Phytoremediation: From the Molecular to the Field Scale

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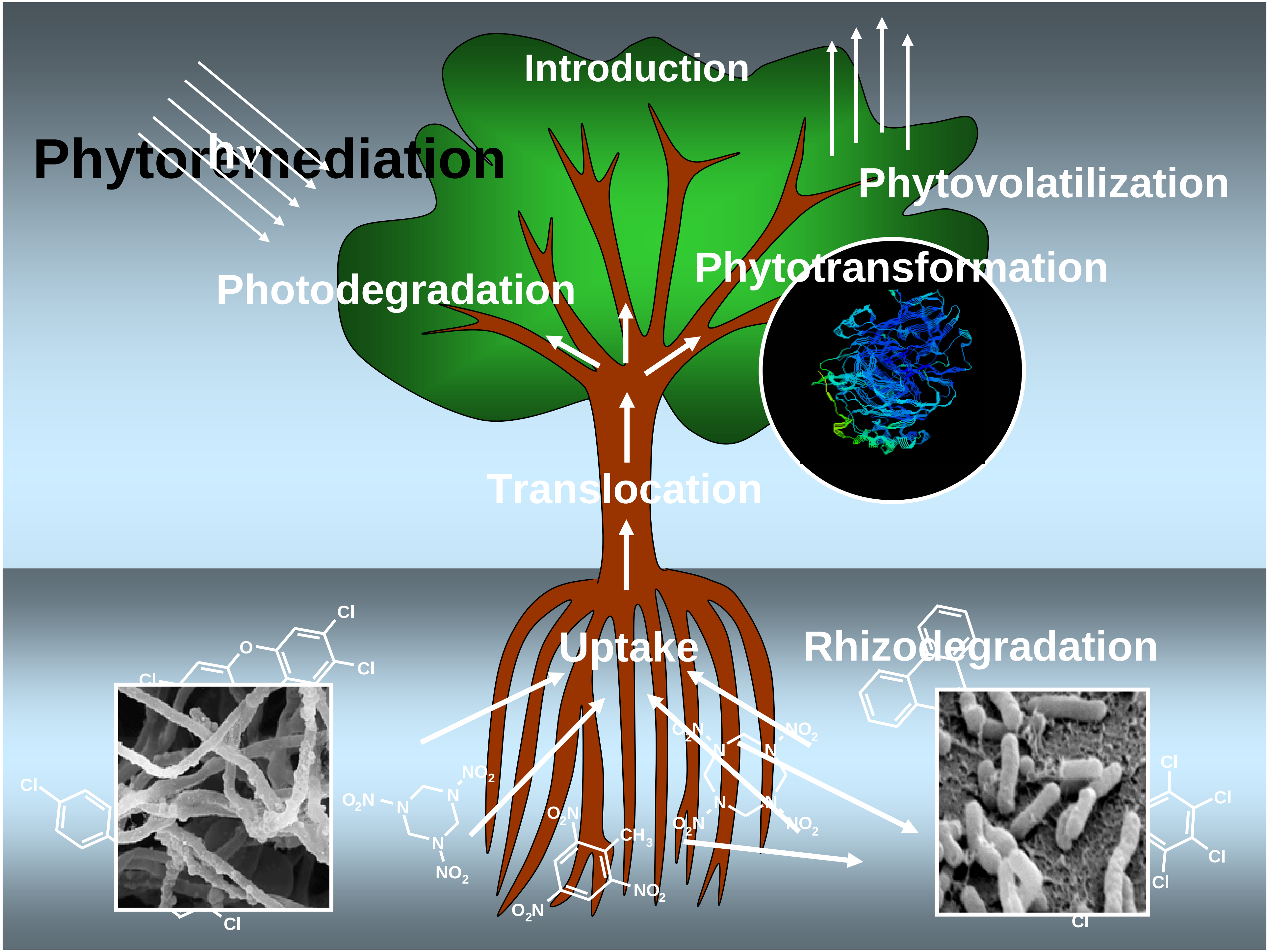
Tree Hugger…



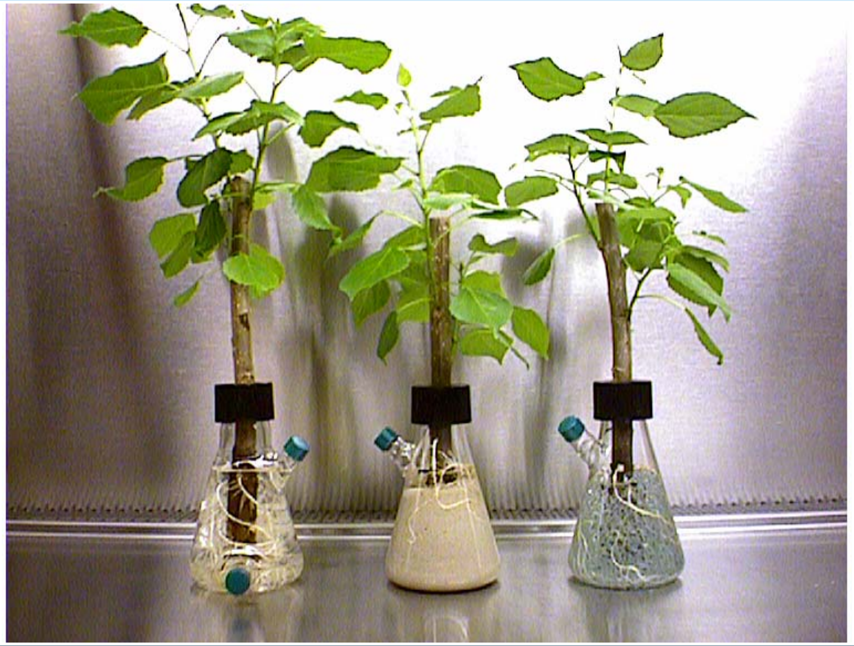
Phytoremediation
hν
Introduction
Phytovolatilization
Photodegradation
Phytotransformation
Translocation
Uptake
Rhizodegradation

Powers of Ten: from the molecular nanoscale (<100 nm) to the field (1000 m), more than 10 orders of magnitude!

