RISKeLearning

Phytoremediation

October 14th, 2008 Session 1:
"Phytoremediation: The Potential is Growing"

David Tsao, BP Corporation North America, Inc., Overview of Phytotechnologies

Jerald Schnoor, SBRP-University of Iowa, Plant Degradation of Airborne PCB Congeners

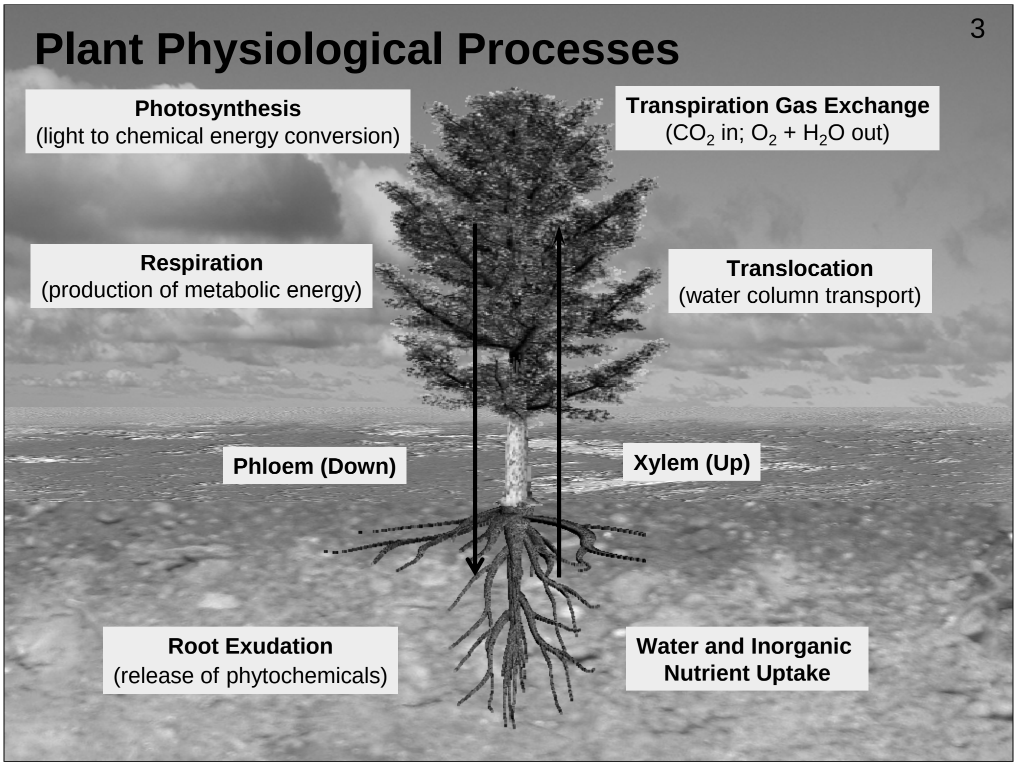
sbrp Superfund Basic Research Program
Balancing Scientific Excellence with Research Relevance

Phytotechnologies

Mechanisms and Applications

Advantages and Limitations

David Tsao, Ph.D
BP Remediation Management


3
Plant Physiological Processes
Photosynthesis
(light to chemical energy conversion)
Transpiration Gas Exchange
(CO_2 in; O_2 + H_2O out)
Respiration
(production of metabolic energy)
Translocation
(water column transport)
Phloem (Down)
Xylem (Up)
Root Exudation
(release of phytochemicals)
Water and Inorganic
Nutrient Uptake

Phyto Picture

What is the Rhizosphere?

-**1-3 mm** of soil surrounding each root

-Contains a **high proliferation** of soil organisms (yeast, fungi, bacteria, viruses, etc.)

- -**General populations:** 1-2 orders of magnitude higher than non-vegetated soil
- -**Specific microbes:** 3-4 orders of magnitude higher; **dominant** organisms

Why there is such a proliferation of microbes?

-**Plants exude chemicals** of all kinds into the subsurface

- -Alcohols, phenols, sugars, carbohydrates, organic acids, inorganic nutrients - NPK

-**Plants produce and release various enzymes**

- -Dehalogenases, nitroreductases, glutathione, phenoloxidases, oxygenases, nitrilases, phosphatases

-**Plants also provide oxygen** and water

- -**Direct production** (0.5 mol O_2 per m^2 surface area)
- -Creating **root channels** for diffusion from the atmosphere

Root Turnover:

-**Annual** event (winter)

-Portion of the root system gets **sloughed off** because it is not needed to maintain the dormant plant

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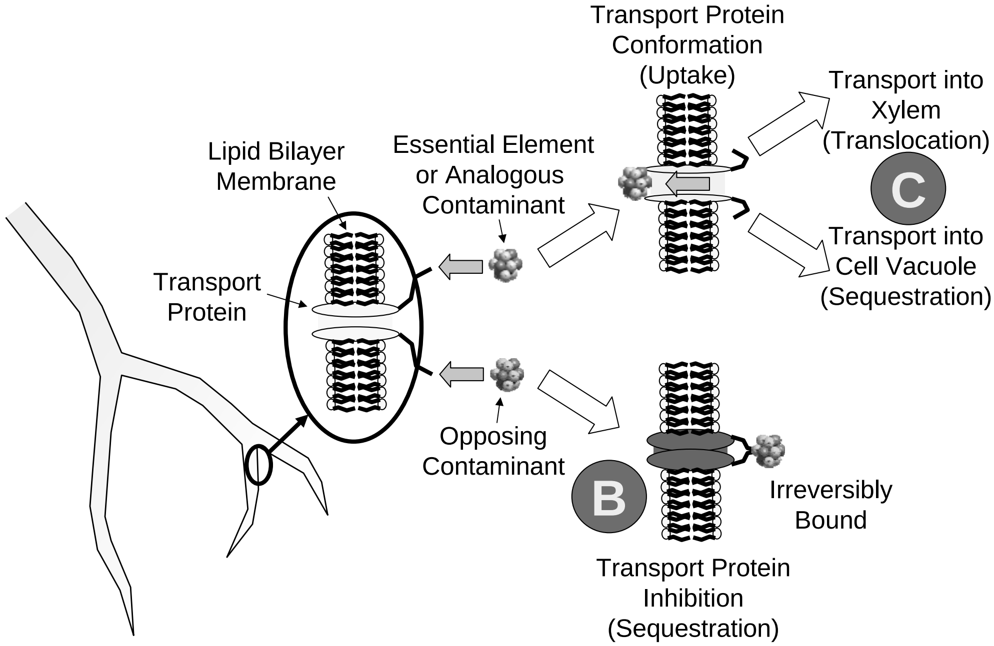
Mechanism: Phytosequestration (PS)

B) Transport Protein Inhibition and C) Vacuolar Storage



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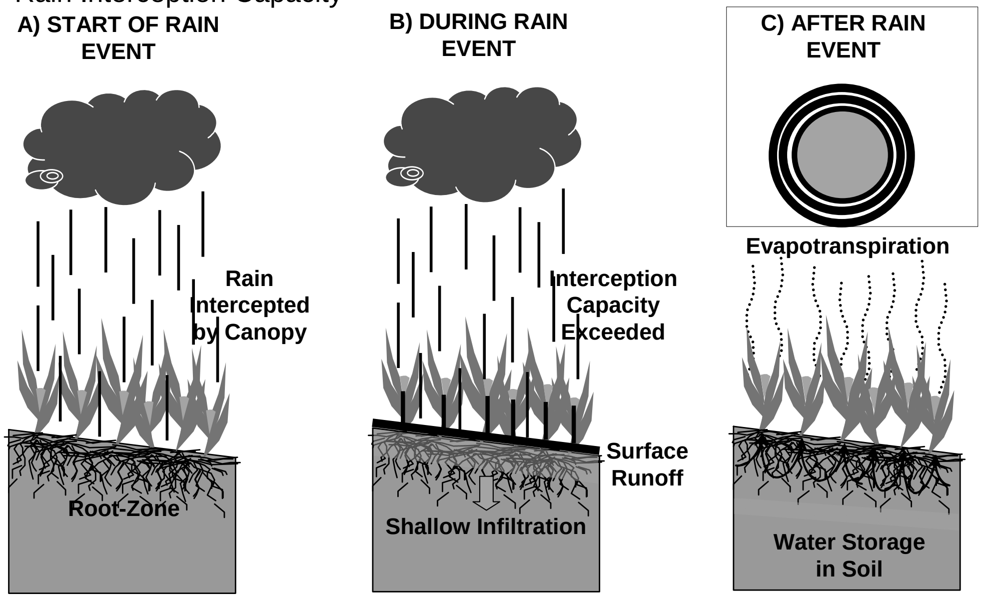
Physiology: Evapotranspiration (ET)
Mechanism: Phytohydraulics (PH)

