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
Phytotechnologies
Mechanisms and Applications
Advantages and Limitations

David Tsao, Ph.D
BP Remediation Management
Plant Physiological Processes

Photosynthesis (light to chemical energy conversion)

Transpiration Gas Exchange (CO₂ in; O₂ + H₂O out)

Respiration (production of metabolic energy)

Translocation (water column transport)

Phloem (Down)

Root Exudation (release of phytochemicals)

Xylem (Up)

Water and Inorganic Nutrient Uptake
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 O₂ per m² 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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Root Turnover:

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- Portion of the root system gets **sloughed off** because it is not needed to maintain the dormant plant
Mechanism: Phytosequestration (PS)

B) Transport Protein Inhibition and C) Vacuolar Storage

- Essential Element or Analogous Contaminant
- Opposing Contaminant
- Irreversibly Bound

Transport Protein Conformation (Uptake)
- Transport into Xylem (Translocation)
- Transport into Cell Vacuole (Sequestration)
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Root Turnover:
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Physiology: Evapotranspiration (ET)
Mechanism: Phytohydraulics (PH)

Rain Interception Capacity

A) START OF RAIN EVENT
- Rain Interception by Canopy
- Root-Zone

B) DURING RAIN EVENT
- Interception Capacity Exceeded
- Shallow Infiltration
- Surface Runoff

C) AFTER RAIN EVENT
- Evapotranspiration
- Water Storage in Soil
Physiology: Transpiration
Contaminant* Taken Up
- Dissolved in Transpiration Water or as Vapor Adsorbed through Roots
- Translocated in Xylem

* Or an intermediate from rhizodegradation

Mechanism: Phytoextraction (PE)
Mechanism: Phytodegradation (PD)

A) Plant Enzymatic Activity and B) Photosynthetic Oxidation

Contaminant* Taken Up
- Dissolved in Transpiration Water or
- as Vapor Adsorbed through Roots
- Translocated in Xylem

* Or an intermediate from rhizodegradation

CO₂ + H₂O
**Mechanism: Phytovolatilization (PV)**

*Contaminant* Taken Up
- Dissolved in Transpiration Water or as Vapor Adsorbed through Roots
- Translocated in Xylem

* Or an intermediate from rhizodegradation
# Mechanisms to Clean Up Goals

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Description</th>
<th>Clean Up Goal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytosequestration</td>
<td>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</td>
<td>Containment</td>
</tr>
<tr>
<td>Rhizodegradation</td>
<td>Exuded phytochemicals can enhance microbial biodegradation of contaminants in the rhizosphere</td>
<td>Remediation by destruction</td>
</tr>
<tr>
<td>Phytohydraulics</td>
<td>The ability of plants to capture and evaporate water off of the plant, and take up and transpire water through the plant</td>
<td>Containment by controlling hydrology</td>
</tr>
<tr>
<td>Phytoextraction</td>
<td>The ability of plants to take up contaminants into the plant with the transpiration stream</td>
<td>Remediation by removal of plants</td>
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<tr>
<td>Phytodegradation</td>
<td>The ability of plants to take up and break down contaminants in the transpiration stream through internal enzymatic activity and photosynthetic oxidation/reduction</td>
<td>Remediation by destruction</td>
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<tr>
<td>Phytovolatilization</td>
<td>The ability of plants to take up, translocate, and subsequently transpire volatile contaminants in the transpiration stream</td>
<td>Remediation by removal through plants</td>
</tr>
</tbody>
</table>
## Containment Applications

<table>
<thead>
<tr>
<th>Media</th>
<th>Application</th>
<th>Potential Mechanisms</th>
<th>Comments</th>
</tr>
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<tbody>
<tr>
<td>Soil/Sediment (impacted)</td>
<td>Phytostabilization Cover (soil/sediment stabilization)</td>
<td>Phytosequestration&lt;br&gt;Phytoextraction (no harvesting)&lt;br&gt;Adsorption (abiotic)&lt;br&gt;Precipitation (abiotic)&lt;br&gt;Settling/Sedimentation (abiotic)</td>
<td>Includes sediment stabilization&lt;br&gt;Also controls soil erosion by wind/water</td>
</tr>
<tr>
<td>Surface Water (clean)</td>
<td>Phytostabilization Cover (infiltration control)</td>
<td>Phytohydraulics (ET)&lt;br&gt;Run-off (abiotic)</td>
<td>Vertical infiltration control&lt;br&gt;Includes alternative (ET) covers</td>
</tr>
<tr>
<td>Surface Water (impacted)</td>
<td>Pond/Lagoon/Basin Riparian Buffer</td>
<td>Phytosequestration&lt;br&gt;Phytoextraction (no harvesting)&lt;br&gt;Evaporation (abiotic)&lt;br&gt;Infiltration (abiotic)</td>
<td>Includes wastewater&lt;br&gt;Also controls soil erosion by water run-off</td>
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<td>Groundwater (clean)</td>
<td>Tree Hydraulic Barrier Riparian Buffer</td>
<td>Phytohydraulics (ET)</td>
<td>Lateral migration control</td>
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### Remediation Applications

