

# Mechanisms of Phytoremediation of Organics

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## Types of phytoremediation of organic compounds

Rhizosphere degradation

By increased microbial biomass

By aeration of the subsurface

Disturbance effect

By induction of specific activities

Petroleum hydrocarbons, PAHs, PCBs, TCE?

By plant uptake and degradation by plant metabolic activities

Cytochrome P450s – Involved in pesticide detoxification

Lignin peroxidases – may be extracellular

Limited to less hydrophobic organics which are available for plant uptake

TCE, pentachlorophenol, TNT, atrazine

## Plant uptake and degradation of trichloroethylene, carbon tetrachloride and perchloroethylene

Laboratory Results at the University of Washington<sup>1-3</sup>

*Oxidation of Chlorinated Hydrocarbons by Pure cultures of Poplar Cells in Pure Culture*

*Populus trichocarpa* x *P. deltoides* H11-11

TCE & CT oxidized to CO<sub>2</sub> and fixed carbon from 14C-TCE, CT, and perchloroethylene (PCE)

Oxidized metabolites were detected in cell cultures exposed to TCE:the

Chloral, trichloroethanol, dichloroacetic acid, trichloroacetic acid

CT was reduced to chloroform, then to CO<sub>2</sub>.

Similar to metabolites from transformation of CT and TCE by mammalian cytochrome P450s.

## Transformation of TCE by Pure Cultures of Poplar Cells

		TCE, μg/g	Trichloro -ethanol, μg/g	Dichloroacetic acid, μg/g	Trichloroacetic acid, μg/g	%Non- extractable*	%CO <sub>2</sub> *
<b>Controls</b>	<b>Pellet</b>	ND	ND	ND	ND	NA	NA
	<b>Supernatant</b>	ND	ND	ND	ND	NA	NA
	<b>Total without cells</b>	NA	NA	NA	NA	NA	ND
<b>Exposed</b>	<b>Pellet</b>	ND	0.07 ±0.01	26 ±19	ND	NA	NA
	<b>Supernatant</b>	1.00 ±1.41	0.44 ±0.46	0.99 ±0.86	0.03 ±0.02	NA	NA
	<b>Total with Cells</b>	NA	NA	NA	NA	0.15 ±0.07	1.5 ±0

Averages of 2 or 3 observations ± standard deviation of the mean.

NA = data not available or not applicable, ND = none detected.

Axenic Poplar Cells Growing in Suspension Cultures Oxidize Carbon Tetrachloride to CO<sub>2</sub> and Fix Its Carbon in Cell Tissue

<b>Fraction</b>	<b>Dead cells</b>	<b>Living cells</b>
Recovered as CO <sub>2</sub>	0.3% ± 0.3	1.4% ± 0.1
In cell biomass	1.1% ± 0.5	3.% ± 0.1
Remaining in media	12.6% ± 5.5	6.4% ± 3.7
Untransformed CCl <sub>4</sub>	87.7% ± 33.	81.1% ± 11.1
Total recovery	101.6% ± 33.5	92.% ± 11.7

### **Chloride Ion Accumulation in Hydroponic Cultures of Poplar Exposed to TCE and TCA**

	Cl <sup>-</sup> with plant & TCE or TCA, mg/L	Cl <sup>-</sup> with plant only, mg/L	Cl <sup>-</sup> with TCE or TCA, no plant, mg/L	Cl <sup>-</sup> from TCE or TCA degradation by plant, mg/L
TCE	0.93	0.25	0.25	0.43
TCA	0.78	0.22	0	0.56