Research into Contaminant Transformations

Plant materials for different purposes:

Whole intact plants

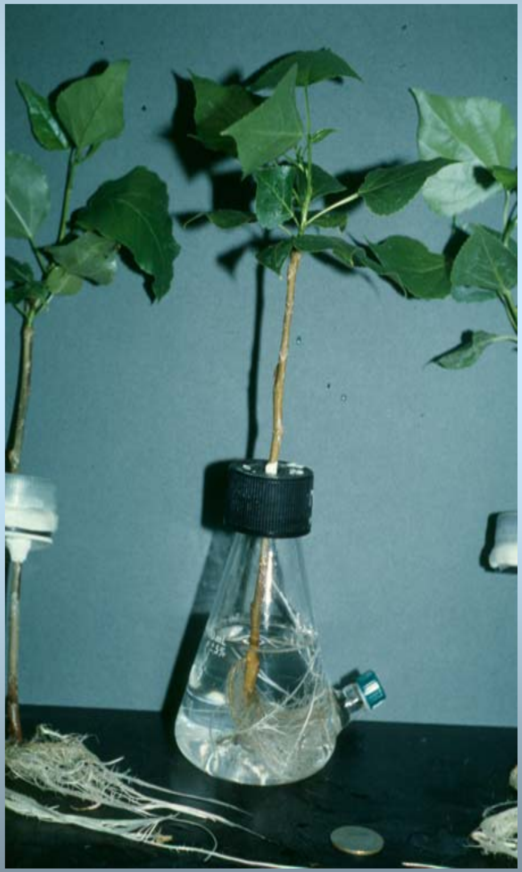


Cell cultures

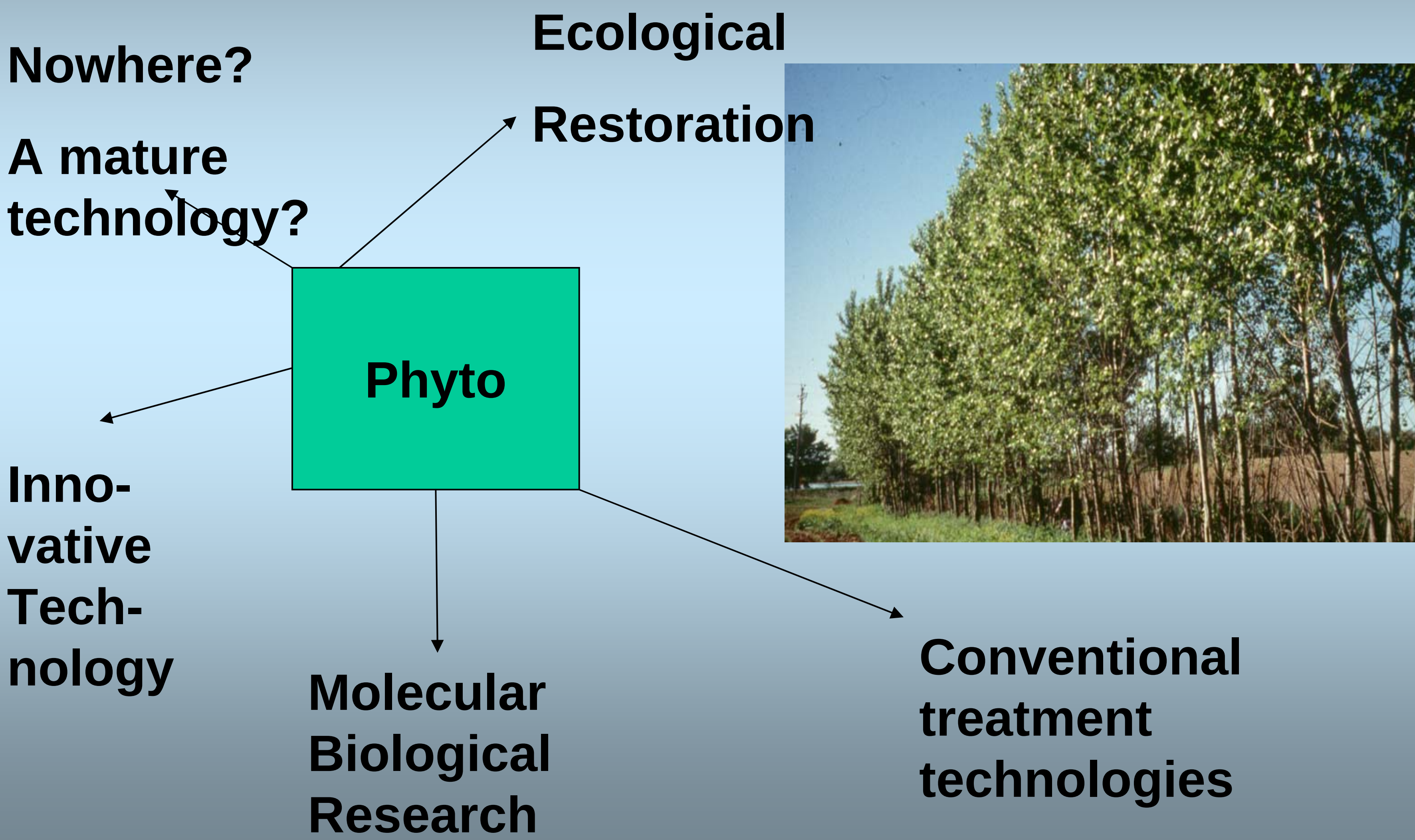
Tissue Cultures

“Nodules”

From the Laboratory to the Greenhouse to the Field and back



Where is Phytoremediation going? What is it?



Phytoremediation—the use of plants to help clean the environment…

- Advantages
  - Natural
  - Green, growing
  - Aesthetically pleasing
  - Cost-effective for large land areas where other technologies are not feasible
  - Sensible, appropriate, sustainable technology
- Disadvantages
  - Long clean-up times
  - Uncertain performance
  - Not for every site (deep wastes, anaerobic soils, etc)
  - Regulatory hurdles

Recent Advances in PhytoTechnology

- New evidence of phytoremediation effectiveness (for PAHs, RDX, ClO_4^-, other)
- Progress with transgenic plants
  - First use in the field for selenium removal in CA (Norman Terry, Gary Banuelos et al., *ES&T*)
  - As and Hg progress also (Meagher, Rugh, others)
- Plume delineation by tree corings (Burken, Compton, and others)

Recent Advances in PhytoTechnology

- Emergence of *Populus* genomic database for plant functional genomics
  - *Populus* EST resource published (Sterky et al., PNAS 101:38, 13951-13956
  - Genome shotgun sequences by DOE (www.jgi.doe.gov
  - International Genome Consortium (www.ornl.gov/ipgc)

Use *Arabadopsis* to learn about poplar

The high similarity between *Populus* and *Arabidopsis* will allow *Populus* to directly benefit from *Arabadopsis* detailed functional genomic information. Enzymes can be identified by molecular techniques…

A. thaliana (At1g17170) GST: 27.5 x increase after TNT exposure (Ekman *et al*., 2003, *Plant Physio* 133:1397-1406)

Blast Corresponding Protein (aa) Sequence in Poplar Genome Project

Arabidopsis → Query; Populus → Sbjct

```
Score =  179 bits (383), Expect(3) = 6e-79
 Identities = 68/109 (62%), Positives = 88/109 (80%)
 Frame = -1
Query: 1   MADEVILLDFWASMFGMRTRIALAEKRVKYDHREEDLWNKSSLLLEMNPVHKKIPVLIHN 60
           M D V LL FW S + MR ++ALAEK ++Y+ RE++L +KS LLLEMNPVHK IPVLIHN
Sbjct: 926 MEDRVTLLIFWPSPWAMRVKVALAEKGIEYESREQNLIDKSPLLLEMNPVHKTIPVLIHN 747
Query: 61  GKPVCESLIQIEYIDETWPDNNPLLPSDPYKRAHAKFWADFIDKKVNVT 109
           GKP+CES   ++YIDE W D +PLLPSDPY+R+ A+FWAD+IDKK +++
Sbjct: 746 GKPICESHNIVQYIDEVWKDKSPLLPSDPYQRSQARFWADYIDKKASIS 600
```

Find DNA poplar sequence corresponding to the *Arabidopsis* protein

Past Applications have shown the promise of phytoremediation for soils and sediments

Amana, Iowa, 1992: Riparian zone buffer strip to control pesticides, nutrients and soil runoff

- Groundwater and soil concs. improved for nitrate and pesticides after only 4 years
- This could still be a growth area!

