Phytoremediation, a novel strategy for the removal of toxic metals from the environment: biochemical and molecular mechanisms

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Outline

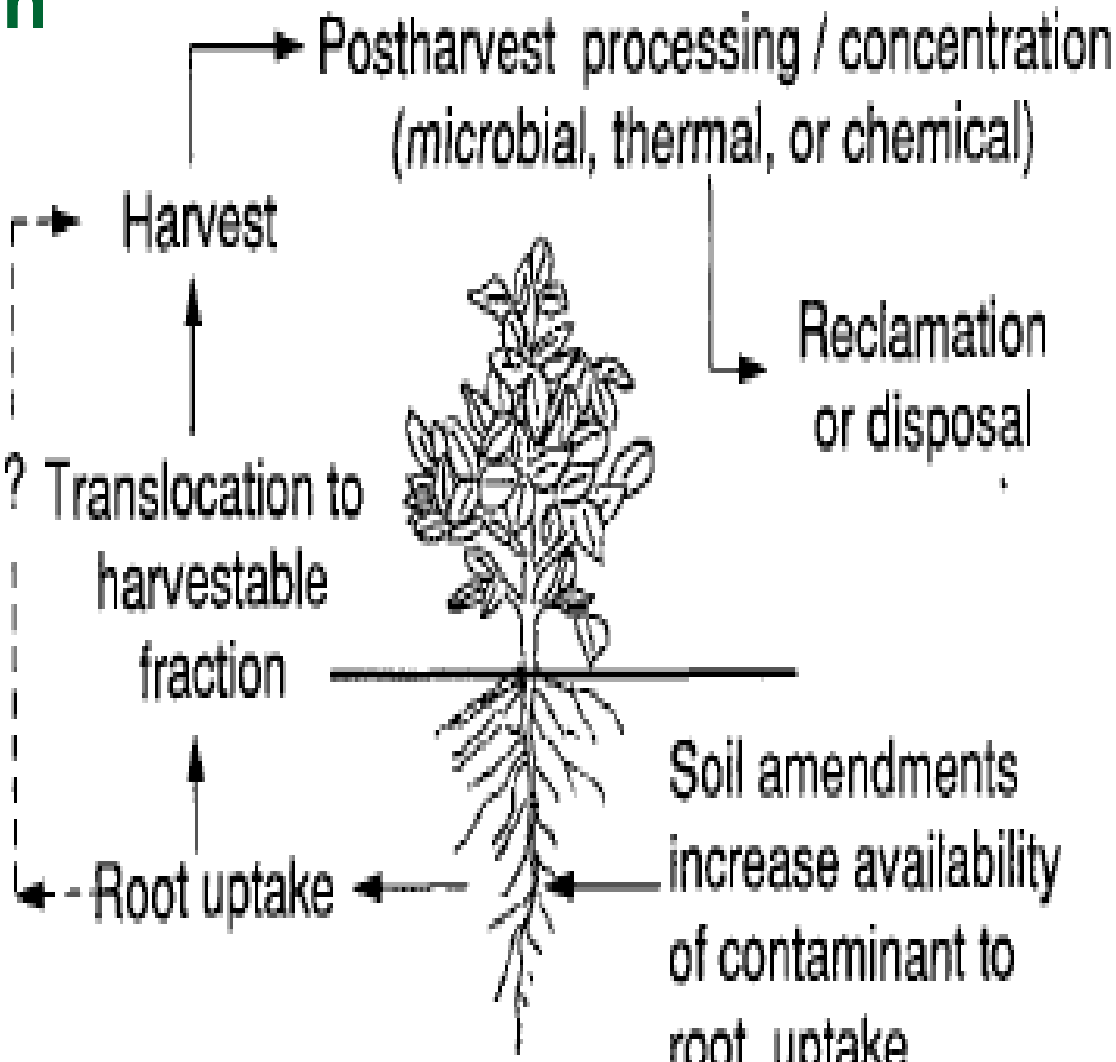
- Introduction of phytoremediation
- *Sesbania drummondii*
  - Metal uptake
  - Microscopic evidence of metal transport
  - Biotransformation of metal compounds
  - Stress enzymes
  - Gene identification/expression
  - Long term goal
  - Conclusion

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

- **Sources of pollution:**
  - Mining and smelting, municipal wastes, sewage sludge, landfill leachates, fertilizers, pesticides, nuclear accidents

- **Dimension of the problem:**
  - 1980 Statute recognized over 40,000 Superfund sites endangering human health
  - >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

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

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

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% (Nature,409,2001)

Aquatic Hyperaccumulators

The water hyacinth (*Eichhornia crassipes*)

Rate of removal of heavy metals from aqueous phase		
Element	mg/g DW biomass/day	g/ha/day
Cd	0.67	400
Co	0.57	340
Pb	0.18	90
Hg	0.15	110
Ni	0.50	300
Ag	0.44	260

Gold Hill Mesa Corp. (CO Springs) - water hyacinth for removal of Au from Au tailings.

Sesbania drummondii

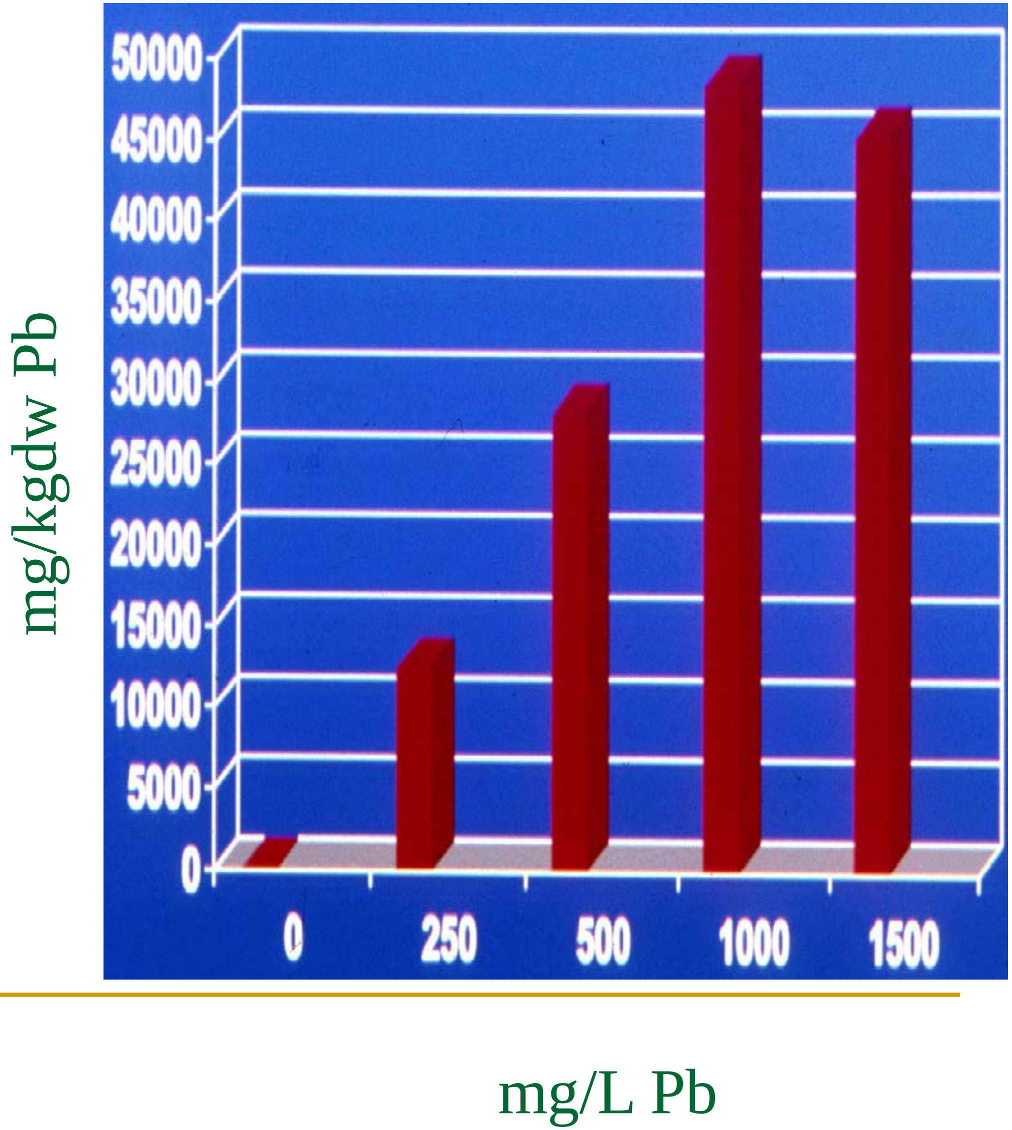
- A high biomass plant
- Common name: Rattlebox
- Native to Southeastern U.S.

Sesbania drummondii & Lead

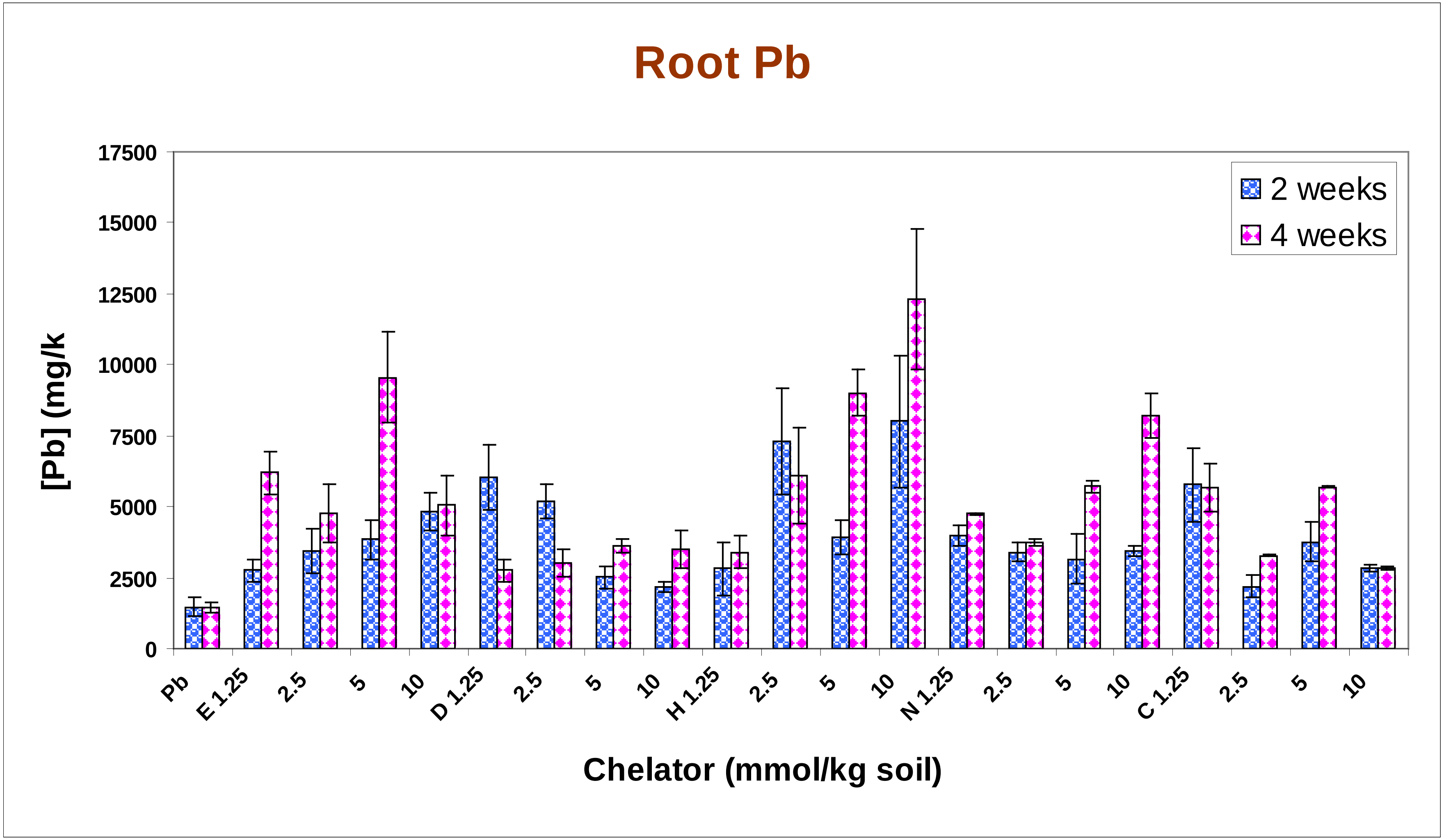
Demonstrated as lead hyperaccumulator

- 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

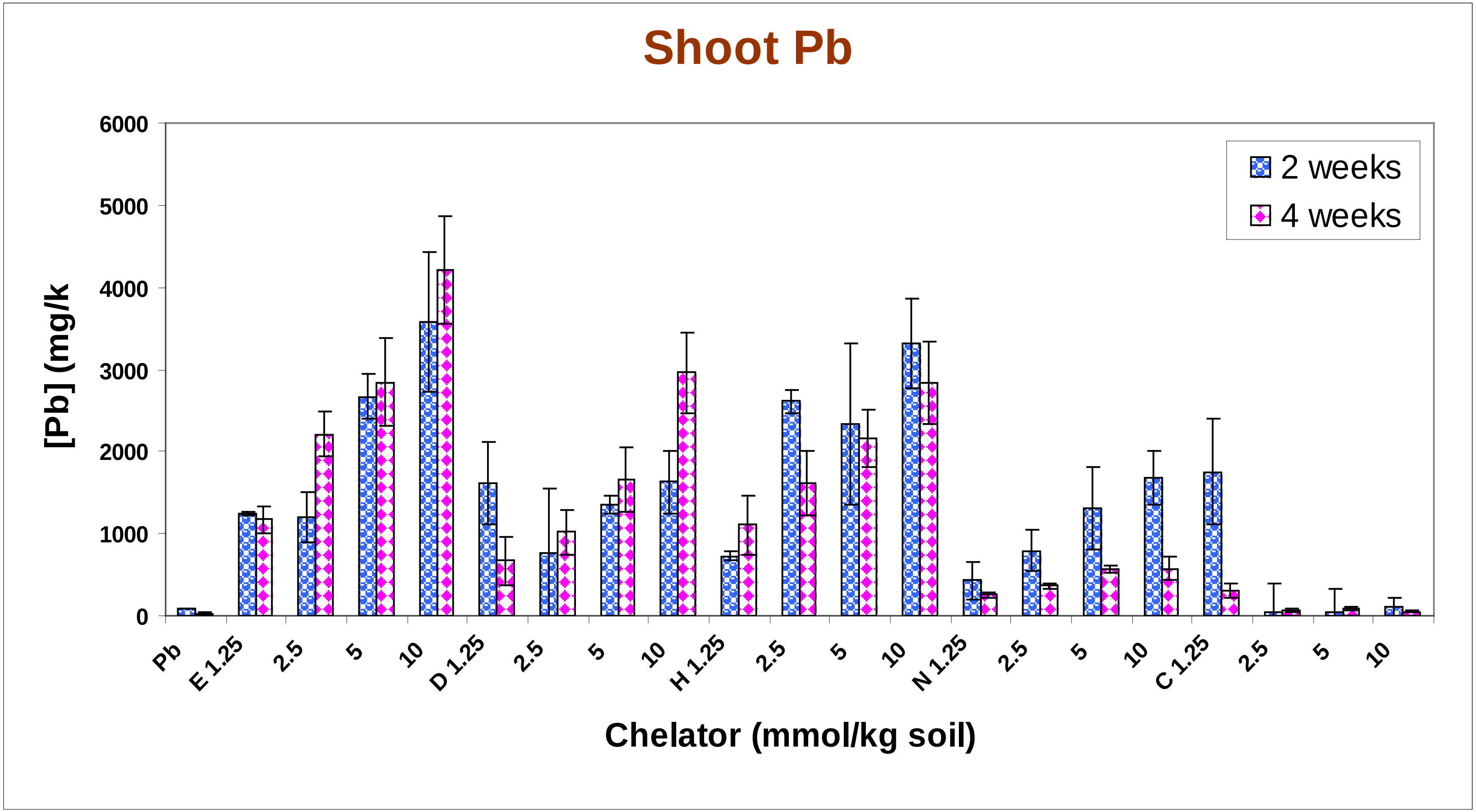
(EST 36, 4676-4680, 2002).