After 13 days incubation, repeated dosing with 15 mg/L TCE or TCA

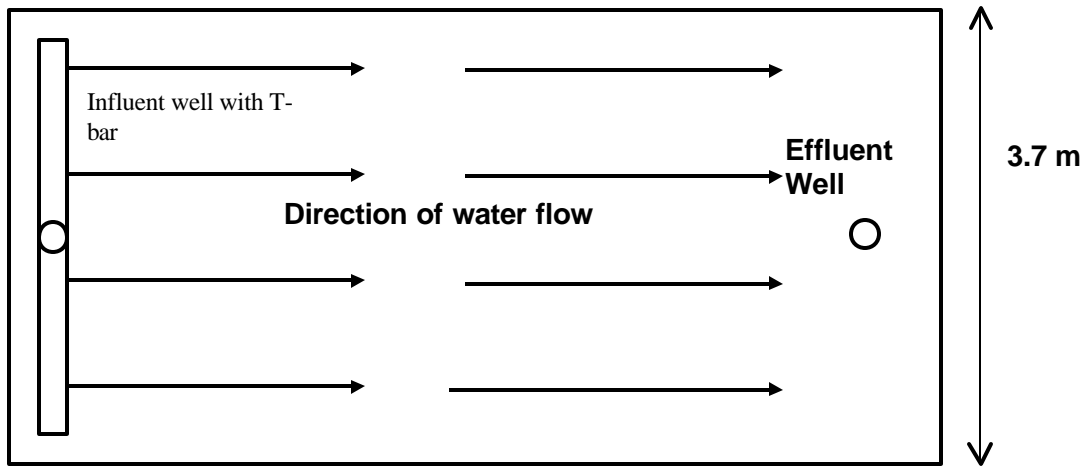
#### Pilot-Scale Experiments with Field Test Beds at UW (Fife WA site)

- Test bed cells with ~12 m<sup>3</sup> soil
- Sand layer with an artificially contaminated groundwater
- Hybrid poplar H11-11, 15 trees per cell
- TCE, CT, or PCE was added to the cells at up to 1 to 100 mg/L, but usually averaging 15 mg/L
- Operation for 4+ years
- Control cells
  - Poplar planted cells with no solvent exposure
  - Cells with TCE or CT exposure but no vegetation.

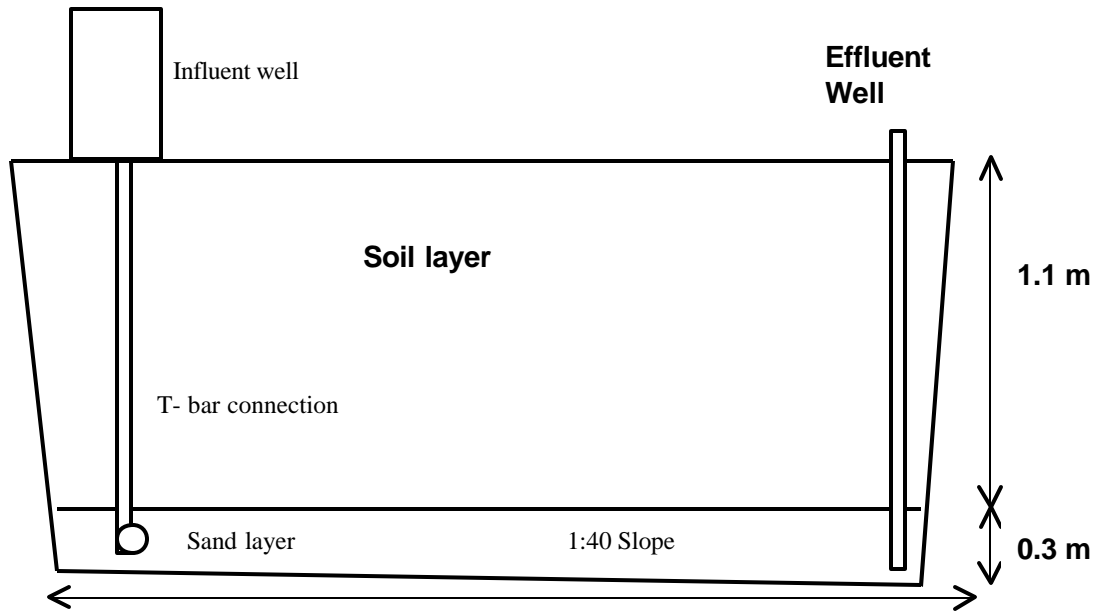
#### Fate of TCE and CT

- Mass removal in the planted cells exceeded 95%
- No enhancement of TCE and CT degradation in the rhizosphere soils
- Transpiration of TCE and CT from poplar leaves measured by two methods
  - Long-path open FTIR
  - By bagging leaves and trapping solvent vapors
  - Air emissions of TCE & CT were <10% of the total TCE & CT removal
- Some chlorinated metabolites (e.g., chloroacetic acids and trichloroethanol) were found in plant tissues
- No significant increase in total organic halides in the tissue of trees exposed to TCE and CT compared to unexposed controls.
- Chloride ion accumulated in the soils

- $111 \pm 8\%$  of the TCE chlorine lost in the TCE-exposed test bed, suggesting complete dechlorination.