Phyto Applications that really work!

- MNA + something more!
- Petrochemical wastes and rhizodegradation
- Plume control and degradation (TCE, MTBE, RDX, ClO_4^-)
- Landfill cap and closures
- Dewatering contaminated sludges/sediments cdfs
- Created wetlands (rhizofiltration)
- Phytostabilization

Former Refinery and Tank Farm, Cabin Creek WV

- Highly contaminated soil >5,000 mg/kg TPH significantly cleaned in 4 years
- Planted with DN-34 hybrid poplar and grasses in 1999
- Growth is shown after 3 seasons; soil concs. improving, g.w. slow

Iowa Army Ammunition Plant

- RDX & TNT concs. in soil/groundwater up to 1 mg/L; excavation followed by phyto…
- Constructed wetlands phytoremediation full scale
- Meets RDX discharge permit of 2 ppb partly due to photolysis

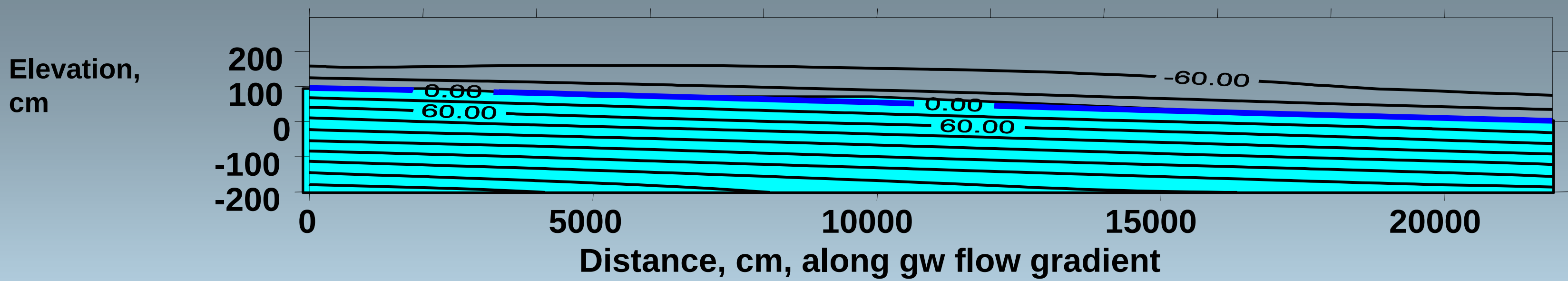
TCE in surficial groundwater plume, at major chip manufacturer, Myrtle Beach SC

- Groundwater plume of TCE was migrating off the plant property
- Two acres of hybrid poplar were planted w/ roots into gw table
- Phytoremediation has decreased the TCE in gw and soils

(a)

(b)

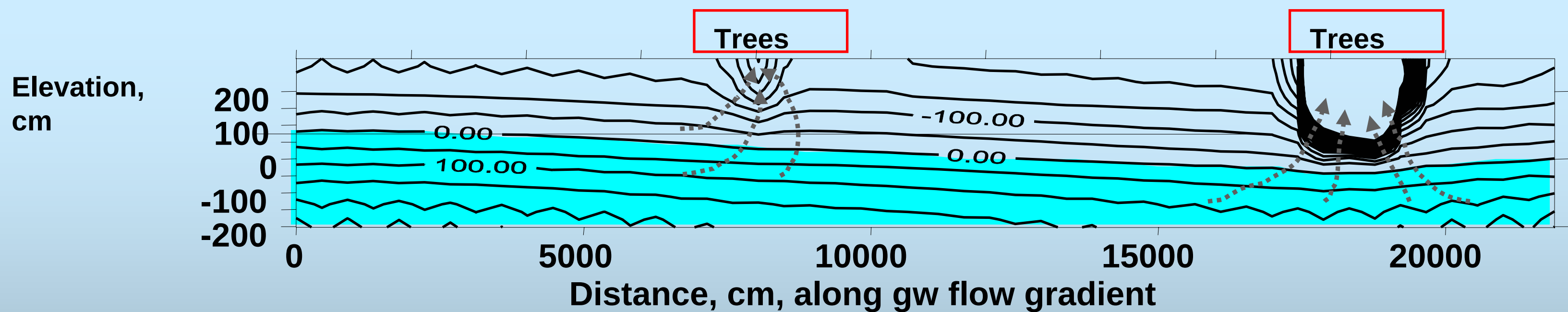
Hydraulic capture of MTBE by hybrid poplars after (a) 1 month and (b) 8 months of growth at Houston, TX, 1999.



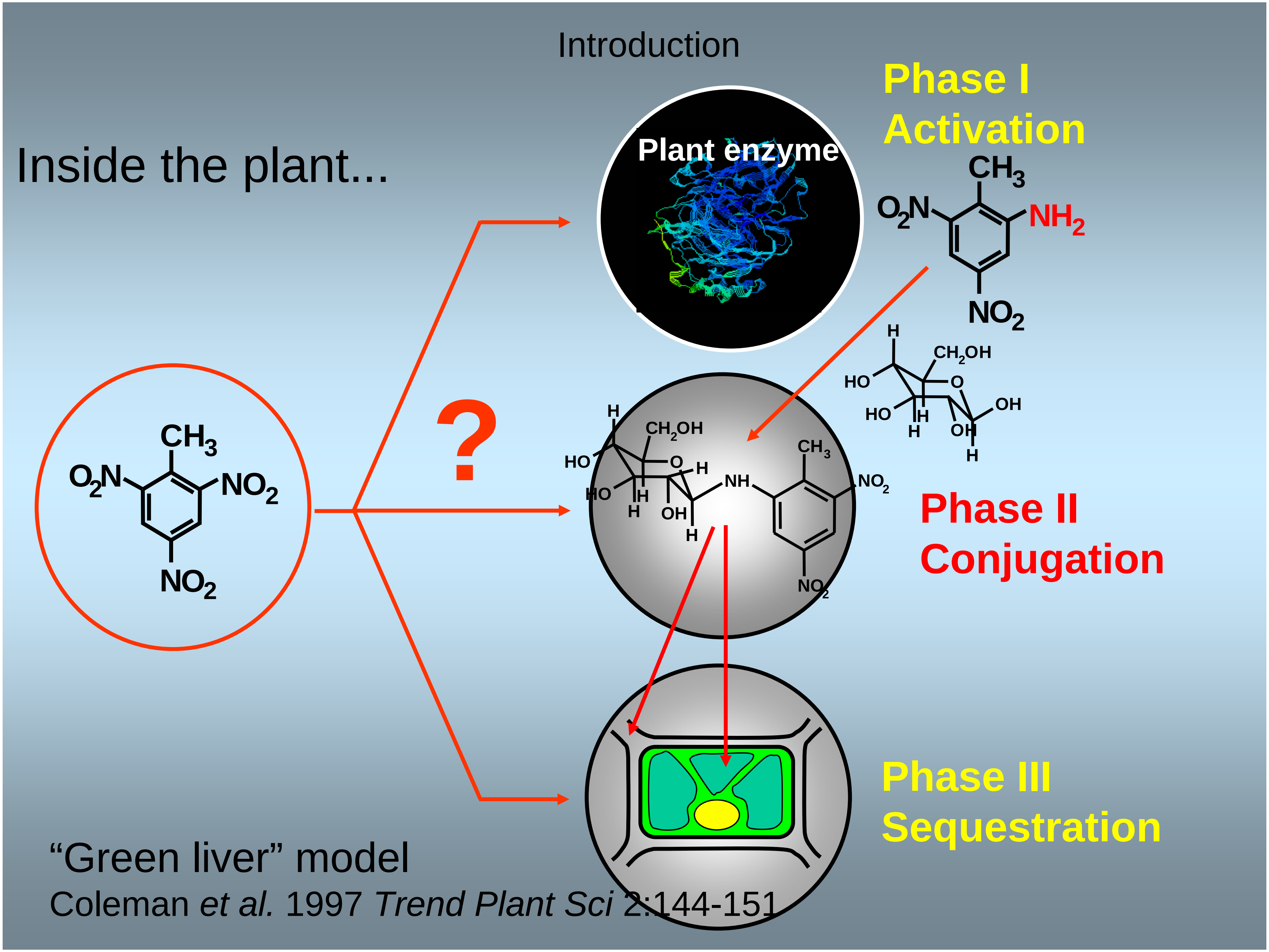
Constant Surface Boundary and PET Conditions

