Toxicity Testing to Evaluate Bioremediation Endpoints at Upstream Oil and Gas Facilities

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Outline

• Background
• Objectives
• Methods
• Results
• Conclusions
• Questions
Petroleum Hydrocarbons

- Petroleum Hydrocarbons (PHC):
  - mixture of organic compounds derived from geological substances (e.g., gas, diesel, crude oil)
  - lower MW PHC: more mobile
  - higher MW PHC: more persistent
- PHC composition at a given site:
  - Function of source, age of release, extent of degradation or transformation, weathering and site-specific factors (geology, hydrogeology etc.)
PHC Contaminated Sites

- Canada (CCME, 2000):
  - >10,000 PHC Contaminated Sites
  - Liability: ~ $10 B
- Canada Wide Standards:
  - 3-Tiered standard
  - Soil/subsoil
  - Human/environment
  - RA/RM
Elements of Risk

- Hazard
- Pathway
- Receptor
- RISK
CCME PHC Canada Wide Standards

- PHC Fractions (CCME, 2000)
  - F1 (C6-C10)-BTEX
  - F2 (>C10-C16)-PAHs
  - F3 (>C16-C34)-PAHs
  - F4 (C34+)

- Standards are a function of land use, exposure pathways, soil texture (fine vs. coarse) and depth of contamination (surface vs. subsurface)
CCME PHC Canada Wide Standards

- PHC Fractions (CCME, 2000)
  - F1 (C6-C10)-BTEX
    - Coarse (HH: vapour inhalation)/Fine-(HH: gw ingestion)
  - F2 (>C10-C16)-PAHs
    - Coarse and fine-grained (HH: groundwater ingestion)
    - Coarse-grained: Aquatic Life (water body adjacent)
  - F3 (>C16-C34)-PAHs: Direct soil contact (EH)
  - F4 (C34-C50)/F4G (C50+): Direct soil contact (EH)
Upstream Oil and Gas Facilities

- Sources of PHCs:
  - Flare pits, Drilling Sumps, Wellheads, Tank Farms and Pipelines

- Hydrocarbons become issue at most Sites, based on assessment data vs. remediation guideline

- Most sources: PHC F3 (>C16-C34) CWS becomes remediation target
The Nature of PHC F3

- PHC F3 (>C16-C34): broad range of solubility, Kow
  - >C16-C21 subfraction (more soluble/toxic)
  - >C21-C34 subfraction (less soluble/toxic)
- Broad range of physico-chemical properties and toxicity
CCME Ecosoil Contact Guideline

- CCME CWS ecosoil contact based on toxicity tests (acute, chronic, subchronic) with distillates of Federated Crude Oil
  - Simulates fresh spill of individual fraction
- Federated Crude Oil (PHC F3):
  - >C16-C21 subfraction (45 %)
  - >C21-C34 subfraction (55 %)
Biodegradation of PHC

- Bioremediation of PHC contaminated media:
  - cost-effective
  - practical: can be used *in situ* or *ex situ*
  - results in mineralization or transformation of PHC
- In many cases, residual PHC concentrations remain in excess of Tier I remediation endpoints particularly for PHC F3
Aging and Bioavailability of PHC

- Biodegradation of more labile (available) PHC leaves a residual PHC fraction that is much less available or mobile (biostabilization)

- **Aging phenomenon**: hydrocarbon availability within geosorbents decreases with time (e.g., earthworm uptake and bacterial mineralization)

- **Chemical extractability versus bioavailability**: how do you define risk-based endpoints??
Soil Matrix Organisms PHCs Bioavailability
The “Aging Effect”

PHC Concentration vs. Time

Remediation Objective
Questions

• Is the addition of freshly added hydrocarbons to soil indicative of toxicity of weathered, aged hydrocarbons?

• Are remediation endpoints that we are trying to achieve appropriate, what is the risk if we don’t meet the goal?

