Successful field and laboratory tests of advanced phytoremediation systems for decontamination of petroleum and salt impacted soils

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Earthmaster Environmental

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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 - $200-600/$m^3$**
- **Soil incineration - On or off site - Organic contamination - $600-800/$m^3$**
- **Chemical extraction - Any type of contamination - $300/$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 - $75/$m^3$**

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 cost effective and technically feasible
4. Plants can provide sufficient biomass for rapid remediation; promote high rhizosphere activity
5. Restoration in a reasonable time frame - 2 to 5 years
6. Can be used effectively at remote sites
7. > 30,000 sites in Canada where such technology is needed, > 300,000 sites in the US

Development and Proof of PGPR Enhanced Phytoremediation Systems (PEPS)

Over 10 years of research with field studies at each stage of development

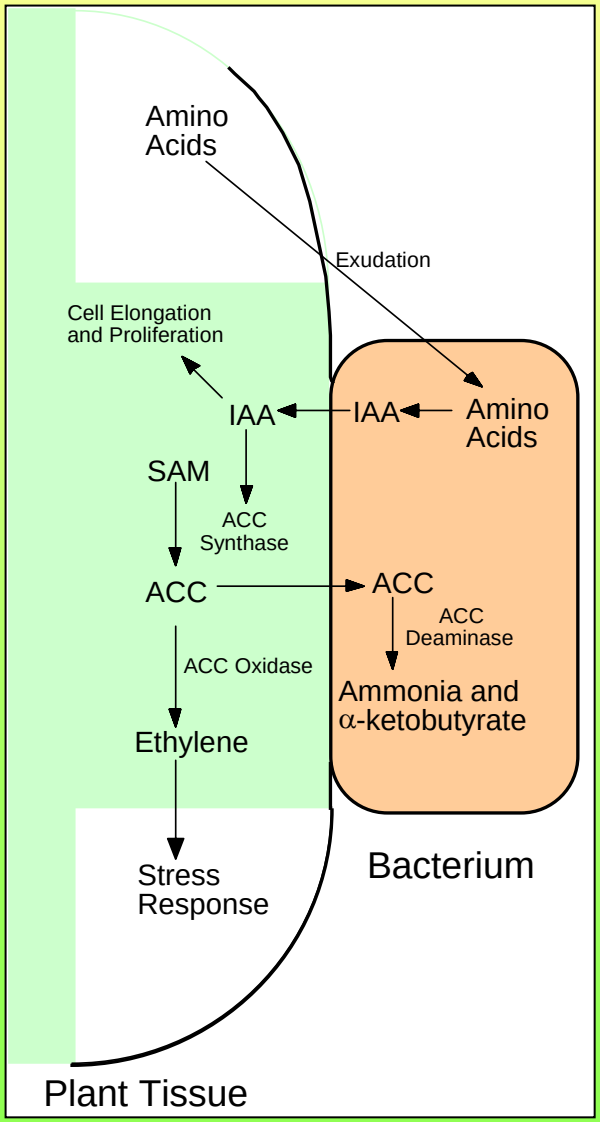
1. **PHC, Imperial Oil Land Farm, Sarnia, ON 2004-08**
2. **PHC, several sites in Alberta 2005-08**
3. **DDT, Simcoe, ON 2005-07**
4. **Brownfield, Toronto, PCBs, PAHs & metals 2007-08**
5. **Fully remediated a gas station site in 1 summer (2007) – Gary Millard - Next talk**
6. **Salt, Saskatchewan, Alberta and Northwest Territories 2007-08**

Description of the PGPR Enhanced Phytoremediation Systems (PEPS)

Physical soil treatment:	**Till the soil: exposure to sunlight and air Exposure to sunlight photooxidizes contaminants**
Bioremediation:	**Inoculation of PAH/PHC degrading bacteria (generally skipped in the field → already present)**
Phytoremediation:	**Growth of plants <u>with PGPR</u>**

- **<u>PGPR:</u> Plant growth promoting rhizobacteria.**
- **Prevent the synthesis of stress ethylene.**
- **<u>PGPR</u> are applied to the seeds prior to sowing → NOT Bioaugmentation**

Interaction of a PGPR containing ACC deaminase with a plant seed or root



Plant growth promoting rhizobacteria (PGPR)

