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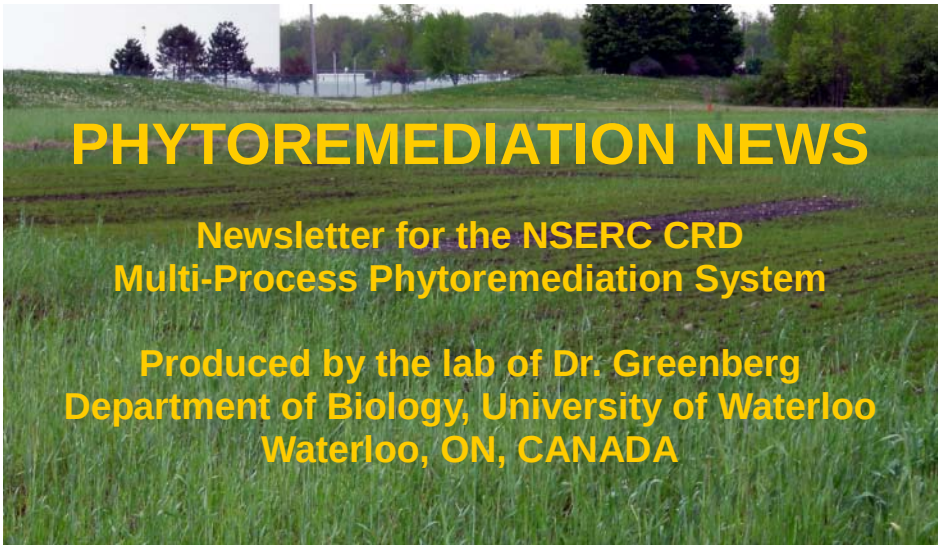

PHYTOREMEDIATION NEWS

Newsletter for the NSERC CRD Multi-Process Phytoremediation System

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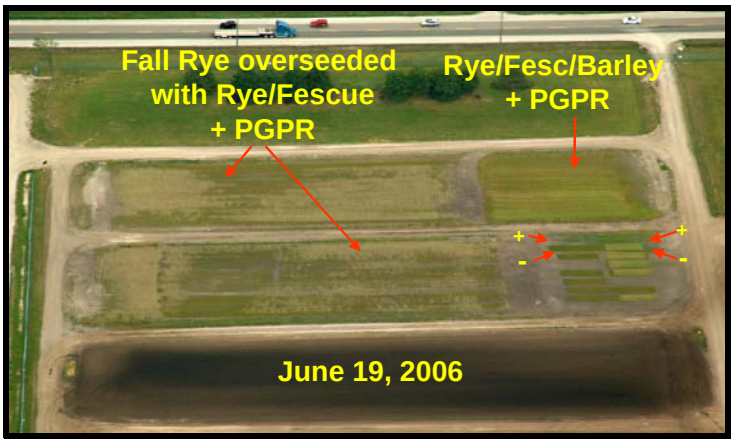
In this issue:

Successful completion of Year 1 of our NSERC CRD for phytoremediation

We completed field trials at several sites for remediation of petroleum and DDT. In all cases, excellent results were realized. We also acquired a Hege 11 seed treater which helped treat the large amounts of seed we put in the field this year. In the greenhouse, we examined metal and salt remediation, with very positive results.

Our second NSERC CRD summer field season is coming to a close

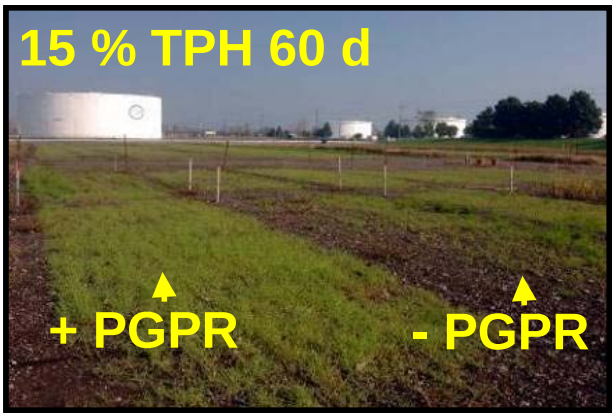
We planted at five sites for petroleum remediation. We had our third year in Sarnia, ON at the Imperial Oil Land Farm. We planted for a second year at the Talisman Energy site in Turner Valley, AB as well as at an auxiliary site in Diamond Valley. We had an additional two petroleum remediation sites in NE AB. We performed remediation experiments at two farm sites near Simcoe, ON. The data from these sites are being processed now.