Deep Infiltration by Precipitation:	~ 6 cm/year (5% of precip.)
Average (Yearly Basis) PET:	~ 3 mm/day
Average (Yearly Basis) AET:	~ 2.2 mm/day (constant LAI, = 2)
Left-Hand Side Boundary Condition	No Flow
Right-Hand Side Boundary Condition	Constant Head

(a)



(b)


Introduction
Inside the plant...
Plant enzyme
Phase I
Activation
Phase II
Conjugation
Phase III
Sequestration
CH3
O2N
NO2
NH2
?
HO
OH
CH2OH
O
H
NH
"Green liver" model
Coleman et al. 1997 Trend Plant Sci 2:144-151

TNT Metabolite Analysis

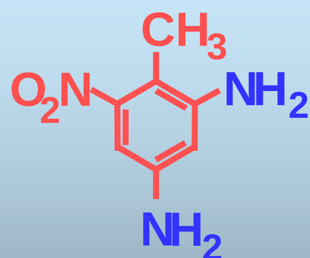
The case of TNT

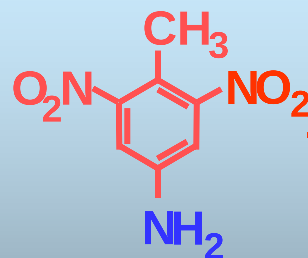
Analysis

- HPLC
- ^{14}C,rad
- ^{15}N,^{13}C,
- GC-MS
- LC-MS
