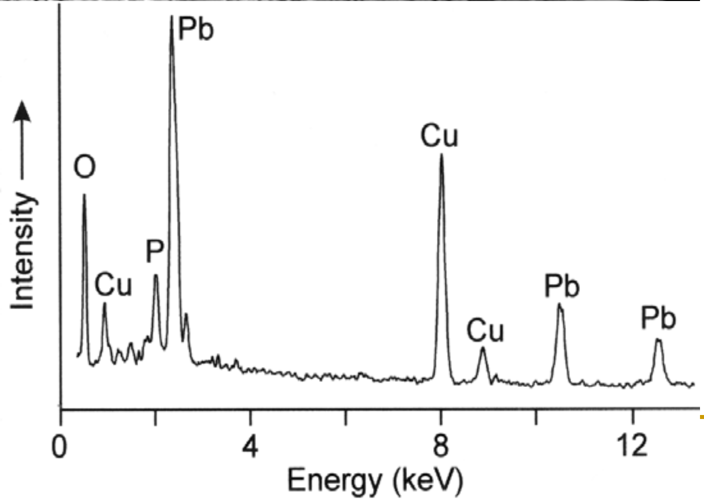
Species	Soil Amendments	Soil Pb (mg/kg)	Shoot Pb (%)	Biomass (t/ha/yr)	Est. total Pb extr. (kg/ha/yr)	Source
Zea mays	5.8 mmol/kg HEDTA	2500	1.06	5-6	53-64	Huang et al. 1997
Pisum sativum	1.34 g/kg EDTA	2450	0.897	3-4	27-36	Huang et al. 1997
Sesbania drummondii	10 mmol/kg EDTA 100 mg/kg EDTA + 10 mg/kg IAA	7500 500	0.42 0.6	10-15 10-15	43-63 60-90	Ruley et al. Unpublished
Brassica juncea	10 mmol/kg EDTA	600	1.6	1-1.5	16-24	Blaylock et al. 1997
Triticum aestivum	5 mmol/kg EDTA+5 mmol/kg acetic acid	2000	0.92	2.5	23	Begonia et al. 2002

EM of *Sesbania* root cells

(Sahi et al., ES & T 36, 4676-4680, 2002)







Pb Transport

- Transport of Pb via different cell types (SEM)
- Pb nanoparticles in intercellular spaces, cell membranes and cell walls

Biotransformation of Metals
(Using XAS Technology)