Rain Interception Capacity

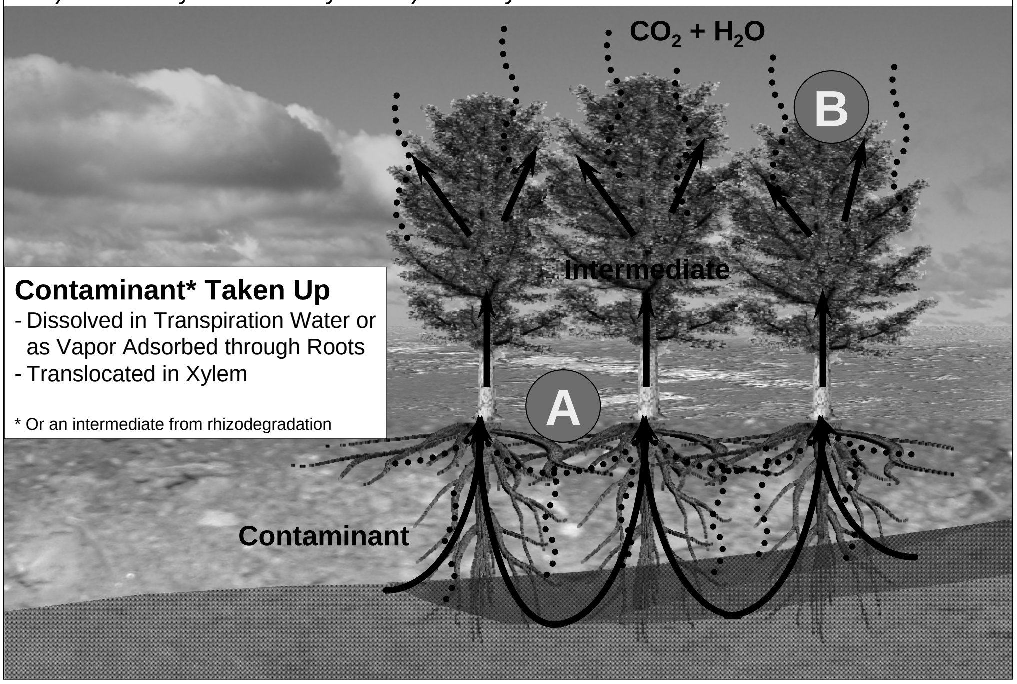


Physiology: Transpiration

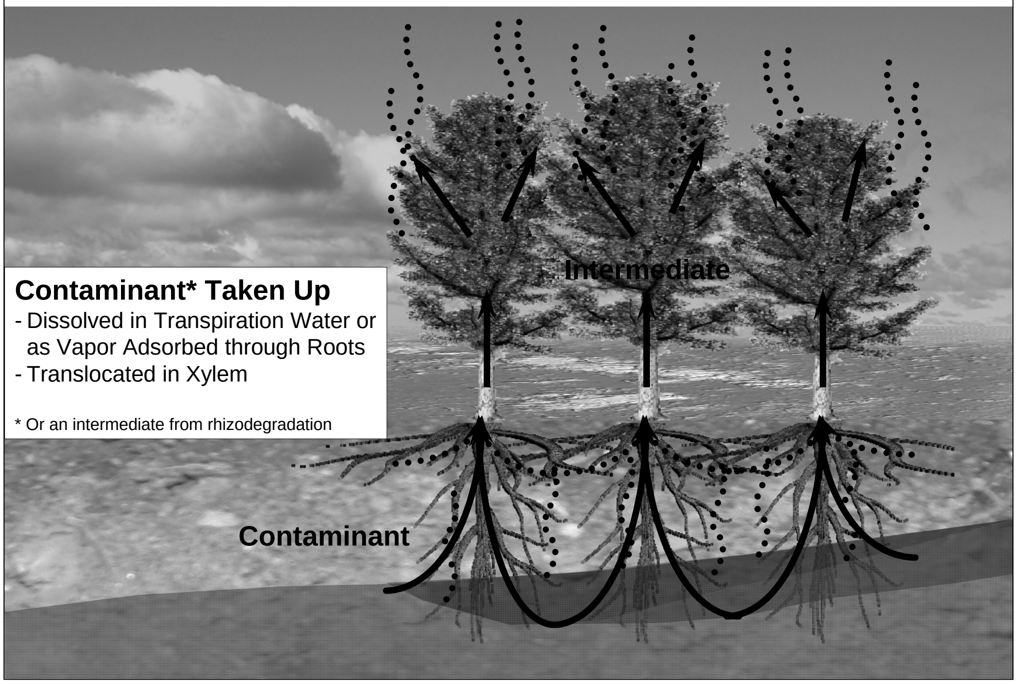
Mechanism: Phytoextraction (PE)

Mechanism: Phytodegradation (PD)

A) Plant Enzymatic Activity and B) Photosynthetic Oxidation



Mechanism: Phytovolatilization (PV)



Mechanisms to Clean Up Goals

Mechanism	Description	Clean Up Goal
Phytosequestration	The ability of plants to sequester certain contaminants into the rhizosphere through exudation of phytochemicals, and on the root through transport proteins and cellular processes	**Containment**
Rhizodegradation	Exuded phytochemicals can enhance microbial biodegradation of contaminants in the rhizosphere	**Remediation by destruction**
Phytohydraulics	The ability of plants to capture and evaporate water off of the plant, and take up and transpire water through the plant	**Containment by controlling hydrology**
Phytoextraction	The ability of plants to take up contaminants into the plant with the transpiration stream	**Remediation by removal of plants**
Phytodegradation	The ability of plants to take up and break down contaminants in the transpiration stream through internal enzymatic activity and photosynthetic oxidation/reduction	**Remediation by destruction**
Phytovolatilization	The ability of plants to take up, translocate, and subsequently transpire volatile contaminants in the transpiration stream	**Remediation by removal through plants**

Containment Applications

Media	Application	Potential Mechanisms	Comments
Soil/Sediment (impacted)	**Phytostabilization Cover** (soil/sediment stabilization)	**Phytosequestration** **Phytoextraction** (no harvesting) **Adsorption** (abiotic) **Precipitation** (abiotic) **Settling/Sedimentation** (abiotic)	Includes sediment stabilization Also controls soil erosion by wind/water
Surface Water (clean)	**Phytostabilization Cover** (infiltration control)	**Phytohydraulics** (ET) **Run-off** (abiotic)	Vertical infiltration control Includes alternative (ET) covers
Surface Water (impacted)	**Pond/Lagoon/Basin** **Riparian Buffer**	**Phytosequestration** **Phytohydraulics** (ET) **Phytoextraction** (no harvesting) **Evaporation** (abiotic) **Infiltration** (abiotic)	Includes wastewater Also controls soil erosion by water run-off
Groundwater (clean)	**Tree Hydraulic Barrier** **Riparian Buffer**	**Phytohydraulics** (ET)	Lateral migration control
Groundwater (impacted)	**Tree Hydraulic Barrier** **Riparian Buffer**	**Phytosequestration** **Phytohydraulics** (ET) **Phytoextraction** (no harvesting)	Lateral migration control

Remediation Applications

Media	Application	Potential Mechanisms	Comments
Soil/Sediment (impacted)	**Phytoremediation Groundcover**	**Rhizodegradation** **Phytoextraction** (with harvesting) **Phytodegradation** **Phytovolatilization** **Biodegradation** (microbial) **Oxidation/Reduction** (abiotic) **Volatilization** (abiotic)	Phytohydraulics (ET) assumed for PE, PD, and PV
Surface Water (impacted)	**Pond/Lagoon/Basin** **Riparian Buffer** **Constructed Treatment Wetland**	**Rhizodegradation** **Phytoextraction** (with harvesting) **Phytodegradation** **Phytovolatilization** **Biodegradation** (microbial) **Oxidation/Reduction** (abiotic) **Volatilization** (abiotic)	Includes wastewater and extracted groundwater Phytohydraulics (ET) assumed for PE, PD, and PV
Groundwater (impacted)	**Phytoremediation Tree Stand** **Riparian Buffer**	**Rhizodegradation** **Phytoextraction** (with harvesting) **Phytodegradation** **Phytovolatilization** **Oxidation/Reduction** (abiotic) **Biodegradation** (microbial)	Phytohydraulics (ET) assumed for PE, PD, and PV

Application: Phytostabilization Cover
Application: Phytoremediation Groundcover

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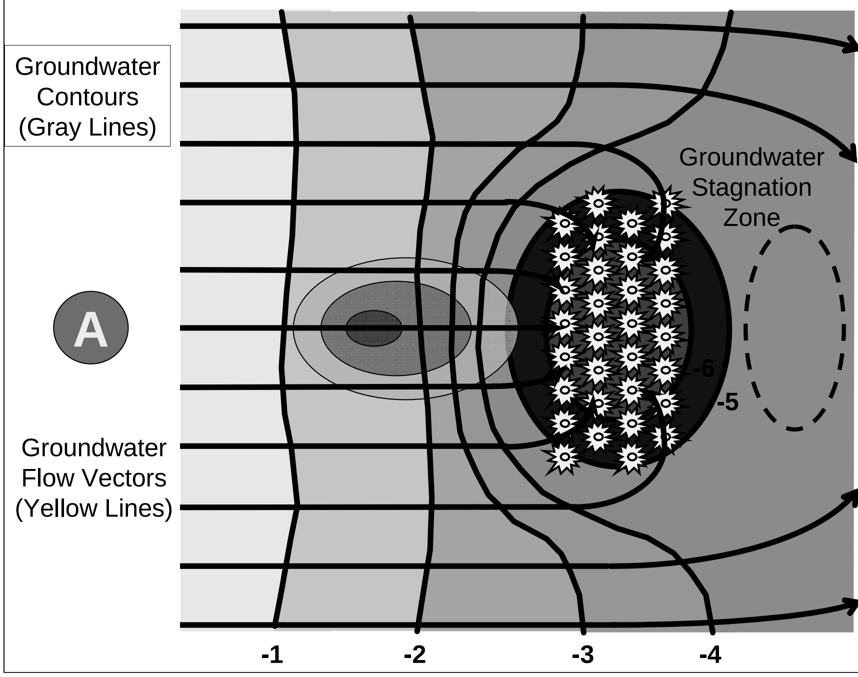
Application: Tree Hydraulic Barrier