Sesbania in soil supplemented with Pb



Sesbania in soil supplemented with Pb

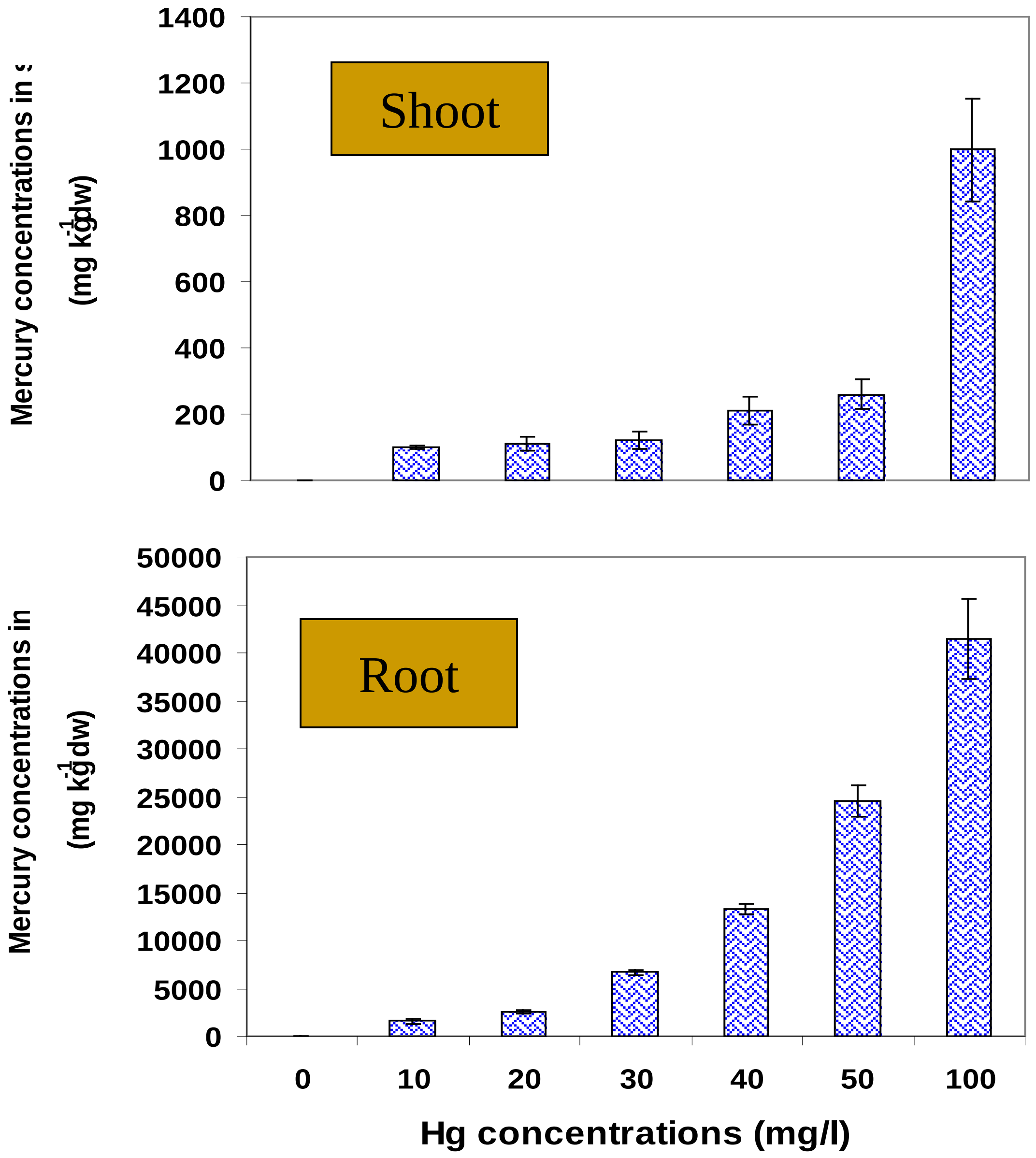


Estimated total Pb removed from soil by several plants

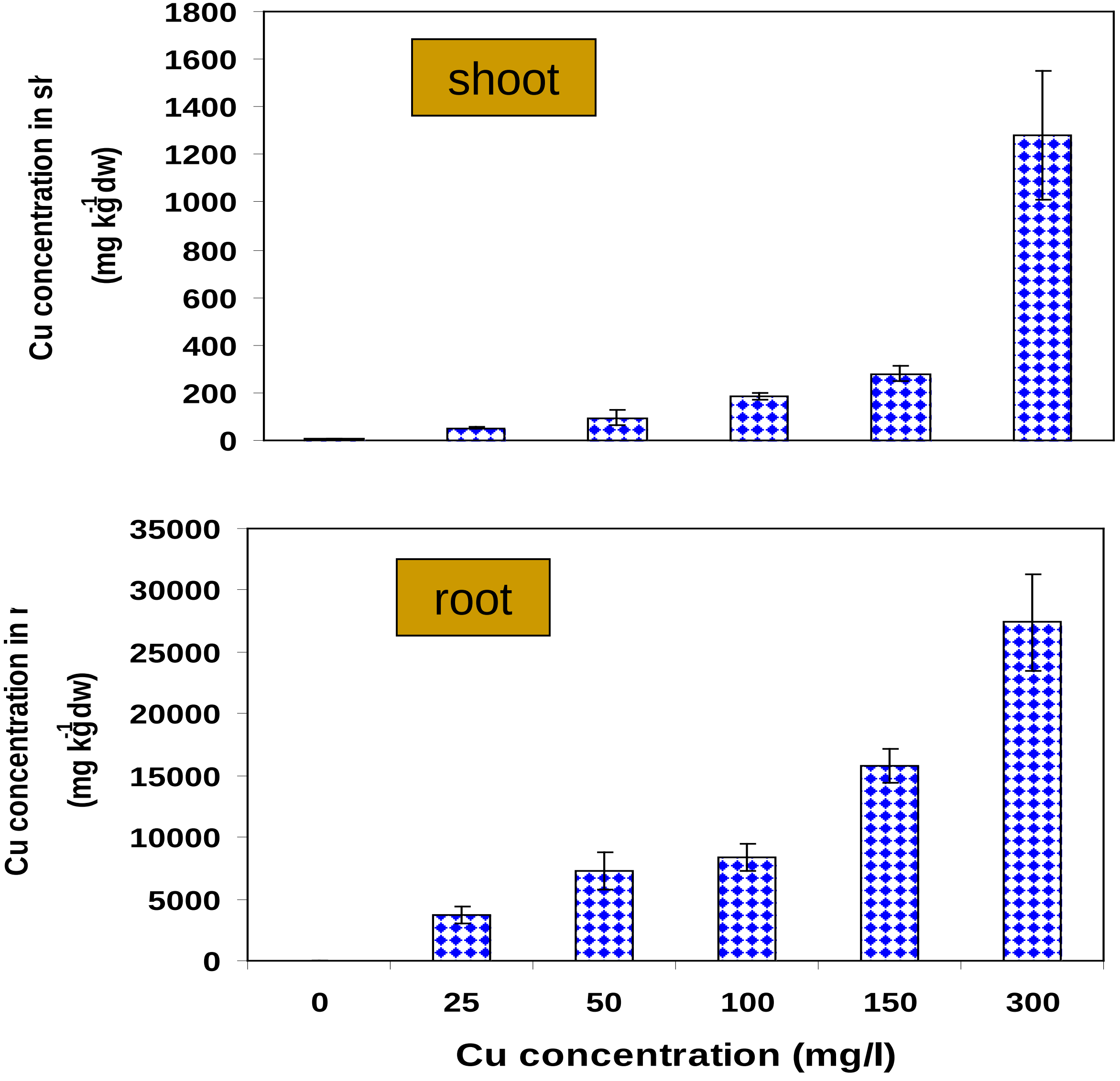
(Ruley 2004)

Species	**Chelators**	**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	7500	0.42	10-15	43-63	
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

