Successful Field and Lab Tests of a Multi-Process Phytoremediation System for Decontamination of Petroleum and Salt Impacted Soils
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Partners: Imperial Oil; Talisman Energy; ConocoPhillips Canada; Northrock Resources; Canetic Resources; BASF; EBA; OAF; Stantec; Ontario Ginseng Growers, CRA; Region of Waterloo; City of Guelph; NSERC
Development and Proof of the Multi-Process Phytoremediation System (MPPS)

1. 6 Years of Lab Studies
   1. Soil spiked with creosote (PAHs)
   2. Total Petrol. Hydrocarbon (TPH) contam. soil
   3. PAH contaminated soil from urban foundry
   4. Plant growth on salt impacted soils

2. 3 Years of Field Studies
   1. TPH, Talisman Energy, Turner Valley, AB 2005-06
   2. TPH, North East Alberta 2005-06
   3. TPH, Imperial Oil Land Farm, Sarnia, ON 2004-06
   4. DDT, Simcoe, ON 2005
Advantages of Phytoremediation

1. Improves the natural structure and texture of soil
2. It is driven by solar energy and suitable to most regions and climates
3. It is low in cost and technically feasible
4. Plants can provide sufficient biomass for rapid remediation; promote high rhizosphere activity
5. Restoration
6. > 30,000 sites in Canada where such technology is needed, > 300,000 sites in the US
### Description of the Multi-Process Phytoremediation System (MPPS)

<table>
<thead>
<tr>
<th>Description</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical soil treatment:</td>
<td>Till the soil: exposure to sunlight and air exposure. Exposure to sunlight photooxidizes contaminants</td>
</tr>
<tr>
<td>Bioremediation:</td>
<td>Inoculation of PAH/TPH degrading bacteria</td>
</tr>
<tr>
<td>Phytoremediation:</td>
<td>Growth of plants alone on the soil</td>
</tr>
<tr>
<td>MPPS:</td>
<td>Land farming the soil for two weeks</td>
</tr>
<tr>
<td></td>
<td>Inoculation of PAH degrading bacteria</td>
</tr>
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<td></td>
<td>Growth of plants with PGPR</td>
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</table>

**PGPR:** Plant growth promoting rhizobacteria. Prevents the synthesis of stress ethylene.
Interaction of a PGPR containing ACC deaminase with a plant seed or root

Plant growth promoting rhizobacteria (PGPR)

Two natural, non-pathogenic strains of *Pseudomonas putida* (UW3 and UW4)
- One has high ACC Deaminase
- One is an auxin producer

Both PGPR are applied to seeds prior to planting
Isolation

Proliferation of selected PGPR
Apply to seed, Plant seeds
Few days later (Tomato)

Control

PGPR Treated
Greenhouse Studies and demonstration of PGPR effects

Use of the Multi-Process phytoremediation System (MPPS) for Removal of Total Petroleum Hydrocarbons from Imperial Oil Soil (~5% TPH)
Plants only
Plants with Degrad. Bacteria
Plants with PGPR
Multi-Process System
Plants from the MPPS on Land-Farmed Contaminated Soil

Plants only

MPPS
Plant Biomass for Phytoremediation and the MPPS

Percentage of no contaminant control

- **phytorem**
- **Multi-system**

Plant Biomass

- Root FW
- Root DW
- Shoot FW
- Shoot DW
Remediation Kinetics of Four Methods Tested

- landfarming
- biorem
- Phytorem only
- MPPS

Remediation time, months

THP remaining in soil, g/kg
Remediation of different fractions of TPH after 2 4-month seasons

Contaminants metabolized/degraded: ~40g TPH/kg soil removed, < 100g DW plants/kg soil. Plants cannot be 40 % TPH.
Field Test at Talisman Energy Biopile (1 % TPH)
Turner Valley Alberta
Year 1: Summer 2005 – Rye/Fescue Growth

Note: Weather cool and wet.
Poor, gravelly soil required fertilizer
Talisman Energy, Turner Valley Site
Summer 2005 Remediation Results
100 d – Year 1