A) Downgradient Control



Application: Tree Hydraulic Barrier

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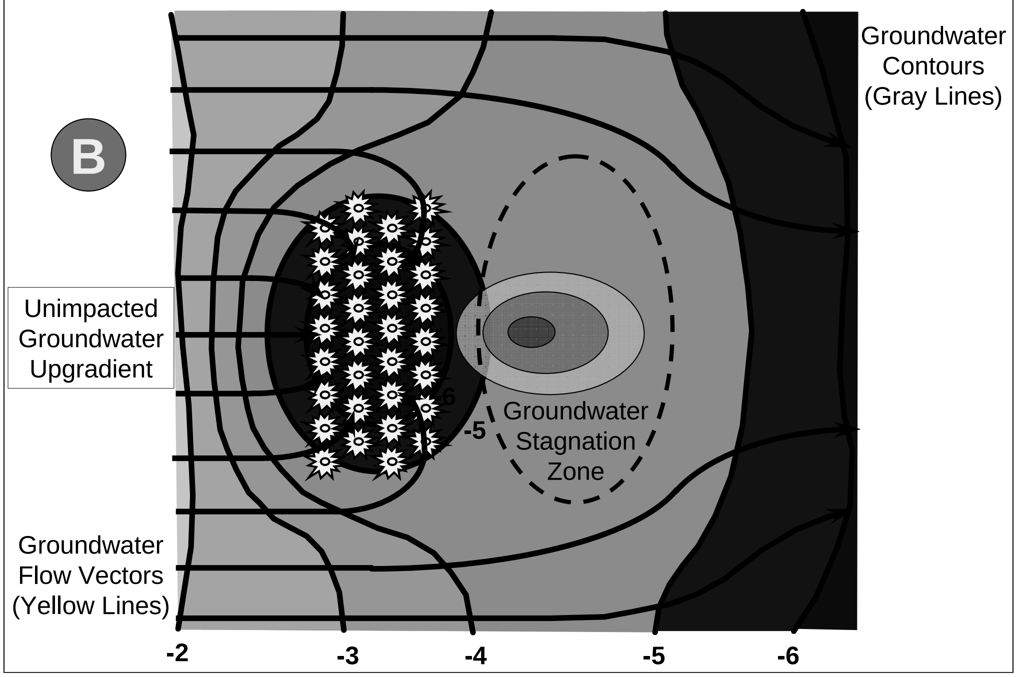
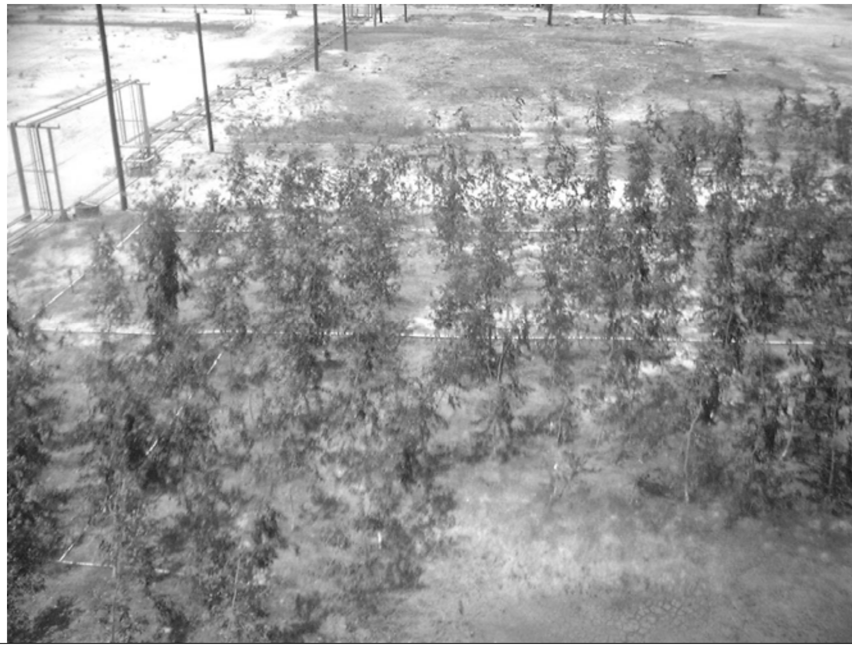
B) Upgradient Control

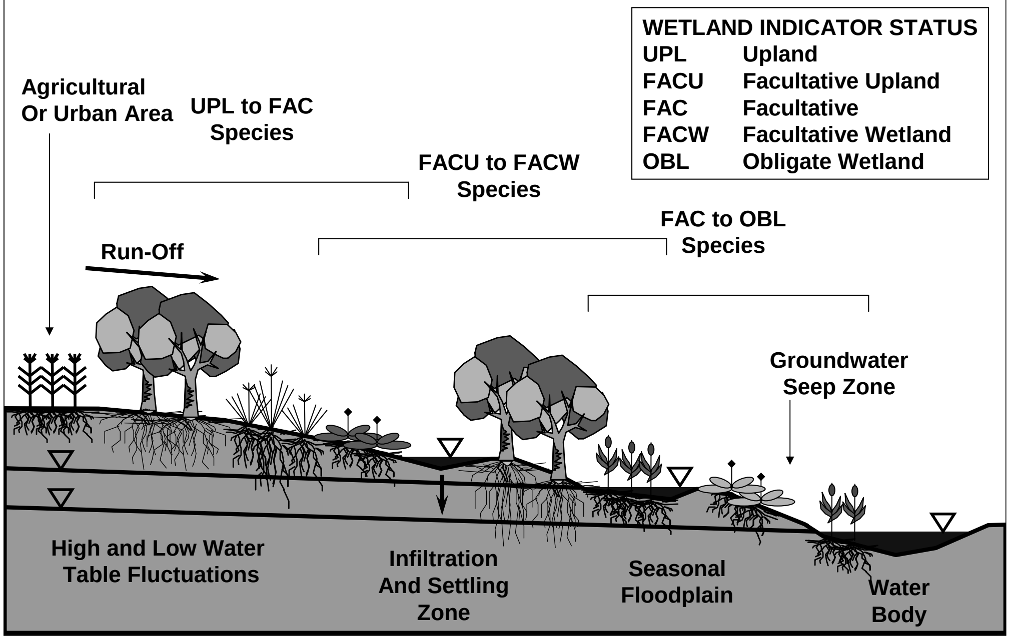


Application: Tree Hydraulic Barrier
Application: Phytoremediation Tree Stand



Application: Pond/Lagoon/Basin
Application: Constructed Treatment Wetland

Application: Riparian Buffer



Riparian Buffers are vegetated area that protect adjacent water resources from non-point source pollution (surface run-off), provide bank stabilization, and habitats for aquatic and other wild life. Wetlands that grow in the edge of a stream also can be considered as Riparian buffers.

Application: Riparian Buffer

List of Advantages

- Considered a green technology and sustainable
- Solar-powered (system itself does not require supplemental energy; although monitoring equipment may)
- Improves air quality and sequesters greenhouse gases
- Minimal air emissions, water discharge, and secondary waste generation
- Inherently controls erosion, runoff, infiltration, and fugitive dust emissions
- Passive and *in-situ*
- Favorable public perception including as an educational opportunity
- Improves aesthetics including reduced noise
- Applicable to remote locations, potentially without utility access (critical utility is a supplemental source of irrigation)
- Can supplement other remediation approaches or as a polishing step
- Can be used to identify and map contamination
- Can be installed as a preventative measure, possibly for leak detection
- Lower maintenance, resilient, and self-repairing
- Creates habitat (can be a disadvantage – attractive nuisance)
- Restores and reclaims land during clean up and upon completion
- Can be cost competitive

List of Show-Stoppers and Limitations

- Space – generally requires large tracts of land
- Depth – limited to rooting depth
- Time – long-term remedial approach
- Contaminant concentration/composition – phytotoxicity
- Fate and Transport – acceptable risk reduction
- Other site growing conditions – plantability
  - Temperature, humidity, precipitation, solar, altitude, season, topography, soil conditions, nutrients, compaction, etc.
  - Suitable species

Plant Degradation of Airborne PCB Congeners

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Dept. of Civil & Environmental Engineering
The University of Iowa