• Is this mixture used in CCME CWS development representative of PHC F3 compositions found in Alberta?
Research Objectives

Using a battery of toxicity tests, we evaluated:

- Toxicity of residual PHC in four biotreated soils
- CCME PHC Standards against the measured toxicity of weathered biotreated PHC contaminated soils
- Composition of PHC F3 within biotreated soils vs. Federated Crude used in CWS development
Experimental Design

- Biotreated PHC Soils Reached Plateau
- Soil Characterization
  - Microtox
  - Seed Germination
  - Risk Characterization
- Earthworms
Materials and Methods: Soils

PHC Contaminated Soils:

- Oil and Gas Facilities (Alberta)
- Soils A, C and D: drilling wastes
- Soil B: crude oil
- Control Soil
- PHC degradation in all soils reached plateau above Tier I
Materials and Methods: Physical-Chemical Characterization

Soil Characterization:

- particle size distribution
- pH, EC, main soluble ions
- Organic Matter
- CCME PHCs
- Polycyclic aromatic hydrocarbons
- Metals and inorganics
Bioassays and Screening Level Assessment of Earthworm Tissues

- Microtox®
- Seed Germination
  - Oat (*Avena sativa*) & radish (*Raphanus sativus*)
- Earthworm (*Eisenia fetida*)
  - acute (14 days)
  - subchronic exposures (10 weeks)
- worm tissues exposed to Soils A, D and control
  - FAC (HPLC), PHC and metabolites (GCMS)
## Results: Physical-Chemical Properties

<table>
<thead>
<tr>
<th>Property</th>
<th>Soil A</th>
<th>Soil B</th>
<th>Soil C</th>
<th>Soil D</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Texture</td>
<td>Fine*(CL)</td>
<td>Fine (CL)</td>
<td>Fine (CL)</td>
<td>Fine (L)</td>
<td>Fine</td>
</tr>
<tr>
<td>S/Si/C (%)</td>
<td>41/33/26</td>
<td>35/38/27</td>
<td>24/41/35</td>
<td>47/30/23</td>
<td>44/38</td>
</tr>
<tr>
<td>OM (%)</td>
<td>4.0</td>
<td>5.8</td>
<td>3.8</td>
<td>2.1</td>
<td>2.7</td>
</tr>
<tr>
<td>pH</td>
<td>7.5</td>
<td>7.5</td>
<td>7.2</td>
<td>7.2</td>
<td>7.1</td>
</tr>
<tr>
<td>EC (dS/m)</td>
<td>1.47</td>
<td>2.19</td>
<td>0.96</td>
<td>2.72</td>
<td>0.53</td>
</tr>
<tr>
<td>Metals</td>
<td>BC</td>
<td>BC</td>
<td>BC</td>
<td>BC</td>
<td>BC</td>
</tr>
</tbody>
</table>

*Defined as having > 50% by mass, particles < 75 um (D_{50} < 75 um)

BC-below criteria
## Results: PHCs following Biotreatment

<table>
<thead>
<tr>
<th>PHC</th>
<th>CWS</th>
<th>Soil A</th>
<th>Soil B</th>
<th>Soil C</th>
<th>Soil D</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1 (C6-C10)</td>
<td>260</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F2 (&gt;C10-C16)</td>
<td>900</td>
<td>289</td>
<td>117</td>
<td>153</td>
<td>279</td>
</tr>
<tr>
<td>F3 (&gt;C16-C34)</td>
<td>800</td>
<td>3693</td>
<td>1127</td>
<td>1113</td>
<td>1993</td>
</tr>
<tr>
<td>F3(C16-C21)</td>
<td>896</td>
<td>261</td>
<td>231</td>
<td>580</td>
<td></td>
</tr>
<tr>
<td>F3(C21-C34)</td>
<td>2797</td>
<td>864</td>
<td>882</td>
<td>1415</td>
<td></td>
</tr>
<tr>
<td>F4 (&gt;C34+)</td>
<td>5600</td>
<td>2180</td>
<td>726</td>
<td>921</td>
<td>894</td>
</tr>
</tbody>
</table>

*Notes: 1) CCME standard for agricultural land use, fine textured surface soil.  
2) Historical data indicated that PHC F1 was below detection (< 5 mg/kg).  
3) All units mg/kg (dw)*
Results: PAHs following Biotreatment

<table>
<thead>
<tr>
<th>PAHs</th>
<th>SQG</th>
<th>Soil A</th>
<th>Soil B</th>
<th>Soil C</th>
<th>Soil D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>0.1</td>
<td>0.09</td>
<td>&lt;0.05</td>
<td>0.07</td>
<td>0.10</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>0.1</td>
<td>0.22</td>
<td>&lt;0.05</td>
<td>0.05</td>
<td>0.13</td>
</tr>
<tr>
<td>Chrysene</td>
<td>-</td>
<td>0.12</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>0.09</td>
</tr>
<tr>
<td>Fluorene</td>
<td>-</td>
<td>0.10</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Other PAHs</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*Note: 1) CCME soil quality guideline (SQG) for agricultural land use applied.  
2) all units mg/kg (dry weight)
Results: PHC F3 Composition Following Bioremediation