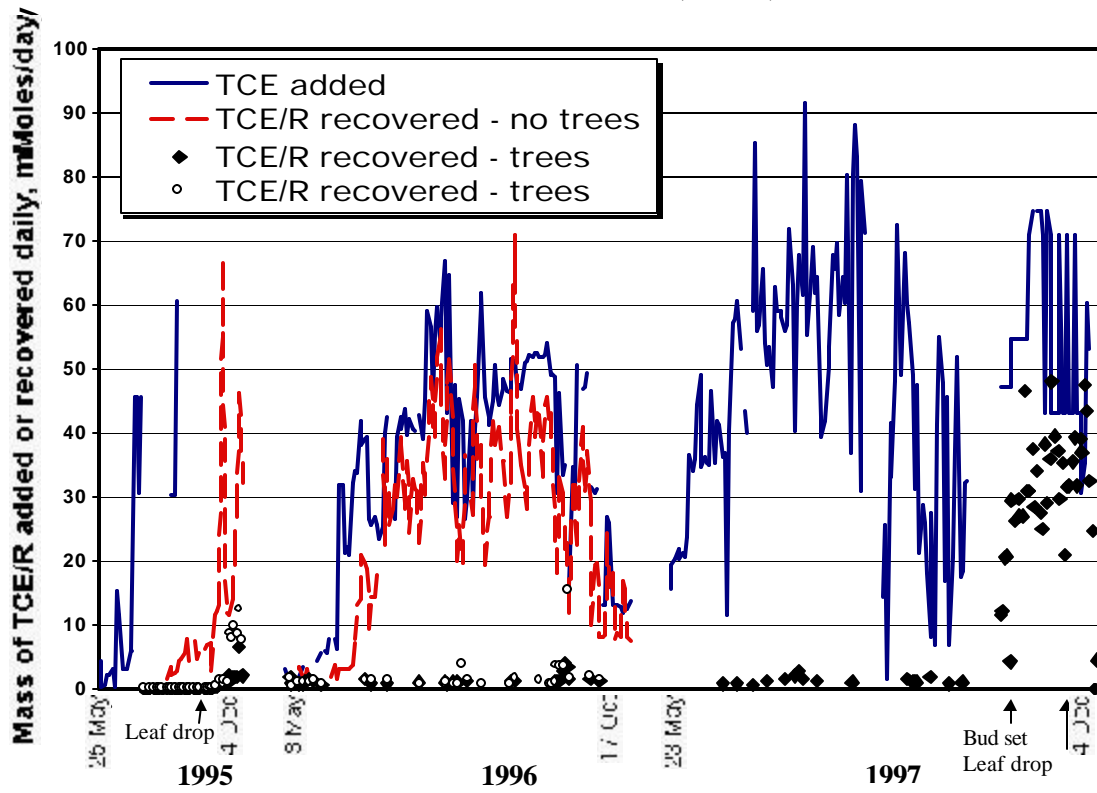


Top view

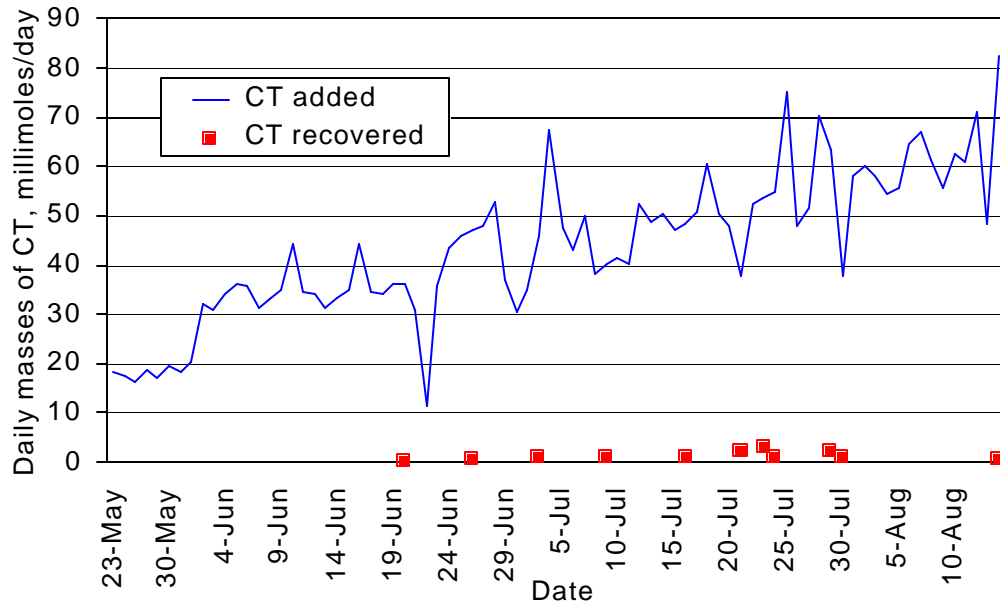


Side view

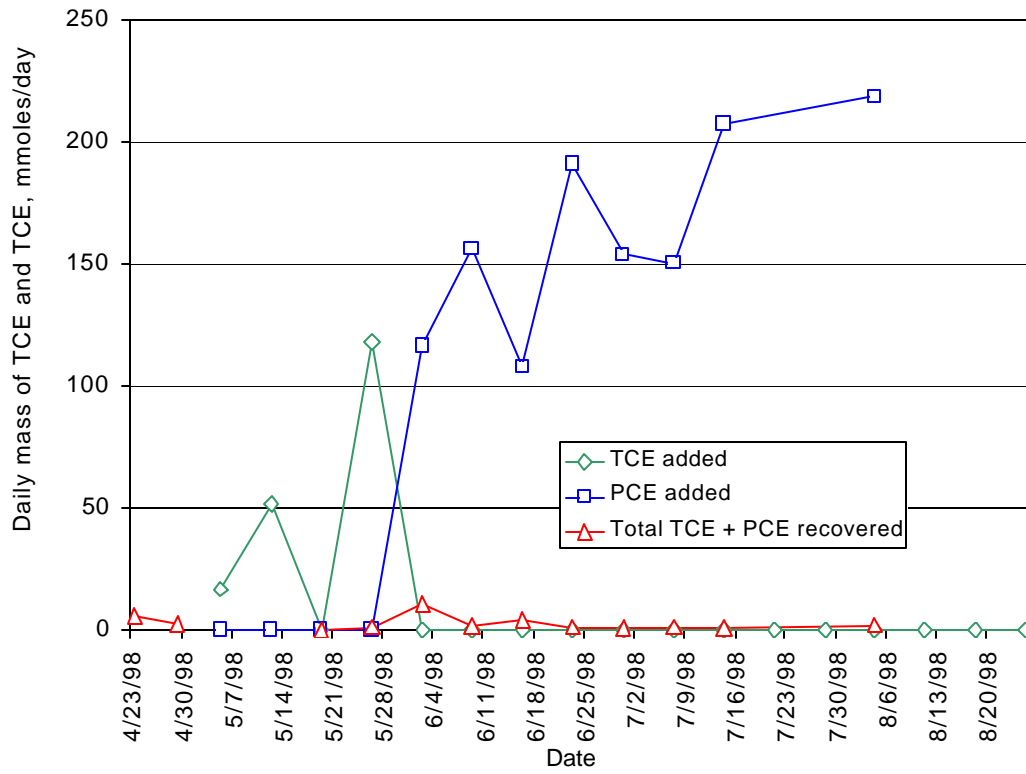
Loss of TCE and Its Reductive Dechlorination Products (TCE/R) in Test Beds



## Loss of CT in Test Beds with Poplar



## Loss of TCE and PCE in Test Beds with Poplar Trees



	Concentration in Air $\mu\text{g}/\text{m}^3$ (average $\pm$ standard error of the mean)	
	Method	
Chlorinated Solvent	Leaf Bags <sup>a</sup>	Open Path FTIR <sup>b</sup>
Trichloroethylene	17 $\pm$ 25	ND 262
Carbon Tetrachloride	ND 12	ND 62

ND = none detected at the indicated detection limits,  $\mu\text{g}/\text{m}^3$

<sup>a</sup>Leaf bag method: Individual leaves were enclosed in Teflon bags with air pumped through at 2 L/mi for 30 min to 2 hr. Air from bag passed through a heated, activated carbon trap. Chlorinated solven were trapped in the carbon and extracted in  $\text{C}_2\text{H}_2$  for analysis by GC-ECD. Recoveries of known amounts added to the bags were 88% for carbon tetrachloride and 90% for TCE.