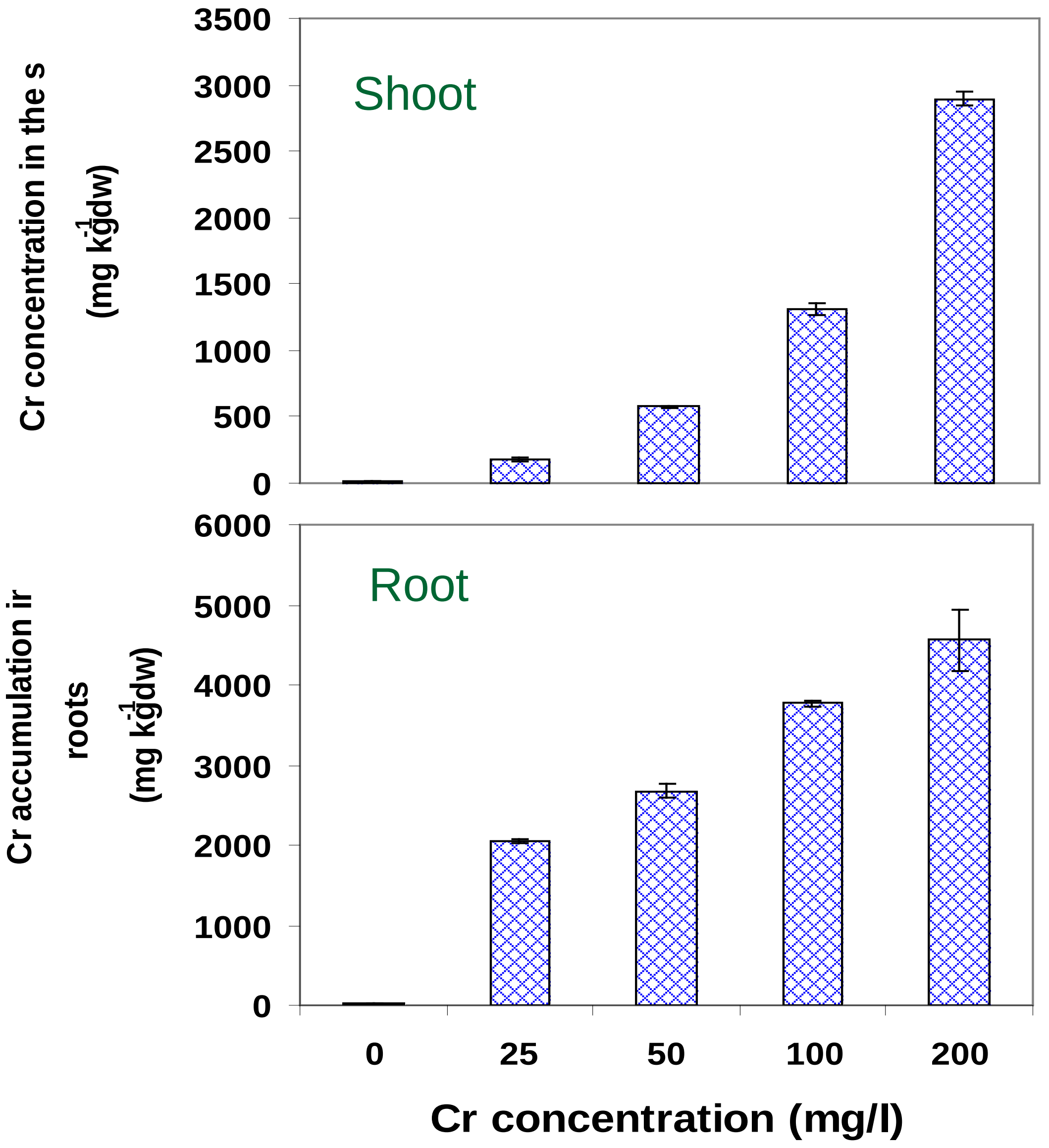
Mercury uptake by *Sesbania*



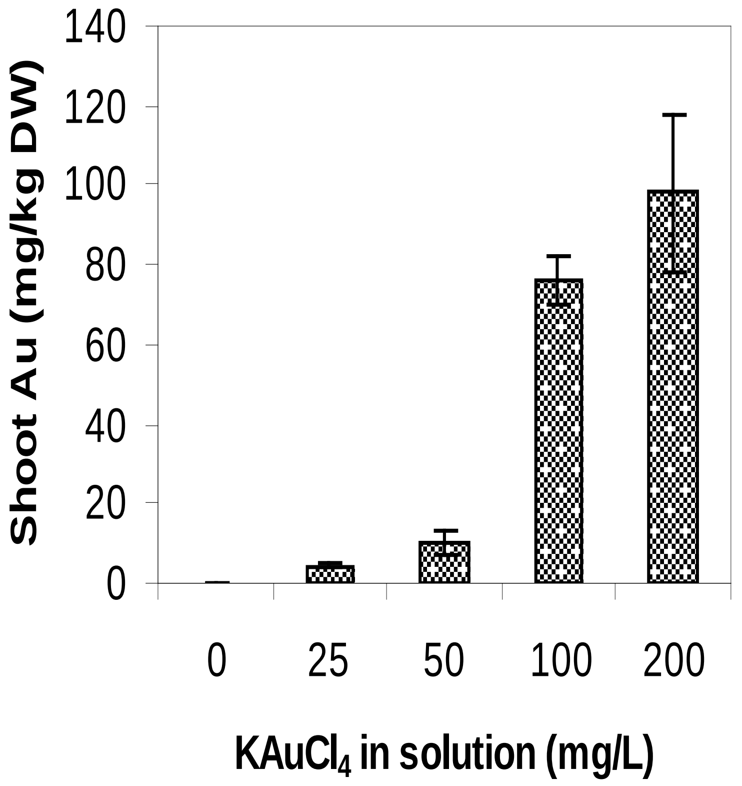
Copper uptake By *Sesbania*

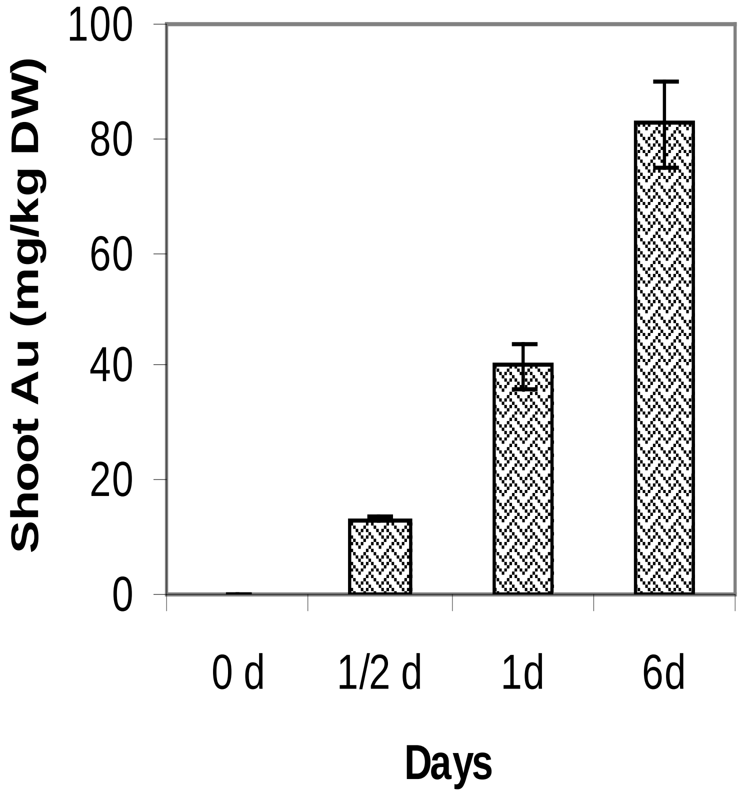


Chromium uptake By *Sesbania*

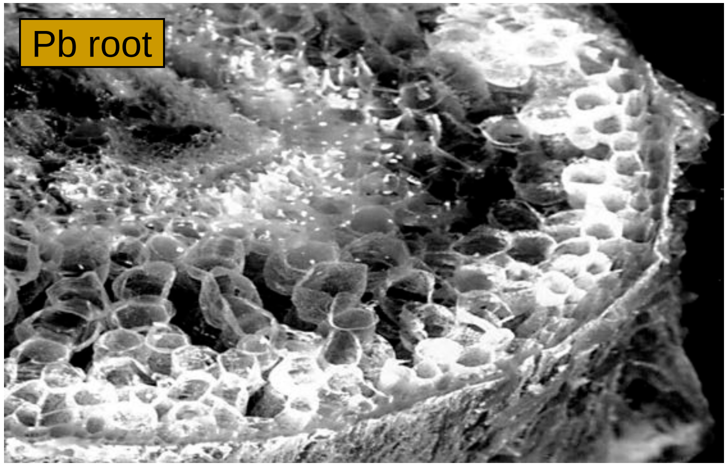


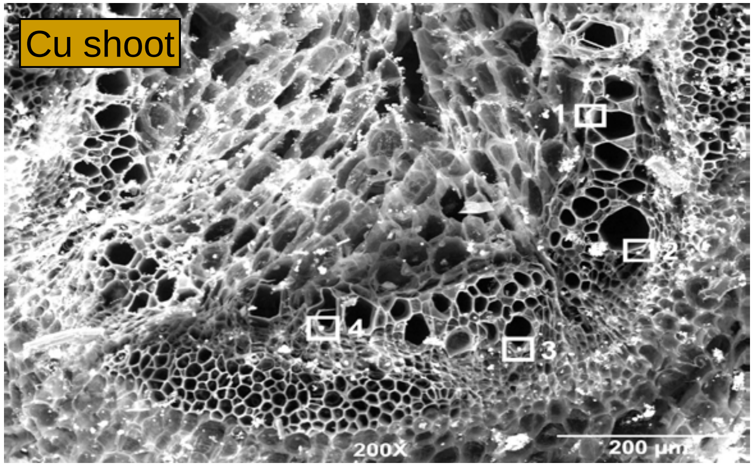
Gold uptake by *Sesbania*

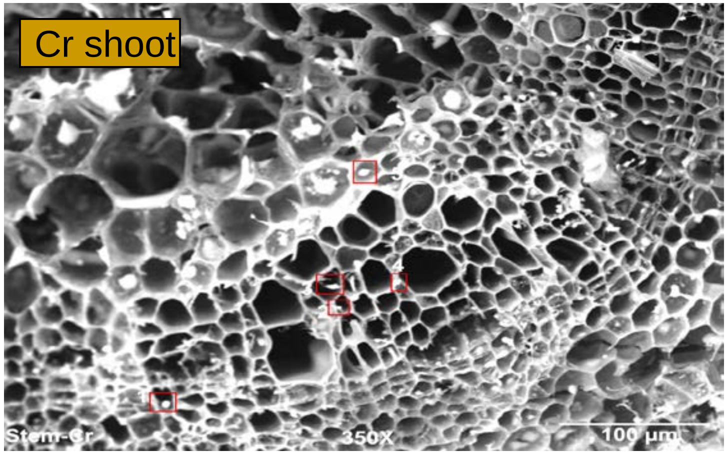


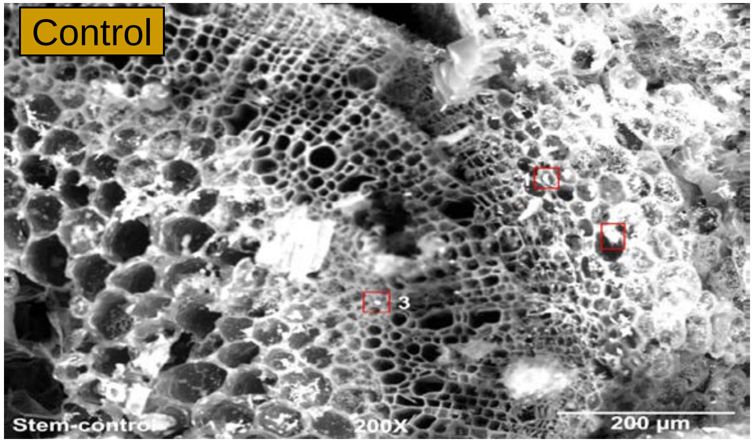


Scanning Electron Microscopy of Plant shoot grown in metals

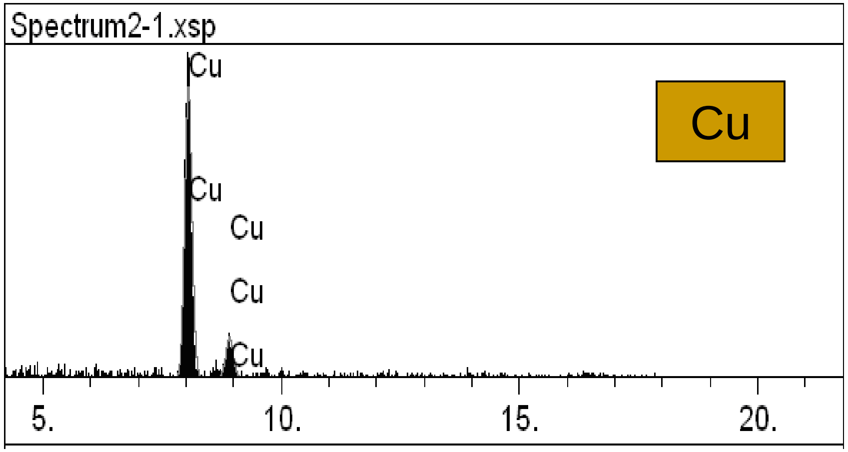

Pb root

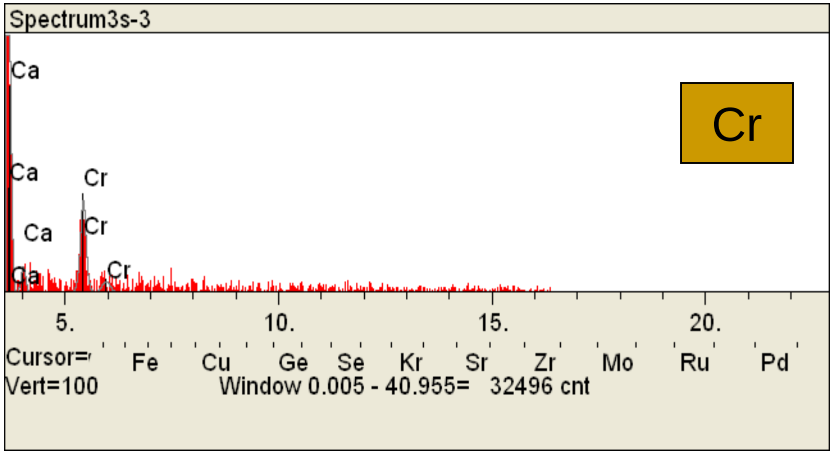

Cu shoot

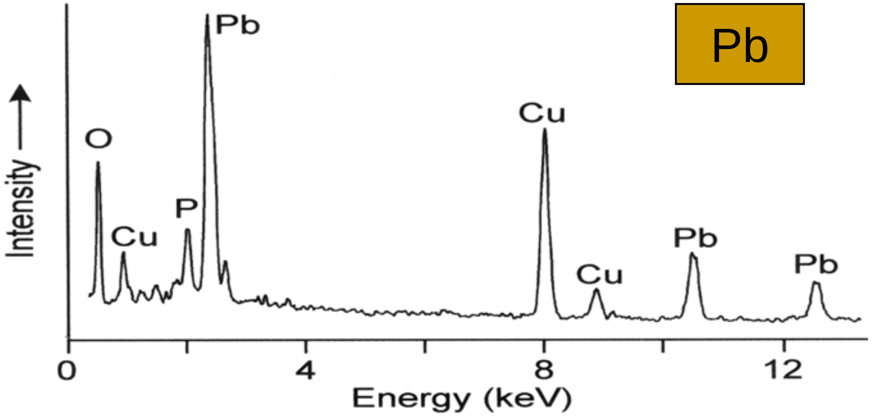

Cr shoot

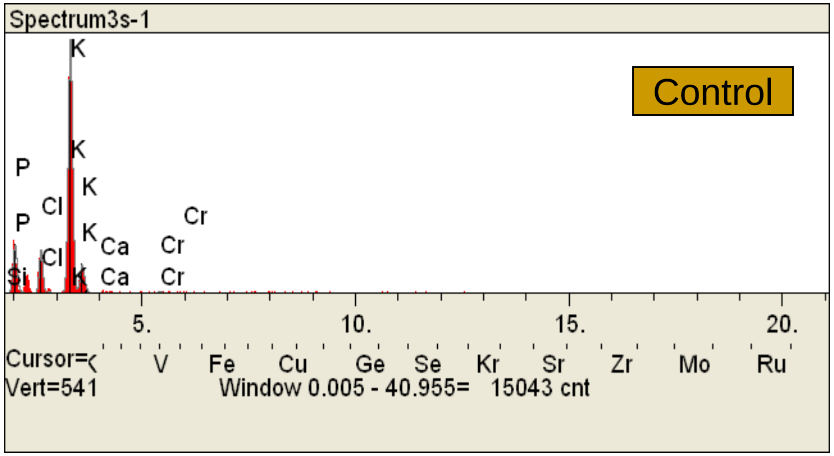

Control

X-ray microanalysis (EDS) of *Sesbania* tissue

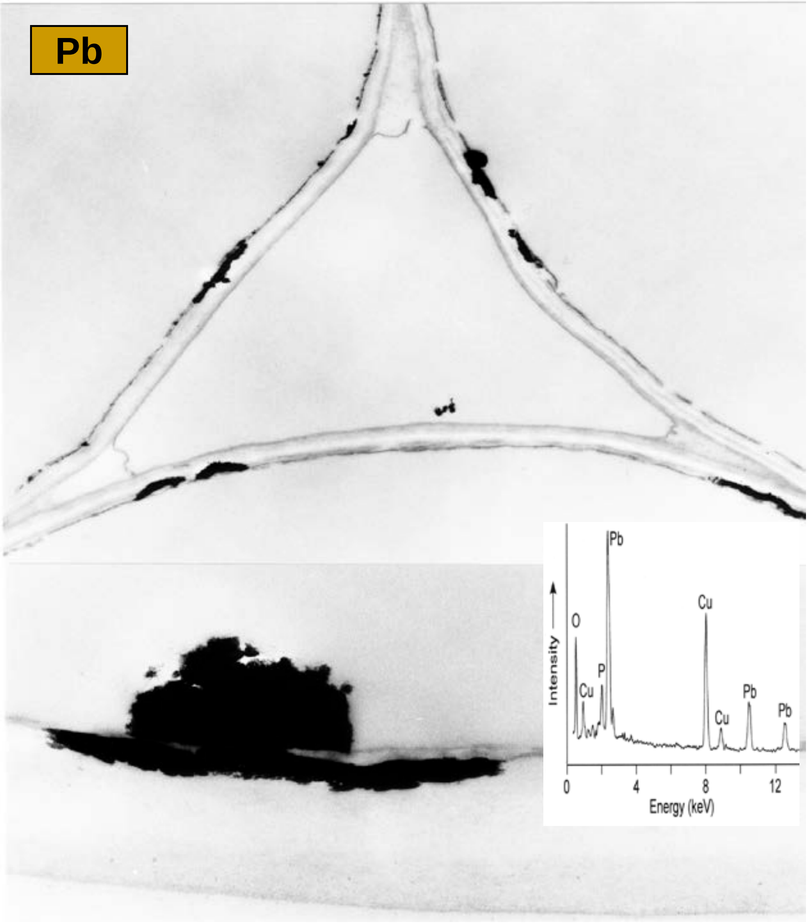


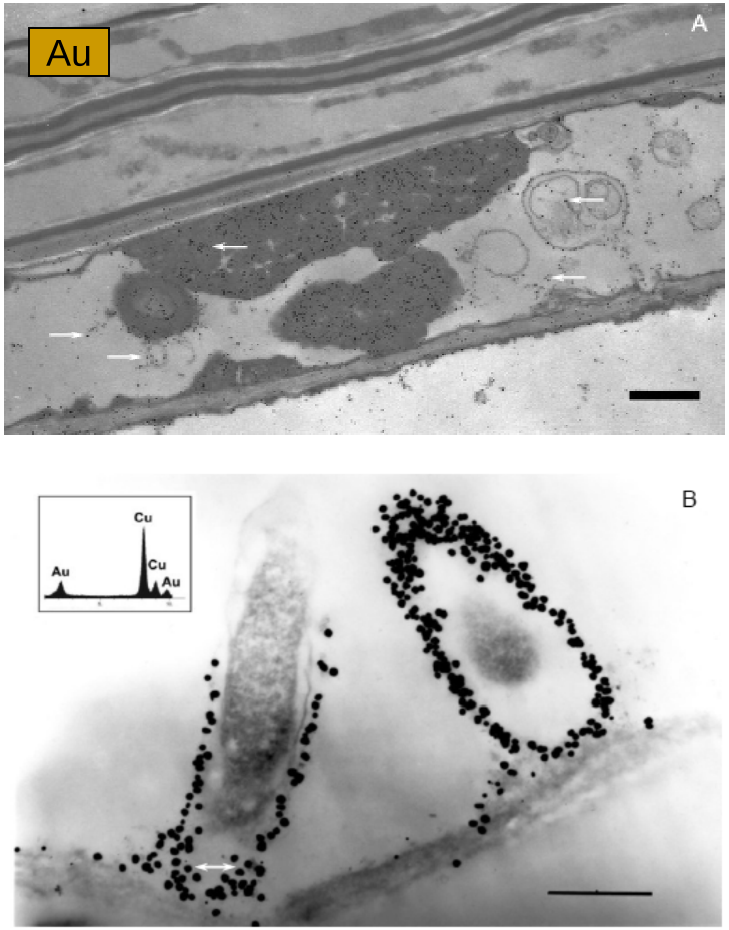




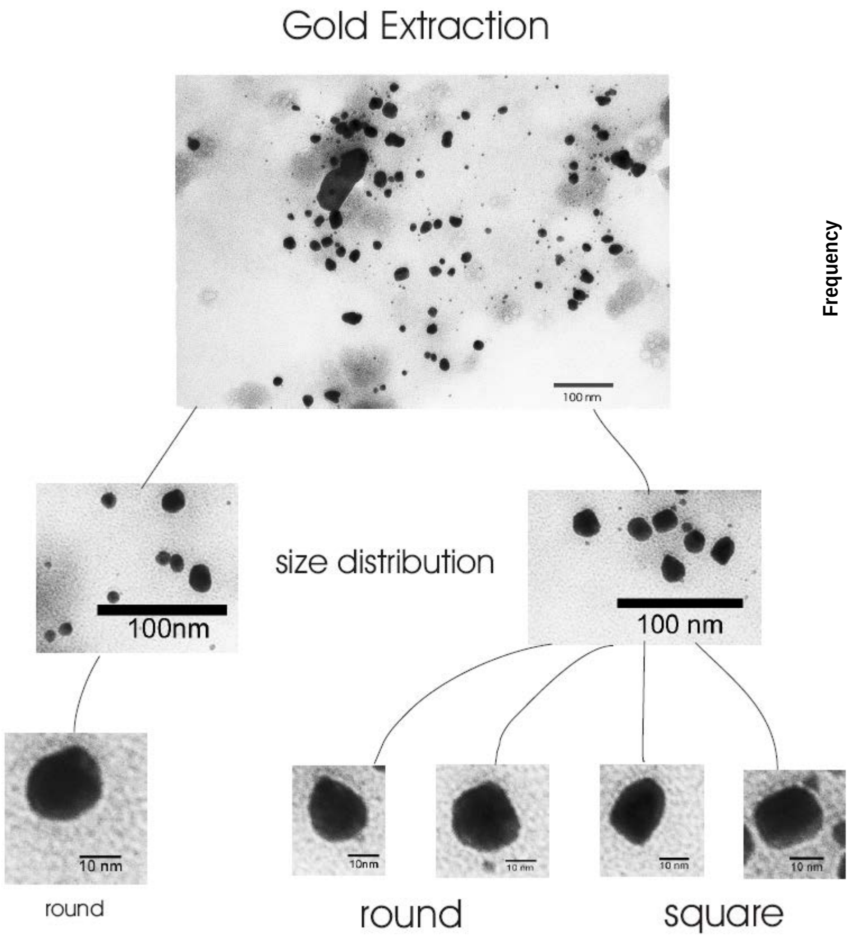


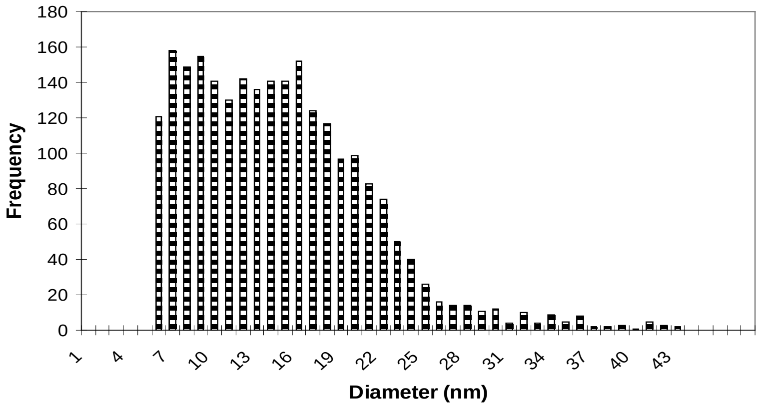