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<td>Soil/Sediment</td>
<td>Phytoremediation</td>
<td>Rhizodegradation</td>
<td>Phytohydraulics (ET) assumed for PE, PD, and PV</td>
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<tr>
<td>(impacted)</td>
<td>Groundcover</td>
<td>Phytoextraction (with harvesting)</td>
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<td></td>
<td></td>
<td>Phytoextraction</td>
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<td></td>
<td></td>
<td>Phytodegradation</td>
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<tr>
<td></td>
<td></td>
<td>Phytovolatilization</td>
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<td></td>
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<td>Biodegradation (microbial)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Oxidation/Reduction (abiotic)</td>
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<tr>
<td></td>
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<td>Volatilization (abiotic)</td>
<td></td>
</tr>
<tr>
<td>Surface Water</td>
<td>Pond/Lagoon/Basin</td>
<td>Rhizodegradation</td>
<td>Includes wastewater and extracted groundwater</td>
</tr>
<tr>
<td>(impacted)</td>
<td>Riparian Buffer</td>
<td>Phytoextraction (with harvesting)</td>
<td>Phytohydraulics (ET) assumed for PE, PD, and PV</td>
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<td></td>
<td>Constructed Treatment</td>
<td>Phytoextraction</td>
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<td>Wetland</td>
<td>Phytoextraction</td>
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<td>Phytodegradation</td>
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<td>Phytohydraulics (ET) assumed for PE, PD, and PV</td>
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<tr>
<td>(impacted)</td>
<td>Tree Stand</td>
<td>Phytoextraction (with harvesting)</td>
<td></td>
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<tr>
<td></td>
<td>Riparian Buffer</td>
<td>Phytoextraction</td>
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<td>Oxidation/Reduction (abiotic)</td>
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</table>
Application: Phytostabilization Cover
Application: Phytoremediation Groundcover
Application: Tree Hydraulic Barrier

A) Downgradient Control

Groundwater Contours (Gray Lines)

Groundwater Flow Vectors (Yellow Lines)

Groundwater Stagnation Zone
Application: Tree Hydraulic Barrier

B) Upgradient Control

Groundwater Flow Vectors (Yellow Lines)

Groundwater Contours (Gray Lines)

Unimpacted Groundwater Upgradient

Groundwater Stagnation Zone
Application: Tree Hydraulic Barrier
Application: Phytoremediation Tree Stand
Application: Pond/Lagoon/Basin
Application: Constructed Treatment Wetland
Riparian Buffers are vegetated areas 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

Jiyan Liu*, C. Krahe, R. Meggo, B. Van Aken, J. Schnoor
W. M. Keck Phytotechnology Laboratory
Dept. of Civil & Environmental Engineering
The University of Iowa

*NIEHS SBRP 5th PCB Workshop
18-21 May, 2008
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
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:

Air Scavenging

Leaf Drop

Phytotransformation

PCB rhizodegradation

PCB uptake
Phyto Processes:

- Air Scavenging
- Leaf Drop
- PCB rhizodegradation
- Uptake

Phytotransformation
Phyto Processes:

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

- Uptake
- Translocation
- Phytovolatilization
- Phytotransformation

Air Scavenging → Leaf Drop → Translocation

PCB rhizodegradation
PCB uptake

30d
Phyto Processes:
- Uptake
- Photodegradation
- Translocation
- Phytovolatilization
- Phytotransformation
- Air Scavenging
- Leaf Drop
- PCB rhizodegradation
- PCB uptake
## PCB congeners of interest