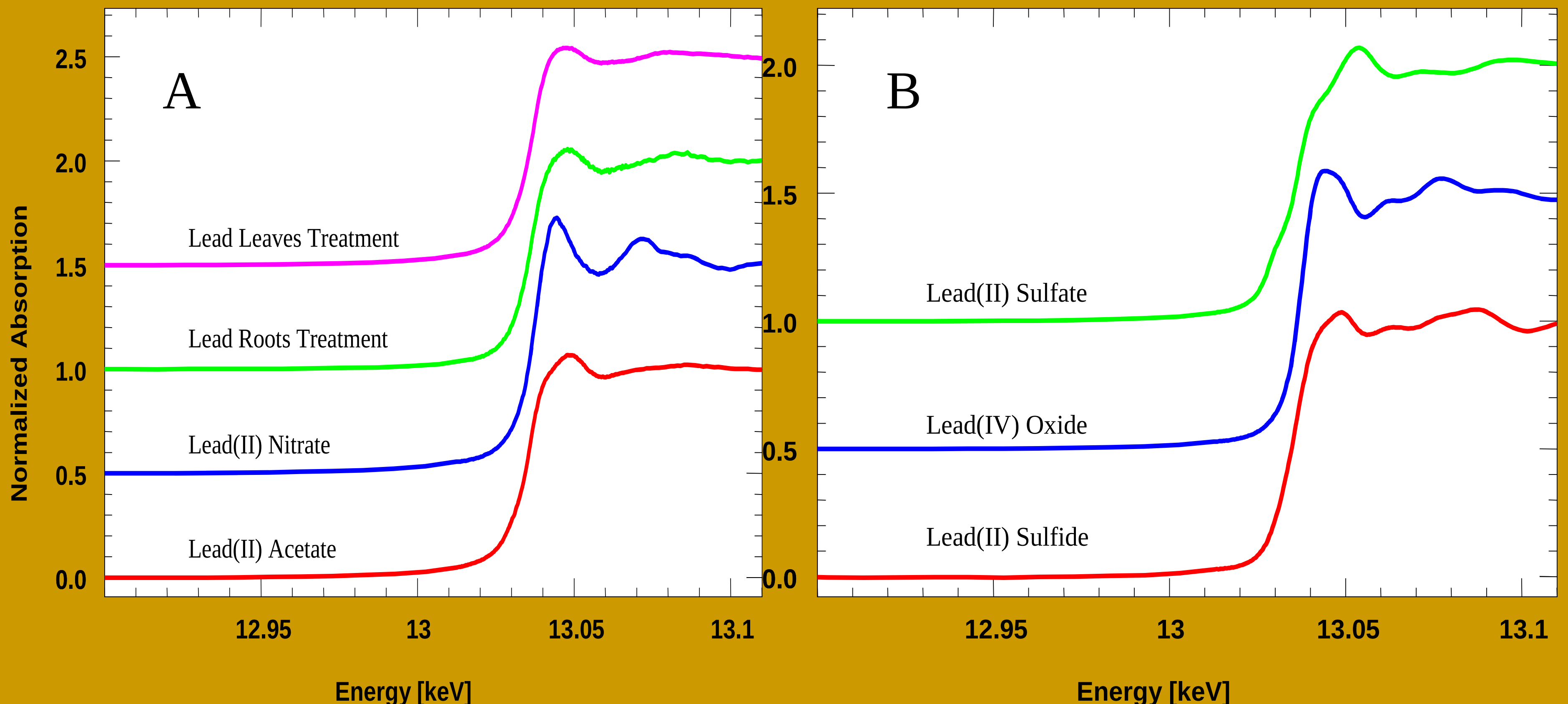
Types of XAS

- **XANES** (X-ray absorption near edge structure) – determines the oxidation state and atomic geometry of a bound metal.

- **EXAFS** (Extended X-ray absorption fine structure) – traces the ligand involved in metal binding by measuring the distance from X-ray-absorbing atom to next nearest atom.

XANES Spectra of *Sesbania*

(ET & C 23, 2068, 2004)



A) L_{III} XANES of Pb-laden plant samples, lead(II) nitrate, and lead(II) acetate. LIII XANES of lead model compounds lead(II) sulfide, lead(II) sulfate, and lead(IV) oxide. **B)**

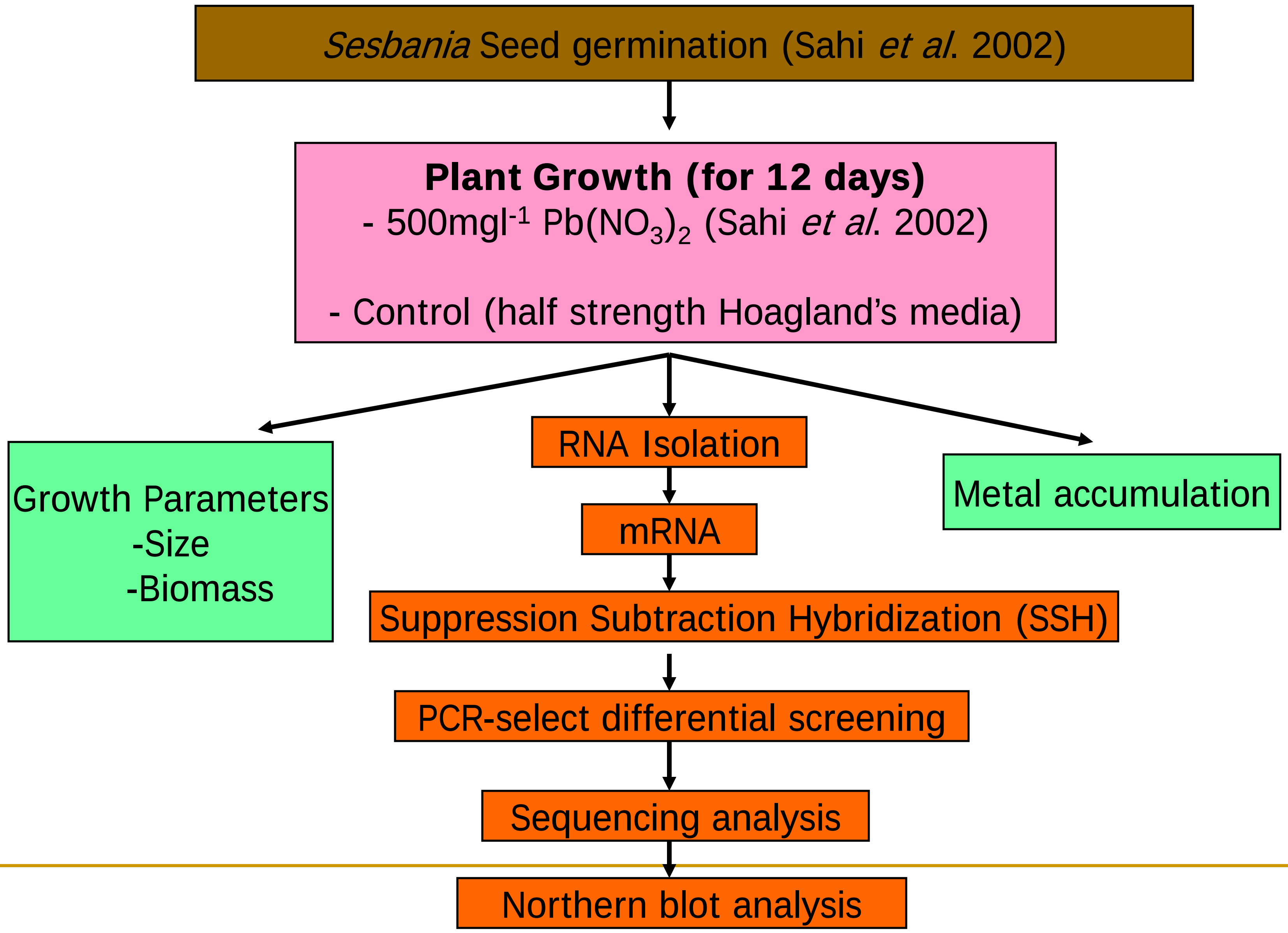
XANES and EXAFS data of Pb-treated *Sesbania*