- LC-MS-MS

Initial pollutant → **Uptake**, **Translocation**, **Storage**

Metabolites → **Phytotransformation**, **Conjugation**, **Enzymes involved**



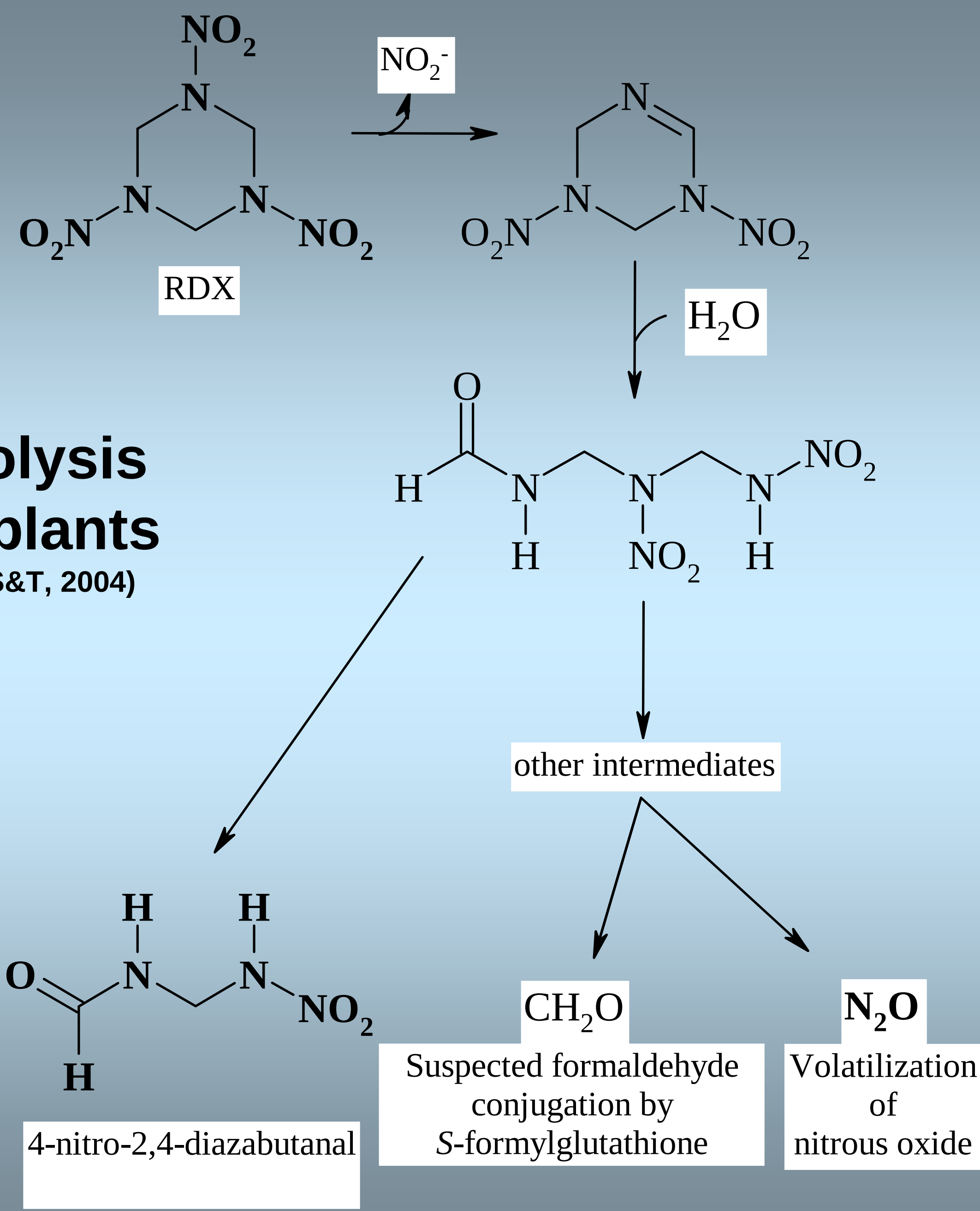


→ **Nitroreductase**

Non-extractable → **Glutathione-*S*-transferase**

Phytophotolysis of RDX by plants

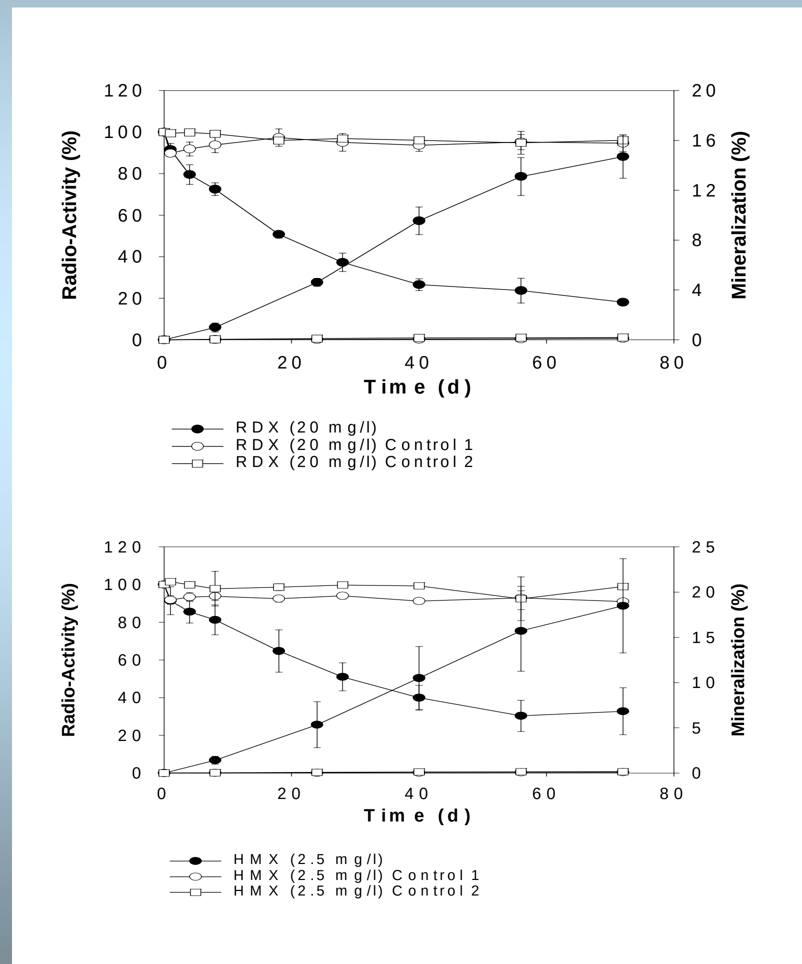
(Just and Schnoor, ES&T, 2004)



Mineralization of ^{14}C-RDX and HMX by DN-34 *Populus deltoides x nigra* Nodules

- Nodules grown in Murashige and Skoog liquid culture medium are capable of mineralizing RDX and HMX to $^{14}CO_2$ with high yields

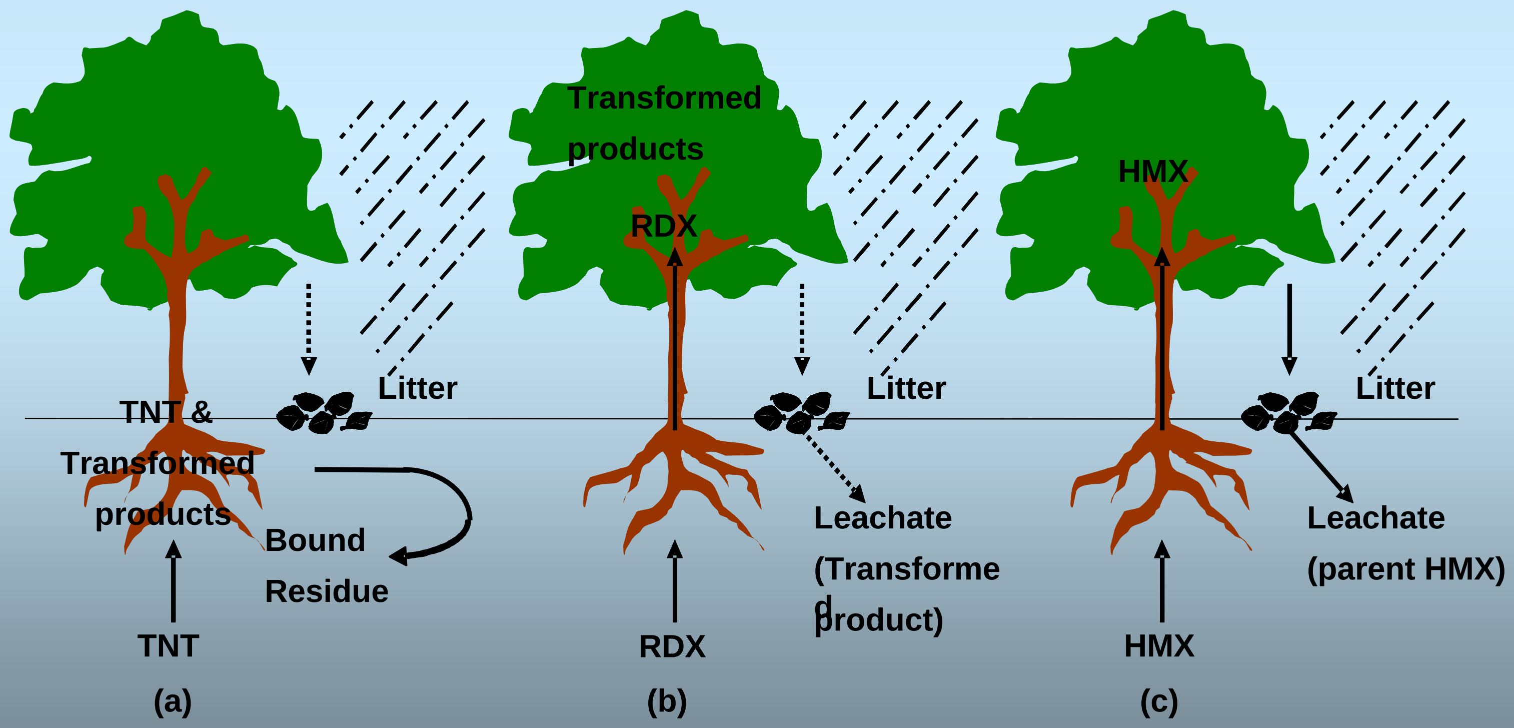
(Van Aken & Schnoor, ES&T, 2004)