Transmission Electron Microscopy of *Sesbania* tissues with metals





Gold nanoparticles in *Sesbania*





ES & T (accepted)

Transport of Pb in *Sesbania*

- Scanning Electron Microscopy
  - Transport of metals via different cell types
- Transmission Electron Microscopy
  - Pb particles in intercellular spaces, cell membranes and cell walls.
  - Au particles (nanoparticles) are inside the cell.
  - Some deposits were also located within the tonoplast.

Biotransformation of Metals
(Using XAS Technology)

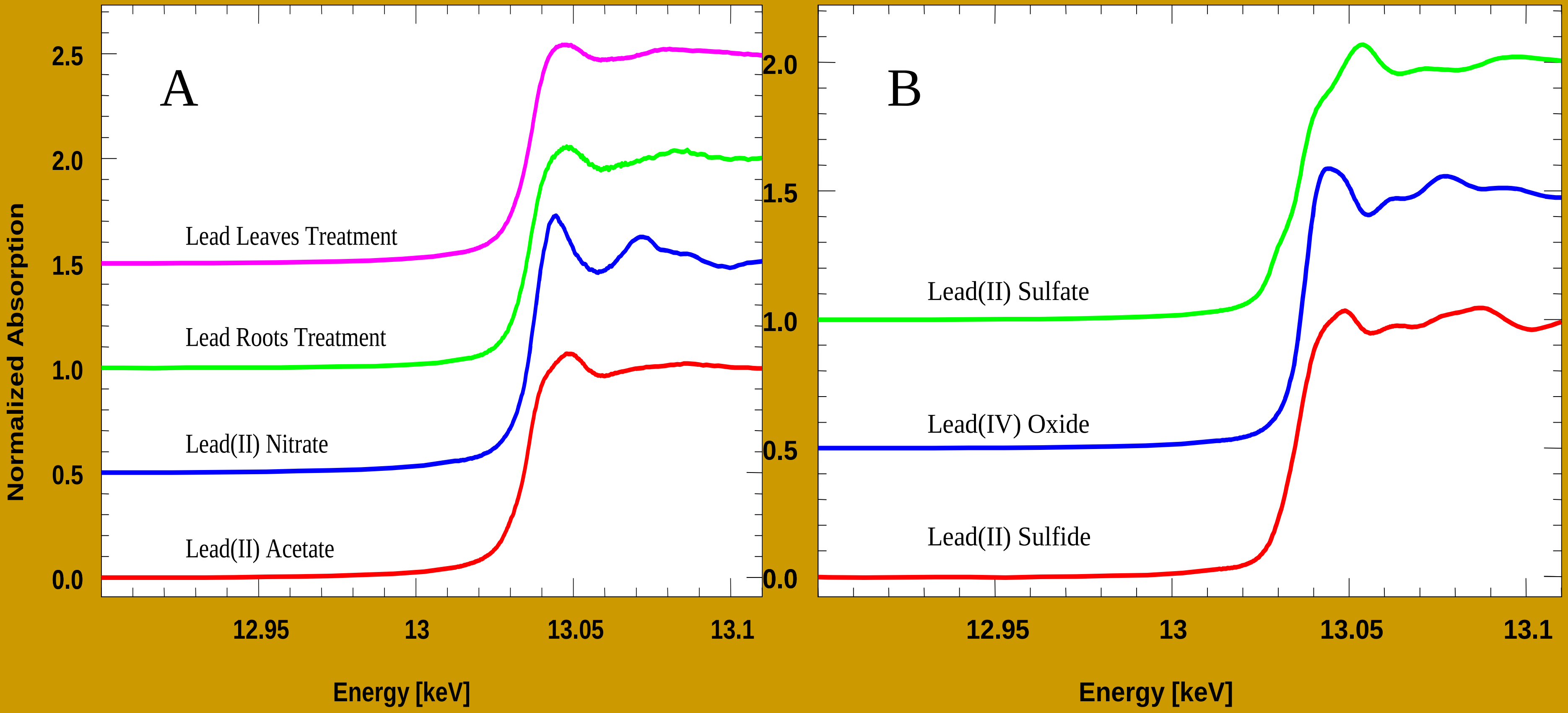
Types of XAS

- **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 the metal binding by measuring the distance from the X-ray-absorbing atom to the next nearest atom.

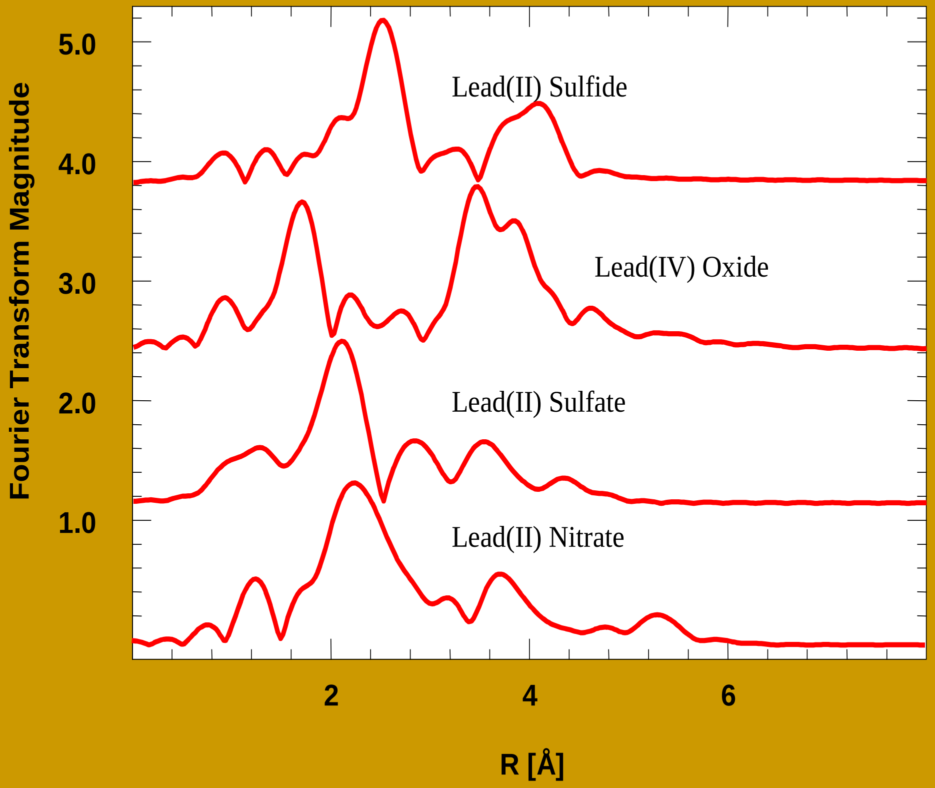
XANES Spectra of *Sesbania*

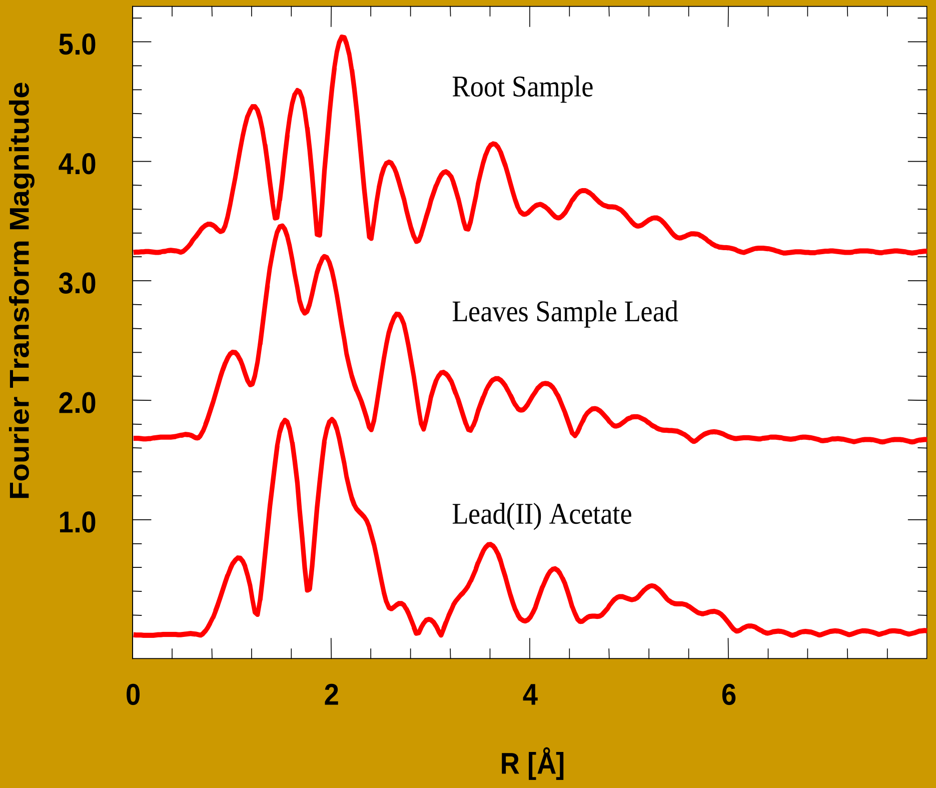
(ET & C 23, 2068, 2004)



A) L$_{III}$ XANES of lead 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)**