NIEHS SBRP 5th PCB Workshop

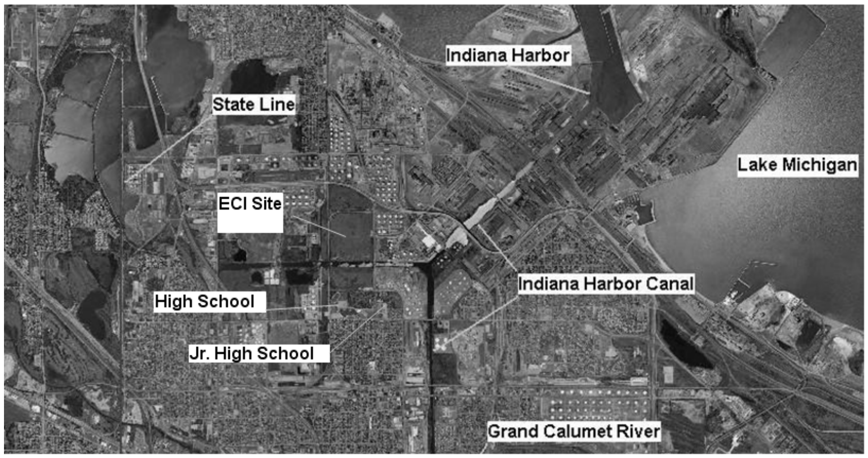
18-21 May, 2008

isbrp
iowa superfund basic research program

Outline of the Talk

1. Introduction
2. PCBs uptake and translocation by plants
3. Evidence of whole plant metabolism of PCBs
4. Gene expression in plants for PCBs
5. Endophytic bacteria

Applications: 1) Intercepting PCBs from Air; 2) Uptake from Dredging Operations and a CDF in East Chicago, IN



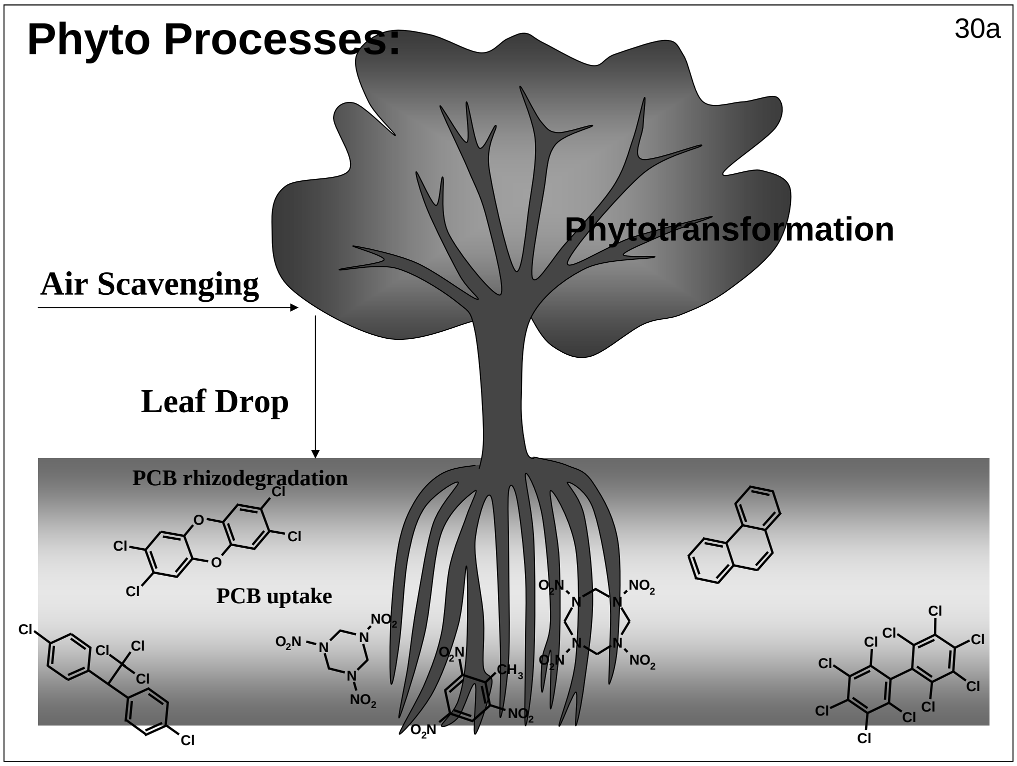
Indiana Harbor

Phytoremediation at a site in Cabin Creek, WV

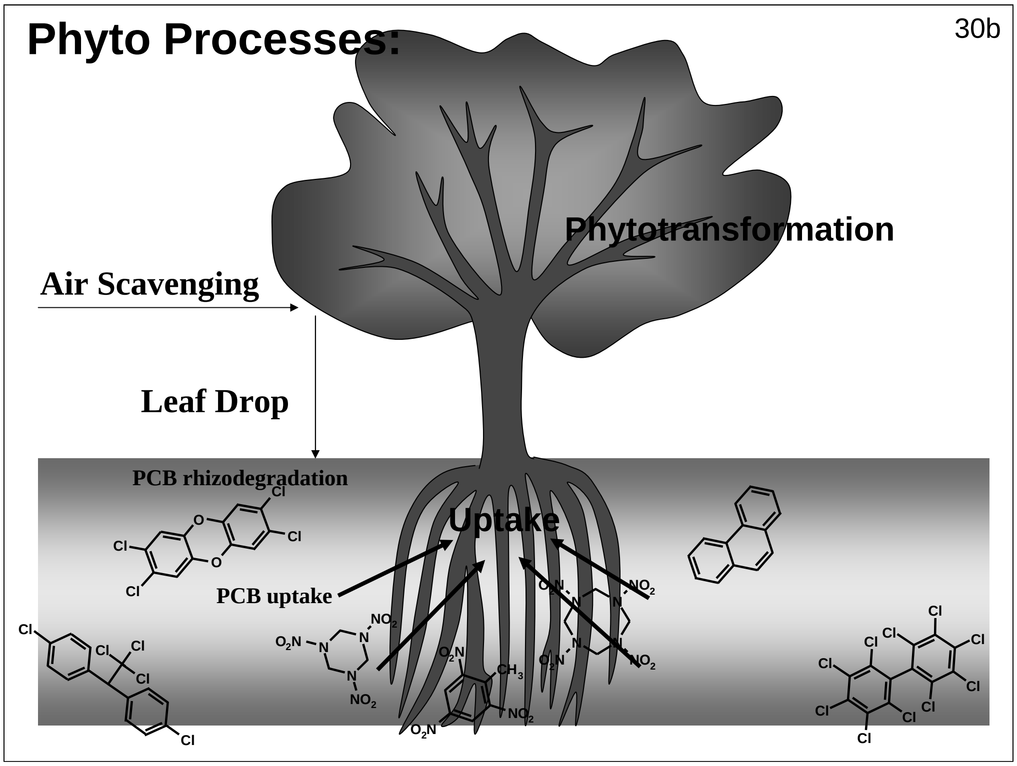
Full Scale Rhizoremediation, 10 ha site

Before: Cabin Creek, West Virginia, 1999 – Former Oil Refinery and Tank Farm contaminated with >5000 mg/kg TPH…

After: in eight years, poplar trees were well established and soil concentrations have decreased by 75%



Phyto Processes:
30a
Phytotransformation
Air Scavenging
Leaf Drop
PCB rhizodegradation
PCB uptake