Ecotoxicology Phyto Research

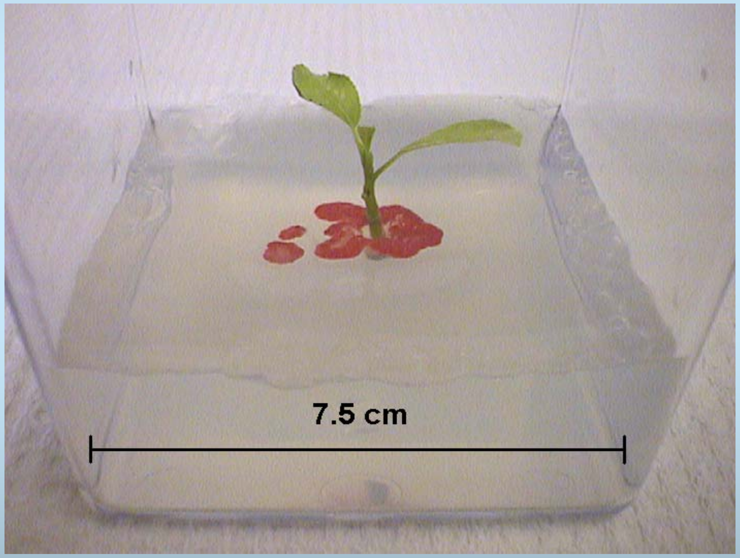
Ecotoxicology of plant materials after phytoremediation using leaching tests, Microtox, earthworm, and C. elegans microarray

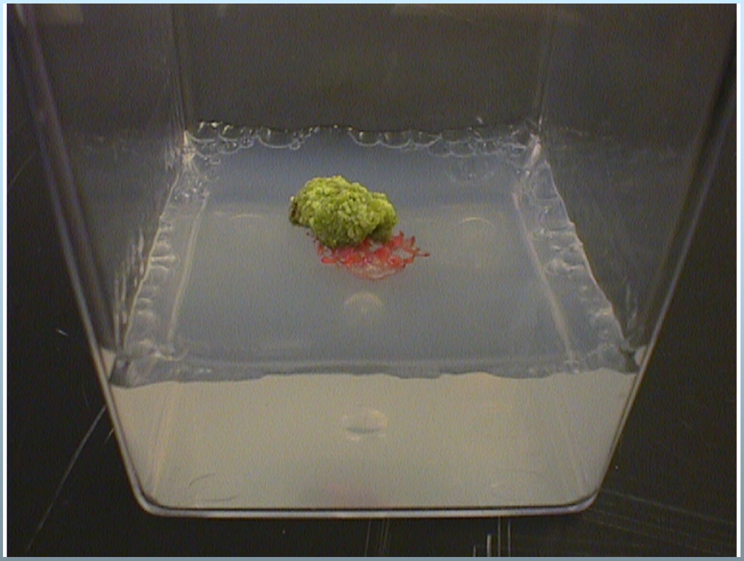
Fate of (a) TNT; (b) RDX; (c) HMX in plants following uptake…



Developments – Ecology of Endophytes

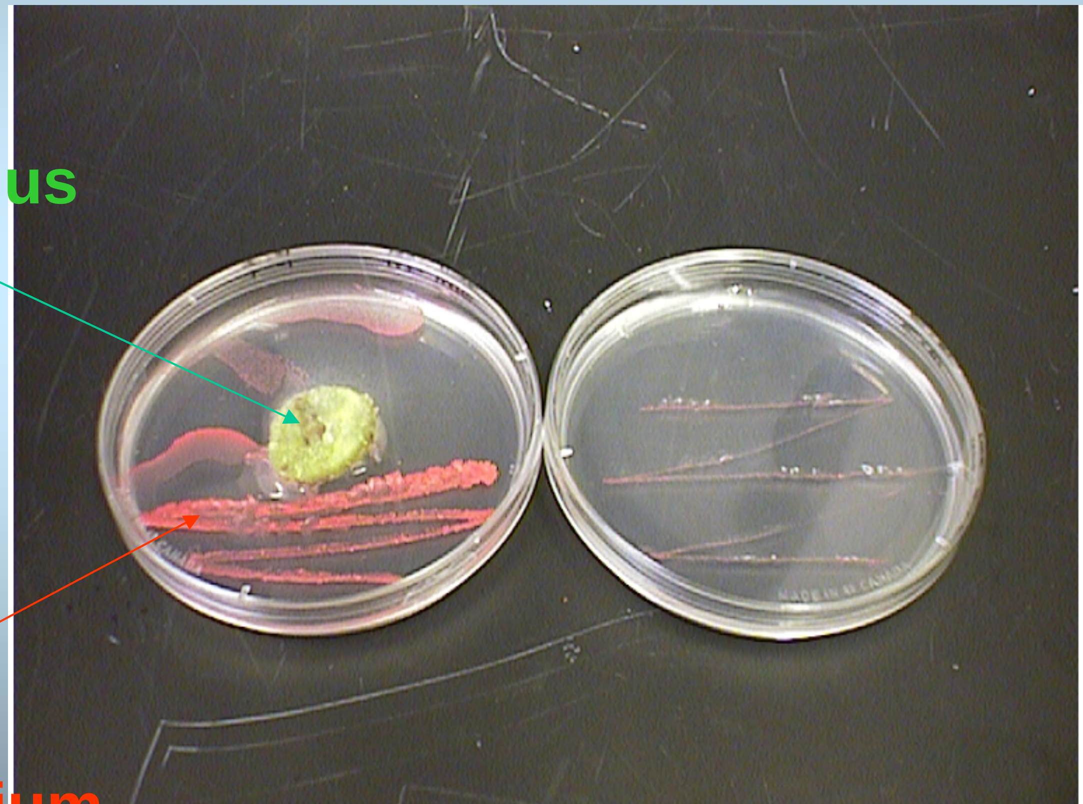
Plants and plant tissues ‘infected’ by *Methylobacterium populi* sp. BJ001



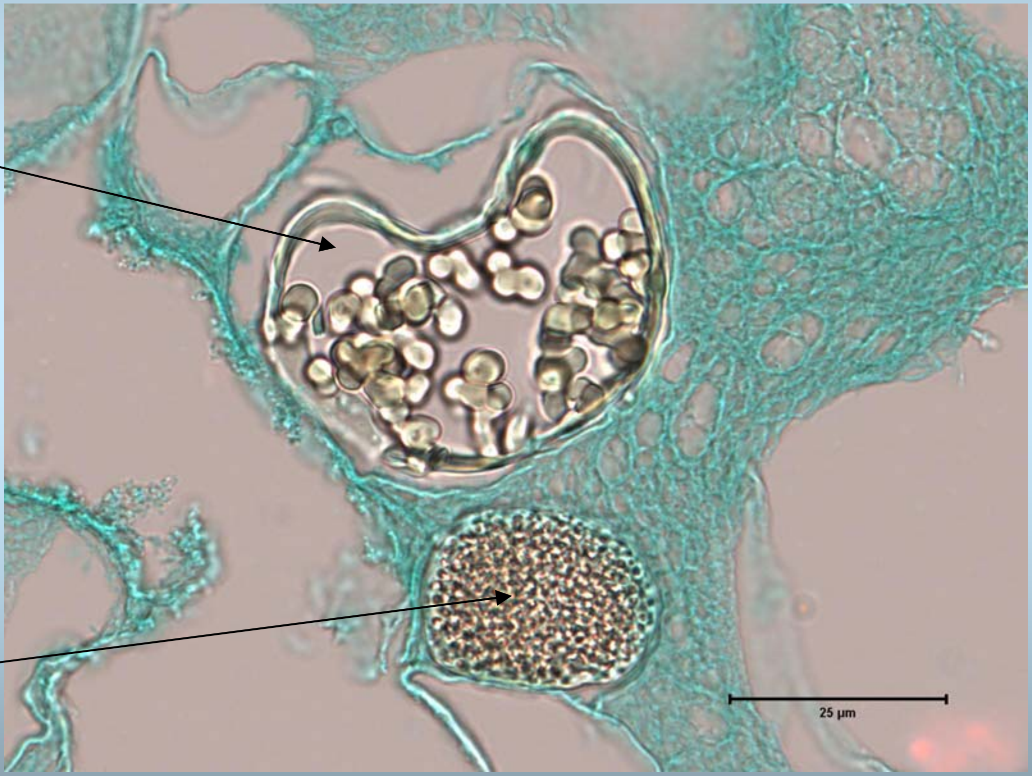


Some remaining research questions…

What do the plants do for the bugs and vice versa? And how does this affect remediation?