(Environ. Toxicol. Chem. 23, 2068-2073, 2004)

Samples	$Pb(NO_3)_2$ %	$PbSO_4$ %	Pb metal %	PbS %	Pb acetate %
Leaves	7.6	25.8	0	14.2	52.4
Roots	10.1	0	8.8	20.2	60.9

Identification of lead responsive genes

Experimental Design



Suppression subtraction hybridization (SSH)

- Based on the technique called suppression PCR
- Compare two populations of mRNA
- Obtain clones of genes that are expressed in one population but not in the other

Sequencing results for Pb samples

- 63 clones corresponds to unigenes
- 49 (78 %) identified as segments of cDNAs contained in GenBank database
- 14 (22 %) were unknown (no similarity)
- Clone # 7 exhibited homology to

  type 2 metallothionein sequences

Clone	Accession number	Length (bp)	Homology[a]	E-value
SSH-1	DQ465754	183	*Acanthopanax sessiliflorus* cDNA library *Eleutherococcus sessiliflorus* cDNA, mRNA sequence (CF923918)	0.0
SSH-2	DQ465755	293	Apple_EST_Mdas Malus x domestica cDNA similar to dbj|BAB33421.1| putative senescence-associated protein [*Pisum sativum*], mRNA sequence (DR993778)	$1e^{-159}$
SSH-3	DQ465756	282	CabSau Flower Stage 12 (FLOu0012) *Vitis vinifera* cDNA clone VVI101F09 5, mRNA sequence (DT015551)	0.0
SSH-4	DQ465757	666	Cold stressed *Glycine clandestina* SSH cDNA clone Gc02 03a05, mRNA sequence (BG838800)	0.0
SSH-5	DQ465758	569	*Phaseolus vulgaris* seedling EST Library inoculated with anthracnose-PVEPSE3029E14 5', mRNA sequence(CB543340)	0.0
SSH-6	DQ465759	565	*Phaseolus vulgaris* seedling EST Library inoculated with anthracnose-cDNA clone PVEPSE3030N16 5', mRNA sequence (CB543682)	0.0
SSH-7	DQ465760	414	Type 2 Metallothionein-Cytochrome P450 like_TBP [*Citrullus lanatus*] (AB182926)	0.0
SSH-8	DQ465761	620	Cytochrome P450 like_TBP [*Nicotiana tabacum*] (BAA10929)	0.0
SSH-9	DQ465762	313	*Glycine max* cDNA clone Gm-c1086-27 5' similar to CYTOCHROME P450 LIKE_TBP mRNA sequence (BM091724)	$1e^{-174}$
SSH-10	DQ465763	366	*Glycine max* cDNA, mRNA sequence (BE660497)	0.0
SSH-11	DQ465764	478	*Glycine max* cDNA, mRNA sequence (BU927378)	0.0
SSH-12	DQ465765	692	*Glycine soja* cDNA clone SOYBEAN CLONE ID: Gm-c1056-3170 5', mRNA sequence (CA799399)	0.0
SSH-13	DQ465766	739	*Glycine max* cold stressed leaves cDNA clone Gm01 16d09, mRNA sequence (BG839363)	0.0
SSH-14	DQ465767	674	*Glycine max* cold stressed leaves cDNA clone Gm01 17a09, mRNA sequence (BG839403)	0.0
SSH-15	DQ465768	471	Gmax SC *Glycine max* cDNA, mRNA sequence (BE660497)	0.0
SSH-16	DQ465769	875	*Gossypium hirsutum* cDNA clone GH_CHX12C18 3', mRNA sequence (DT462491)	0.0
SSH-17	DQ465770	840	hemolysin [*Acanthamoeba polyphaga*] (AAA58585)	0.0
SSH-18	DQ465771	666	*Heterobasidion annosum* - Scots pine infection stage (HAGE) subtraction cDNA clone hage001aD09, mRNA (BQ789710)	$2e^{-85}$
SSH-19	DQ465772	295	Leafy spurge subtractive cDNA libraries *Euphorbia esula* cDNA clone RTP5O15 5', mRNA sequence (DT639472)	$1e^{-158}$
SSH-20	DQ465773	633	*Lotus japonicus* nodule library 5 and 7 week-old *Lotus corniculatus* var. japonicus cDNA 5', mRNA sequence (AW720640)	0.0
SSH-21	DQ465774	299	*Medicago truncatula* cDNA clone MtTA01F19S6, mRNA sequence (AJ847433)	$1e^{-149}$
SSH-22	DQ465775	341	*Medicago truncatula* cDNA clone MtTA09L24S6, mRNA sequence (AJ847823)	$1e^{-177}$
SSH-23	DQ465776	522	Methyl Jasmonate-Elicited mRNA sequence from Root Cell Suspension Culture *Medicago truncatula* (CX533136)	0.0
SSH-24	DQ465777	137	*Mimulus guttatus* cDNA clone 0048P0008Z, mRNA sequence (CV515336)	$9e^{-45}$
SSH-25	DQ465778	314	*Phaseolus vulgaris* leaf EST library cDNA clone PV_GEa0013C_C03.b1 5', mRNA sequence (CV530371)	$1e^{-165}$
SSH-26	DQ465779	628	*Phaseolus vulgaris* leaf EST library cDNA clone PV_GEa0015C_G10.b1 5', mRNA sequence (CV531021)	0.0
SSH-27	DQ465780	229	*Populus trichocarpa* cDNA clone WS02553_I06 3', mRNA sequence (DT493138)	$1e^{-127}$
SSH-28	DQ465781	899	Potato abiotic stress cDNA library *Solanum tuberosum* cDNA clone POAD792 5' end, mRNA sequence (CK272883)	0.0
SSH-29	DQ465782	900	Water stressed gmrtDrNS01 32 Glycine max cDNA 3', mRNA sequence (CX711410)	0.0