Phyto Processes:
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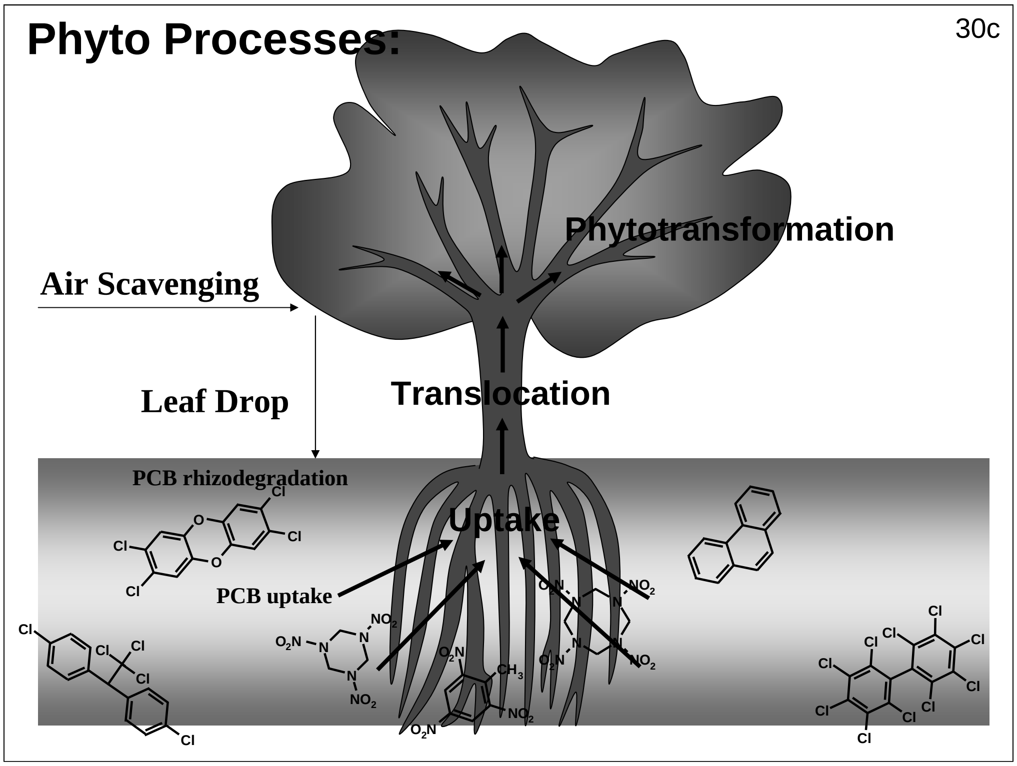
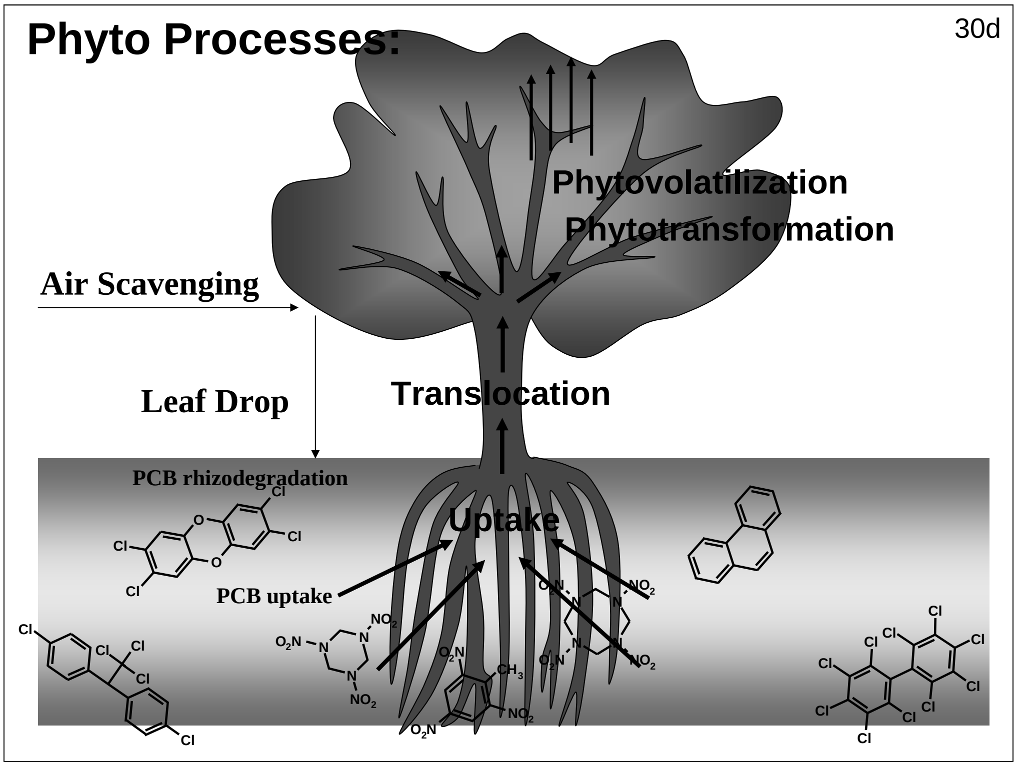
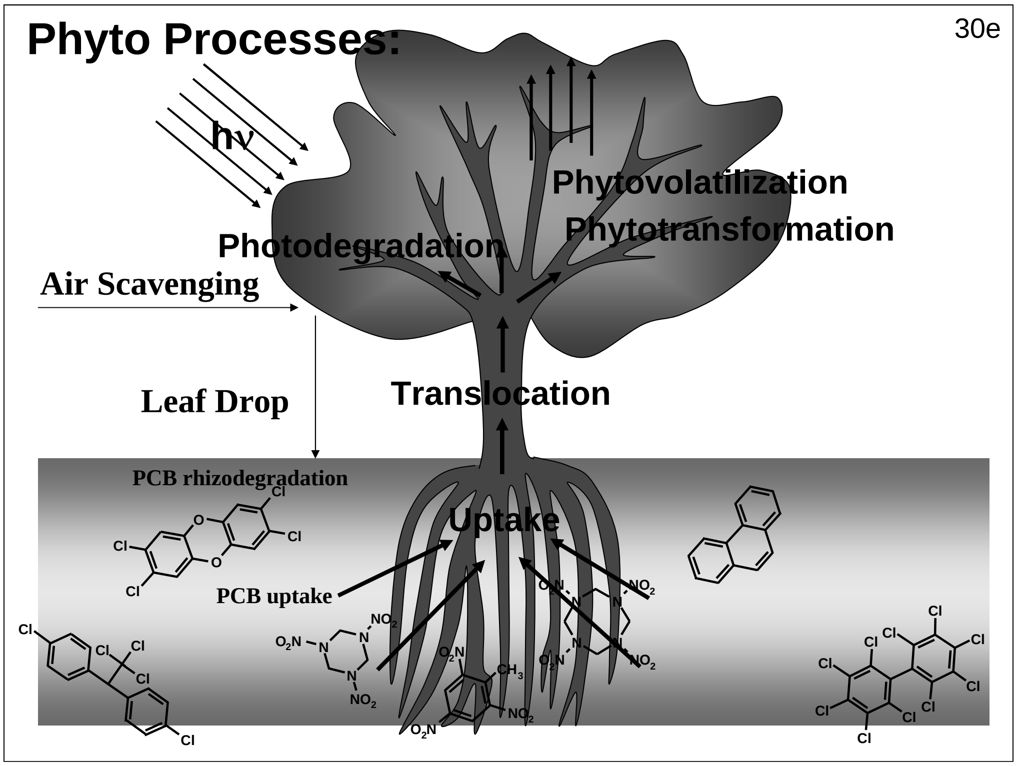
Uptake
PCB uptake



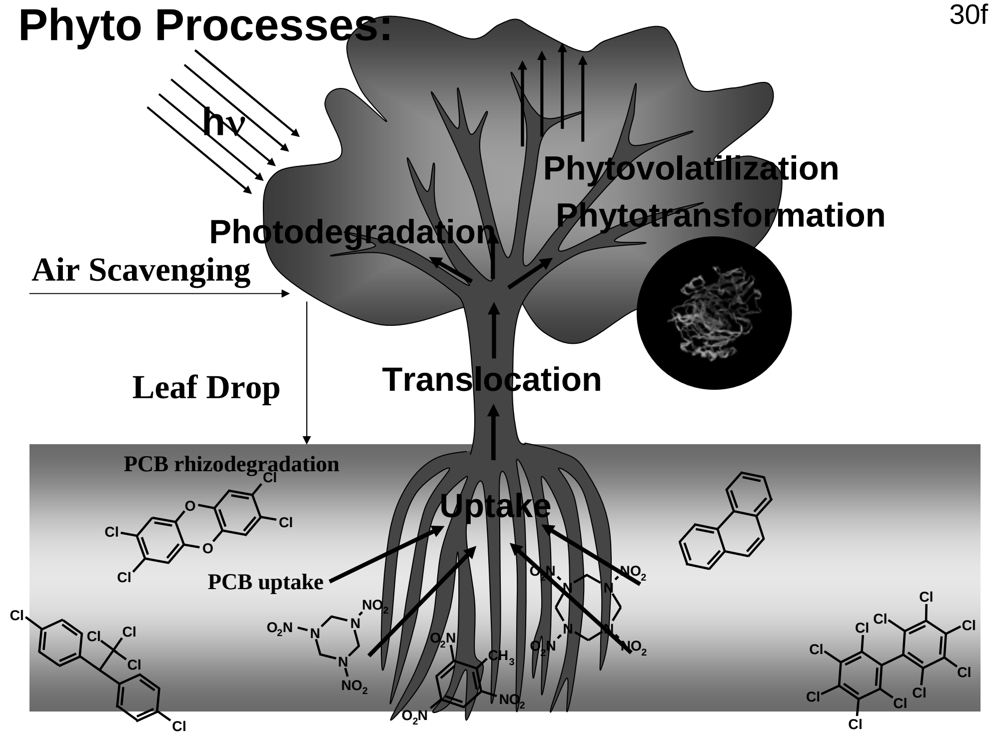
Phyto Processes:
30c
Phytotransformation
Air Scavenging
Leaf Drop
Translocation
PCB rhizodegradation
Uptake
PCB uptake


Phyto Processes:
30d
Phytovolatilization
Phytotransformation
Air Scavenging
Leaf Drop
Translocation
PCB rhizodegradation
Uptake
PCB uptake


Phyto Processes:
30e
hν
Phytovolatilization
Phytotransformation
Photodegradation
Air Scavenging
Leaf Drop
Translocation
PCB rhizodegradation
Uptake
PCB uptake

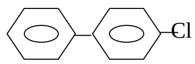
Phyto Processes:

30f



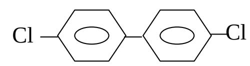
PCB congeners of interest

PCB 3



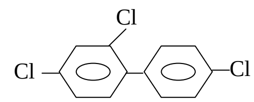
Mono, para

PCB 15



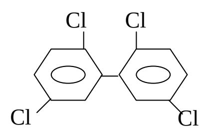
Di-, Chicago air, p-p’ may be easy to degrade

