Successful full-scale deployments of advanced PGPR enhanced phytoremediation systems (PEPS) for decontamination of petroleum and salt impacted soils.

Bruce Greenberg, Xiao-Dong Huang & Perry Gerwing

**Partners:** Talisman; PennWest; Canadian Forest Oil; Devon; Baytex; ConocoPhillips; Solaction; Shell; Seaway Energy Services; Questerre Beaver River; Cenovus; Imperial Oil; URS; NorthWind; SNC Lavalin; MWH; NSERC
Outline

1. Advantages of phytoremediation
2. Overview of our phytoremediation system
3. Field tests of our phytoremediation system for petroleum and salt remediation
Examples of Remediation Methods

- Dig and dump - Any contaminant type - $100-500/m^3
- Soil incineration - On or off site - Organic contamination - $200-600/m^3
- Chemical extraction - Any type of contamination - $250/m^3
- Electrokinetic separation - Metals/Salts - $200/m^3
- Soil flushing/fracturing - Any contaminant type - $250/m^3
- Land farming - Natural attenuation - Small organics - $50/m^3
- Bioremediation - Organics - $100/m^3
- Phytoremediation - Any contaminant type - $25-50/m^3
Process of Phytoremediation

- Volatilization
- Phytodegradation
- Chelation/compartment in leaves

Salt
- Translocation: root symplast $\rightarrow$ xylem
- Chelation/compartment in roots
- Plant uptake soil $\rightarrow$ root
- Rhizosphere Processes
- Bioavailability particle $\rightarrow$ water
Advantages of Phytoremediation

1. Improves the quality of soil
2. It is driven by solar energy and suitable to most regions and climates
3. It is cost effective and technically feasible
4. Plants provide **sufficient biomass** for rapid remediation; promote high rhizosphere activity
5. Restoration in a reasonable time frame - 2 to 3 years
6. Can be used effectively at **remote sites**
7. Effective for remediation of PHC and salt – relevant to the energy industry
Development, Proof, and Application of PGPR Enhanced Phytoremediation Systems (PEPS)

Over 10 years of research with full-scale field studies at each stage of development and application

1. PHC: sites in AB, BC, QC, and ON (2004-10)
2. Gas station: site fully remediated in 1 summer (2007)
3. Salt: sites in SK, AB and NWT (2007-10)
Description of the PGPR Enhanced Phytoremediation System (PEPS)

Physical soil treatment: Till the soil: exposure to sunlight and air
Exposure to sunlight photooxidizes contaminants

Bioremediation: Inoculation of PAH/TPH degrading bacteria
(generally skipped in the field → already present)

Phytoremediation: Growth of plants with PGPR

- **PGPR**: Plant growth promoting rhizobacteria.
- Prevent the synthesis of stress ethylene.
- **PGPR** are applied to the seeds prior to sowing
  → NOT Bioaugmentation
- Grass species used generally
- Effect depth of remediation ~ 0.5 m
Interaction of a PGPR Containing ACC Deaminase with a Plant Seed or Root

Plant growth promoting rhizobacteria (PGPR)

Natural, non-pathogenic strains of PGPR (usually *Pseudomonads*)

We have isolated PGPRs from ON, AB, SK and the NWT

PGPR are applied to seeds prior to planting
Research and Development of the PEPS for PHC Remediation

1. Sarnia, ON – land farm – 4 year study
   Oil sludge – PHC contaminated soil
   (15% w/w – 60% F3 (C16-C34), 30% F4 (C34 – C50))

2. Turner Valley, AB – 3 year study

3. Hinton, AB – 2 year study
Sarnia, ON – Land Farm

- Planted barley/fescue/ryegrass
- Plants were treated with PGPR (UW3 and UW4) using a mechanical seed treater
- PHC remediation from:
  - 15% - 3% in 4 years with PEPS
  - 15% - 8% in 4 years for plants w/o PGPR
  - 15% - 11% in 4 years w/o plants
Conclusions on Development of the PEPS

1. 100% increase in plant biomass due to PGPR, root growth to 50 cm below ground level.
2. 30 to 40% remediation per year with PEPS; 100% faster than plants without PGPR.
3. Rhizosphere microbes (esp. PHC degraders) elevated 10 to 100 fold with the PEPS - microbes and plants consume PHC.
4. Very low $^{14}$C detected in soil microbial fatty acids – Carbon came from PHC metabolism (PHC has no $^{14}$C).
5. Very low $^{14}$C in CO$_2$ that evolves from soil – PHC has been mineralized to CO$_2$.
6. No PHC detected in plant tissue as it disappears from the soil.
7. CCME PHC analytical method used effectively to show extent of remediation.
Phytoremediation of PHC