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

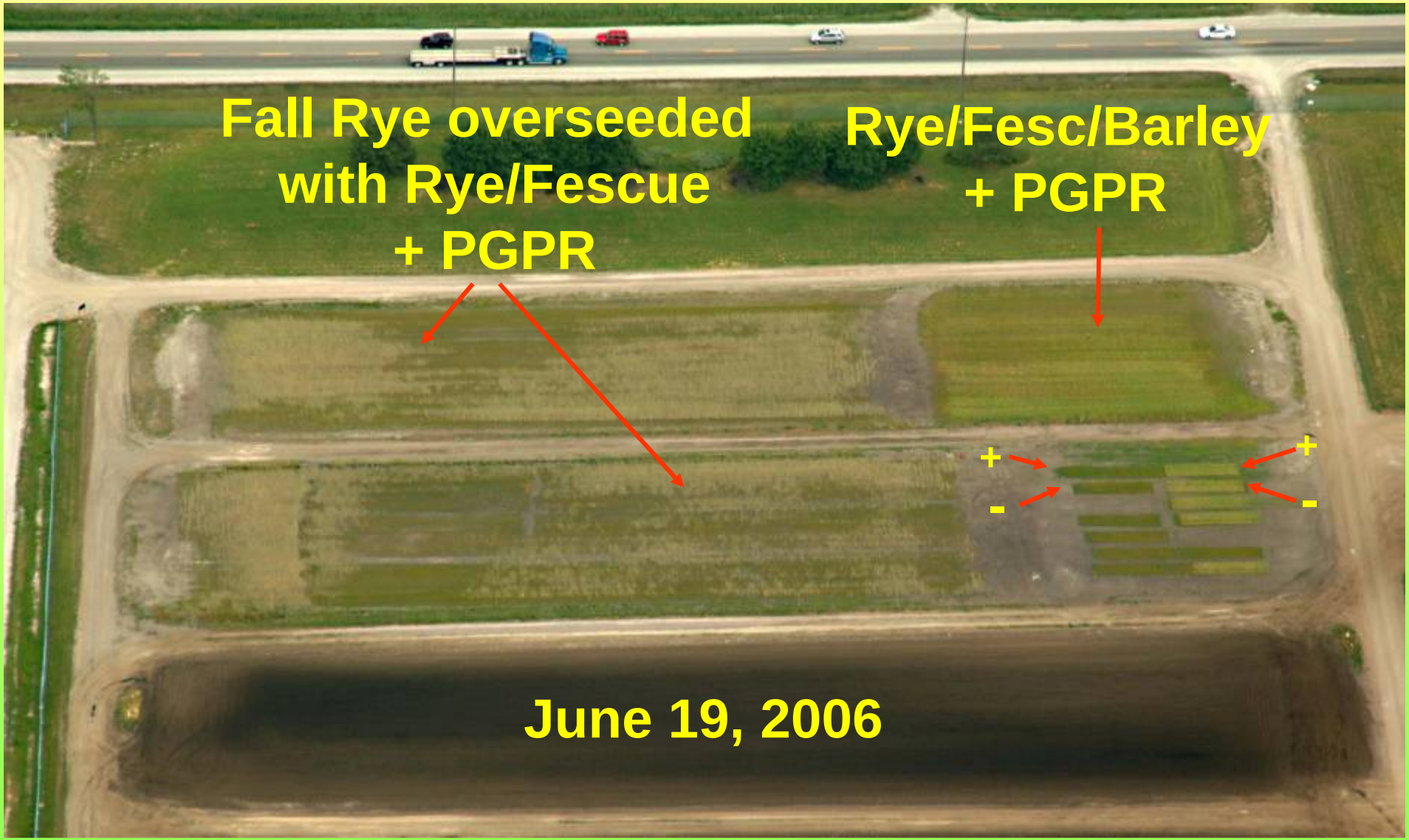
We have isolated PGPRs from ON, AB, SK and the NWT

PGPR are applied to seeds prior to planting

Research and Development of the PEPS for PHC Remediation

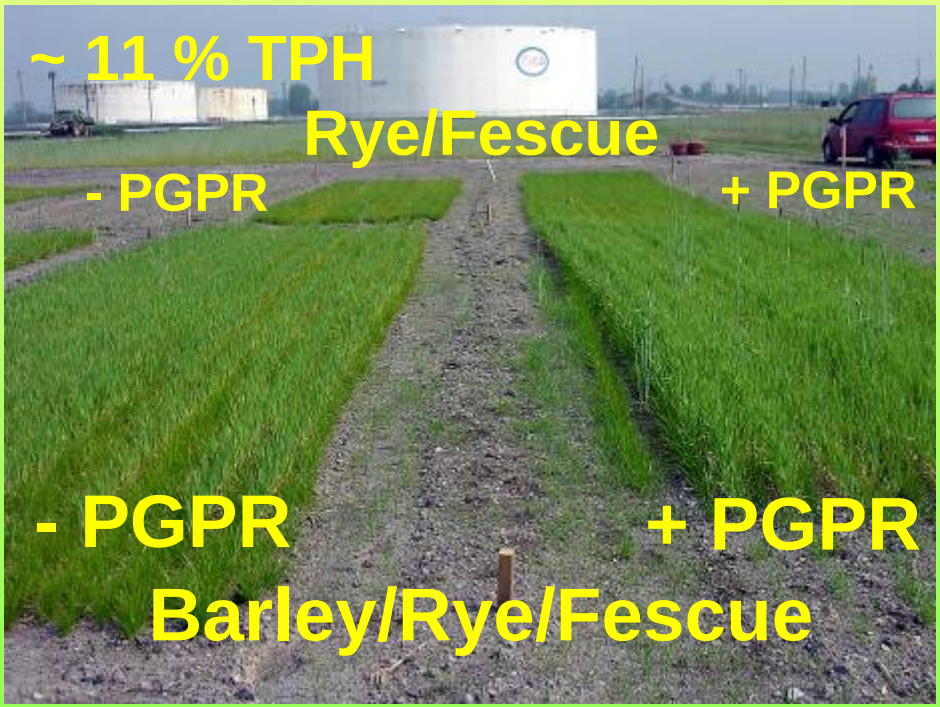
1. Sarnia, ON – Land farm – 4 year study
2. Turner Valley, AB – 3 year study
3. Hinton, AB – 2 year study