SSH-30	DQ465783	565	Probable cytochrome P450 monooxygenase - maize (fragment) (T02955)	0.0
SSH-31	DQ465784	666	Putative ACC synthase/oxidase gene (BAB33421)	0.0
SSH-32	DQ465785	255	rRNA promoter binding protein [*Rattus norvegicus*] (NM147136)	$1e^{-143}$
SSH-33	DQ465786	657	*Sesbania rostrata* root primordia cDNA clone SSH-10, mRNA sequence (AJ301742)	0.0
SSH-34	DQ465787	162	Subtracted cDNA library of maize inbred line H95-Rp1-Kr1N *Zea mays* cDNA clone Kr1N-4_D09, mRNA sequence (CA452627)	$1e^{-26}$
SSH-35	DQ465788	531	Unknown protein (*Schistosoma japonicum*) (AAX30301)	0.0
SSH-36	DQ465789	889	Water stressed gmrtDrNS01_28 Glycine max cDNA 3', mRNA sequence (CX711160)	0.0
SSH-37	DQ465790	874	Water stressed gmrtDrNS01_30 Glycine max cDNA 3', mRNA sequence (CX548993)	0.0
SSH-38	DQ465791	446	Water stressed gmrtDrNS01_31 Glycine max cDNA 3', mRNA sequence (CX707998)	0.0
SSH-39	DQ465792	289	Unnamed protein product [Kluyveromyces lactis NRRL Y-1140] (CAH00932)	$5e^{-91}$
SSH-40	DQ465793	648	CYTOCHROME P450 monooxygenase (EC 1.14.14.1) - common tobacco (	0.0
SSH-41	DQ465794	881	Hypothetical protein [*Oryza sativa* (japonica cultivar-group)] (BAD46202)	0.0
SSH-42	DQ465795	357	26S ribosomal protein	0.0
SSH-43	DQ465796	288	Hypothetical protein GLP_748_1200_211 [*Giardia lamblia* ATCC 50803] (XP767406)	$1e^{-137}$
SSH-44	DQ465797	371	Hypothetical protein UM05244.1 [Ustilago maydis521] (XP761391)	$1e^{-162}$
SSH-45	DQ465798	293	Unknown protein	$1e^{-173}$
SSH-46	DQ465799	286	Unknown protein	$1e^{-165}$
SSH-47	DQ465800	330	Unknown protein	$1e^{-162}$
SSH-48	DQ465801	285	Unknown protein	$1e^{-153}$
SSH-49	DQ465802	292	Unknown protein	$1e^{-167}$
SSH-50	DQ465803	178	No homology[b]	---
SSH-51	DQ465804	499	No homology	---
SSH-52	DQ465805	404	No homology	---
SSH-53	DQ465806	472	No homology	---
SSH-54	DQ465807	478	No homology	---
SSH-55	DQ465808	561	No homology	---
SSH-56	DQ465809	578	No homology	---
SSH-57	DQ465810	646	No homology	---
SSH-58	DQ465811	377	No homology	---
SSH-59	DQ465812	547	No homology	---
SSH-60	DQ465813	293	No homology	---
SSH-61	DQ465814	352	No homology	---
SSH-62	DQ465815	368	No homology	---
SSH-63	DQ465816	630	No homology	---