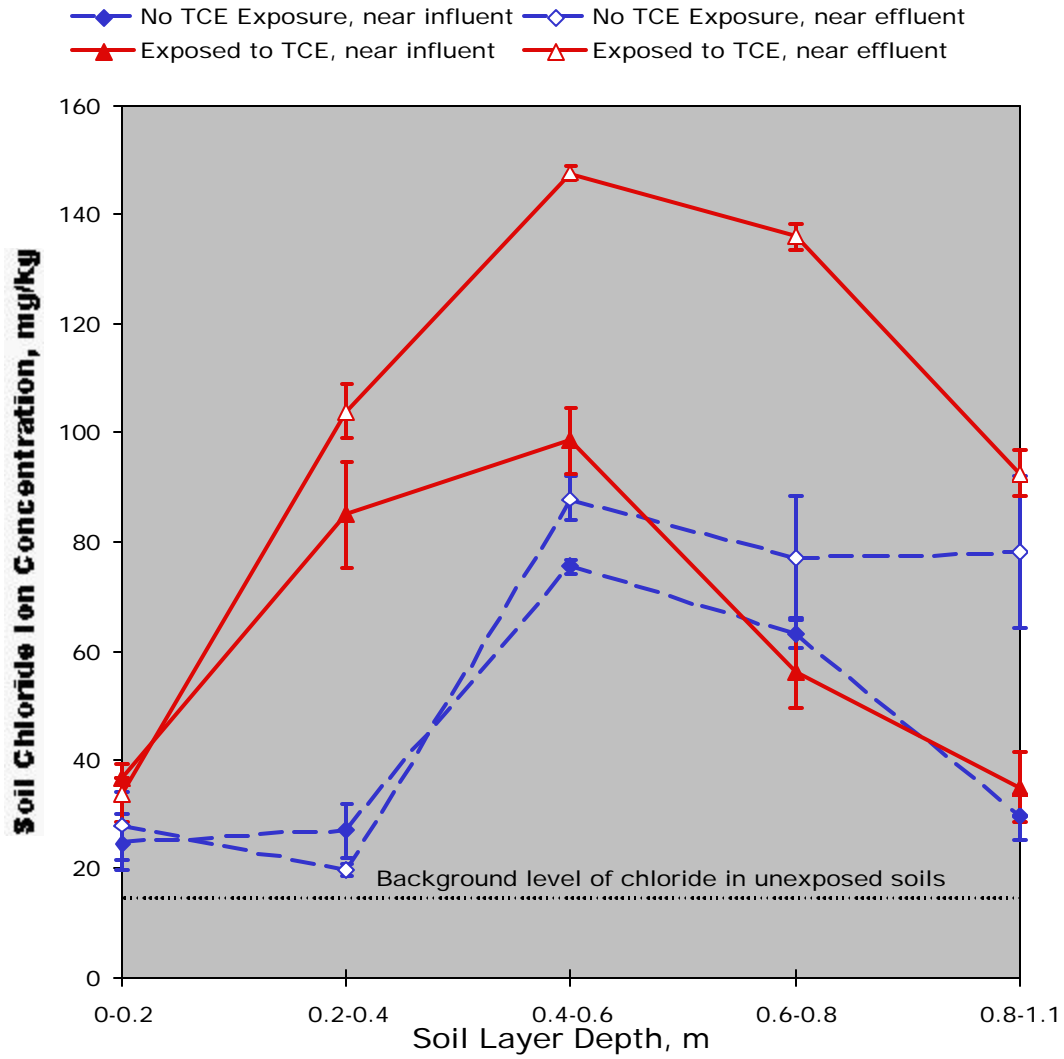
<sup>b</sup>Open Path FTIR method: Environmental Technologies Group Air-Sentry FTIR system was used. The IR source beam was sent from a telescope through the tree canopy to a retroreflector mounted on scaffolding and back to the detector. Optical path length = 14 m.