Imperial Oil Sarnia Land Farm – 2004-07



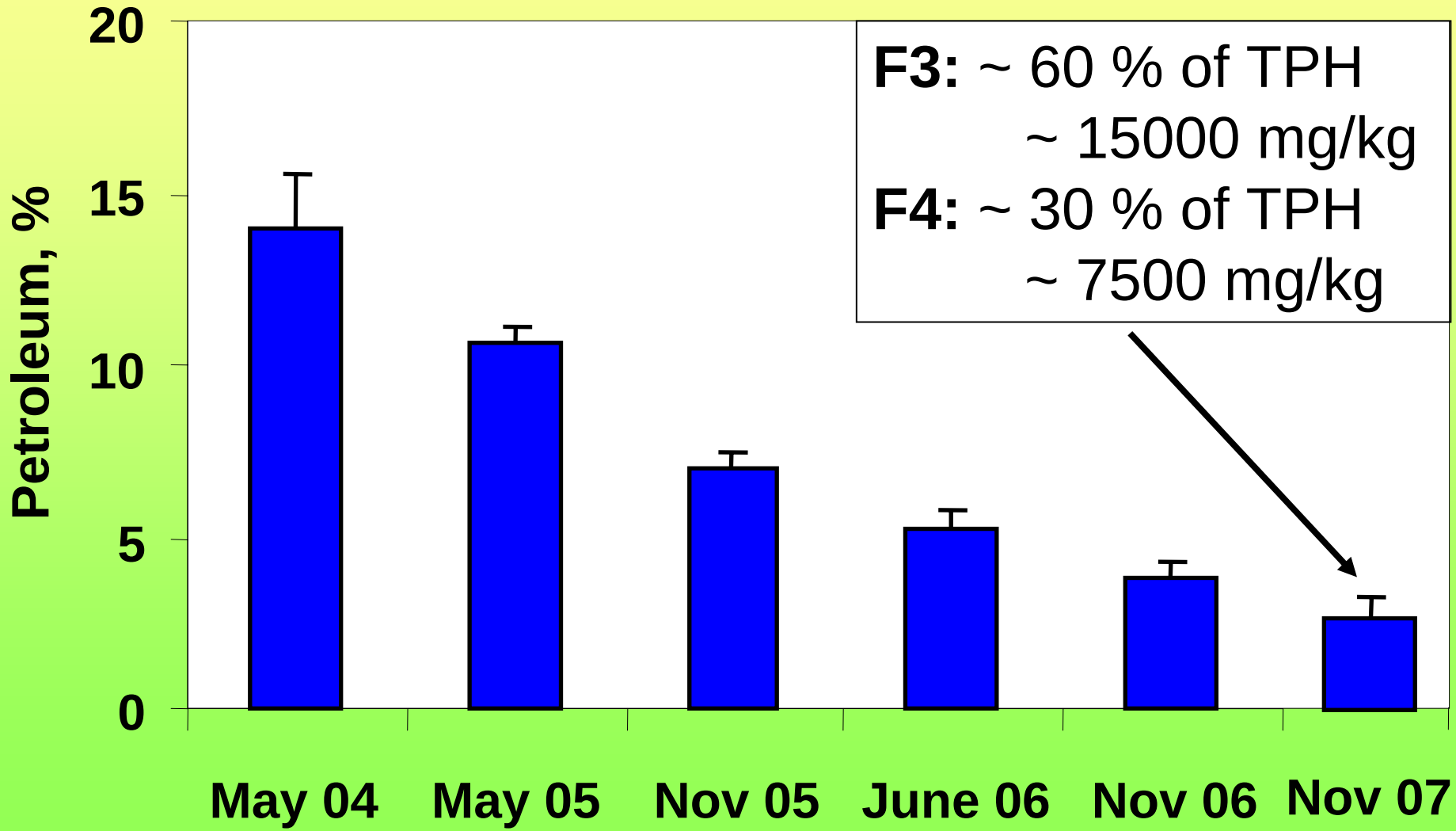
Imperial Oil Sarnia Land Farm

- **Planted Barley/Fescue/Rye Grass**
- **Plants were treated with PGPR (UW3 and UW4) using a mechanical seed treater**