(A) Bioavailability of PHC

(B) General processes affecting rhizoremediation

(C) Microbial aerobic PHC degradation – rhizosphere supported by plants

(D) Possible microbial oxygenation pathway of PHC to form a fatty acid

Hydrophobic oil droplet trapped between grains of soil

Aqueous micropore

Grains of soil

Roots penetrate into soil grains freeing oil droplets

Respiration

\[ \text{CO}_2 \rightarrow \text{affects soil pH} \]

\[ \text{O}_2 \rightarrow \text{redox reactions} \]

Respiration affects contaminant bioavailability

\[ \text{H}_2\text{O} \rightarrow \text{affects plant growth} \]

\[ \text{small organic contaminants} \]

Plant enzymes – oxidases and hydrolases that can degrade contaminants (phytodegradation)

Release of \( \text{H}^+ \) and \( \text{OH}^- \)
- affects pH, acid/base reactions, bioavailability

Microbial enzymes - affect plant growth/physiology (e.g. PGPR with ACCD can diminish ethylene stress)

Ion uptake - plant growth

Exudates - substrates that can stimulate microbial growth

Microbial chelators deliver plant nutrients

Cₙ PHC

[O] P450?

OH

Cₙ F.A.

Cₙ PHC

[O] DEHYD

OH

Cₙ F.A.

Rhizo cell membrane (width C₃₀ to C₄₀)

Cytosol
Application of PEPS for PHC Remediation – 1st Generation Full Scale Sites for Proof of Concept (2007-09)

All sites planted with oats, tall fescue and ryegrass treated with PGPR – All sites met applicable criteria

1. Hinton 2, AB – Complete remediation in 2 years – Diesel invert drilling waste

2. **Edson, AB** – Complete remediation in 2 years – Diesel invert drilling waste

3. Peace River, AB – Complete remediation in 3 years – Flare pit material

4. Steinbach, MB – Complete remediation in 1 year – Gas station site

5. **Quebec City, QC** – Tier 1 criteria met in one year
Edson, AB – Site and Sampling Map (2008)
Soil Impact – PHC (Diesel Invert; 85% F3)

Area:
120 m x 100 m
= 12,000 m²
= 3 acres
Edson, AB – Beginning and Mid-Season (2008)
Soil Impact – PHC (Diesel Invert; 85% F3)

June 5, tilling and planting

July 31
In June 2007, 9 of 13 sampling points above Tier 1 criteria (F3 > 1300 mg/kg)
Gravimetric Total PHC vs Analytical Lab F3

\[ y = 1.0067x + 0.0142 \]

\[ R^2 = 0.8914 \]

- Can follow F3 remediation with a less expensive method
- Final remediation is confirmed using accredited lab
Edson, AB – PHC Remediation (2007-08)
Soil Impact – PHC (Diesel Invert; 85% F3)

- Remediation goals were met
- No points over Alberta Tier 1 criteria
Quebec City, QC — End of Season (2009)
Soil Impact – PHC
Quebec City, QC — End of Season (2009)
Soil Impact – PHC

Root depth to 50 cm
Quebec City, QC – End of Season (2009)
Soil Impact – PHC

Meets Tier 1 criteria for QC
Second Generation Full Scale Sites

1. Three sites near Dawson Creek, BC
2. One site near Swan Hills, AB
3. One site near Hinton, AB
4. One site near Edson, AB
5. One site near Red Earth Creek, AB
6. One site in Northern BC

All sites worked with very similar results
All sites planted with tall fescue, ryegrass, and/or oats treated with PGPR.
Northern BC near NWT Border — Mid/End of Season (2010)
Soil Impact — PHC (Diesel Invert)

Mid-Season

End of Season
Northern BC near NWT Border – Site Map (2010)

Soil Impact – PHC (Diesel Invert)

Site Area:
- 2400 m²
- 0.6 acres

Area of lower PHC met applicable criteria after 1 year of PEPS treatment

Area of higher PHC with wood chips

EPH_{C10-C19} Remediation (Maxxam)
- 6 out of 8 sampling points showed a decrease in EPH_{C10-C19} levels over two months
- All sampling points had EPH_{C10-C19} level above 1000 mg/kg criteria
- At the end of the season the average EPH_{C10-C19} level decreased by 29%, from 3659 mg/kg in July to 2608 mg/kg in September