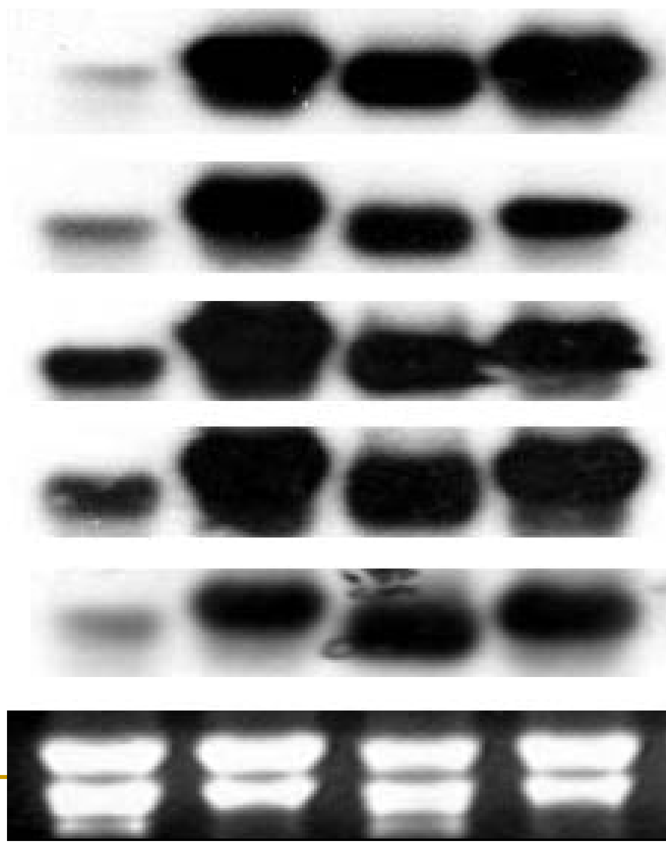
bNo significant sequence homology found in genome, EST, and protein database.

Northern blot analysis (Pb)

(Srivastava et al. Planta 2007)

Control **Pb treated**

Shoot Root Shoot Root



Water-stress induced gene (Clone # 29, 36, 37, 38)

Cold stress-induced gene (Clone # 4, 13, 14)

ACC synthase/oxidase gene (Clone # 31)

Abiotic stress-induced gene (Clone # 28)

Metallothionein gene (Clone # 7)

EtBr stained RNA

Conclusion

- Phytoremediation is a slow process
- *Sesbania* is effective for sites with shallow contaminated soils.
- Lead accumulated in form of nanoparticles.
- *Sesbania* transforms toxic compounds
- A type II metallothionein gene identified - may be involved in heavy metal detoxification
- Interdisciplinary approach

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Thank you

Prerequisites for Phytoremediation

Hyperaccumulators

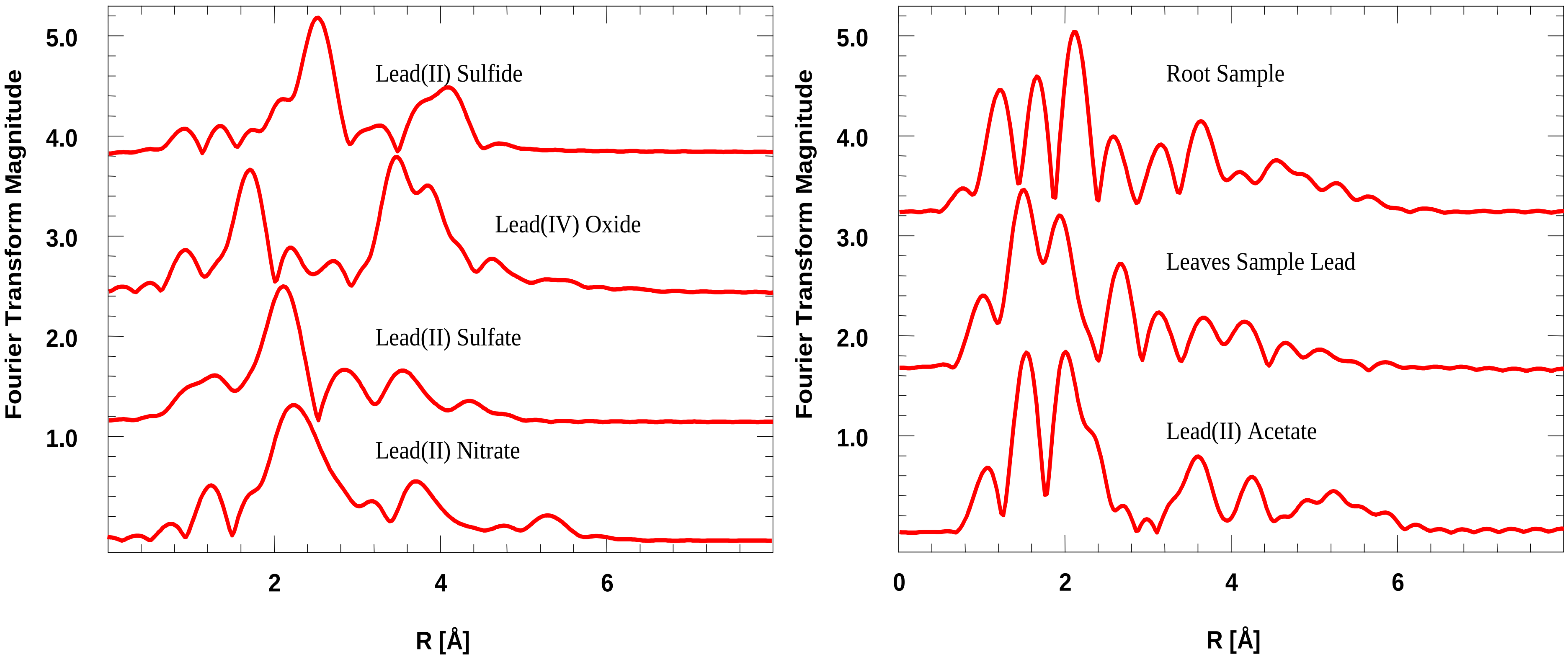
- Accumulate 100 times more metals than the non-accumulators
  - Conc. Criterion (% Shoot DW)