### Total Organic Halides in Tissue of Poplar Exposed to TCE and CT

	TOX, $\mu\text{g}/\text{g}$		
	Roots	Stem	Leaves
Control, unexposed trees	17 $\pm$ 2	16	96 $\pm$ 43
TCE-exposed trees	27 $\pm$ 22	20 $\pm$ 5	99 $\pm$ 40
CT-exposed trees	21	22	91 $\pm$ 28

Distribution of Chloride Ion in Soil of Test Beds with Poplar Exposed to TCE

Soil Chloride Concentration, mg/kg



## Chloride Balance for Field Test of Phytoremediation of TCE

Chlorine added in the form of TCE-chlorine was balanced against the amount of TCE-chlorine, metabolite-chlorine & free chloride ion recovered from the system. Masses given cover the 3 years the experiment ran.

	1995	1996	1997	3-year total los	3-yr total recovered
	Moles of chlorine or chloride ion.				
TCE-chlorine lost from the irrigation water	2.75	13.4	11.32	27.47	
TCE-chlorine recovered from transpiration	0.28	0.87	0		1.15
TCE-chlorine recovered as metabolites in leaf	$0.03 \times 10^{-3}$	0.006	0.002		0.008
branch	$0.03 \times 10^{-3}$	0.005	0.002		0.007
trunk	$0.05 \times 10^{-3}$	0.01	0.003		0.013
root	$0.03 \times 10^{-3}$	0.006	0.002		0.008
Excess chloride ion in soil	ND	ND	18		18
	Chloride balance			27.5	19.2
ND: Not determined	Recovery efficiency				70%

### Applications of Phytoremediation to groundwaters contaminated with chlorinated solvents

- Pump and irrigate
  - Subsurface drip irrigation prevents volatilization
  - Reduces land requirements
  - Removes depth limitations
- Savings estimated to range from 50-90%
- Socially acceptable
- Environmentally restorative
- Destructive treatment with minimal transfer to other media

### Limitations of Phytoremediation

- Contaminated water taken up along with pollutant
- Requires large, arable land areas over or near the contaminated aquifer
- Passive uptake requires especially large areas since water taken up only from top of the aquifer
- Limited to contaminants within reach of the roots, ~ 10-20 m

### Summary

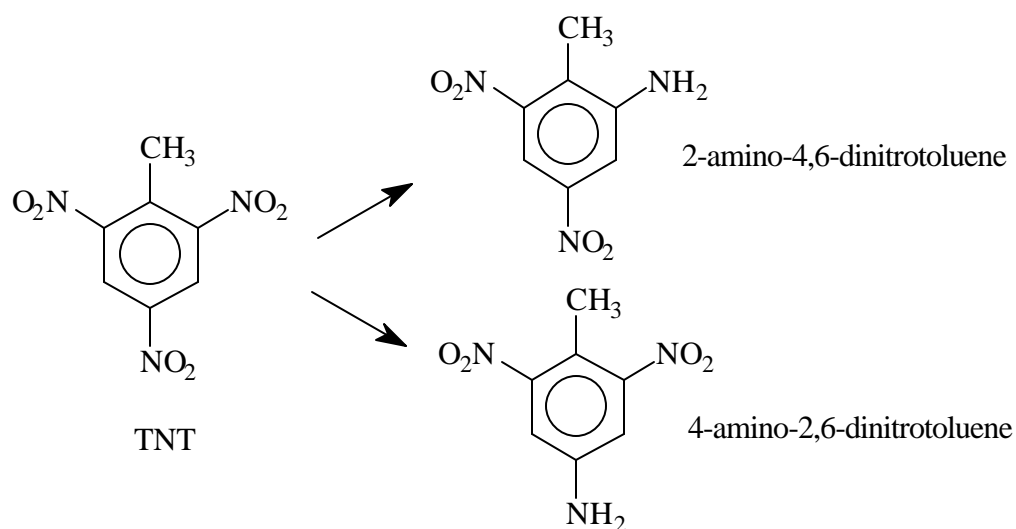
- Poplar trees growing in the field can take up TCE and CT with low air emissions and no accumulation of toxic residues
- TCE is primarily destroyed, with quantitative recovery of chloride ion
- Presence of oxidative metabolites of TCE and CT in plant tissues suggest involvement of oxygenase activity

### Other types of organic pollutant degradation in plant tissue

#### TNT

2,4,6-Trinitrotoluene (TNT) is taken and transformed up by a variety of aquatic and terrestrial plants. The disappearance of TNT in aquatic systems can be quite rapid (days). Terrestrial plants such as bush beans have also been observed to take up and transform TNT<sup>4</sup>.