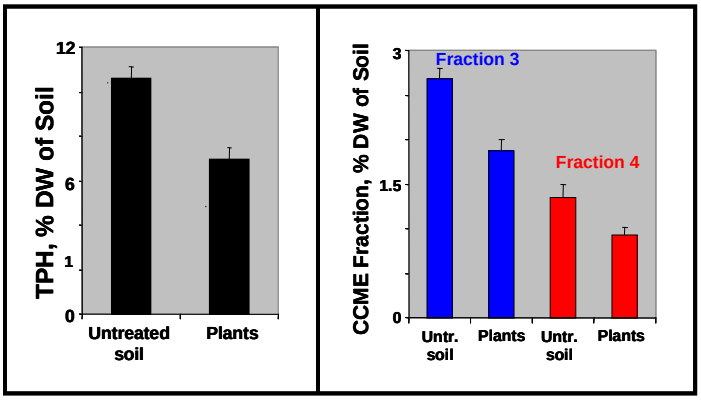
Aerial photo of Imperial Oil Sarnia Land Farm in Year 3 of phytoremediation.

2005 results for TPH remediation of an Imperial Oil Land Farm in Sarnia, ON

In the 2005 field season, we planted 2 fields. One field (10 East) was also planted in 2004; another field (10 West) was planted for the first time in 2005. Due to hot, dry weather in 2005, the first planting on 10 West did not thrive. However, the plants on 10 East performed well due to remediation the previous year. 10 West was planted with fall rye later in the summer and this grew well. For 10 East, remediation was very good (35% overall), with equal remediation of both F3 and F4.



Imperial Oil Land Farm, Sarnia, ON. Fall rye growth after 60 days (Summer 2005, Year 2 of remediation.)

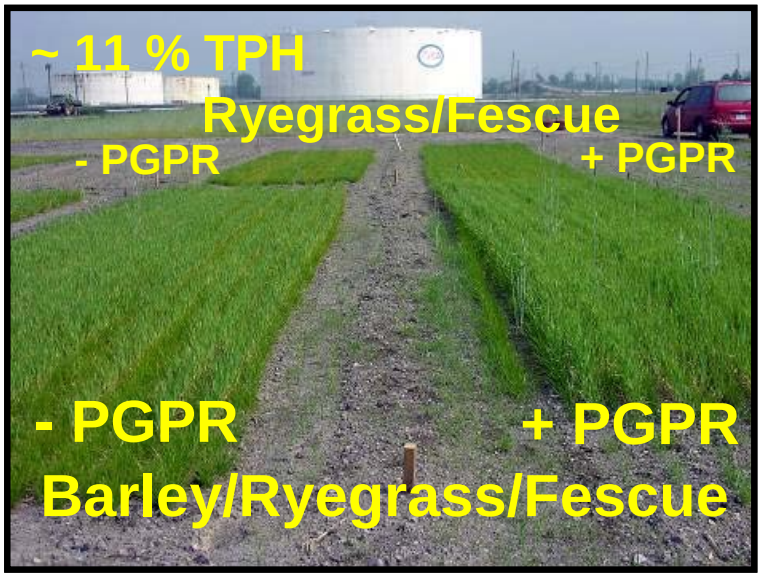


TPH removal from Imperial Oil Land Farm in Sarnia, ON. Ryegrass/fescue was grown for 120 days (2005, Year 2 of remediation). "Plants" indicates soil that was planted with ryegrass/ fescue.

"2 years of successful field tests at the Sarnia Land Farm *in situ*. MPPS removed 30% - 60% of recalcitrant TPHs per year."