    Cd (>0.01), Co, Cu, Cr and Pb (>0.1),

    Ni and Zn (>1), Hg (0.001)

- Should have good biomass

EXAFS Spectra of *Sesbania*



Phytoremediation approaches

1. **Phytoextraction**: to remove contaminants directly from soil/water
2. **Phytostabilization**: use of vegetation and soil amendments to reduce contaminant availability and movement.
3. **Rhizofiltration**: plant root system is directed to extract pollutants from water bodies
4. **Phytomining**: for extraction and concentration of valuable metals

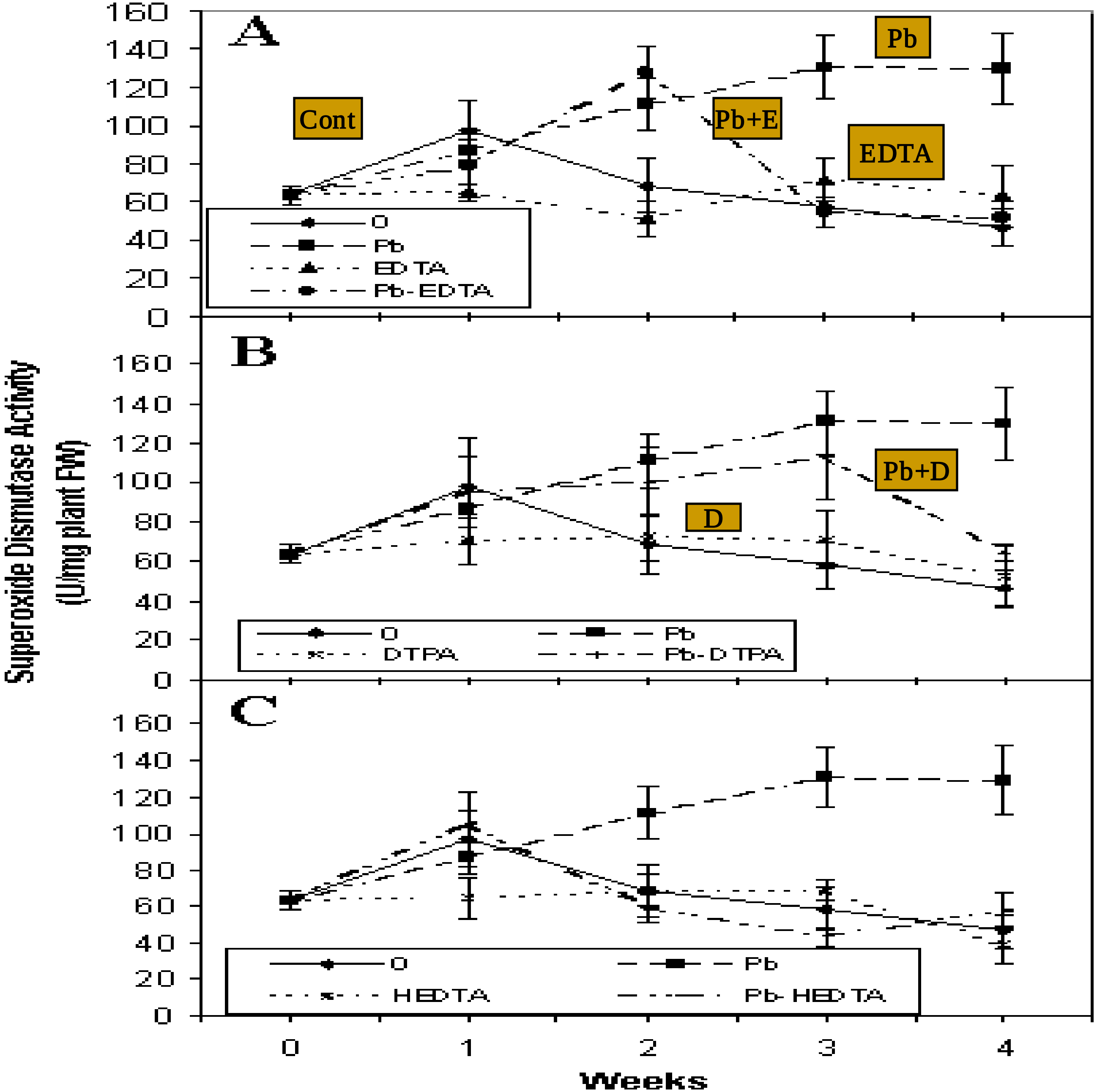
Antioxidant Reactions & metal Stress in *Sesbania*