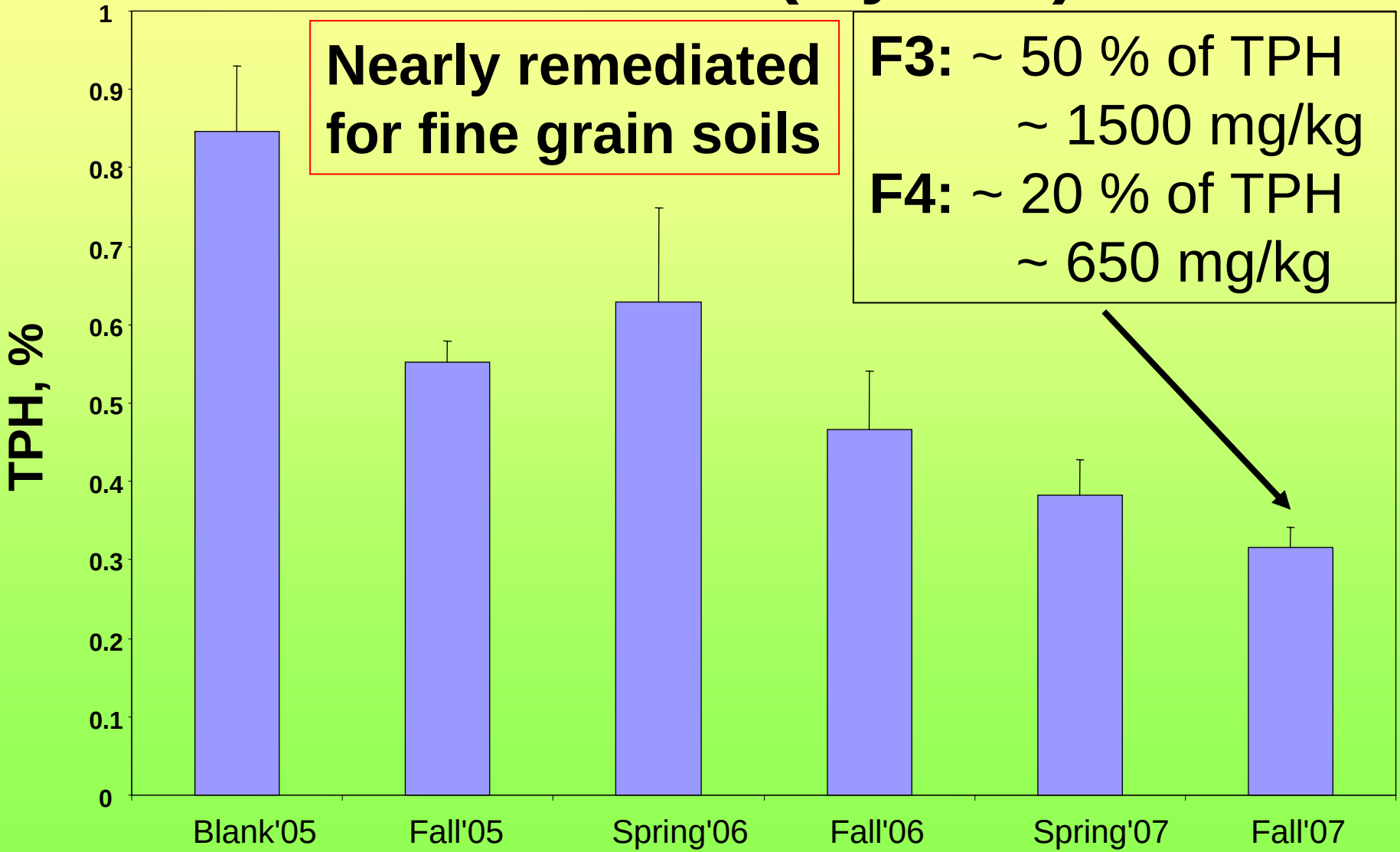
40 days after planting

Petroleum Remediation 2004 to 2007 at the Sarnia Land Farm



Turner Valley, AB
Phytoremediation of a biopile 2005-07

Turner Valley TPH remediation from 2005 to 2007 (3 years)



Conclusions on Development of the PEPS

- 30 to 100 % improvement in plant growth with PEPS
- 30 to 40 % remediation per year
- Rhizophere activity (esp. PHC degraders) elevated 10 to 100 fold with the PEPS
- Very low ^{14}C in detected in soil microbial fatty acids – Carbon came form PHC metabolism as PHC has no ^{14}C
- Very low ^{14}C in CO_2 that evolves from soil – PHC has been mineralized to CO_2
- No PHC is detected in plant tissue as it disappears from the soil
- Developing advanced GC-MS techniques – Tracking of biomarkers as measures of PHC remediation – e.g. showed hopanes and chrysenes are degraded