EPH_{C19-C32} Remediation (Maxxam)
- 6 out of 8 sampling points showed a decrease in EPH_{C19-C32} levels over two months
- Only 3 out of 9 sampling points had EPH_{C19-C32} level above 1000 mg/kg criteria in September
- At the end of the season the average EPH_{C19-C32} level decreased by 27%, from 1335 mg/kg in July to 979 mg/kg in September
Swan Hills, AB — End of Season (2009 – 10)

Soil Impact – PHC

Impacted Plot

Planted un-impacted plot

Soil sampling point

Un-impacted Plot

**Soil Impact – PHC**

20 m
6 m
3 m

2 m 2 m 2 m

3.5 m 3.5 m 3.5 m

35 m

EARTHMASTER ENVIRONMENTAL STRATEGIES INC.
Swan Hills, AB – End of Season (2009 – 10)
Soil Impact – PHC

Impacted Plot

Un-impacted Plot
<table>
<thead>
<tr>
<th>Sample date</th>
<th>Soil-type</th>
<th>Plant Biomass (g/m²) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>October 2009</td>
<td>Impacted</td>
<td>427.7 ± 88.06</td>
</tr>
<tr>
<td>October 2009</td>
<td>Un-impacted</td>
<td>438.1 ± 53.56</td>
</tr>
</tbody>
</table>
Swan Hills, AB – F2/F3 Results

F2 & F3 analysis performed by Maxxam. Method used: cold shake extraction, single silica-column clean-up

EARTHMASTER ENVIRONMENTAL STRATEGIES INC.
Phytoremediation Cost analysis for a typical PHC impacted site - Edson

- Collaborative project between Earthmaster Environmental and Waterloo Environmental Biotechnology
- Volume of impacted material – 460 m$^3$ of diesel invert drilling mud was originally spread over 1.07 ha
- 1.07 ha impacted to a depth of 0.3 m or 3,210 m$^3$ of PHC impacted material
- The costs for the entire project was: $104,000 or $32.50/m$^3$
  - Includes all Earthmaster, WEBi and 3$^{rd}$ party costs
- Landfilling this material would have cost $80/m^3$
  - Assumes a 2 h truck turnaround time
  - No backfill required – if backfill was required the cost would rise to $90/m^3$
Conclusions for PHC Remediation

SUCCESS
• Achieved PHC remediation: 4 sites brought to closure, 6 second generation sites progressing well towards closure

PERFORMANCE PREDICTIONS FOR PEPS
• PHC up to 10,000 mg/kg – Remediation in 2 to 4 years
• PHC > 10,000 mg/kg – Remediation in 3 to 6 years
• In all cases, soil becomes non-toxic before regulatory criteria are met – Site specific risk assessment possible

COST
• Actual cost for the Edson site (3,400 m³) was $33/m³
• Cost to landfill (landfill 1 h from site) would have been > $80/m³
• Cost differential increases as site become more remote or volume of impacted material increases
Development of PEPS for Salt Impacted Sites
Plant responses to salinity

- Inhibited germination
- Decreased water uptake $\rightarrow$ Low water potential (drought)
- Unbalanced sodium/potassium ratios
- Inhibition of photosynthesis
- Increased reactive oxygen species (ROS)
- Increased ethylene production

<table>
<thead>
<tr>
<th>Salinity effects mostly negligible (or salt deprived)</th>
<th>Yields of very sensitive crops may be restricted</th>
<th>Yields of many crops diminished</th>
<th>Only tolerant plants grow</th>
<th>Only a few very tolerant plants can grow</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>16</td>
</tr>
</tbody>
</table>

$EC_e$ (dS/m)
Sites for Development of PEPS for Salt Remediation

1. Cannington Manor, SK
2. Alameda, SK
3. Kindersley, SK
4. Brazeau, AB
5. Norman Wells, NWT
6. Weyburn, SK (7 sites)
7. Provost, AB
8. Red Earth, AB
Lab Research Summary of PEPS for Salt Impacted Soils

- 50 to 100% increases in plant growth due to PGPR with root growth to 50 cm
- Plants can grow on soils with $EC_e \sim 25$ dS/m
- ON, SK, and NWT PGPRs all worked well
- PGPRs protected against inhibition of photosynthesis and plant membrane damage
- Levels of salt up-take to plant foliage: 50 – 75 g NaCl/kg dry weight
- Remediation can be based on up take of salt into foliage
- Phytoremediation is feasible for soils with $EC_e$ of 15 to 20 dS/m in about 5 years
Norman Wells, NWT – End of Season (2010)

Soil Impact – Salt
Plants used: slender wheatgrass and red fescue
No soil conditioning
High shale area has filled in due to soil conditioning