EXAFS Spectra of *Sesbania*





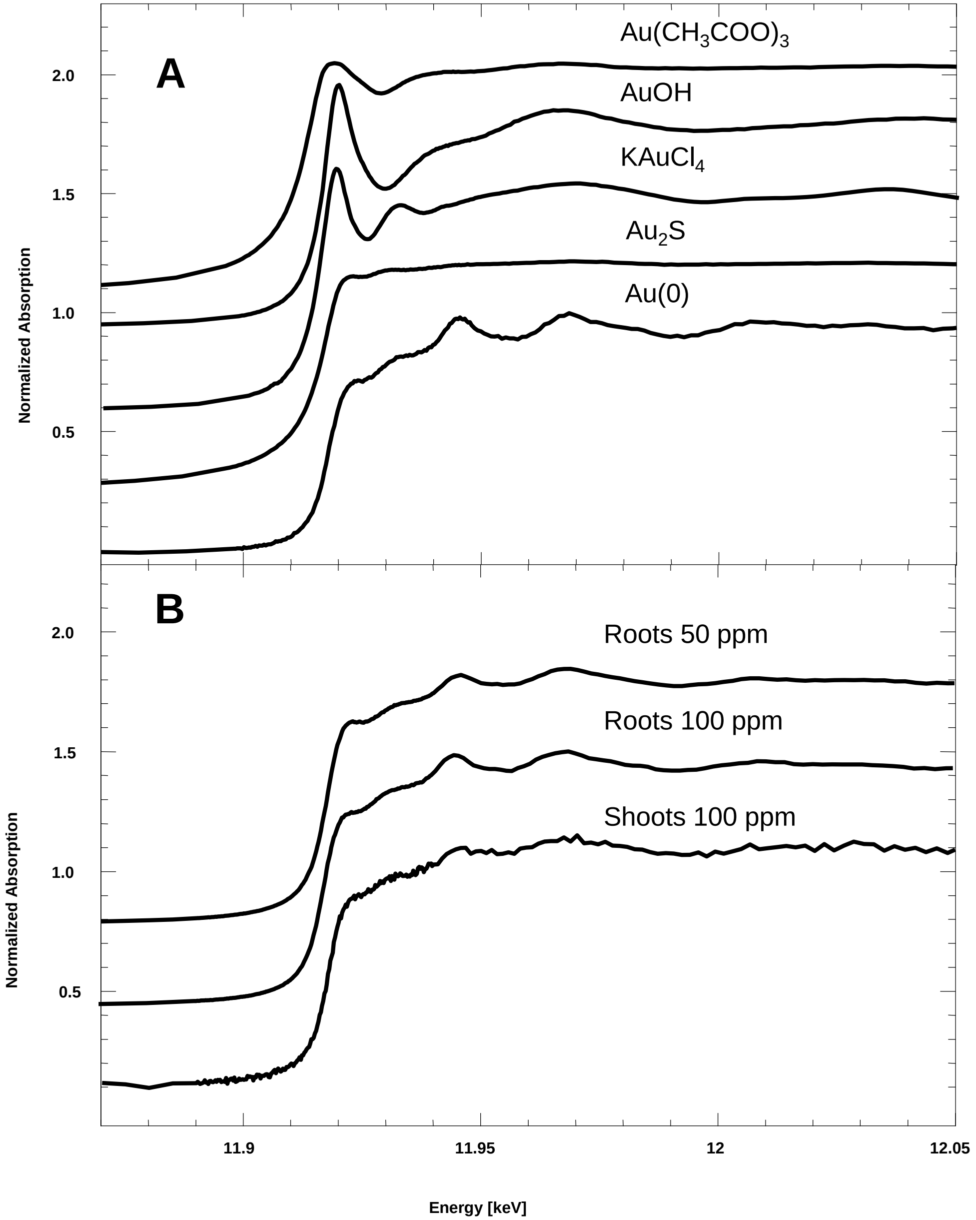
XANES and EXAFS data of Pb-treated *Sesbania*

(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

A. XANES of gold model compounds: gold acetate, gold hydroxide, potassium tetrachloroaurate, gold sulfide, and gold metal.

B. XANES of gold-laden plant samples.



XANES of gold accumulated in *Sesbania*

Samples	% $KAuCl_4$	% $Au(CH_3COO)_3$	% Au_2S	% Au(0)	% AuOH
Au **50 ppm roots**	0	0	18.4	81.6	0
Au **100 ppm roots**	0	0	16.4	83.6	0
Au **100 ppm shoots**	0	0	14.2	84.4	1.4

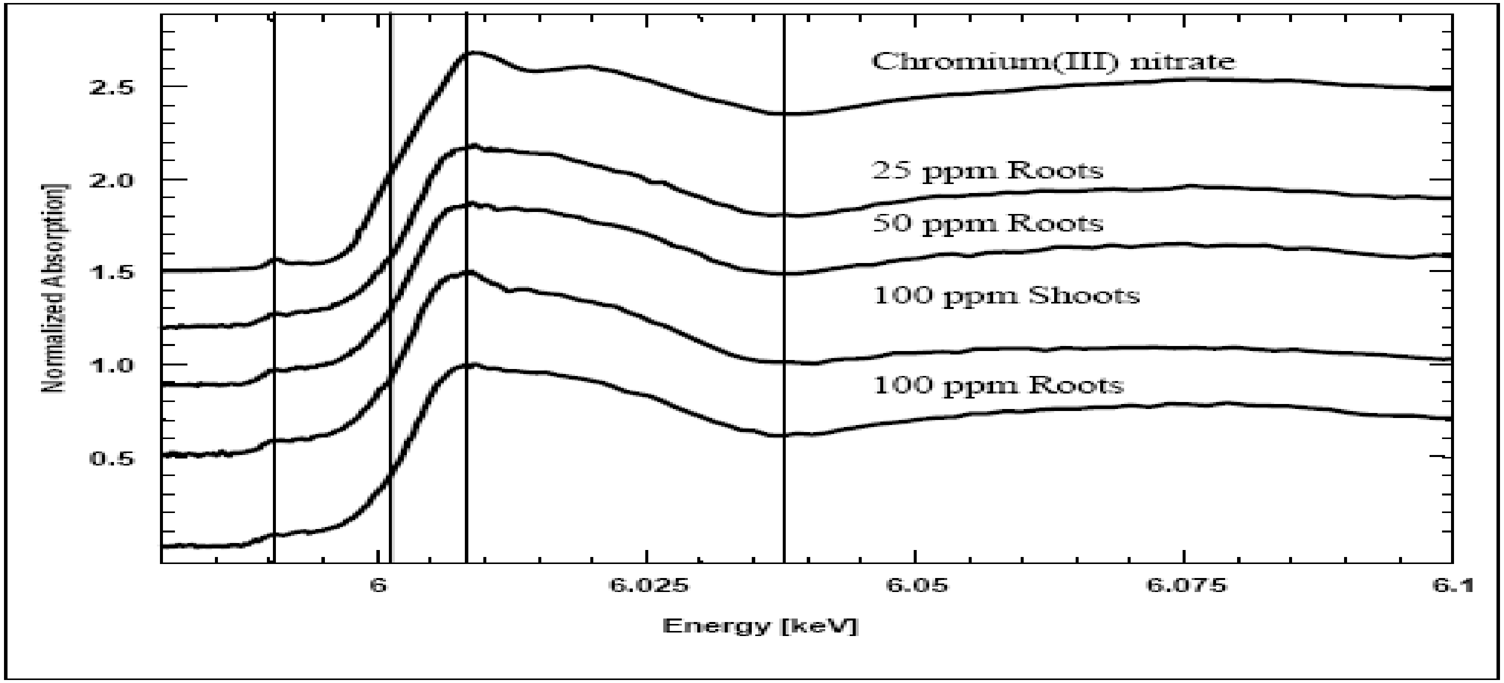
XANES of Cu accumulated in *Sesbania*

(Chemosphere 67, 2257-66, 2007)

Samples	% copper(II) acetate	% copper(II) nitrate	% copper(II) phthalocyanine	% copper(II) gluconate	% copper(II) Oxide
Cu 25 mg l^{-1}	0	20	2	57	21
Cu 100 mg l^{-1}	30	14	5	18	33

Biotransform cupric sulfate to copper(II) sugar/small organic acid complex and acetate in its tissue

XANES analysis of Cr in *Sesbania*

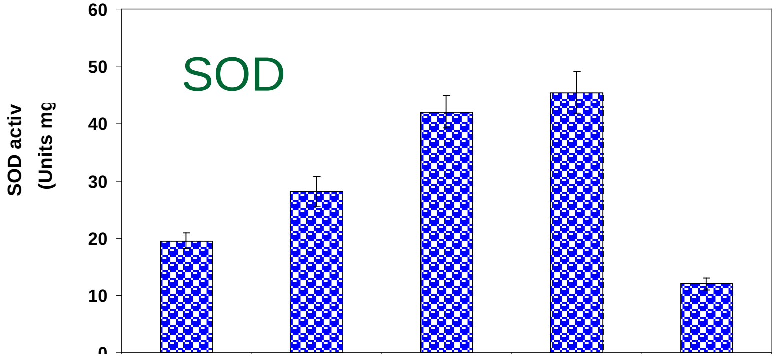


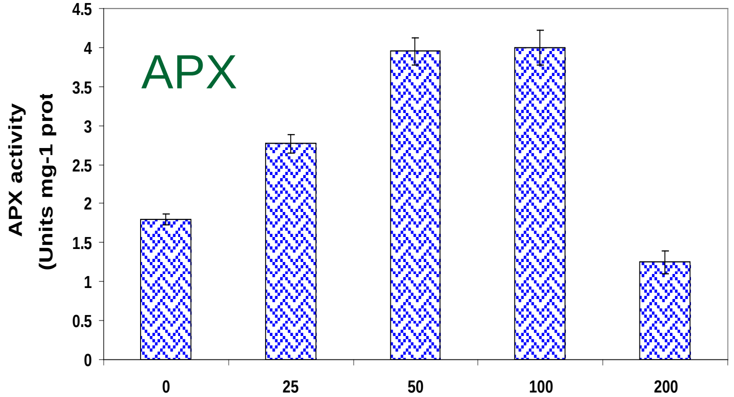
Sesbania has capability to biotransform Cr(VI) in to Cr(III) in its tissue

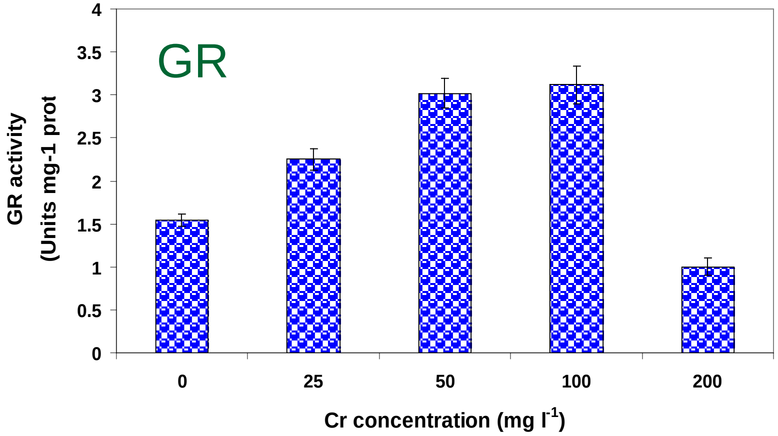
Antioxidant Reactions & metal Stress in *Sesbania*

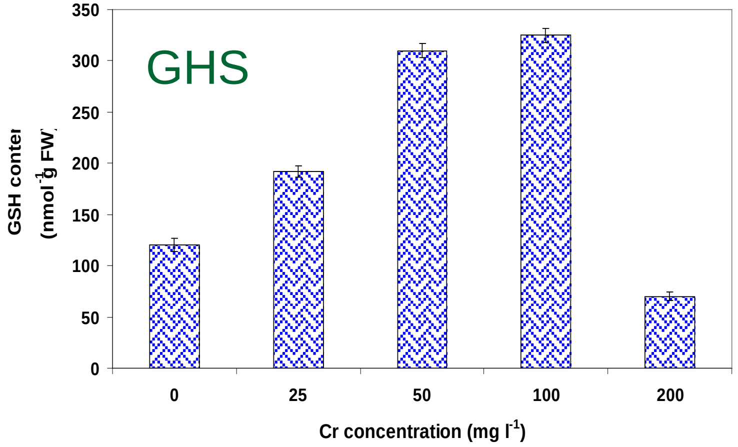
- Generally metal exposure triggers an increase in activity of antioxidant enzymes.
  - **Superoxide dismutase (SOD) –** catalyze dismutation of superoxide radicals to hydrogen peroxide & oxygen
  - **Catalase (CAT)** – catalyzes decomposition of hydrogen peroxide to water and oxygen
  - **Ascorbate 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