Note: Every plot with PGPR had 15 to 20% superior remediation

Excellent remediation of recalcitrant contamination on poor soil to a depth of 20 cm in 1st year

+ PGPR
Avg 0.55%
SE: 0.01%
35% REM

- PGPR
Avg 0.61%
SE: 0.06%
28% REM

Blank
Avg 0.85%
SE: 0.03%
CCME Fractions remaining in Turner Valley Soil after 100 d – Year 1 (rye/fescue + PGPR)
Remediation Kinetics For Turner Valley Soil
Greenhouse vs. Field

Field 100 days
Both at 35 to 40 % Remediation in 100 d

MPPS: Greenhouse 100 days
Turner Valley Year 2 2006
Planted May 15, 2006

Site plan May 13th, 2006

- Quality control
- Ryegrass treated with seed treater
- UW3 + UW4 + Me-Celluose
- Test of PGPR efficacy on 3 % Creosote
- Plants grew well in the field
Turner Valley, Year 2 2006

- Planted May 15, 2006
- Treated with seed treater
- UW3 + UW4 + Me-Celluose
- Good positive PGPR effect
- Excellent growth

June 8, 2006
- PGPR
+ PGPR
Rye Fescue

July 28, 2006
Rye Fescue + PGPR

Sept 5, 2006
Rye Fescue + PGPR

Timothy Brome Alfalfa + PGPR
Turner Valley, Year 2 Remediation

9 % Remediation in 2 months

30 % Remediation in 3.5 months

Same as the remediation kinetics observed in previous years
Turner Valley 2006 – 60 cm deep plot on clay pad - Planted May 15, 2006

- Controlled experiment
- Test depth of remediation
- UW3 + UW4 + Me-Celluose
- Spring ryegrass and tall fescue
- Plants have grown well
- Positive PGPR effect
- Remediation Sept 5:
  
  15 % w/ PGPR, 6 % w/o PGPR
Field Test at Imperial Oil Land Farm, Sarnia, ON

Year 1: Summer 2004 – Rye grass
Oil Sludge Total Petroleum Hydrocarbon (TPH)
Contaminated Soil (15 % w/w)

MPPS (+ PGPR)  Plants alone (- PGPR)

60 after planting
Contaminants metab and/or degraded:
- ~75 g TPH/kg soil removed
- All fractions removed
- 100 g DW plants/kg soil.
- Plants cannot be 75% TPH.

Note: Summer 2004 was a cool wet summer.
### Sarnia Land Farm – 10E and 10 W – 2005 Planting

**Year 2**

<table>
<thead>
<tr>
<th>T</th>
<th>PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fall rye + PM</td>
<td>Fall rye + PGPR + PM</td>
</tr>
</tbody>
</table>

- **Fall Rye + PGPR + Peat Moss**
- **Fall Rye - PGPR + Peat Moss**

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- **10 W**
- **25 m**
- **~ 130 m**

<table>
<thead>
<tr>
<th>T = Treated with PGPR</th>
<th>PM = Peat moss coated with PGPR</th>
<th>Beads = Alginate beads containing PGPR</th>
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</thead>
<tbody>
<tr>
<td>Fall rye - PGPR</td>
<td>Blank</td>
<td>Barley/Rye + PGPR</td>
</tr>
<tr>
<td>Triticale + PGPR</td>
<td>Blank</td>
<td>Barley/Rye - PGPR</td>
</tr>
<tr>
<td>Triticale - PGPR</td>
<td>Blank</td>
<td>Corn - PGPR</td>
</tr>
<tr>
<td>F Rye + PM</td>
<td>Blank</td>
<td>Corn + PGPR</td>
</tr>
<tr>
<td>F Rye - PM</td>
<td>Blank</td>
<td>F Rye + PGPR</td>
</tr>
<tr>
<td>F Rye + AC1</td>
<td>Blank</td>
<td>F Rye - PGPR</td>
</tr>
<tr>
<td>F Rye – AC1</td>
<td>Blank</td>
<td>Fall Rye + PGPR</td>
</tr>
<tr>
<td>F Rye + Beads</td>
<td>Blank</td>
<td></td>
</tr>
<tr>
<td>F Rye - Beads</td>
<td>Blank</td>
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</tr>
</tbody>
</table>