PCB 28



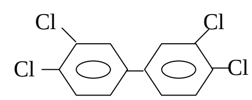
Tri-, Chicago air

PCB 52



Tetra-, Chicago air, PXR, non-coplanar, CYP 3A inducer

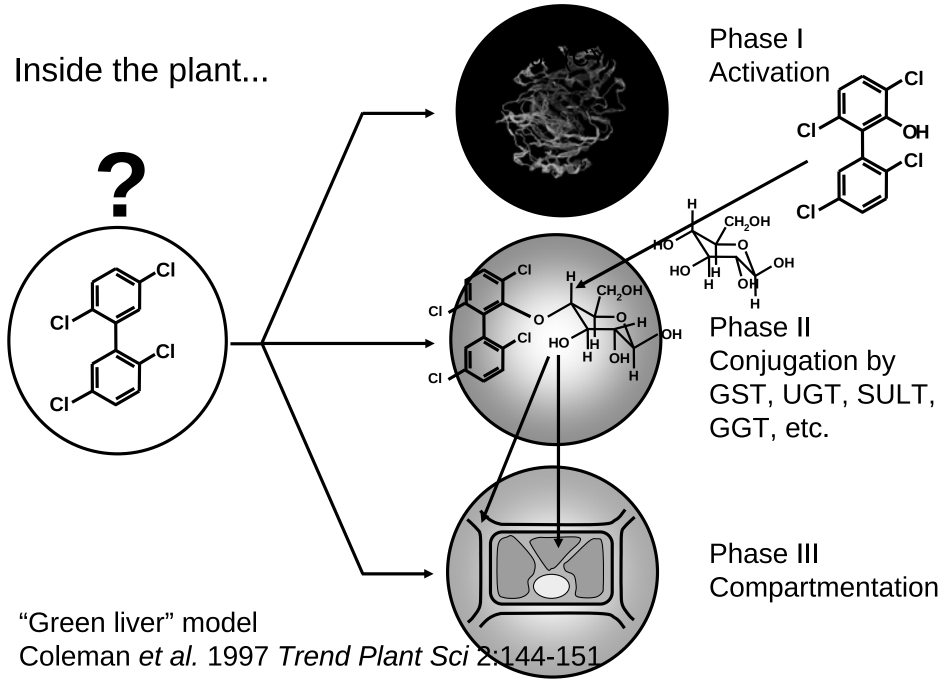
PCB 77



Tetra-, coplanar, toxic, CYP 1A inducer, AhR

PCB Phytoremediation Mechanism – Green Liver Model

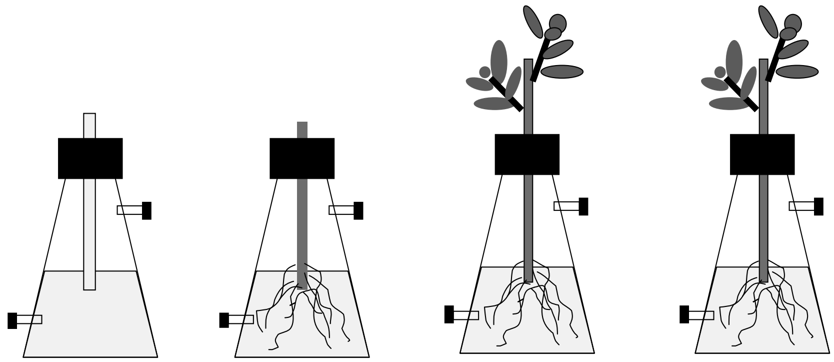
Inside the plant...



"Green liver" model
Coleman *et al.* 1997 *Trend Plant Sci* 2:144-151

33a

Exposure experiment design

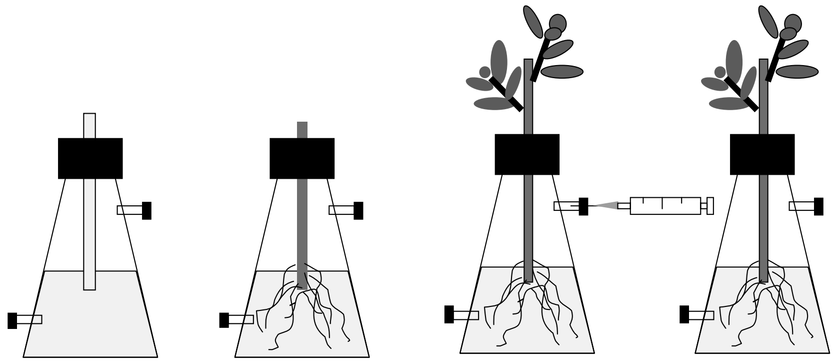


Unplanted controls	Excised controls	Treatments	Controls
PCBs spiked Glass rod	PCBs spiked Cutting tops were cut off	PCBs spiked Whole plants	No PCBs Whole plants

	PCB 3	PCB 15	PCB 28	PCB 52	PCB77
Exposure C (mg/kg)	1	0.1	0.05	0.05	0.01

Exposure time: 2, 5, 10, 15, 20 days

Exposure experiment design



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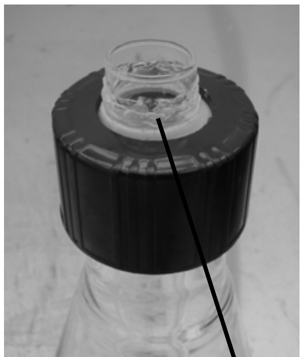
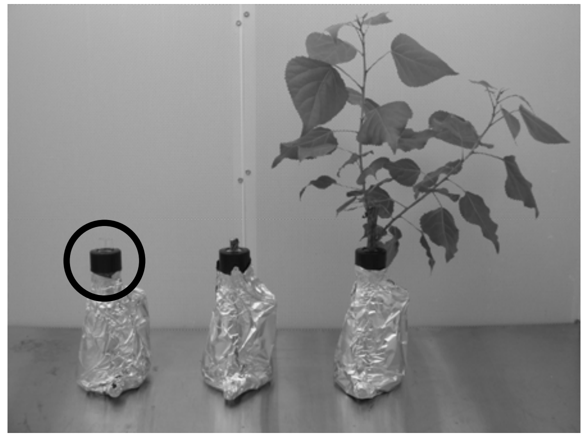
Hydroponic exposure system





100% silicon sealant

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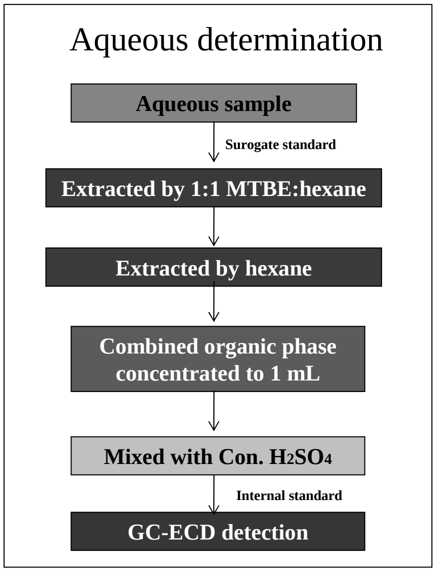
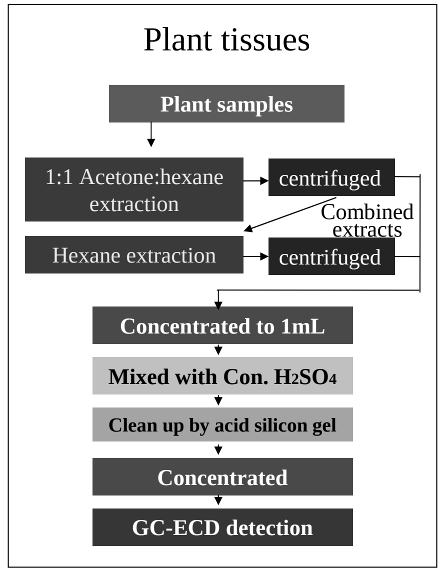
Analysis methods for PCBs in aqueous and plant samples