- CCME (HH)
- CCME (EH)
- Soil A
- Soil B
- Soil C

Hydrocarbon %

- >C16-C21
- >C21-C34
# Toxicity Results: Microtox Bioassay (% Survival)

<table>
<thead>
<tr>
<th></th>
<th>Soil A</th>
<th>Soil B</th>
<th>Soil C</th>
<th>Soil D</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC&lt;sub&gt;50&lt;/sub&gt; (5 min)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>EC&lt;sub&gt;20&lt;/sub&gt; (5 min)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>EC&lt;sub&gt;50&lt;/sub&gt; (15 min)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>EC&lt;sub&gt;20&lt;/sub&gt; (15 min)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

*Note: 50% diluted soil data not shown, however, data are the same as 100% contaminated soils.*
### Results: Earthworm/Plant Bioassays

#### Worms (% Survival)

<table>
<thead>
<tr>
<th></th>
<th>Soil A</th>
<th>Soil B</th>
<th>Soil C</th>
<th>Soil D</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 days</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10 weeks</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

#### Plants (% Germination)

<table>
<thead>
<tr>
<th></th>
<th>Soil A</th>
<th>Soil B</th>
<th>Soil C</th>
<th>Soil D</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radish</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Oats</td>
<td>100</td>
<td>100</td>
<td>93.3</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*Note: 50% diluted soil data not shown, however, data are the same as 100% contaminated soils.*
Results: Seed Germination - Oats

<table>
<thead>
<tr>
<th>Soil</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>84</td>
<td>94</td>
<td>88</td>
</tr>
<tr>
<td>B</td>
<td>86</td>
<td>96</td>
<td>90</td>
</tr>
<tr>
<td>C</td>
<td>88</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td>D</td>
<td>90</td>
<td>94</td>
<td>96</td>
</tr>
<tr>
<td>Control</td>
<td>92</td>
<td>96</td>
<td>98</td>
</tr>
</tbody>
</table>
Screening Level Assessment of Earthworm Uptake

HPLC/Fluorescence Detection

- No clearly distinguishable FACs
- Could not discriminate earthworms exposed to PHC from control

GC/MS Analysis

- Complex but similar total ion chromatograms for all samples
- Trimethylnaphthalene and two isomers of ethoxyphenylacetone found in all samples.
Discussion

- Residual PHC F3 concentrations in all soils in excess of standard: *chemically extractable PHC*

- No observed toxicity (Microtox®, seed germination, earthworms) from exposure to PHC contaminated soils: *bioavailable fraction of PHC*

- Incorporation of weathered hydrocarbon data should be considered

- Results of screening level assessment indicate better controls (e.g., OECD Soil) necessary for evaluation of earthworm tissues
Discussion

• Achieving PHC F3 (>C16-C34) standard may prove difficult through bioremediation; however measured toxicity not observed

• CWS for PHC F3 may not accurately represent risk to ecological health:
  • Assumes fresh spill of distilled fraction
  • Assumes F3 composition with high level of C16-C21 subfraction vs. study soils
  • Federated Crude not representative of all hydrocarbon mixtures
  • Does not consider interactions amongst fractions, weathering and limitation in bioavailability
Conclusions

• Risk of toxicity from weathered PHC (F3) in biotreated loam to clay loam soils may be overestimated by current CCME Tier I PHC standards.

• Coupling site-specific toxicity data with chemical characterization can assist in achieving protective, and obtainable remediation endpoints.
Future Directions

- The refinement of standards requires data relating PHC concentrations in aged samples containing complex hydrocarbon mixtures to measured toxicity in soil organisms
- Evaluating CCME PHC standards (particularly F3) versus additional weathered biotreated PHC data
  - *PTAC in process of completing*
- Inventory of other products (e.g., crude oils) to see if Federated Crude Oil composition representative
- Appropriate controls for earthworm toxicity studies (OECD)
Acknowledgments

- Komex International Ltd., Edmonton, Alberta
- Norwest Labs- for performing chemical and physical analyses of soils
- EnviroTest Labs- for performing earthworm analyses
- Bill McGill (UNBC) and Sumithrai Vasanthan (University of Alberta)- who performed seed germination and earthworm bioassays
BIOREMEDIATION

Just when you thought it was safe to go back into the topsoil.