Stress enzymes in *Sesbania* in Cr

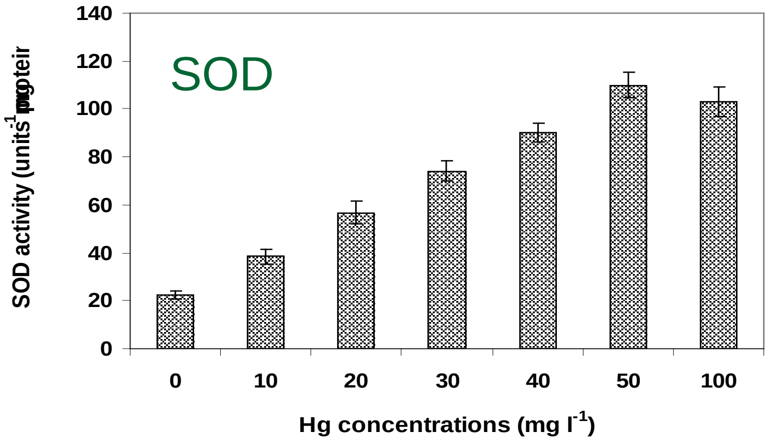


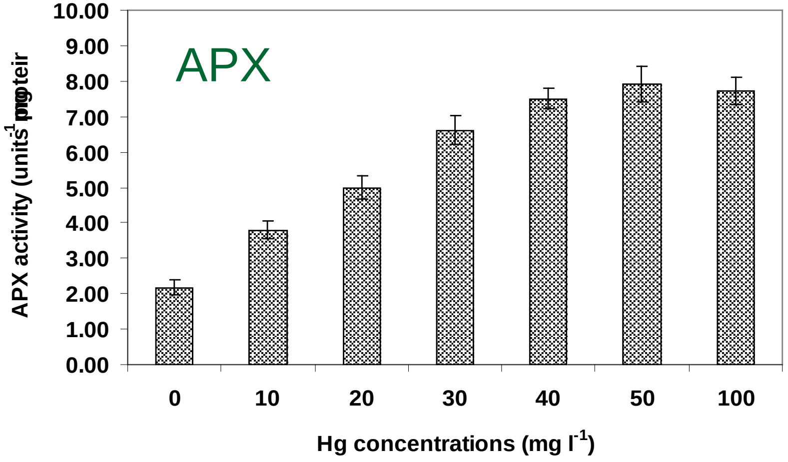


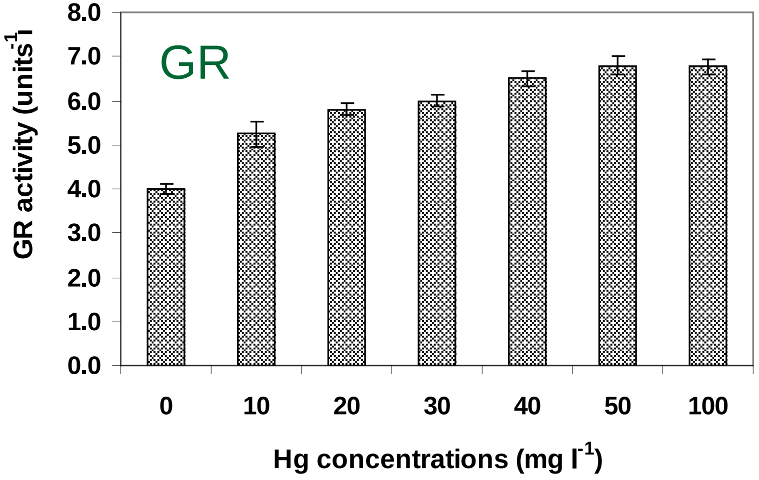




Stress enzymes in *Sesbania* in Hg

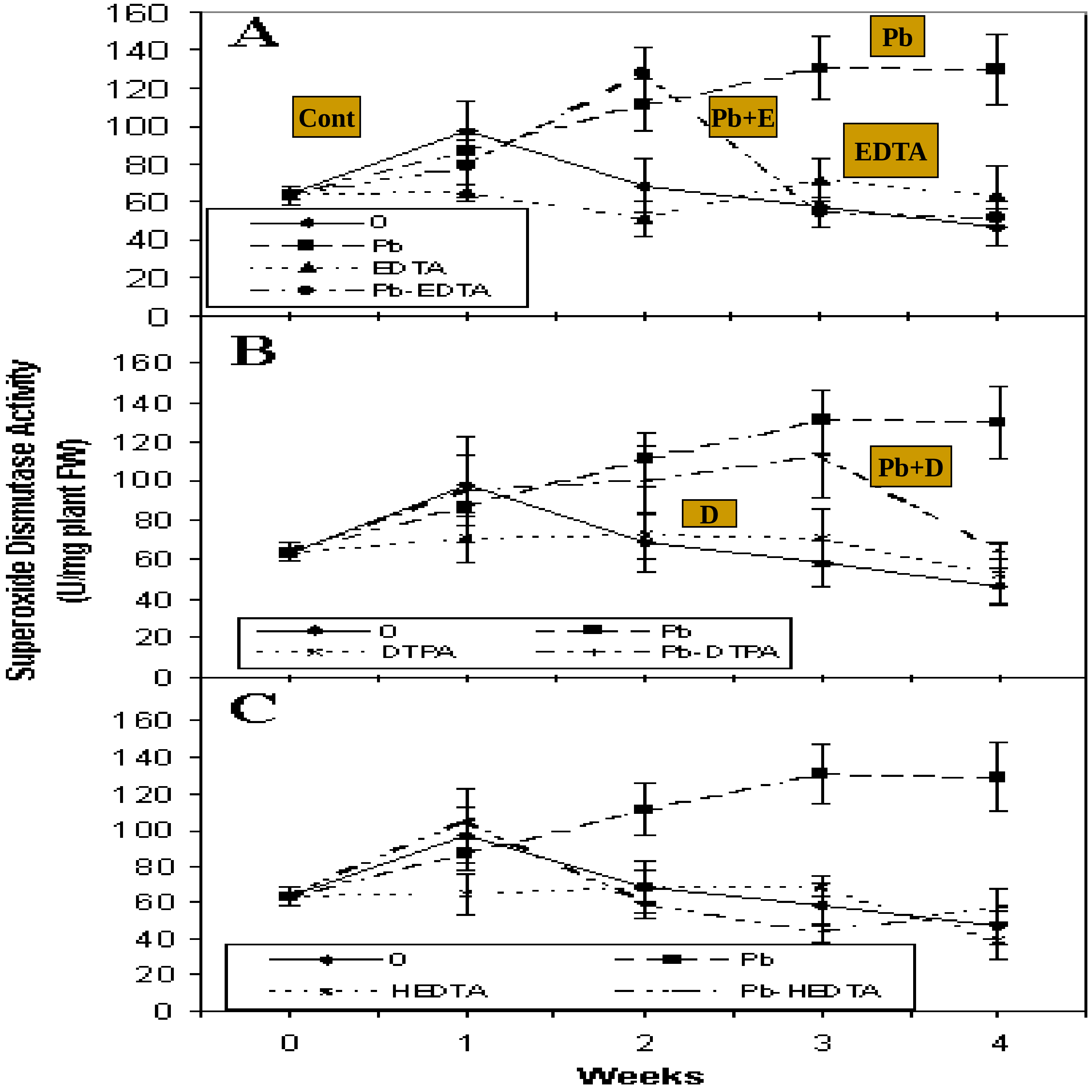






Superoxide dismutase

(SOD)



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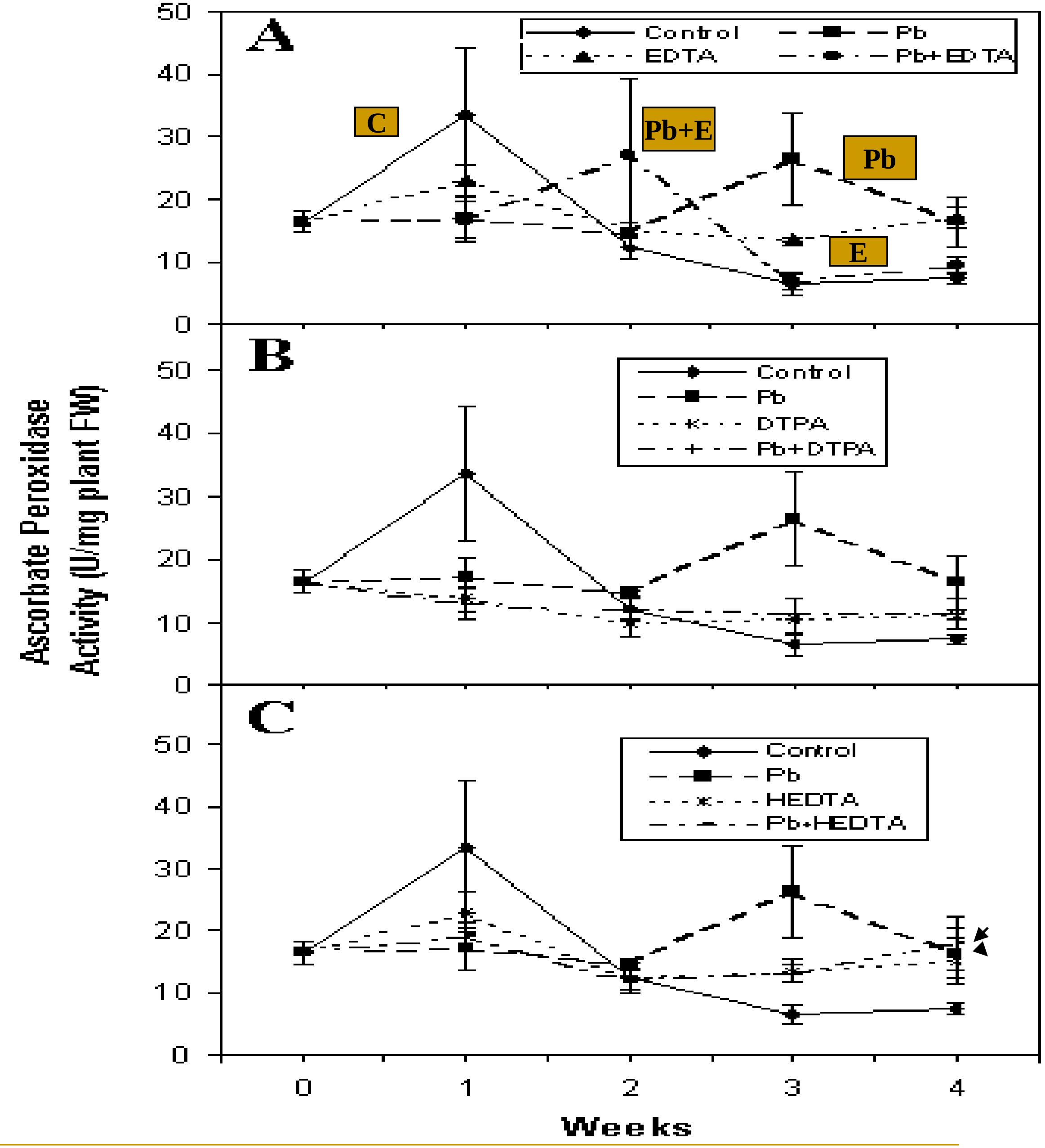
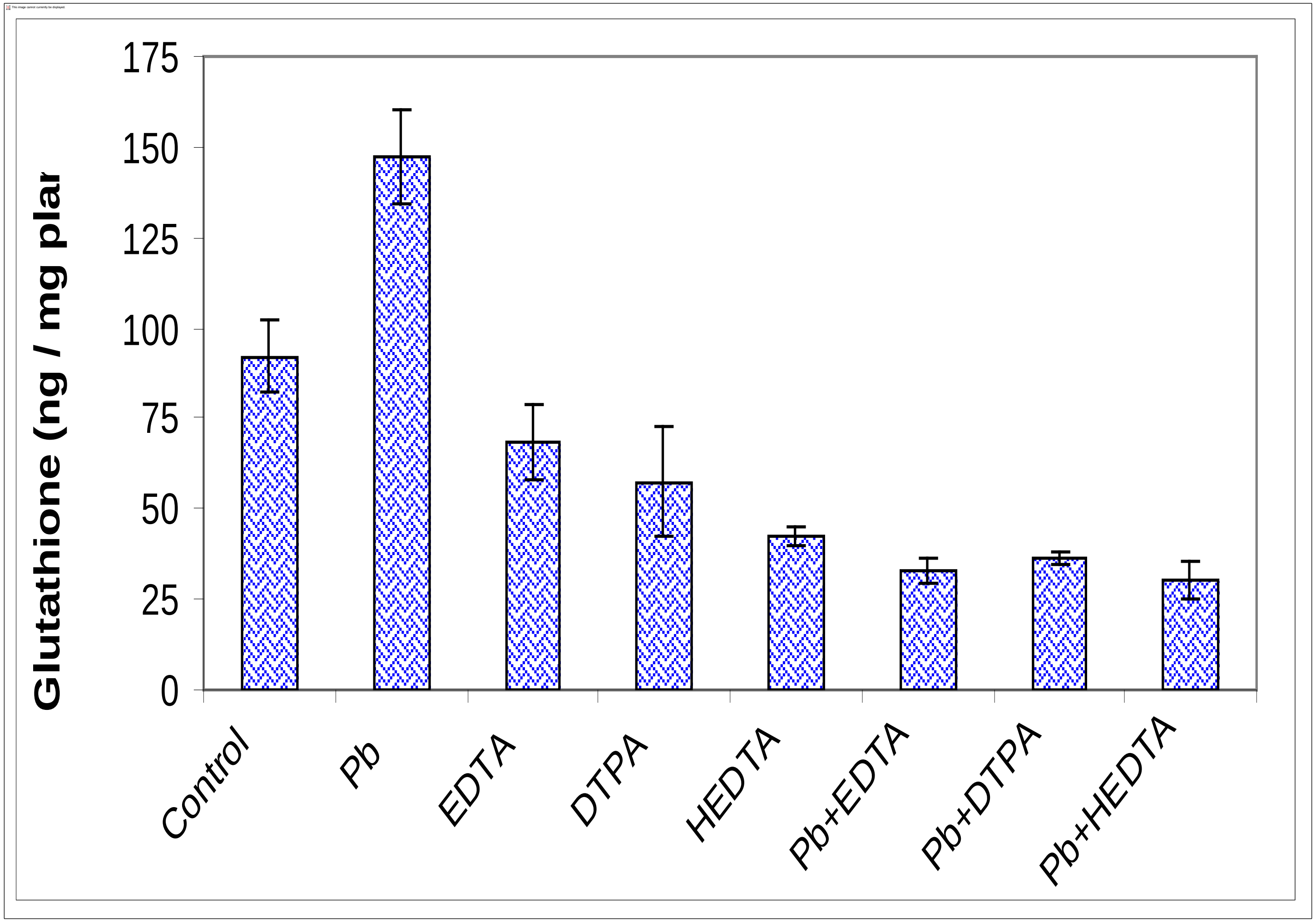
Ascorbate Peroxidase

(APX)