PCB14, recovery 85-115%

PCB14, recovery 75-104%

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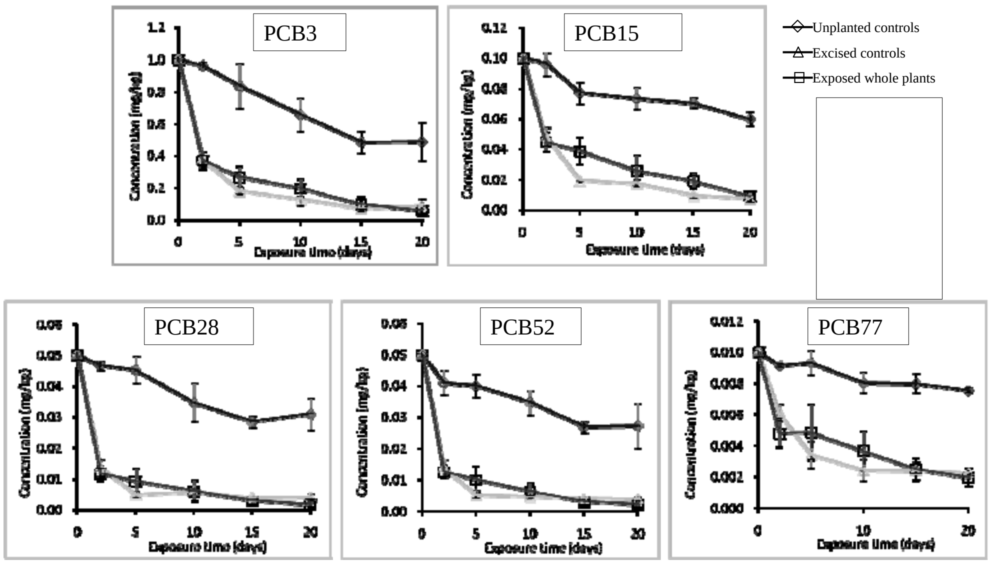
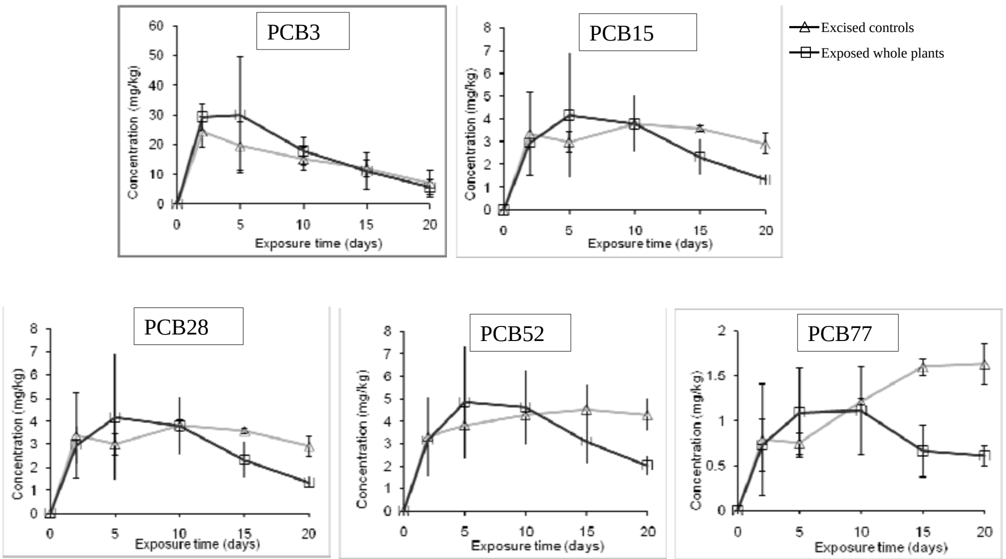
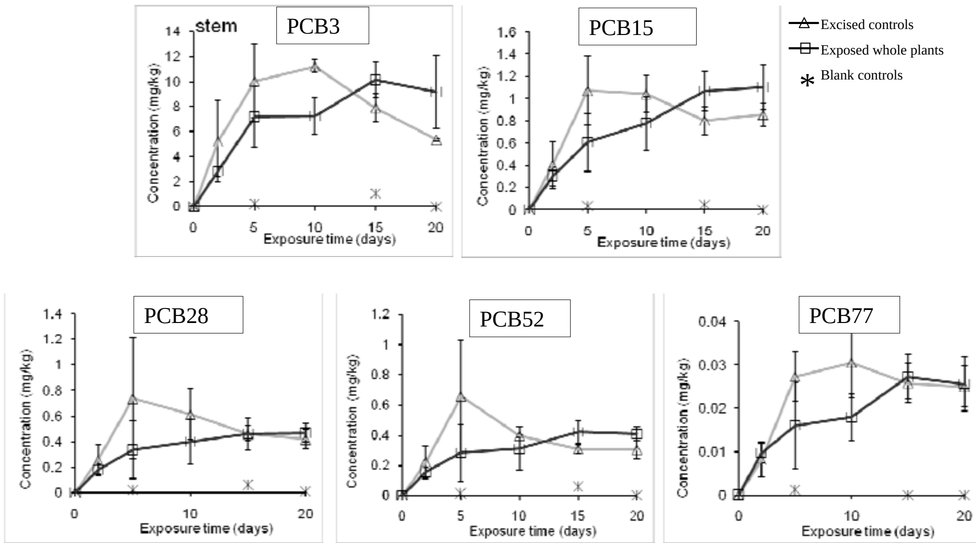
Removal of PCBs from hydroponic solution by whole plants



Uptake and Degradation of PCBs by Roots (whole plants and excised roots)



Uptake and Sorption of PCBs associated with main stem



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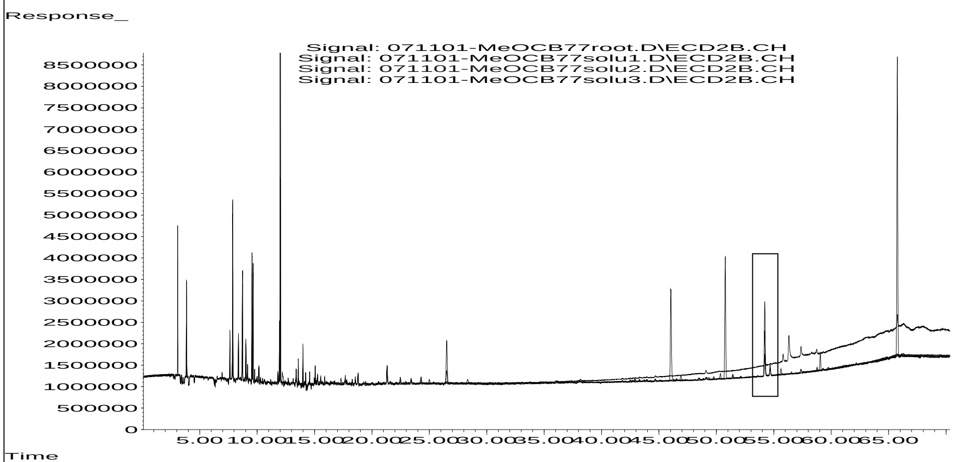
Hydroxylation of PCB 77 by poplar plant



All samples including solution and roots have this peak. Compared with the OH-PCB77 standards, it has same retention time as that 4OH-PCB79 and 6OH-PCB77.

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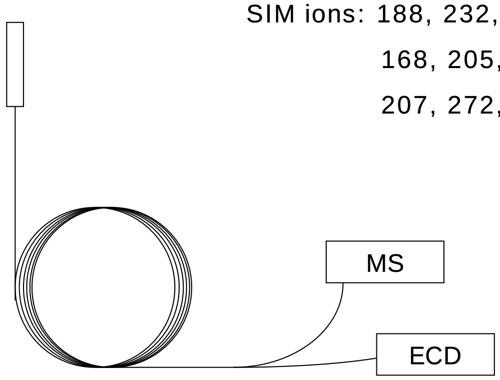
GC/ECD/MS

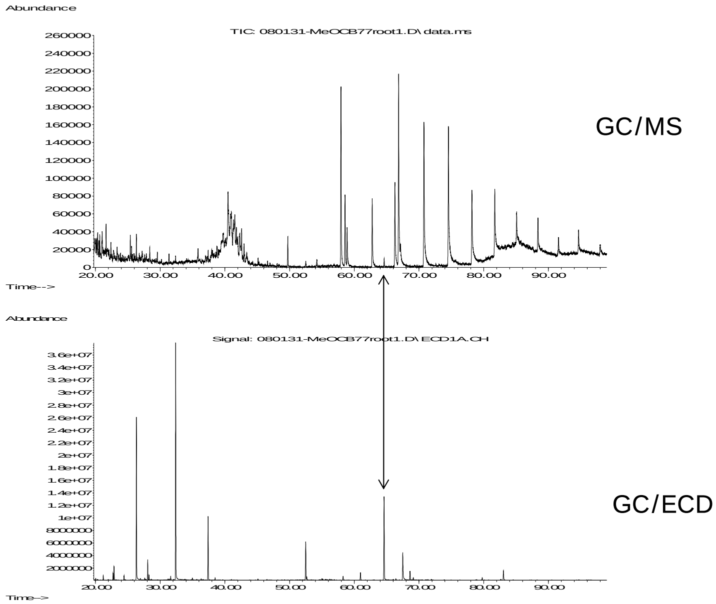
MS SIM mode

SIM ions: 188, 232, 255.95, 289.90
168, 205, 220, 248, 233,
207, 272, 270, 279, 307, 322

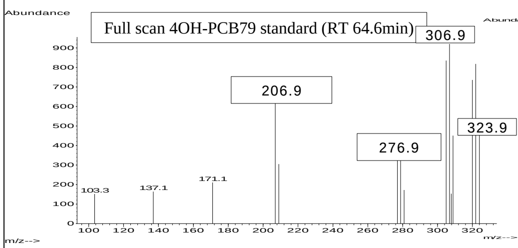
GC injector

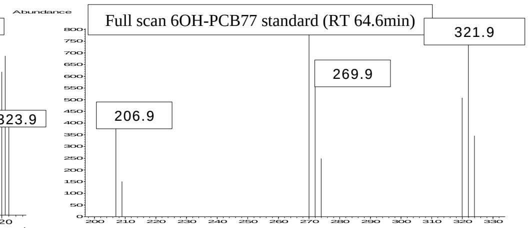


Hydroxylation of PCB 77 by poplar plant

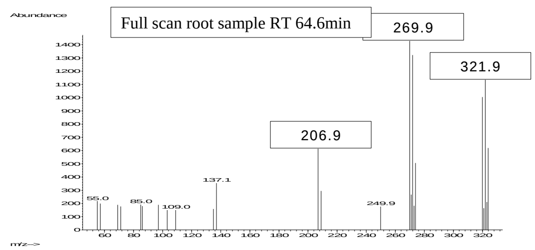


Hydroxylation of PCB 77 by poplar plant





The Mass spectrum of roots sample is match with 6OH-PCB77



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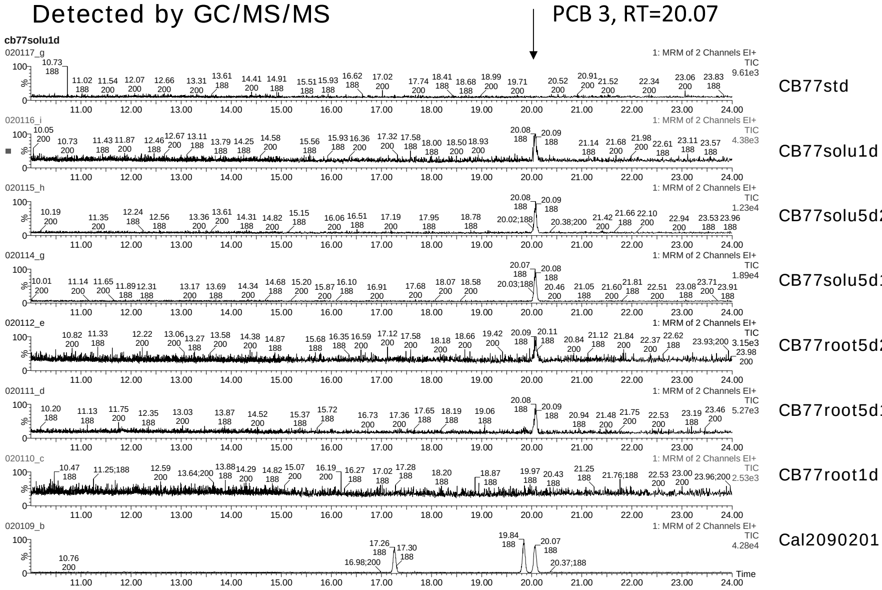
Dechlorination of PCB 77 → PCB 3