Results from Spring/Summer 2006 at the Imperial Oil Land Farm are encouraging

In 2006, we again planted 2 fields: 10 East and 10 West. The weather was excellent and plants grew extremely well. In August 2005, both fields were planted with fall rye. In 2006, these plants re-emerged after over-wintering. They were also overseeded with a combination of ryegrass/fescue/barley. Growth was excellent and good PGPR responses were observed. Remediation is now being assessed; results so far are showing excellent remediation.



Imperial Oil Sarnia Land Farm, Year 3 (2006). Barley/ ryegrass/fescue were planted on April 20, 2006. Seeds were treated with PGPR (UW3 and UW4) using the Hege 11 seed treater. Excellent plant growth was observed after 40 days of good weather, with a clearly visible PGPR effect.

2005 results from Talisman Energy, AB biopile site

Summer 2005 was the first season for a field test at a Talisman Energy biopile in Turner Valley, AB. We achieved good plant growth and excellent remediation in 2005 at this site. This site had a low level of recalcitrant contamination (~1% TPH) in poor quality, gravelly soil. A variety of plants ± PGPR were planted to assess remediation in this type of soil, over a 120 day period. As with previous greenhouse experiments and field work, 35% - 40% remediation of TPH was achieved with superior remediation (15% - 20%) attributable to the addition of PGPR. Approximately 50% of the total CCME fractions 3 and 4 were removed from the soil.

Plant growth at the Talisman Energy biopile (0.85 % TPH) in Turner Valley, AB. Ryegrass/fescue growth after 60 days (Summer 2005, Year 1 of remediation).

TPH removal by the MPPS at a petroleum-contaminated constructed biopile in the Turner Valley, AB. A mixture of ryegrass/tall fescue + PGPR was used. The TPH level at the onset of the experiment was 8.5 g/kg DW of soil.

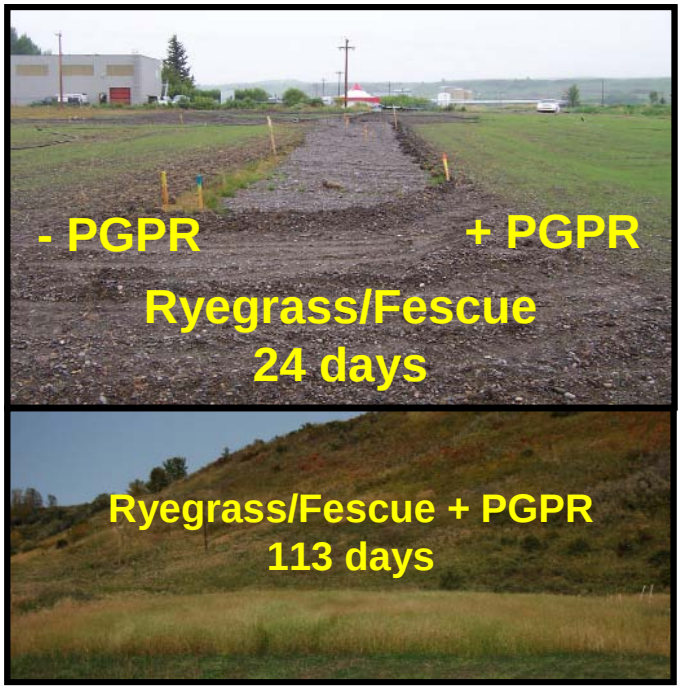
% Remediation* for 2005

Gravimetric	CCME Fraction 3	CCME Fraction 4
35 ± 3	44 ± 16	56 ± 15

* % remediation is the decrease in % TPH of soils remediated for 100 d with the MPPS relative to % TPH present in blank samples (untreated soil control). Values are ± SE, n = 10.

Preliminary results from Spring/ Summer 2006 at Talisman Energy, AB

We planted a mixture of annual ryegrass and tall fescue, barley, and a mixture of Timothy grass + Brome grass + alfalfa ± PGPR (UW3 and UW3). In our second season at this site we achieved much better plant growth than in 2005. This is likely due to increased bio-organic content in the soil from last year's biomass. Again, a clear PGPR response was observed. Preliminary remediation data indicated ~ 30% remediation as of September, based on gravimetric and F4G analysis.