<table>
<thead>
<tr>
<th>PCB 3</th>
<th>PCB 15</th>
<th>PCB 28</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="PCB 3" /></td>
<td><img src="image2" alt="PCB 15" /></td>
<td><img src="image3" alt="PCB 28" /></td>
</tr>
<tr>
<td>Mono, para</td>
<td>Di-, Chicago air, p-p' may be easy to degrade</td>
<td>Tri-, Chicago air</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PCB 52</th>
<th>PCB 77</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image4" alt="PCB 52" /></td>
<td><img src="image5" alt="PCB 77" /></td>
</tr>
<tr>
<td>Tetra-, Chicago air, PXR, non-coplanar, CYP 3A inducer</td>
<td>Tetra-, coplanar, toxic, CYP 1A inducer, AhR</td>
</tr>
</tbody>
</table>
Inside the plant...

Phase I
Activation

Phase II
Conjugation by GST, UGT, SULT, GGT, etc.

Phase III
Compartmentation

“Green liver” model
Exposure experiment design

<table>
<thead>
<tr>
<th>PCBs spiked</th>
<th>PCBs spiked</th>
<th>PCBs spiked</th>
<th>PCBs spiked</th>
<th>No PCBs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass rod</td>
<td>Cutting tops were cut off</td>
<td>Whole plants</td>
<td>Whole plants</td>
<td>Whole plants</td>
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<tr>
<th>PCB 3</th>
<th>PCB 15</th>
<th>PCB 28</th>
<th>PCB 52</th>
<th>PCB77</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>0.05</td>
<td>0.05</td>
<td>0.01</td>
</tr>
</tbody>
</table>

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

Unplanted controls
- PCBs spiked
- Glass rod

Excised controls
- PCBs spiked
- Cutting tops were cut off

Treatments
- PCBs spiked
- Whole plants

Controls
- No PCBs
- Whole plants

<table>
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<tr>
<th>Exposure C (mg/kg)</th>
<th>PCB 3</th>
<th>PCB 15</th>
<th>PCB 28</th>
<th>PCB 52</th>
<th>PCB 77</th>
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Exposure time: 2, 5, 10, 15, 20 days
Hydroponic exposure system

100% silicon sealant
Analysis methods for PCBs in aqueous and plant samples

**Aqueous determination**
- Aqueous sample
  - Surogate standard
  - Extracted by 1:1 MTBE:hexane
  - Extracted by hexane
  - Combined organic phase concentrated to 1 mL
  - Mixed with Con. H₂SO₄
  - Internal standard
  - GC-ECD detection

**Plant tissues**
- Plant samples
  - 1:1 Acetone:hexane extraction
  - Centrifuged
  - Combined extracts
  - Centrifuged
  - Hexane extraction
  - Concentrated to 1 mL
  - Mixed with Con. H₂SO₄
  - Clean up by acid silicon gel
  - Concentrated
  - GC-ECD detection

PCB14, recovery 85-115%
PCB14, recovery 75-104%
Removal of PCBs from hydroponic solution by whole plants

- PCB3
- PCB52
- PCB28
- PCB15
- PCB77

Unplanted controls
Excised controls
Exposed whole plants
Uptake and Degradation of PCBs by Roots (whole plants and excised roots)

- PCB3
- PCB15
- PCB28
- PCB52
- PCB77

- Exposed whole plants
- Excised controls
Uptake and Sorption of PCBs associated with main stem

- PCB3
- PCB15
- PCB28
- PCB52
- PCB77

*Excised controls*  
*Exposed whole plants*  
*Blank controls*
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.
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
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
Dechlorination of PCB 77 → PCB 3

Detected by GC/MS/MS

PCB 3, RT=20.07

CB77std
CB77solu1d
CB77solu5d2
CB77solu5d1
CB77root5d2
CB77root5d1
CB77root1d
Cal2090201
*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

Poplar callus

Symbiotic red bacterium
**Methylobacterium populi** sp. BJ001

Sequencing *M. populi* BJ001 is ongoing by DOE

Van Aken and Schnoor 2004
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.
Endophyte was purified from surface sterilized poplar leaf extracts.
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.
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

Many thanks to:
Hans Lehmler (Synthesis Core);
Keri Hornbuckle, Craig Just, Collin Just, and Dingfei Hu (Analytical Core)
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.