Detected by GC/MS/MS



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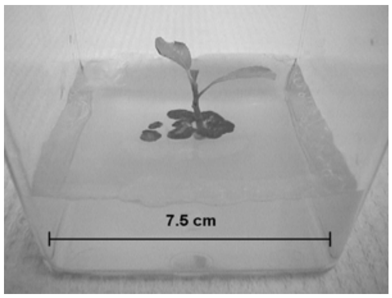
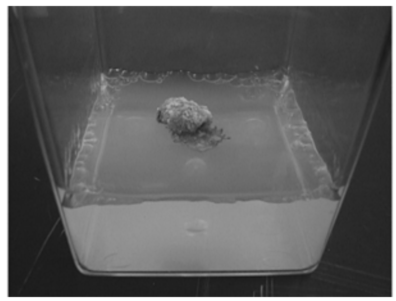
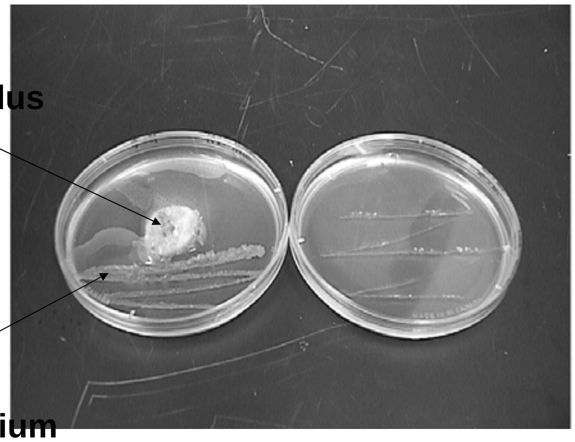
Can endophytic bacteria be exploited for phytoremediation?

Plant tissue cultures show bacterial contaminant which proves to be a novel organism (Van Aken and Schnoor, *AEM*, 2004)

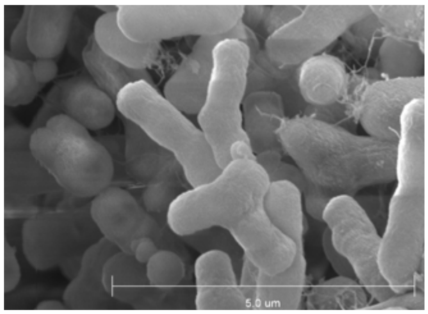


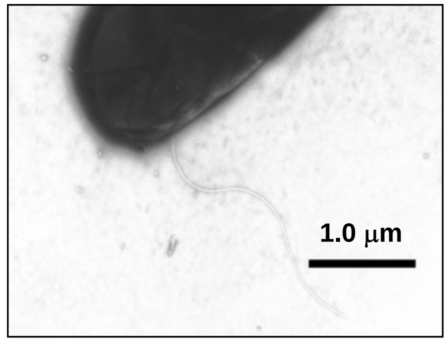


Symbiosis of plants with endophytic bacteria



Methylobacterium populi sp. BJ001





Van Aken and Schnoor 2004

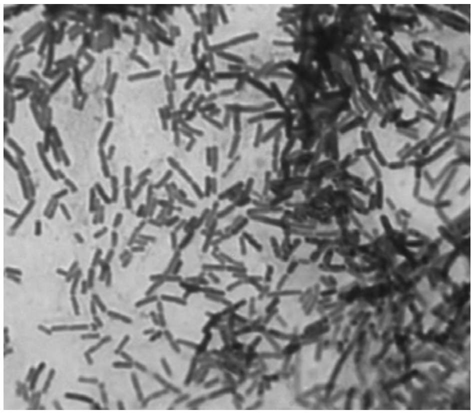
Sequencing *M. populi* BJ001 is ongoing by DOE

Another endophytic bacteria isolated from poplar plants

Microorganism from surface sterilized poplar leaf tissues.

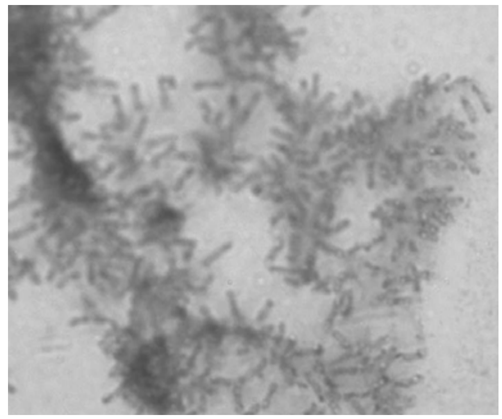
Grows on “NS (non-specific) media”

C-source: glucose, fructose, succinate.



100x, gram stain, endophyte mixed with BJ001 pure culture. Endophyte was purified from surface sterilized poplar leaf extracts.

100x, gram stain, BJ001 only.



Conclusions (1)

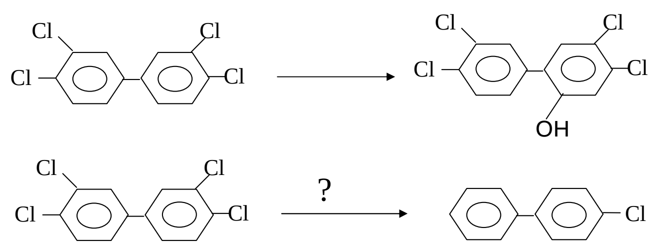
Phytoremediation may be a useful method to uptake and degrade PCBs from soil and groundwater at cdfs or other “hot spot” locations

- *Populus* uptakes and translocates lightly chlorinated PCBs (PCB3 and PCB15 translocated to shoots) but not the more chlorinated (high log K_{ow}) congeners
- Accumulation of PCBs on roots is linearly correlated with log K_{ow}, but not with transpiration
- Woody stems accumulate more PCBs than leaves or xylem; roots seem to degrade PCB congeners
- CYP 189, 567 and GST 173 genes in poplar may be involved in the metabolism of PCBs.

Conclusions (2)

The roots of hybrid poplar can *in vivo* biotransform co-planar PCB77. Hydroxylated metabolite 6OH-PCB77 and dechlorinated metabolite PCB 3 were detected in roots (and hydroponic solution).

Switchgrass can not hydroxylate PCB77.



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Conclusions (3)

Endophytic bacteria (and rhizosphere bacteria) may be useful in speeding the rate of degradation of PCBs in phytoremediation

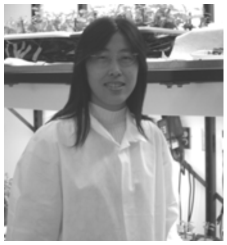
-- *Methylobacterium populum* BJ001

-- *Bacillus licheniformis* strain

-- other bacteria

-- fungal species

Acknowledgments



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Thank you!

Registration opens November 1st for the second and third Phytoremediation web seminars:

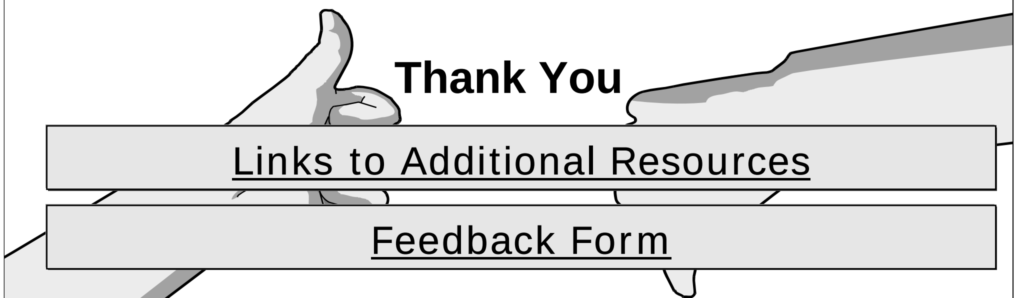
"Phytoremediation of Organics" – November 12th, and

"Phytoremediation of Metals" – November 25th

For more information and archives of this and other Risk e Learning web seminars please refer to the Superfund Basic Research Program Risk e Learning web page:

http://tools.niehs.nih.gov/sbrp/risk_elearning/

After viewing the links to additional resources, please complete our online feedback form.



Thank You

Links to Additional Resources

Feedback Form

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