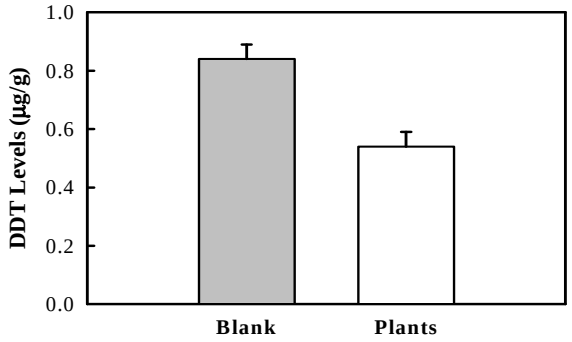
Talisman Energy biopile, Year 2 (2006). Ryegrass/fescue seeds were treated with UW3 + UW4 + Me-Cellulose using the Hege 11 seed treater. Seeds were planted on May 15. Excellent growth, and a positive PGPR effect, were observed at this site again this year.

"1% TPH soils (mainly CCME F3 and F4) were remediated by 30-35% in both years of remediation at 2 field sites in AB."

2005 results for DDT remediation of an OGGS tobacco field

The Ontario Ginseng growers have a problem with latent DDT in fields that were previously used for tobacco crops. We did a trial remediation in 2005 using millet and fall rye. There was little effect of the PGPR at these sites because the soils were excellent farm soils and thus, there was no plant stress. Due to plant growth, ~ 30% DDT remediation was observed. However, we do not know at this juncture whether DDT is being degraded in the soil or in the plants.

> "35% DDT was removed by rye and millet after 90 days in a field with low level DDT contamination near Simcoe, ON."



DDT removal from a tobacco field using phytoremediation. Blank: untreated soil control. Plants: soil after 90 d plant growth. Data were generated from HPLC analyses of soil samples collected after remediation. Rye and millet were planted independently, and DDT concentration data were pooled, disregarding plant species and PGPR treatment. Values are ± SE, n = 20.

Summer/Fall 2006 experiments at an OGGS tobacco field

This season, we planted sugar beets. Due to the large tap roots, this species can sequester DDT, and plants can subsequently be removed from the soil. We planted Fall rye and millet, to determine if degradation of DDT (an organochlorine) occurs as per TPH degradation in phytoremediated soil. If degradation does not occur, only sugar beets will be used in the future.

Recent MPPS publications

Greenberg, B.M., X.-D. Huang, D.G. Dixon, and B.R. Glick, "An Integrated Multi-Process Phytoremediation System (MPPS) for Removal of Persistent Organic Contaminants from Soil", in *Proceedings of the 8th International In Situ and On-Site Bioremediation Symposium*, B. Alleman and M. Kelly, (eds.), Batelle Press, Columbus, OH, Published on CD (ISBN 1-57477-152-3), Paper K-08, 8 pp, 2005.

Huang, X.D., Y. El-Alawi, J. Gurska, B.R. Glick, and B.M. Greenberg, "A Multi-Process Phytoremediation System for Decontamination of Persistent Total Petroleum Hydrocarbons from Soils", *Microchemical Journal,* 81, pp. 139-147, 2005.

Greenberg, B.M., X.D. Huang, Y. Gurska, K.E. Gerhardt, W. Wang, M.A. Lampi, C. Zhang, A. Khalid, D. Isherwood, P. Chang, H. Wang, D.G. Dixon and B.R. Glick, "Successful Field Tests of a Multi-Process Phytoremediation System for Decontamination of Persistent Petroleum and Organic Contaminants", *Proceedings of the 29th Arctic and Marine Oilspill Program (AMOP) Technical Seminar*, Vol. 1, pp. 389-400, 2006.