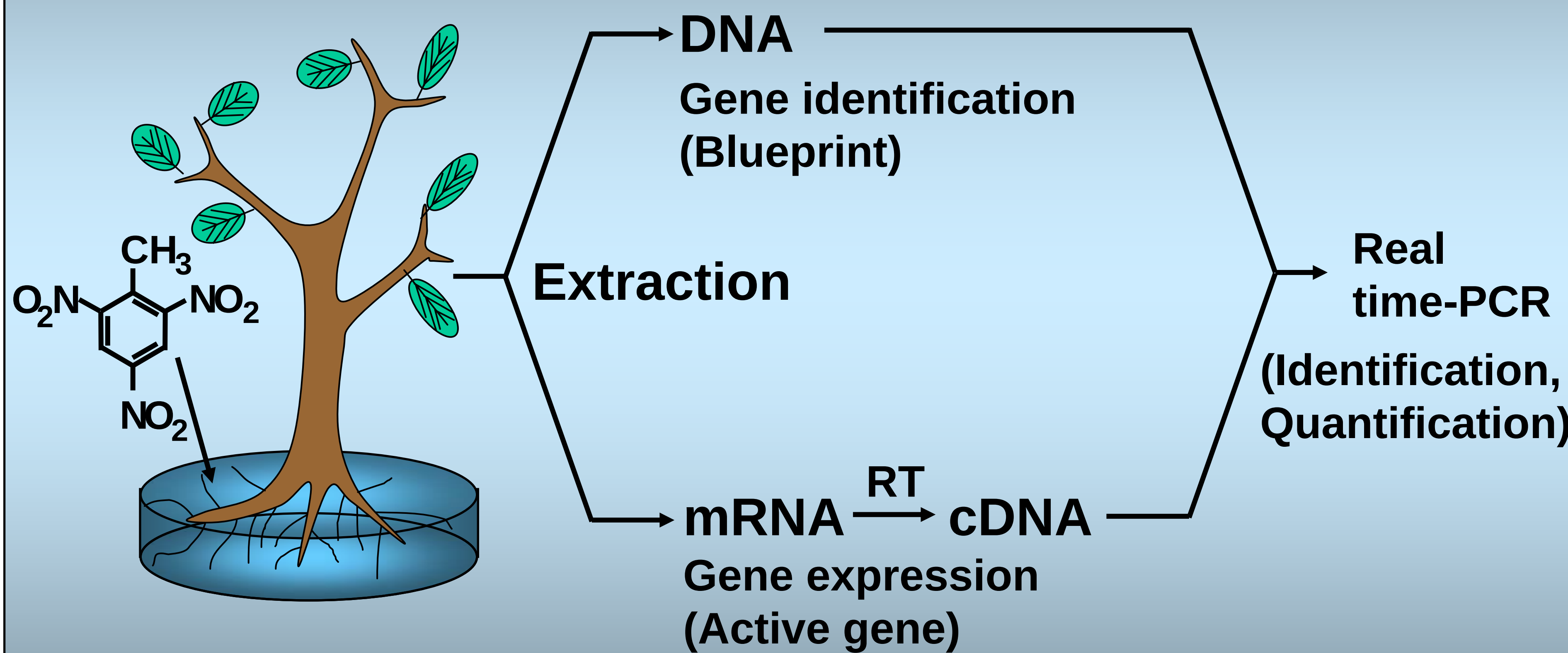
Plant cell ‘infected’ by *Methylobacterium populi* sp. BJ001



Optical microscope (1,000x), safranine

DoD EPA DOE SERDP
Strategic Environmental Research and Development Program

Gene Expression Research



Exposed plants show induced genes…

Populus trichocarpa genes induced by TNT and identified

Glutathione S-Transferase (2 isozymes)
Nitrilase 2
Monodehydroascorbate Reductase
Isocitrate Lyase
Indole-3-Acetate B-Glucosyl Transferase
RAP2-like Transcription Factor
12-Oxophytodienoate Reductase (OPR1)
Cyto P450 - CYP71A12
Cyto P450 - CYP706A2
Cyto P450 - C4H - CYP73A5
Cyto P450 - CYP89A6
Cyto P450 - CYP81D11

Calibrators

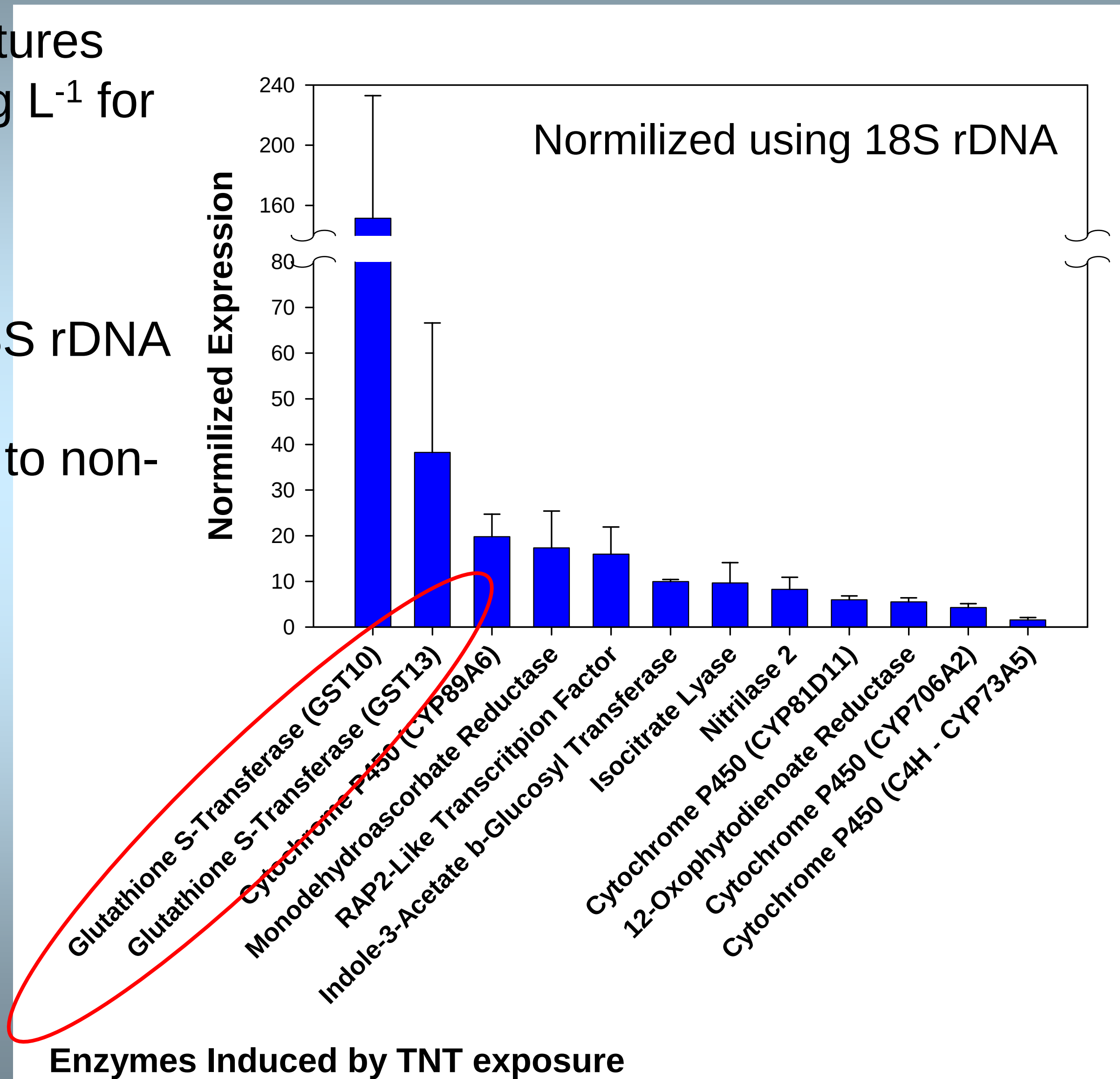
Actin
Cyclophilin
Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH)
18S rDNA