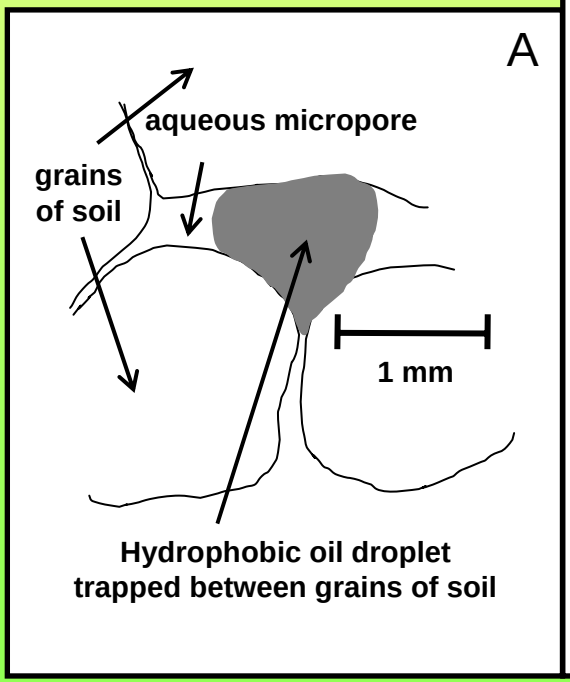
Phytoremediation of PHC

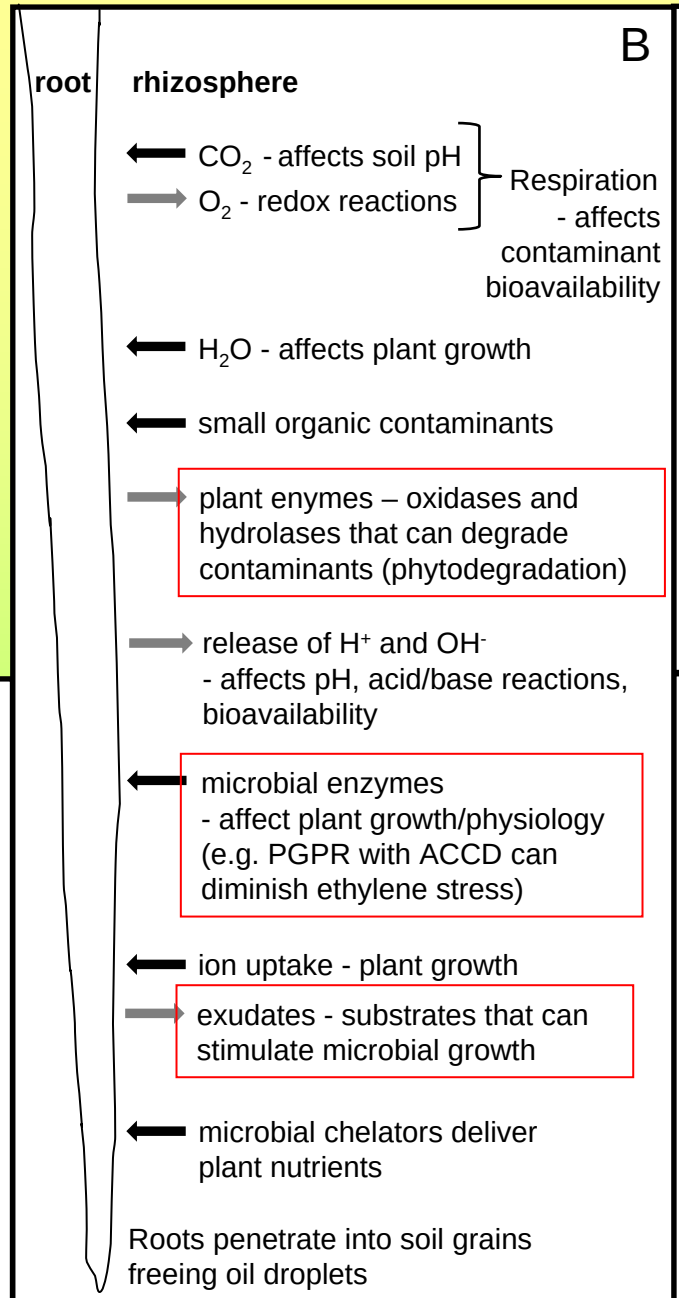
(A) Bioavailability of PHC

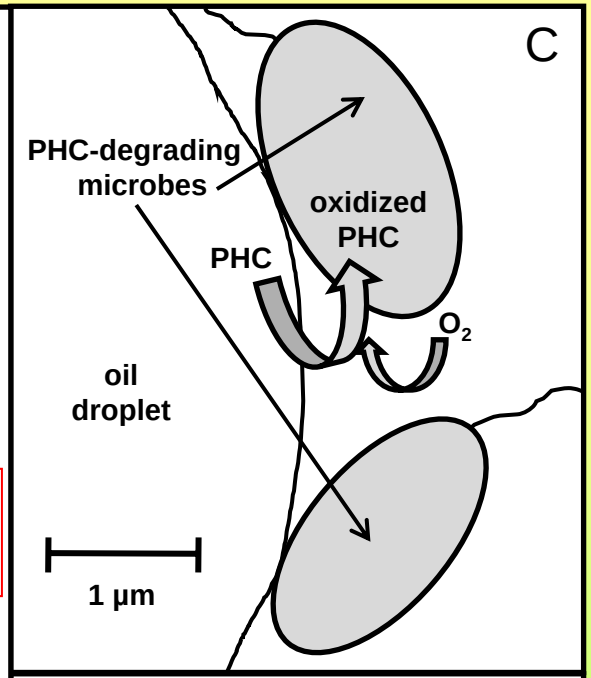
(B) General processes affecting rhizoremediation