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Not to scale
Imperial Oil Land Farm, Sarnia, ON
Year 2: 2005 – Fall Rye Growth

Note: Summer 2005 was unusually hot and dry.
Imperial Oil Land Farm, Sarnia, ON
Summer 2005 – Rye/Fescue
Results

Root depth (+ PGPR) after 90 d was > 40 cm

Extent of Remediation

<table>
<thead>
<tr>
<th>+ PGPR</th>
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</thead>
<tbody>
<tr>
<td>30 d</td>
<td>9 % ± 1.5</td>
</tr>
<tr>
<td>60 d</td>
<td>17 % ± 2</td>
</tr>
<tr>
<td>120 d</td>
<td>35 % ± 4</td>
</tr>
</tbody>
</table>

- PGPR remediation was about 50 % slower
TPH Removal from Sarnia Year 2

TPH, % DW of Soil

Blank | Rye/Fescue

CCME Fraction, % DW of Soil

Blank | Plants | Blank | Plants

Fraction 3

Fraction 4
Fall Rye overseeded with Rye/Fescue + PGPR

Rye/Fesc/Barley + PGPR

June 19, 2006
Imperial Oil Sarnia Land Farm
Year 3 (2006)

- Planted Barley/Fescue/Rye Grass on April 20, 2006
- Plants were treated with PGPR (UW3 and UW4) using a mechanical seed treater

40 days after planting – Weather good
Imperial Oil Sarnia Land Farm Year 3

Fall Rye overseeded with Rye/Fescue + PGPR

40 d after planting

Over seeded Rye/Fescue field, after fall rye mowed

60 d after planting

Rye/Fescue, Test plots

100 d after planting

Remediation data on Aug 15: 13 % ± 3%
HPLC assay for TPH uptake by Rye/Fescue roots

~ 35% of the TPH removed from the soil

Root data rescaled; Qualitatively different HPLC profile

Remediated TPH does not appear in the roots
What happened to the TPH?

- TPH removed from the soil, but not in the plant roots
- TPH must have been degraded
- Where was it degraded?
- Perhaps in rhizosphere by bacteria, fungi and roots
- Soil fungi and bacteria have very active and diverse metabolic activities
- In soils with PGPR treated plants, bacteria and fungal counts are 5 to 10 fold higher
Possible degradation pathway of TPH in Soil

Aliphatic hydrocarbon

\[
\begin{align*}
\text{R} & \quad \text{R'} \\
\quad \downarrow [\text{O}] \text{ P450?} \\
\quad \text{R} & \quad \text{R'} \\
\quad \downarrow \text{H}_2\text{O} \\
\quad \text{R} & \quad \text{R'} \\
\quad \downarrow [\text{O}] \\
\quad \text{R} & \quad \text{R'} \\
\quad \downarrow [\text{O}] \text{ P450?} \\
\text{R} & \quad \text{R'} \\
\end{align*}
\]

2 Fatty Acids
PGPR Can Degrade Oil

Saturated TPH in Phos-Buff-Saline

TPH sole reduced carbon source

No Inoculation  Inoculation w/ PGPR

Grow for 12 h on shaker

Control  Oil consuming bact.  Oil consuming bact.
Microbes in Sarnia Land Farm Soil From Remediation Field Trial 2006

Rye Fescue Barley Soils

- Plants - PGPR
- Plants - PGPR
+ Plants + PGPR

Total Bacteria

Total Fungi

PGPR

Petro Deg Bacteria

27.4 29.5 21.7 13.8
Enhancement of Plant Growth on Salt Contaminated Soils Using PGPR
Effect of PGPR on Plant growth in Alberta Salt Impacted Soil
EC = 2.2 dS/m, SAR = 27, Cl = 260 mg/kg

Oat – 10 d

Shoots – 219% increase
Roots – 356% increase

Barley – 10 d

Shoots – 24% increase
Roots – 4.1% increase
Sask Salt Soil: EC = 14 dS/m, SAR = 11, Cl = 1880 mg/kg