Gene Expression – Results

P. trichocarpa tissue cultures exposed to TNT 20.0 mg L^{-1} for 24 h

Normalized using "housekeeping" gene 18S rDNA

Expressed by reference to non-exposed tissues

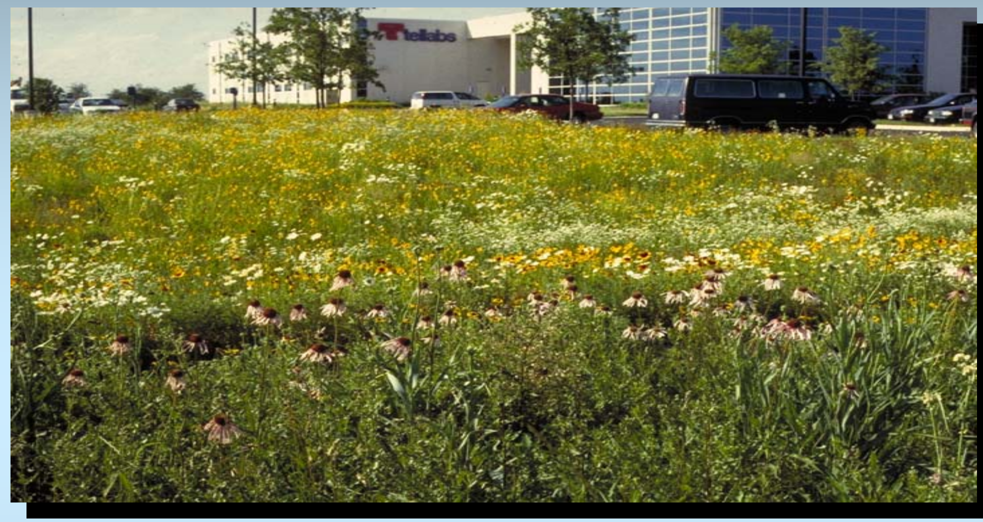


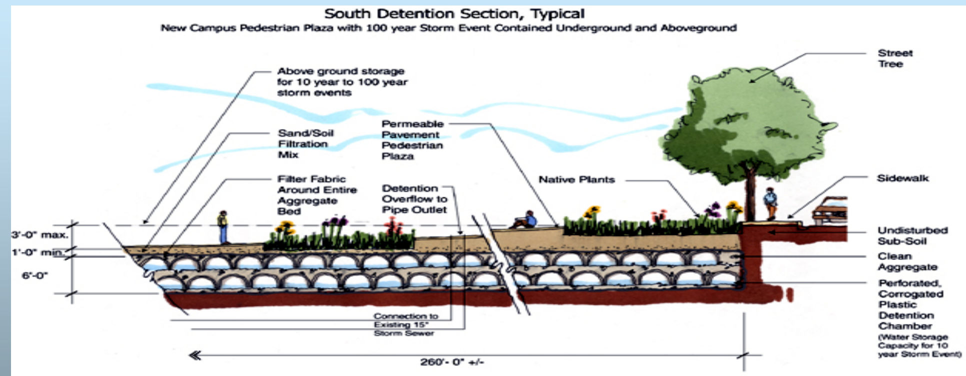
Gene Expression – Results

P. trichocarpa tissue cultures exposed to TNT 20.0 mg L^{-1} show a higher expression (induction) of potential "detoxification" genes

-Glutathione *S*-transferases (conjugation with GSH)
-Cytochromes P-450 (oxidative transformation, e.g. hydroxylation)
-Reductases, nitrilase (nitro group reduction)
-Glucosyl transferase (conjugation with sugar)

Phyto has “morphed” into many innovative technologies including green roofs and low impact development..





James Patchett, President
Conservation Design Forum, Inc.
Elmhurst, Illinois
February 4, 2004

Before

After

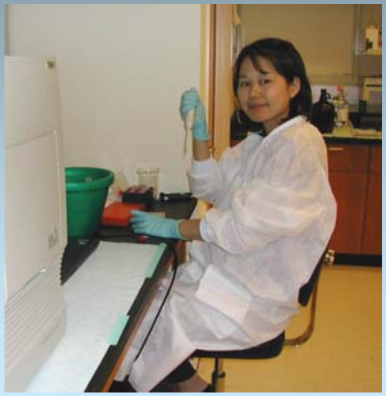
Chicago City Hall 1999


Conclusions

- PhytoTechnologies are evolving…
  - It's innovative but also becoming part of mainstream treatment technologies (constructed wetlands, landfill caps, and ecological restoration)
  - Research is becoming more molecular to understand which plants might be used to degrade which chemicals opening new sustainable development technologies

Acknowledgements

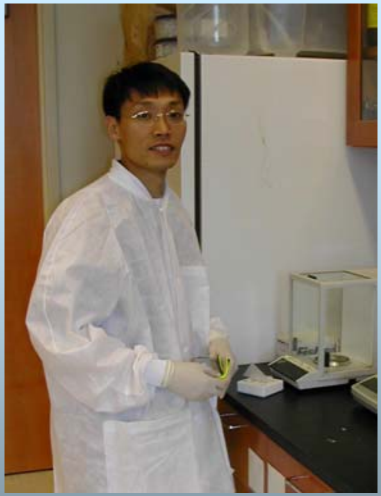
The Great Students:



Sachiyo Tanaka

Laura Brentner

Brittany Folkstra



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