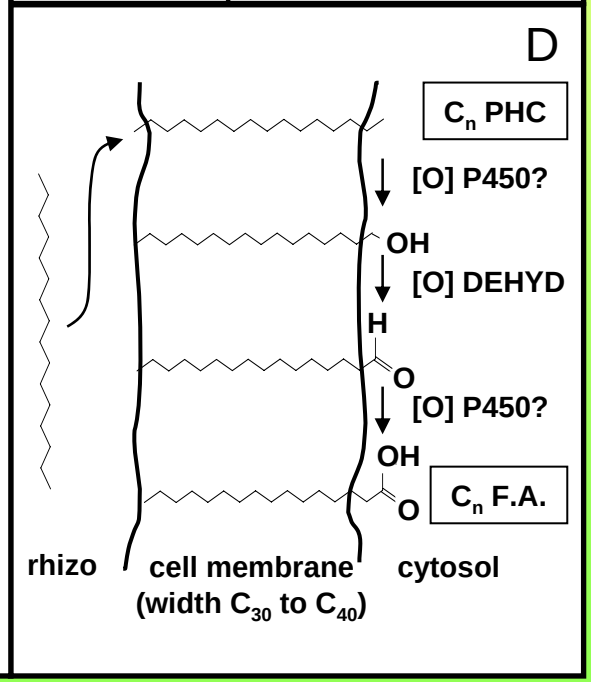
(C) Microbial aerobic PHC degradation – rhizosphere supported by plants

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









Application of the PEPS for PHC Remediation – Proof of Concept

All sites planted with Oats, Tall Fescue and Rye grass treated with PGPR

1. Hinton 1, AB – 1st year of a full scale remediation
2. Hinton 2, AB – 2nd year of a full scale remediation
3. Edson, AB – 2nd year of a full scale remediation
4. Peace River, AB – 2nd Year of a full scale remediation