- Generally metal exposure triggers an increase in activity of antioxidant enzymes.
  - **Superoxide dismutase (SOD) –** catalyze dismutation of superoxide radicals to hydrogen peroxide & oxygen
  - **Catalase (CAT)** – catalyzes decomposition of hydrogen peroxide to water and oxygen
  - **Ascorbate deroxidase (APX)** – detoxifies hydrogen peroxide to water using ascorbate as substrate
  - **Glutathione reductase (GR)** – reduces oxidized glutathione (GSSG) to reduced glutathione (GHS)

    - maintains high GHS/GSSH to sustain role of GHS as anti-oxidant
    - also incorporating into phytochelatins
    - GSH also function as free radical scavenger

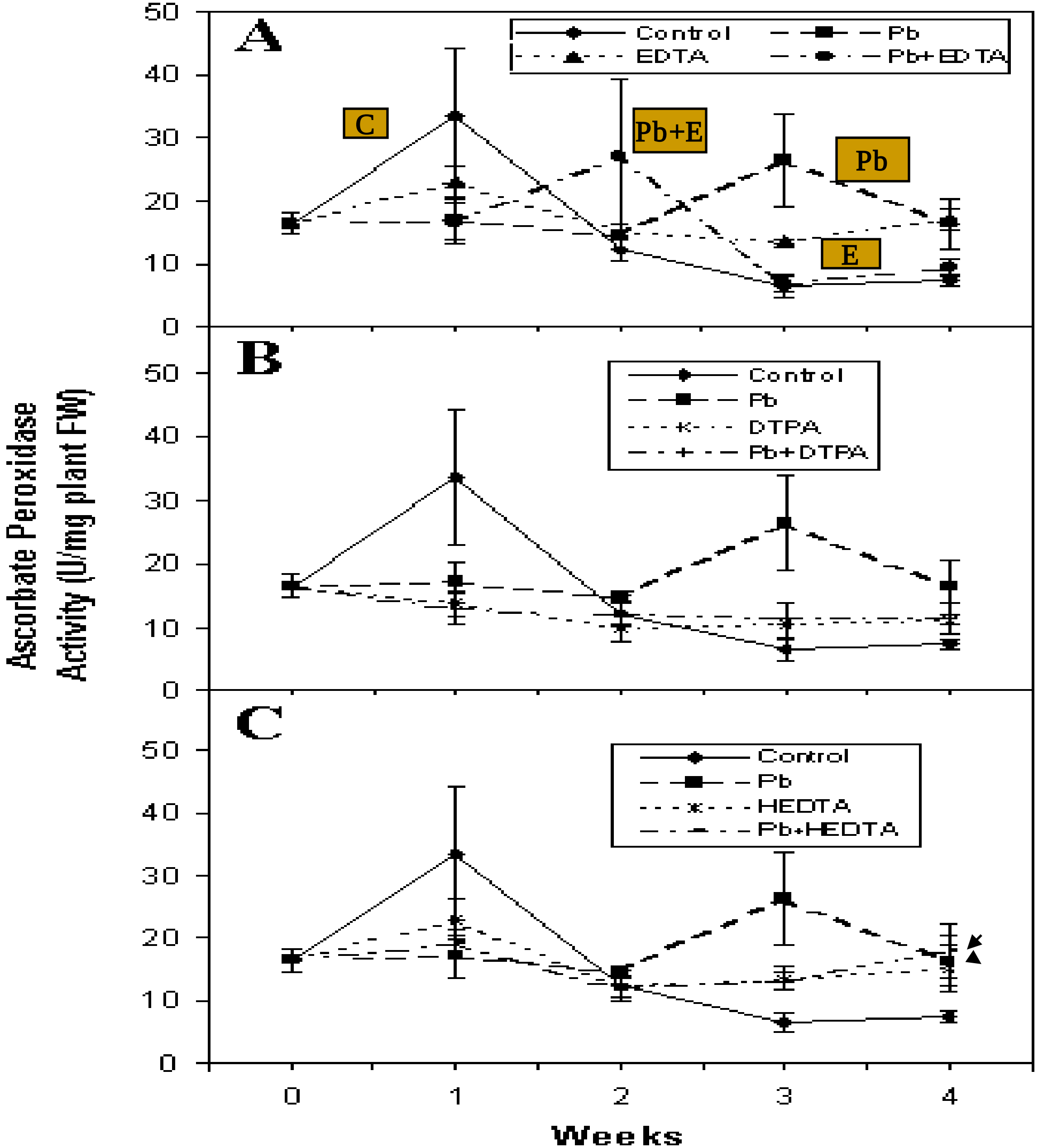
Superoxide dismutase

(SOD)