Oat

### Oat Low 21d Fresh Weight

- **Promix**
- **-PGPR UW3+UW4 Fungus PGPR +Fungus**

- **Shoots and roots 100% improved**

- **Oat Low 21d Fresh Weight**

- **Wheat**

### Wheat Low 21d Fresh Weight

- **Promix**
- **-PGPR UW3+UW4 Fungus PGPR +Fungus**

- **Wheat Low 21d Fresh Weight**

- **Shoots and roots 60 to 80% improved**
CONCLUSIONS

• MPPS has great potential for efficient remediation of organic, salt and metal contaminated sites
• PGPR is the key: healthy plants with vigorous roots in PAH, TPH, DDT, salt and metal contam. soils
• PGPR alleviate stress and promote growth: Low ethylene and high auxin
• 8 Months in the greenhouse: MPPS removed 90 % of recalcitrant TPHs and PAHs
• 3 years of fields tests successful: MPPS removed 30 % to 60 % of recalcitrant TPHs and DDT per year
• Contaminants metabolized and/or degraded
• Great promise for restoration of oil and salt impacted sites, and brownfields
Future Work

- Continued field testing of the MPPS
- Increasing research on salt remediation
- Ready to deploy at new sites
- Proposals for new sites are being entertained
- TPH sites can be remediated in 2 to 4 years
- Further research on salt and metal remediation underway
Colleagues and partners

• The people that do all the work
  - Karen Gerhardt
  - Yola Gurska
  - Mark Lampi
  - Pearl Chang
  - Wenxi Wang
  - Haitang Wang
  - Aaron Khalid
  - David Isherwood
  - Shan Shan Wu
  - Julie Nykamp
  - Anabel Ueckermann
  - Xiao-Ming Yu

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  - Xiao-Dong Huang
  - Bernie Glick
  - Perry Gerwing
  - George Dixon
  - Ron Pitblado

• Partners
  - D Bristow, L Lawlor, M Young, Imperial Oil
  - J Gordon, Talisman Energy
  - A Hopf, BASF
  - G Surgeoner, OAFI
  - G Stephenson, Stantec
  - G Brooks, CRA
  - D Hoffman, Ginseng Growers
  - Region of Waterloo
  - City of Guelph
  - NSERC
Phytoremediation of TPH Contaminated Soil Early in Tall Fescue Growth

Plants only

Plants + PAH Deg. bacteria

Plants + PGPR

MPPS
Effectiveness of Different Methods on TPH Remediation - Second 4 month season (i.e., 8 months total)
Remediation Kinetics For Land Farm Soil
Greenhouse vs. Field

- THP remaining in soil, g/kg
- Remediation time, months
- landfarming
- biorem
- Phytorem only
- MPPS

Field 120 days
Both at 50 to 60 % Remediation in 120 d

MPPS: Greenhouse 120 days
Sarnia Land Farm – 2005

15 % TPH

Barley/Rye 30 days
- PGPR  + PGPR

Barley/Rye 40 days
- PGPR  + PGPR

Barley/Rye + PGPR - PGPR

Fall rye 30 d
- PGPR  + PGPR
Remediation of Metals
PGPR Effect on Plant Growth with 2% Salt in Irrigation Water

Control w/ PGPR  Salt w/ PGPR  Salt w/o PGPR
Dundas foundry soil – PAH contamination ~ 500 ppm

MPPS: germinated earlier and grew faster in the contaminated soil
Dundas Foundry: 30 d growth, MPPS has 300% more plant biomass in roots and shoots than plants alone.
Effectiveness of the MPPS for Remediation From Dundas Foundry Soil: 90 days

![Bar chart showing the effectiveness of different remediation methods for PAH remaining in soil. The methods compared are Biorem, Phytorem, MPPS, and Control soil. The bars indicate the percentage of PAH remaining, with MPPS showing the least remaining PAH.]
Multi-Process System for Remediation of PAH Contaminated Brownfield: 90 d remediation leads to ~ 40 % removal of organics

Untreated brownfield soil
Phytoremediation for 90 days

Bioremediation for 90 days
MPPS for 90 days
DDT Remediation with Millet
Summer 2005, Simcoe, ON