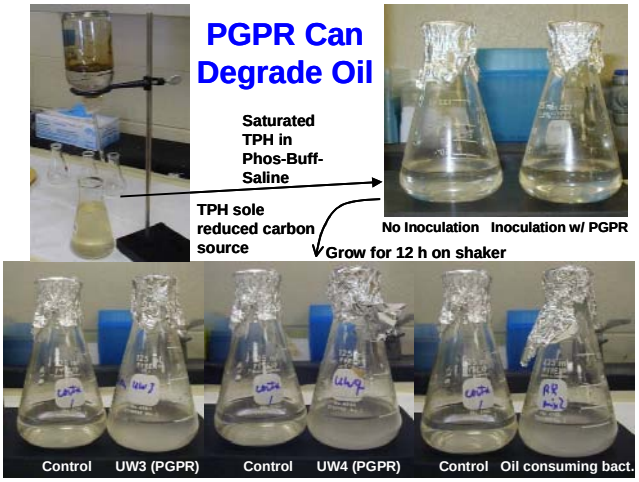
Greenberg, B.M. "Development and Field Tests of a Multi-Process Phytoremediation System for Decontamination of Soils", Canadian Reclamation, Spring/Summer, Issue 1, pp. 27-29, 2006.

Greenberg, B.M., Huang, X.-D., Gurska, J., Gerhardt, K.E., Lampi, M.A., Khalid, A., Isherwood, D., Chang, P., Wang, W., Wang, H., Dixon, D.G., and Glick, B.R. 2006. Development and successful field tests of a multi-process phytoremediation system for decontamination of persistent petroleum and organic contaminants in soils. In: CLRA 2006: Reclamation and Remediation: Policy and Practice. B. Tisch, K. Zimmerman, P. White, P. Beckett, L. Guenther, A. Macleod, S. Rowsome and C. Black, eds., Canadian Land Reclamation Association (CLRA), Calgary, AB, pp. 124-133.

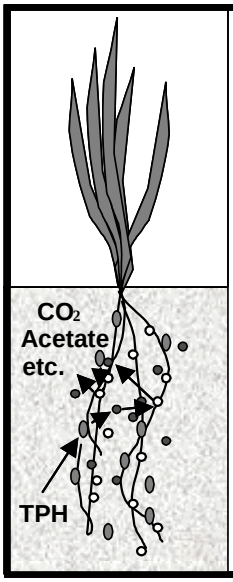
Greenhouse results

In addition to field experiments, we have been performing complementary greenhouse/lab experiments that have given us more insight into the MPPS. Highlights are presented below.

- Seeds can be treated more than 30 d in advance of sowing without any negative impact on remediation.
- We established an operating procedure for the application of PGPR to seeds using a Hege 11 Liquid Seed Treater. This allows a large volume of seeds to be treated rapidly and results in homogenous PGPR-treated seeds.
- Use of a polymer in the seed treatment mix facilitates adhesion of PGPR to the seeds which decreases the loss of PGPR due to dusting off.
- Use of a colorant satisfies the safety regulations requiring all treated seeds to be visibly coloured to avoid consumption by livestock.
- QA/QC procedures were established to ensure quality control of each batch of PGPR-treated seeds.
- Strong evidence that PGPR can degrade oil was obtained using crude oil as the only source of biological energy for the growth of *P. putida* UW3 and UW4 (see right).
- New PGPR strains were isolated from Imperial Oil Ontario Sarnia Land Farm soil, from a TPH-impacted field in NE AB, and from the Talisman Energy, AB TPH-impacted biopile.



Growth of PGPR with crude oil as the only source of biological energy. UW3 and UW4 were tested. Oil consuming bact. is a commercial bacterial mixture known to be able to grow on and consume crude oil. The two PGPR were found to grow (cloudy solutions) on oil.



What Happened to the TPH?

- TPH was removed from the soil, but was not in the plant roots
- F3 and F4 TPH are too hydrophobic to be transported to shoots
- TPH must have been degraded
- Where were they degraded?
- Likely in the rhizosphere by bacteria, fungi and root exudates
- Soil fungi and bacteria have very active and diverse metabolic activities
- In soils with plant growth, bacterial and fungal counts are 10 to 100 fold higher than in soil without plants (data not shown)
- PGPR can use oil as a carbon source (see figure above in "Greenhouse results")

○ **PGPR** ● **Soil bacteria** ⬮ **Fungi**

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