Glutathione content



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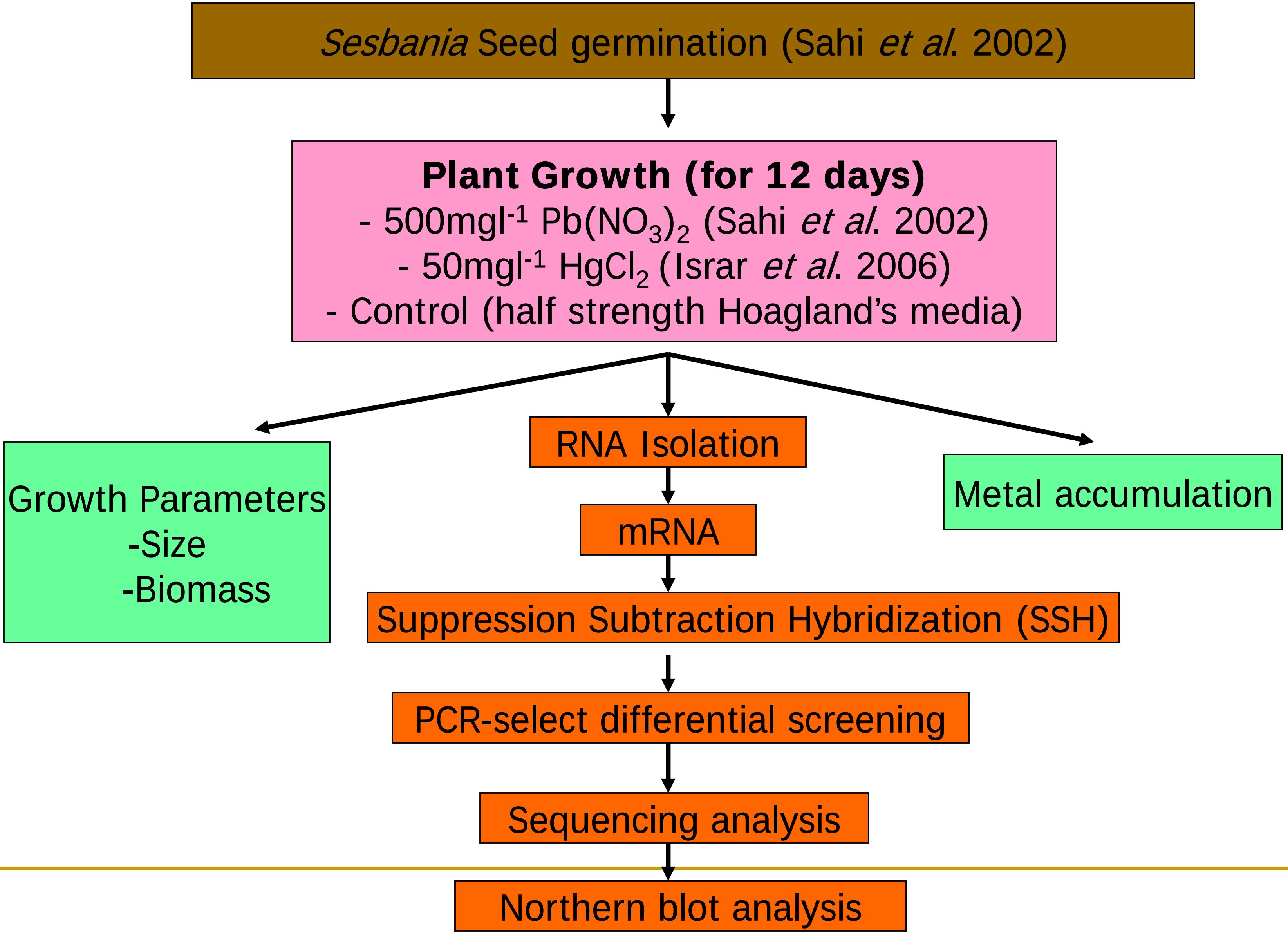
Identification of lead and mercury responsive genes

Experimental Design



Sesbania Seed germination (Sahi *et al*. 2002)

Plant Growth (for 12 days)

- 500mgl^{-1} $Pb(NO_3)_2$ (Sahi *et al*. 2002)
- 50mgl^{-1} $HgCl_2$ (Israr *et al*. 2006)
- Control (half strength Hoagland's media)

Growth Parameters
-Size
-Biomass

RNA Isolation

mRNA

Suppression Subtraction Hybridization (SSH)

PCR-select differential screening

Sequencing analysis

Northern blot analysis

Metal accumulation

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

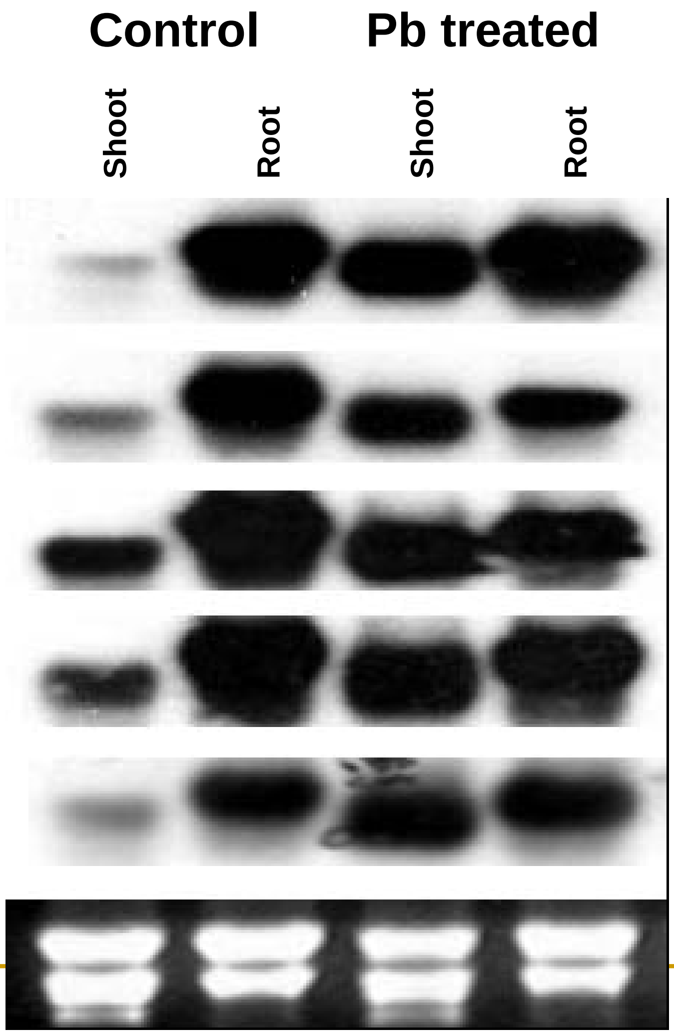
Clone	Accession number	Length (bp)	Homology[a]	E-value
SSH-1	DQ465754	183	*Acanthopanax sessiliflorus* cDNA library *Eleutherococcus sessiliflorus* cDNA, mRNA sequence (CF923918)	0.0
SSH-2	DQ465755	293	Apple_EST_Mdas Malus x domestica cDNA similar to dbj|BAB33421.1| putative senescence-associated protein [*Pisum sativum*], mRNA sequence (DR993778)	$1e^{-139}$
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`)	$1e^{-28}$
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	---

[b] No significant sequence homology found in genome, EST, and protein database.

Northern blot analysis (Pb)

Planta 2007 (in press)



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

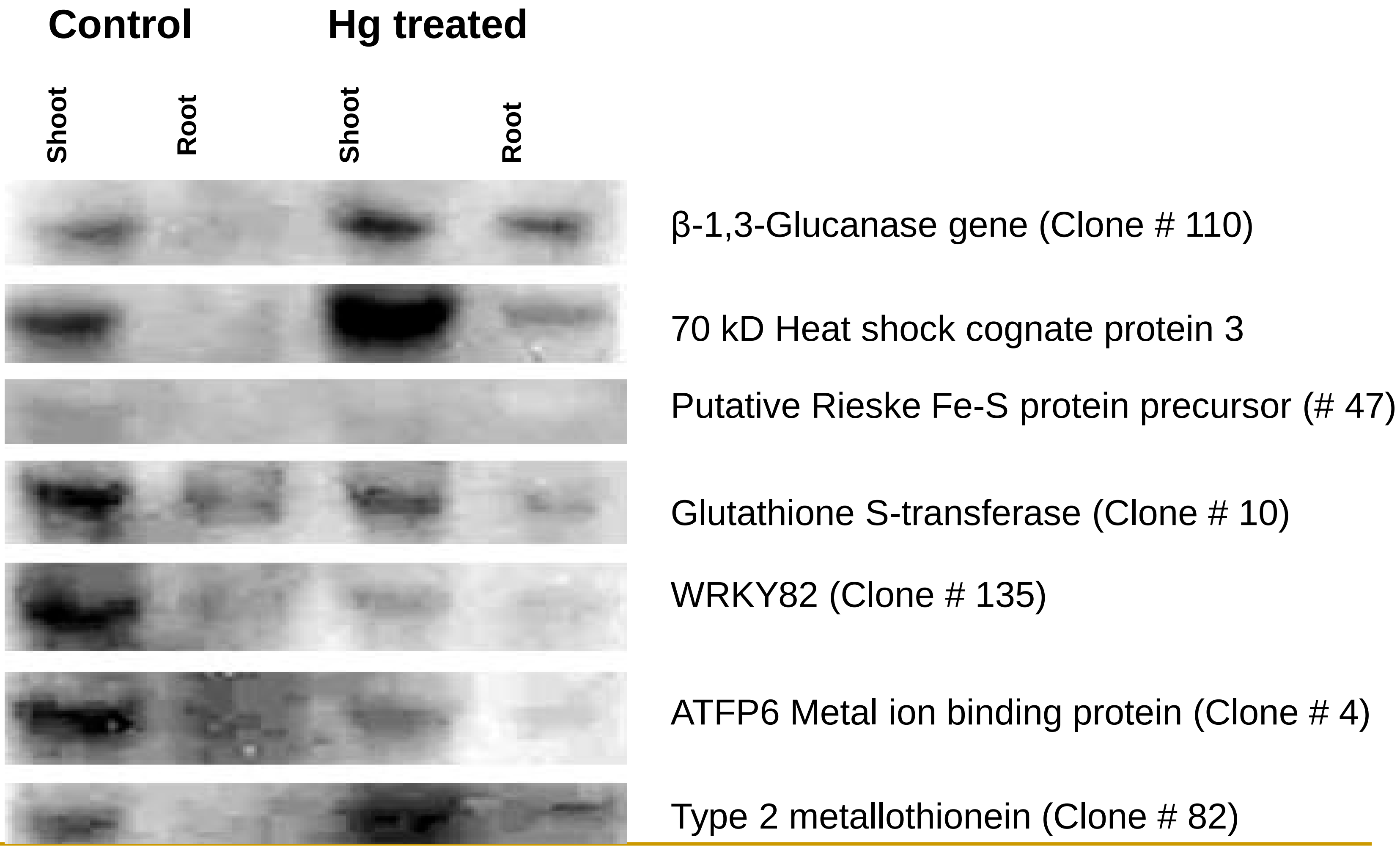
Sequencing results (Hg)

- 87 clones corresponds to unigenes
- Clone # 31 had homology to type 2 metallothionein (MT)
- Clone # 252- 70 kD Heat shock cognate protein 3
- Clone # 4- ATFP6-metal ion binding protein

1. Vses-forward_016- gb|AAM94615.1|putative hydrolase [Glycine max] 333 7e-90
2. Vses-forward_031- ref|NP_190489.1|unknown protein [Arabidopsis thaliana] 58.5 7e-08
3. Vses-forward_032- ref|NP_195570.1|ATFP6 (FARNESYLATED PROTEIN 6); metal ion binding [Arabidopsis thaliana] 92.4 5e-18
4. Vses-forward_047- gb|AAG24883.1|ribulose-1,5-bisphosphate carboxylase small subunit rbcS2 [Glycine max] 206 6e-52
5. Vses-forward_048- emb|CAA68848.1|hin1 [Nicotiana tabacum] 186 1e-45
6. Vses-forward_063- dbj|BAA77604.1|plastidic aldolase NPALDP1 [Nicotiana paniculata] 394 1e-108
7. Vses-forward_064- gb|AAT80649.1|lipid transfer protein precursor [Malus x domestica]139 3e-32
8. Vses-forward_079- emb|CAA85354.1|alpha-1,4 glucan phosphorylase, L isoform precursor [Vicia faba var. minor] 353 7e-96
9. Vses-forward_080- gb|AAG34805.1|glutathione S-transferase GST 15 [Glycine max] 337 2e-91
10. Vses-forward_095- emb|CAB17075.1|cysteine proteinase precursor [Phaseolus vulgaris] 297 3e-79
11. Vses-forward_030- sp|O65194|RBS_MEDSA Ribulose bisphosphate carboxylase small chain, chloroplast precursor (RuBisCO small subunit) 276 6e-73
12. Vses-forward_045- emb|CAA32197.1|chlorophyll a/b-binding protein [Lycopersicon esculentum] 376 1e-103
13. Vses-forward_046- gb|AAG24882.1|ribulose-1, 5-bisphosphate carboxylase small subunit rbcS1 [Glycine max] 283 6e-75
14. Vses-forward_061- gb|AAC16403.1|early light-induced protein [Glycine max192 3e-48
15. Vses-forward_062- ref|NP_190490.1|unknown protein [Arabidopsis thaliana] 42.4 0.006
16. Vses-forward_043- emb|CAA96570.1|CP12 [Pisum sativum] 189 5e-47
17. Vses-forward_060- emb|CAA05979.1|adenine nucleotide translocator [Lupinus albus] 117 1e-25
18. Vses-forward_091- ref|NP_974774.1|HAP2A; transcription factor [Arabidopsis thaliana] 152 1e-35
19. Vses-forward_025- sp|P93508|CRTC_RICCO Calreticulin precursor 358 9e-98
20. Vses-forward_058- sp|O22518|RSSA_SOYBN 40S ribosomal protein SA (p40) 170 1e-41
21. Vses-forward_089- emb|CAA45151.1|chloroplast Rieske FeS protein [Pisum sativum] 300 2e-80
22. Vses-forward_090- ref|NP_182172.1|unknown protein [Arabidopsis thaliana] 88.2 9e-17
23. Vses-forward_023- emb|CAA67696.1|23 kDa oxygen evolving protein of photosystem II [Solanum tuberosum] 260 1e-68
24. Vses-forward_055- ref|XP_467654.1|MutT/nudix-like [Oryza sativa (japonica cultivar-group)] 84.0 4e-15
25. Vses-forward_087- gb|AAD28640.2|geranylgeranyl hydrogenase [Glycine max] 372 1e-102
26. Vses-forward_037- ref|NP_186761.1|MTO1 (METHIONINE OVERACCUMULATION 1) [Arabidopsis thaliana] 155 6e-37
27. Vses-forward_085- ref|XP_469854.1|putative dehydrogenase precursor [Oryza sativa (japonica cultivar-group)] 293 3e-78
28. Vses-forward_003-emb|CAA33557.1|unnamed protein product [Pisum sativum] 124 8e-28
29. Vses-forward_020-emb|CAA43590.1|Type I (26 kD) CP29 polypeptide [Lycopersicon esculentum] 211 7e-54
30. Vses-forward_067-emb|CAB79860.1|putative zinc finger protein [Arabidopsis thaliana] 166 2e-40
31. Vses-forward_068-dbj|BAD18379.1|type 2 metallothionein [Vigna angularis] 110 2e-23
32. Vses-forward_083-ref|NP_177596.1|DNA binding [Arabidopsis thaliana] 88.6 7e-17
33. Vses-forward_084-emb|CAA32429.1|unnamed protein product [Arabidopsis thaliana] 43.5 0.002
34. Vses-forward_001-gb|AAM94806.1|rubisco activase alpha [Gossypium hirsutum] 105 5e-22
35. Vses-forward_002-emb|CAA81078.1|glycine hydroxymethyltransferase [Flaveria pringlei] 399 1e-109
36. Vses-forward_034-gb|AAQ72789.1|60S ribosomal protein L5 [Cucumis sativus] 358 1e-97
37. Vses-forward_050-dbj|BAB86847.1|elongation factor EF-2 [Pisum sativum] 183 2e-45
38. Vses-forward_066-gb|AAK25800.1|rubisco activase [Zantedeschia aethiopica] 43.9 0.002
39. Vses-forward_081-emb|CAA81082.1|glycine hydroxymethyltransferase [Solanum tuberosum] 408 1e-112
40. Vses-forward_031-dbj|D78130.1|Homo sapiens mRNA for squalene epoxidase, complete cds 89.7 6e-15
41. Vses-forward_032-ref|NM_120019.1|Arabidopsis thaliana ATFP6 (FARNESYLATED PROTEIN 6); metal ion binding AT4G38580 (ATFP6) mRNA, complete cds 79.8 6e-12
42. Vses-forward_047-gb|AF303941.1|AF303941 Glycine max ribulose-1,5-bisphosphate carboxylase small subunit rbcS3 mRNA, complete cds 454 1e-124
43. Vses-forward_048-gb|AY279310.1|Malus x domestica enolase-like mRNA, partial sequence 63.9 9e-07