70 d growth

+ PGPR
+ Peat Moss w/PGPR

+ PGPR
- Peat Moss
Remediation of DDT

- Approximately a 0.5 Hectare site
- SW Ontario farmland
- Homogeneous levels of DDT at ~ 0.8 mg/kg (ppm)
- Half planted with Millet and half planted with fall rye
- 40 % overall DDT remediation after 90 d
- DDT breakdown products (DDE or DDD) not found
- Chlorinated compounds can be degraded by phytoremediation
Effect of PGPR on Plant Growth during Flooding or Drought Stress

- **Flooding**
- **Flooding + PGPR**
- **Drought**
- **Drought + PGPR**

*Time, days: 0, 6, 12, 17, 23*

*Biomass, g:
- 0
- 500
- 1000
- 1500
- 2000
- 2500
- 3000

The diagram illustrates the biomass growth over time for different conditions: flooding, drought, and their respective treatments with PGPR.
Growth of rye grass in salt contaminated soil

EP3 (a salt tol. PGPR) - PGPR UW4+EP3 (2 PGPRs)
Survival 50% higher with PGPR

Salt impacted clay-loam soil Fenn-Big Valley.
Preliminary greenhouse experiment.
Cl: 1200-2000 mg/ml, SAR: 11-15, ECe: 9-15 dS/m
Growth of fall rye in salt contaminated soil from Fenn-Big Valley

**Fall rye shoots**
- Uncontam. Control soil
- No PGPR
- UW4
- MG3
- EP3
- UW4 + EP3
- UW4 + MG3

**Fall rye roots**
- No PGPR
- UW4
- MG3
- EP3
- UW4 + EP3
- UW4 + MG3

**MG3/EP3: PGPR from salted soils**

- 50% more survival + 20 - 30% more growth
- ≅ 100% more biomass
Triticale/Barley Mix
Year 1: Summer 2005, North East, AB
~ 1 % TPH on a 7 Hectare site

- PGPR
+PGPR
70 d growth on good soil
Remediation of the NE Alberta Site

- A very large site: > 7 hectares
- Overall ~1 % TPH, with high variability in TPH levels (0.3 to 3 % TPH)
- 6 Hectares planted: Large scale pilot remediation; All plants (barley/fall rye mix, 2000 kg of seeds) were PGPR treated
- 1 Hectare was used as a random block test for PGPR efficacy. Barley, fall rye and triticale were used
- Pilot area: 15% to 30 % remediation was observed in 70 d
- Random block test (70 d), % remediation:
  
<table>
<thead>
<tr>
<th></th>
<th>+ PGPR</th>
<th>- PGPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>F3</td>
<td>2.8 ± 0.5</td>
<td>- 4.2 ± 0.7</td>
</tr>
<tr>
<td>F4</td>
<td>8.9 ± 1.3</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>Total</td>
<td>21.9 ± 4.7</td>
<td>16.7 ± 2.3</td>
</tr>
<tr>
<td>F4G</td>
<td>32.6 ± 10.9</td>
<td>28.7 ± 5.3</td>
</tr>
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</table>

- Consistent rates of remediation observed
- Evidence of PGPR improvement, PGPR impact on par with Turner Valley
- 2006 season: Planted only + PGPR Barly/Rye on June 20.
Multi-Process Phytoremediation System (MPPS)

1. Complicated mixtures of contaminants are present in the environment
2. Many techniques based on an individual process failed or were ineffective
3. Contaminants are too toxic to plants and bacteria for remediation
4. Use and understanding of different remediation mechanisms
5. Multiple remediation kinetics resulting in effective and efficient remediation
Field Test at Talisman Energy Biopile (1 % TPH)  
Turner Valley Alberta  
Summer 2005 – Rye/Fescue Plant Growth

Average root depth: 21 cm
Field Test at Imperial Oil Land Farm, Sarnia ON
15 % Total Petroleum Hydrocarbon (TPH)
Year 1: Summer 2004 - 60 d after planting

Rye Grass + PGPR

Rye Grass - PGPR
Field Test at Imperial Oil Land Farm, 120 d