Ascorbate Peroxidase

(APX)



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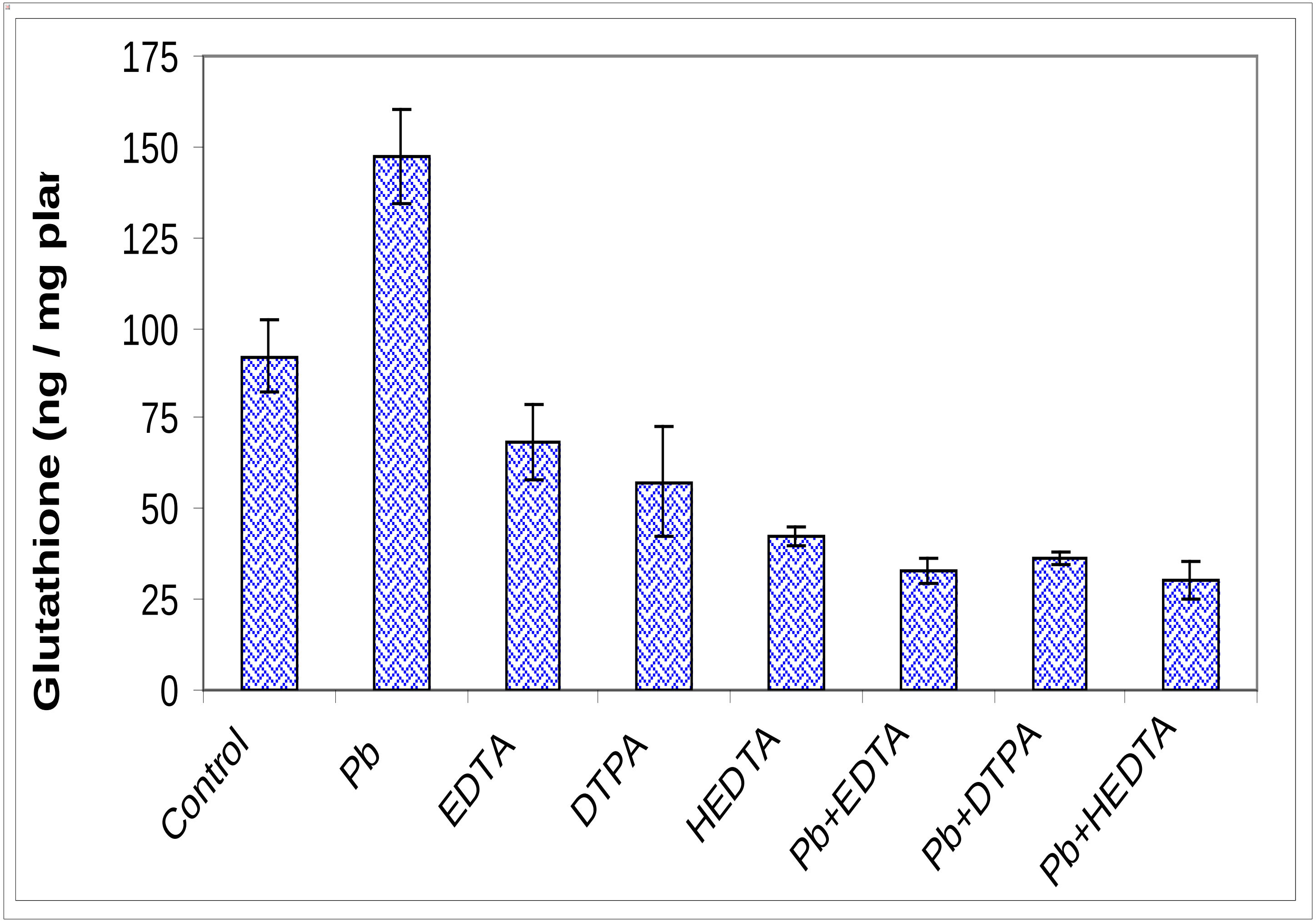
Glutathione content



Antioxidant Reactions and Pb Stress in *Sesbania drummondii*

(Plant Physiol. Biochem. 42, 899-906, 2004)

- Generally Pb exposure triggers an increase in activity of antioxidant enzymes.
- Significant Increased activity of these enzymes not observed in *S. drummondii* up to 1,000 mg/L $Pb(NO_3)_2$.
- Either *Sesbania drummondii* does not experience stress at these levels of Pb treatment, or antioxidant enzyme activities are not an indicator of stress in this plant.