44. Vses-forward_063-gb|M97476.1|PEAALDIA Pisum sativum L. aldolase gene, 3' end cds 694 0.0
45. Vses-forward_064-gb|DQ122797.1|Medicago sativa clone QB12 lipid transfer protein precursor, mRNA, partial cds 105 1e-19
46. Vses-forward_080-gb|AC161864.9|Medicago truncatula clone mth2-69d21, complete sequence 256 9e-65
47. Vses-forward_095-dbj|AP006110.1|Lotus corniculatus var. japonicus genomic DNA, chromosome 1, clone:LjT39G11, TM0195b, complete sequence 500 1e-138
48. Vses-forward_014-emb|V00458.1|GMRUBP Glycine max gene encoding ribulose-1,5-bisphosphate carboxylase small subunit 240 3e-60
49. Vses-forward_030-gb|AF056315.1|AF056315 Medicago sativa ribulose-1,5-bisphosphate carboxylase small subunit mRNA, nuclear gene encoding chloroplast protein, complete cds 252 1e-63
50. Vses-forward_045-emb|X81962.1|PSLHAB P.sativum mRNA for type II chlorophyll a/b binding protein 400 1e-108
51. Vses-forward_060-emb|AJ003197.1|LAAJ3197 Lupinus albus mRNA for adenine nucleotide translocator 260 2e-66
52. Vses-forward_009-gb|AC125095.3|Mus musculus BAC clone RP24-179H12 from chromosome 14, complete sequence 52.0 0.003
53. Vses-forward_025-gb|U74630.1|RCU74630 Ricinus communis calreticulin mRNA, complete cds 466 1e-128
54. Vses-forward_058-emb|AJ006759.1|CAR6759 Cicer arietinum mRNA for ribosome-associated protein p40 216 3e-53
55. Vses-forward_089-dbj|AB025003.1|Cicer arietinum mRNA for plastoquinol-plastocyanin reductase, partial cds 317 2e-83
56. Vses-forward_090-gb|AC109247.14|Mus musculus chromosome 9, clone RP23-462C14, complete sequence 56.0 9e-05
57. Vses-forward_008-gb|U74630.1|RCU74630 Ricinus communis calreticulin mRNA, complete cds 513 1e-142
58. Vses-forward_087-gb|DQ013361.1|Lotus corniculatus var. japonicus geranylgeranyl hydrogenase (GGH) mRNA, complete cds 652 0.0
59. Vses-forward_005-gb|AF220405.1|AF220405 Vitis riparia transcription factor (Rev136-2) mRNA, complete cds 75.8 2e-10
60. Vses-forward_037-gb|AF097180.1|AF097180 Nicotiana tabacum cystathionine gamma-synthase precursor (metB) mRNA, complete cds 69.9 8e-09
61. Vses-forward_085-gb|BT009463.1|Triticum aestivum clone wr1.pk0004.c11:fis, full insert mRNA sequence 198 1e-47
62. Vses-forward_003-dbj|AB236819.1|Trifolium pratense RNA for putative PSII-P protein, partial cds, clone: C214 244 2e-61
63. Vses-forward_020-gb|BT014450.1|Lycopersicon esculentum clone 133776F, mRNA sequence 230 2e-57
64. Vses-forward_067-dbj|AP004913.1|Lotus corniculatus var. japonicus genomic DNA, chromosome 5, clone:LjT02A14, TM0072a, complete sequence 206 4e-50
65. Vses-forward_083-ref|XM_466397.1|Oryza sativa (japonica cultivar-group), mRNA 75.8 7e-11
66. Vses-forward_084-gb|AC129090.21|Medicago truncatula clone mth2-14i8, complete sequence 113 2e-22
67. Vses-forward_002-gb|M87649.1|PEASHMTA Pisum sativum serine hydroxymethyltransferase mRNA, complete cds 763 0.0
68. Vses-forward_034-dbj|AB049724.1|Pisum sativum ssa-15 mRNA for putative senescence-associated protein, complete cds 460 1e-126
69. Vses_06_A06_T3 gb|AAQ87663.1|translationally controlled tumor protein [Elaeis guineensis] 224 1e-57
70. Vses_06_A08_T3 gb|AAX94836.1|Major Facilitator Superfamily, putative [Oryza sativa (japonica cultivar-group)] 115 4e-25
71. Vses_06_A12_T3 sp|P27774|KPPR_MESCR Phosphoribulokinase, chloroplast precursor (Phosphopentokinase) (PRKase) (PRK) 64.7 1e-09
72. Vses_06_B02_T3 ref|NP_192718.1|RPS18C (S18 RIBOSOMAL PROTEIN); RNA binding / structural constituent of ribosome [Arabidopsis thaliana] 93.6 2e-18
73. Vses_06_C01_T3 gb|AAB36543.1|DnaJ-like protein [Phaseolus vulgaris] 52.0 7e-06
74. Vses_06_C07_T3 ref|NP_187886.2| oxidoreductase [Arabidopsis thaliana] 170 1e-41
75. Vses_06_C08_T3 sp|P34921|G3PC_DIACA Glyceraldehyde-3-phosphate dehydrogenase, cytosolic 219 2e-56
76. Vses_06_C10_T3 gb|AAM93434.1| 40S ribosomal S4 protein [Glycine max] 142 3e-33
77. Vses_06_C11_T3 gb|AAG33924.1| auxin-repressed protein [Robinia pseudoacacia] 169 5e-41
78. Vses_06_E08_T3 gb|AAS57914.1| 70 kDa heat shock cognate protein 3 [Vigna radiata] 343 6e-93
79. Vses_06_D03-g_025-1672 gb|DQ322696.1| Glycine max WRKY82 mRNA, complete cds 119 8e-24
80. Vses_06_D08_T3 gb|AAA65011.1| similar to Atriplex nummularia chaperone ANJ1 protein, Swiss-Prot Accession Number JQ2142 76.6 3e-13
81. Vses_06_B09_T3 emb|AJ749797.1| Photobacterium damselae subsp. piscicida trpB gene for putative transposase, clone pRDA16 50.1 0.005
82. Vses_06_C08_T3 gb|DQ355800.1| Glycine max glyceraldehyde-3-phosphate dehydrogenase (GAPC1) mRNA, complete cds 323 3e-85
83. Vses_06_C11_T3 gb|AY009094.1| Robinia pseudoacacia auxin-repressed protein mRNA, complete cds 375 1e-100
84. Vses_06_C12_T3 emb|AJ006764.1|CAR6764 Cicer arietinum mRNA for putative cytidine or deoxycytidylate deaminase, partial 176 1e-41
85. Vses_06_D03_T3 gb|AY109342.1| Zea mays CL3469_4 mRNA sequence 167 4e-38
86. Vses_06_D04_T3 dbj|AB089677.1| Prunus persica PpNRT1 mRNA for nitrate transporter, complete cds 107 5e-20
87. Vses_06_F08-g_054-1672 dbj|AB242265.1| Sesbania rostrata Srglu64 mRNA for beta-1,3-glucanase, complete cds 795 0.0

Northern blot analysis (Hg)



Bioengineering of Plants for Efficient Remediation

- ***Arabidopsis*** transgenics constructed to express bacterial genes merB and merA. (PNAS 93, 1996)

  $$(CH_3)_2Hg \xrightarrow{\mathit{merB}} Hg^{2+} \xrightarrow{\mathit{merA}} Hg$$

- **Indian mustard** transformed with *ATP Sulfurylase* (*APS*) genes demonstrate 4-fold increase in APS activity and accumulated 3X Se than the wild type.
- **Transgenic tomato** over expressing the bacterial gene

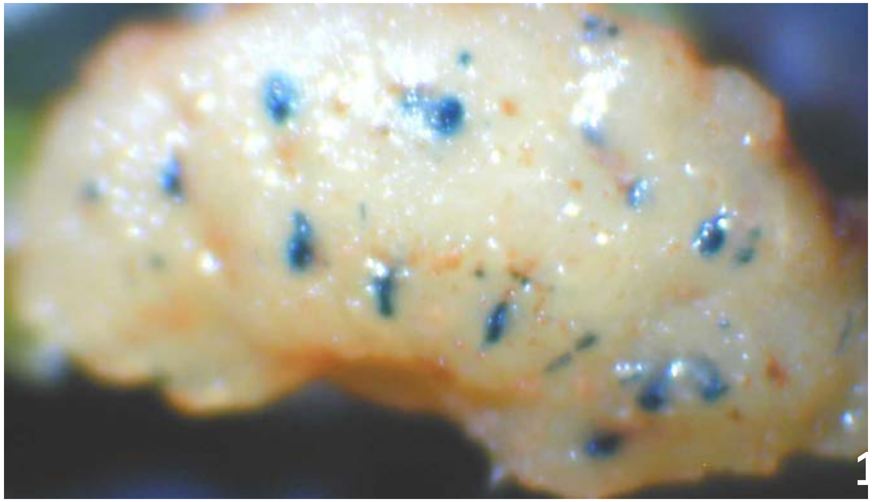
  1-aminocyclopropane-1-carboxylic acid (*ACC*) *deaminase* demonstrated enhanced tolerance for and accumulation of Co, Cu, Ni, Pb and Zn (J. Biotech 81, 2000).
- **Transgenic tobacco** expressing *citrate synthase* showed enhanced tolerance to Al toxicity (Science 276, 1997).
- **Transgenic *Arabidopsis*** expressing *phytochelatin synthase* from wheat

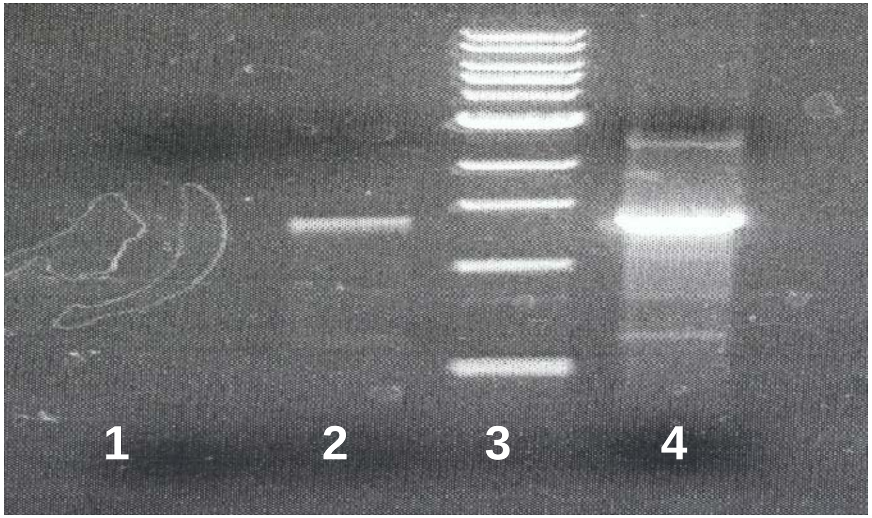
  demonstrated enhanced accumulation of Cd (PNAS 100, 2003)

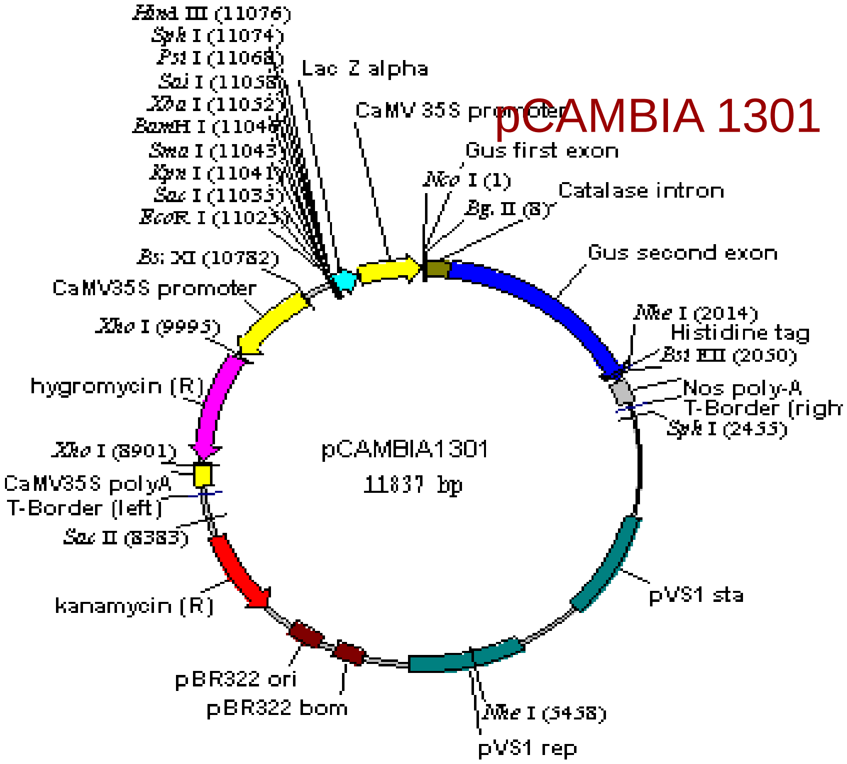
Sesbania Transformation

- Developed *in vitro* regeneration system using nodal explants
- *Sesbania* callus infected with *Agrobacterium* containing pCambia 1305
- pCambia has *GUS* gene which produces beta-glucuronidase
- *GUS* histochemical assay to check gene expression.
- Expression confirmed by PCR

Sesbania callus showing transformation







pCAMBIA 1301

Lane 1: *S. drummondii* transformed tissue with strain K289 (without plasmid), **Lane 2:** transformed tissue with strain K289pCAMBIA 1305.1. **Lane 3:** 1 kb ladder; **Lane 4:** *GUS* gene amplified from pCAMBIA 1305.1 plasmid

Sesbania Regeneration

General Conclusion

- Phytoremediation by *Sesbania* is effective against a wide variety of contaminants.
- Effective for sites with shallow contaminated soils.
- A type II metallothionein gene identified - may be involved in heavy metal detoxification
- Pb and Hg in *Sesbania* also induced other stress related genes
- Slow process
- Interdisciplinary approach
- More research to manipulate metals accumulation efficiency of *Sesbania.*

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