Plants used: slender wheatgrass, ryegrass and red fescue
Soil conditioned
Norman Wells, NWT – End of Season (2010)
Soil Impact – Salt

High shale area has filled in
High shale area has filled in


Plot B

Year 3 (2010)

Plot C

Year 3 (2010)

Salt Remediation

Norman Wells, NWT – End of Season (2010)
High salt plant material was mowed and removed from the site.
High salt plant material was mowed and removed from the site.
Weyburn, SK – 1 of 7 sites: Soil Salinity (ECe) Map (2010, Year 1)

Soil Impact – Salt

Field Averages:
= 8.35 dS/m (0-60 cm)
= 5.67 dS/m (0-15 cm)
= 7.48 dS/m (15-30 cm)
= 11.89 dS/m (30-60 cm)

Site Area:
• 4500 m²
• 1.1 acres
Weyburn, SK – 1: End of Season (2010, Year 1)
Soil Impact – Salt

5 cm
Weyburn, SK – 1: End of Season (2010, Year 1)
Soil Impact – Salt

Roots

5 cm
Weyburn, SK – 2 of 7 sites: Soil Salinity (EC$_e$) Map (2010, Year 1)

Soil Impact – Salt

Site Area:
- 12 acres

Field Averages:
- $4.61$ dS/m (0-60) cm
- $3.25$ dS/m (0-15 cm)
- $3.43$ dS/m (15-30 cm)
- $3.82$ dS/m (30-60 cm)

Soil Salinity Map:

<table>
<thead>
<tr>
<th>Surface soil EC$_e$ at each sample point</th>
</tr>
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<tbody>
<tr>
<td>7.63</td>
</tr>
<tr>
<td>4.58</td>
</tr>
<tr>
<td>3.06</td>
</tr>
<tr>
<td>2.72</td>
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<td>6.35</td>
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<td>1.92</td>
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<td>2.06</td>
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<td>5.33</td>
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<tr>
<td>3.86</td>
</tr>
<tr>
<td>8.72</td>
</tr>
<tr>
<td>5.77</td>
</tr>
<tr>
<td>3.58</td>
</tr>
</tbody>
</table>

20m
Weyburn – 2, SK: End of Season (2010, Year 1)
Soil Impact – Salt
Weyburn – 2, SK: End of Season (2010, Year 1)
Soil Impact – Salt
High salt hot spots and poor soil from pipeline construction: $EC_e: 13-17 \text{ dS/m}$
Provost, AB: End of Season (2009, Year 1)
Soil Impact – Salt

- High salt spots have filled in with plants

- $EC_e$ (2009): decreased from 13-17 to 4-12 dS/m
- $EC_e$ (Spring 2010): all sampling points were below applicable targets
- **Successful remediation was achieved in 1 year**
Red Earth, AB: 2009 - 2010
Soil Impact – Salt and Herbicide (Arsenal®)

Great improvement in plant growth, therefore, can remediate herbicides
Why Use Phytoremediation?

- It works for PHC and salt remediation.
- Remediation at all sites (> 20) successful.
- Costs of PHC and salt remediation will be similar.
- Unit cost drops as the volume of material increases.
- Phytoremediation costs (all in) < half the cost of landfilling.
- Liability is reduced, not transferred to a landfill.
- Costs are spread over more than one year (2 to 4 yrs).
- The price differential relative to landfilling increases when sites become more remote.
- Purchase of backfill not required. Soils are reused.
- Tier 2 approach - if required only marginal cost increase.
- Green technology: Good PR and environmentally friendly.
Colleagues and Partners

- The people that do all the work
  - Karen Gerhardt
  - Jola Gurska
  - Xiao-Ming Yu
  - Wenxi Wang
  - Mark Lampi
  - Shan Shan Wu
  - Julie Nykamp
  - Nicole Knezevich
  - Greg MacNeill
  - Xiaobo Lu
  - Scott Liddycoat
  - Han Zheng
  - Brianne McCallum
  - Jing Ma
  - Peter Mosley
  - Conrad Neufeld
  - Xiao-Dong Huang

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- Partners
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  - B Moore, Devon
  - G Millard, Shell
  - J Budziak, Seaway
  - P Coldham, Questerre Beaver River
  - E Harrison, Cenovus
  - L Lawlor, Imperial Oil
  - K Cryer, M Metzger, S Brown, C Chattaway, Earthmaster
  - D McMillan, SNC
  - G Stephenson, Stantec
  - T Chidlaw, MWH
  - S Steed, NorthWind
  - A Traverse, Baytex
  - G Adams, URS
  - B Chubb, Maxxam