Until recently it was unclear whether TNT was transformed in plant tissue after uptake or in the rhizosphere. In the last year work with axenic cultures of parrot feather, *Myriophyllum spicatum*, has shown that TNT is transformed reductively by intracellular activity to low levels of aminated nitrolouenes and other, soluble but unidentified metabolites, though mineralization was not observed<sup>5</sup>.



#### Pentachlorophenol

Glucoside formation has been observed for PCP taken up in an aquatic plant, *Eichhornia crassipes*<sup>6</sup>. Glutathione S-transferase (GST), a major conjugating enzyme, superoxide dismutase, and ascorbate peroxidase activities were also increased. In a study of

several aquatic plants at the UW we have found a wide variation in the uptake of PCP carbon by aquatic plants.

### **Atrazine**

The herbicide atrazine is taken up and dealkylated by poplar in a manner similar to bacterial dealkylation pathways.

### **PCB**

Di- and trichlorinated biphenyls were metabolized by hydroxylation and glycosylation by the action of mixed function oxidases in rose cell cultures <sup>7, 8</sup>.

## **Enhancement of microbial degradation in rhizosphere**

### **PCBs**

Growth of PCB degraders, *Alcaligenes eutrophus* H850 and *Pseudomonas putida* LB400, was supported by root exudate phenolic compounds, apigenin, naringin, phloridzin, catechin, maclurin, and myricetin <sup>9</sup>. Degradation of PCBs with up to 4 Cls was supported as shown for LB400 in following table:

Table 4. Metabolism of PCBs by *Pseudomonas putida* LB400 following growth for 3 transfers on different sole carbon sources.

PCB Congener	Biphenyl	Catechin	Malicinn	Myricetin
			(%) <sup>a</sup>	
<u>Open 2,3 and 3,4 sites</u>				
2,3	●	●	●	●
2,4'	●	●	●	●
2,5,4'	●	●	●	●
2,2'	●	●	●	●
2,3,2',3'	●	●	●	●
2,5,2'	●	●	●	●
2,3,2',5'	●	●	●	●
2,4,5,2',3	●	●	●	●
2,5,3',4'	●	●	●	●
2,3,4,2',5'	●	●	●	●
<u>Open 2,3 sites</u>				
4,4'	●	●	●	●
2,4,4'	●	●	●	●
2,4,3',4'	●	●	●	●
2,4,2',4'	●	●	●	●
3,4,3',4'	●	●	●	●
<u>Open 3,4 sites</u>				
2,5,2',5'	●	●	●	●
2,4,5,2',5'	●	●	●	●
<u>Blocked 2,3 and 3,4 sites</u>				
2,4,6,2',4'	●	●	●	●
2,4,5,2',4',5'	●	●	●	●

<sup>a</sup>Percent of congener removed following a 24 h incubation of the bacterium in a PCB mixture containing 5 mM of each congener. % Metabolism: ● 20-39, ● 40-59, ● 60-79, ● 80-100

Usefulness of plant-enhanced degradation of PCBs in soils has not been demonstrated.

### Petroleum hydrocarbons and PAHs

Most experiments have found degradation of petroleum hydrocarbons and PAHs to be slightly enhanced in rhizosphere soils compared to unvegetated soils <sup>10</sup>, as in the following figure:

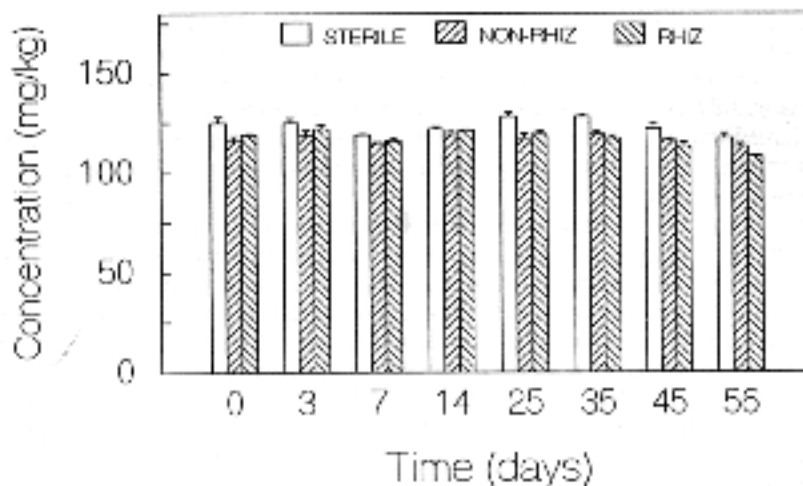


Figure 1. Concentrations of anthracene in soil as a function of time for samples that were kept at 4 °C to minimize biological degradation. Error bars represent one standard deviation. NON-RHIZ refers to non-rhizosphere soil, and RHIZ refers to rhizosphere soil.

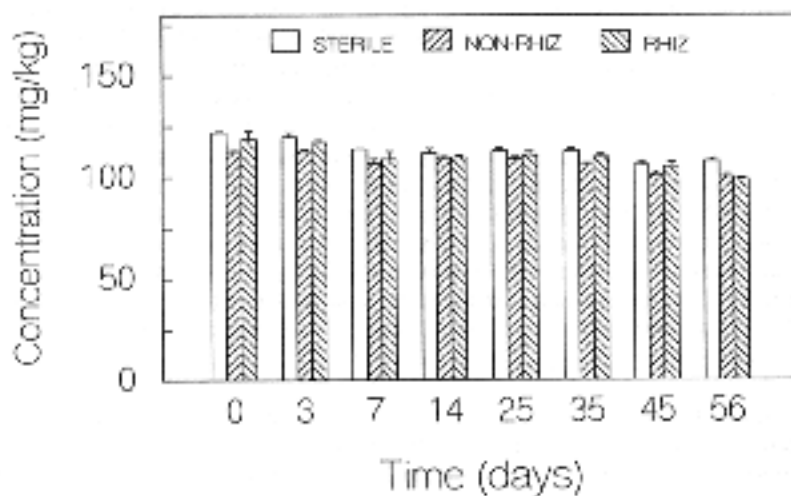


Figure 2. Concentrations of pyrene in soil as a function of time for samples that were kept at 4 °C to minimize biological degradation. Error bars represent one standard deviation. NON-RHIZ refers to non-rhizosphere soil, and RHIZ refers to rhizosphere soil.

Low molecular weight PAHs may be enhanced in rhizospheres, but the more important high molecular weight PAHs usually are not affected by enhanced degradation in the rhizosphere of most plants. In one study degradation of HMW PAHs was enhanced only with one grass species, verde klein <sup>11</sup>.

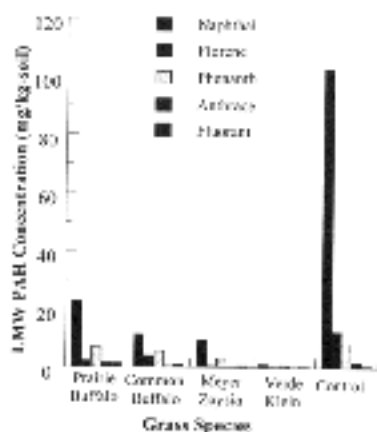


Figure 7. Comparison of LMW PAH concentrations in root zone soils of the four top-grading grasses against unvegetated control]

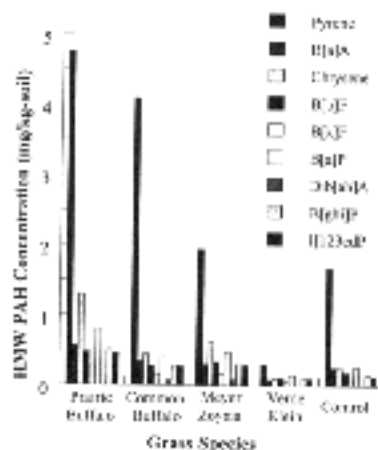


Figure 8. Comparison of HMW PAH concentrations in root zone soils of the four top-grading grasses against unvegetated control]

Most studies to date have been performed with grasses. Some anecdotal reports suggest that trees, such as mulberry, may enhance increased PAH degradation.

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