Hinton - Full Scale Use of the PEPS,

Invert Drilling Mud – Wood chips

With Neil Reid at EBA

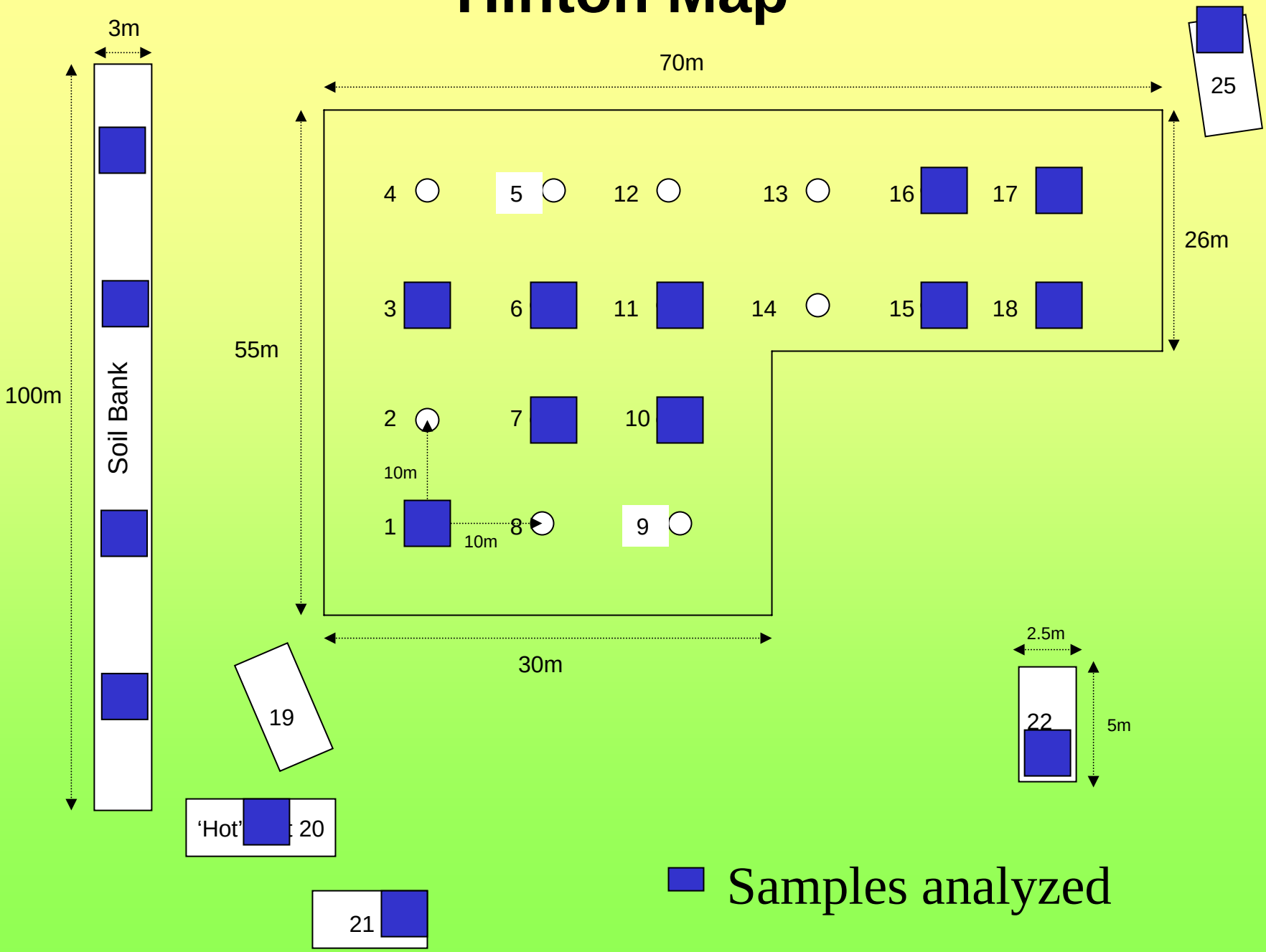
Hinton

Hinton

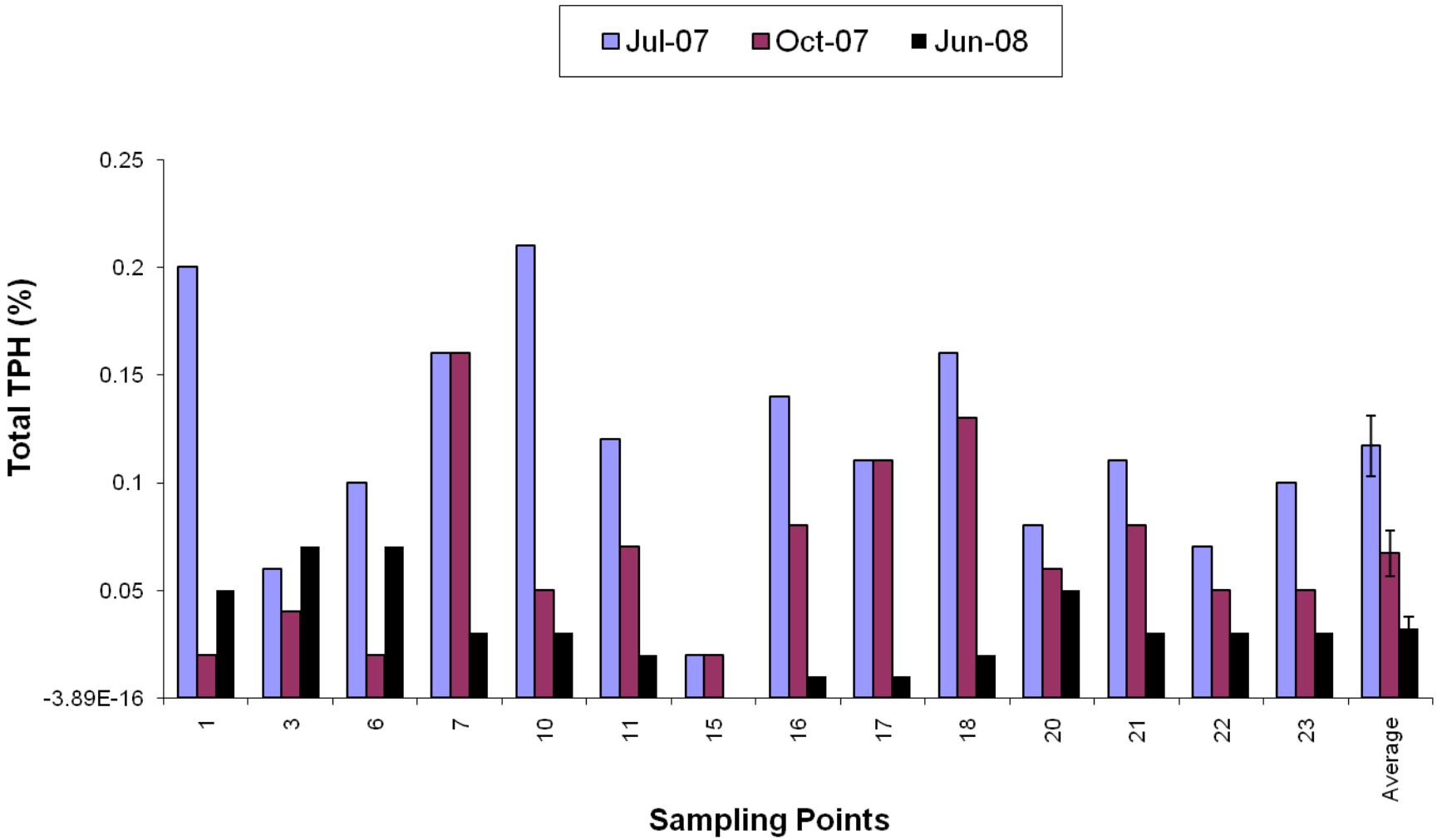
June 25, 2008

August 7, 2008

Hinton Map

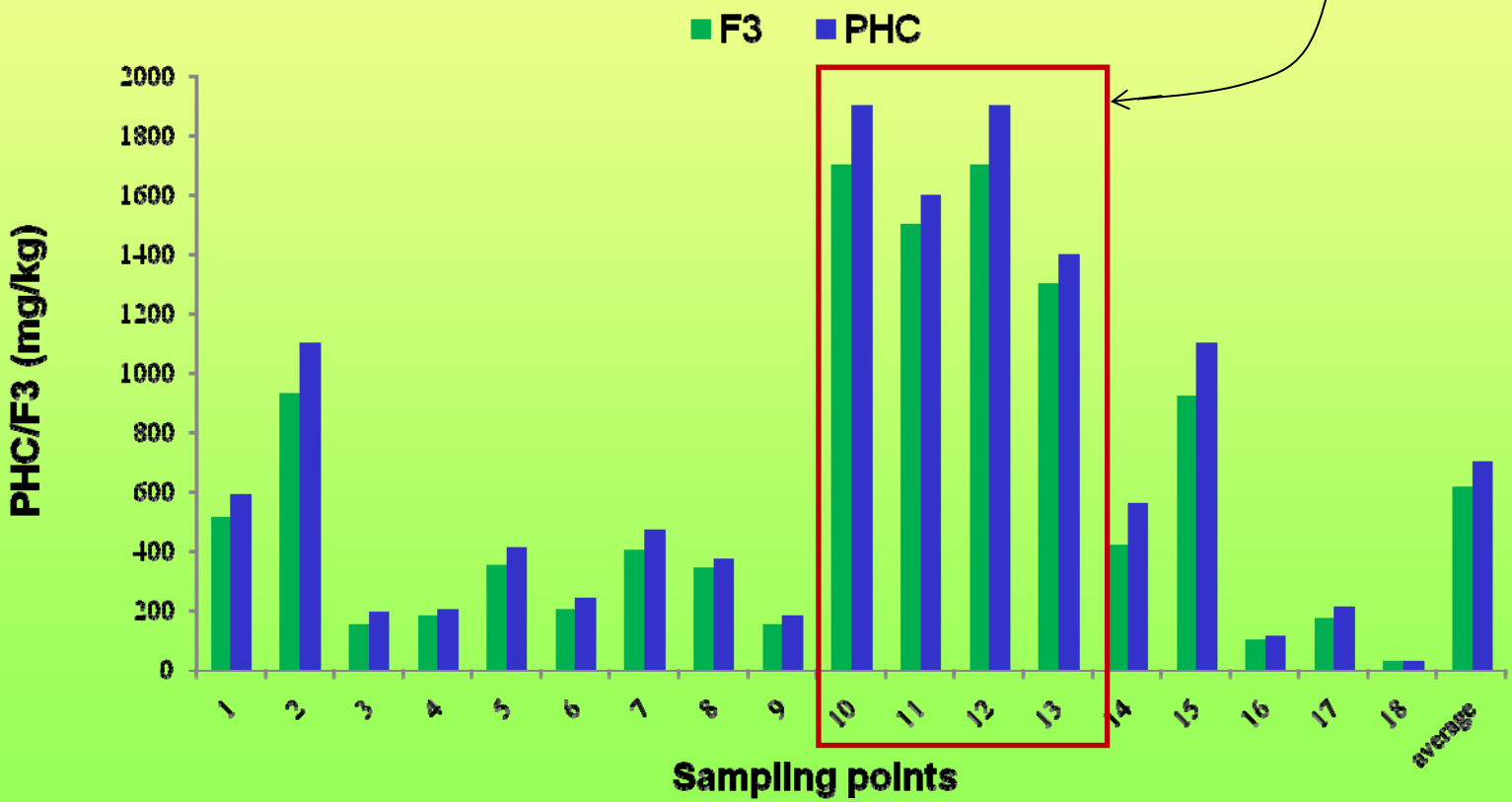

3m
70m
25
100m
Soil Bank
55m
4
5
12
13
16
17
26m
3
6
11
14
15
18
2
7
10
10m
1
10m
8
9
30m
2.5m
22
5m
19
‘Hot’
20
21
Samples analyzed

Phytoremediation at Hinton 2



F3 Phytoremediation at Hinton 2 (Start of season, June 2008)

These 4 points are located in the site soil bank
Only 2 above Tier 1 standards in June 08
Should reach Tier 1 standards at end of 2008



Edson – Diesel Invert

June 5, 2008 Tilling Planting

July 31, 2008

With Perry Gerwing and Glen Pullishy at Earthmaster

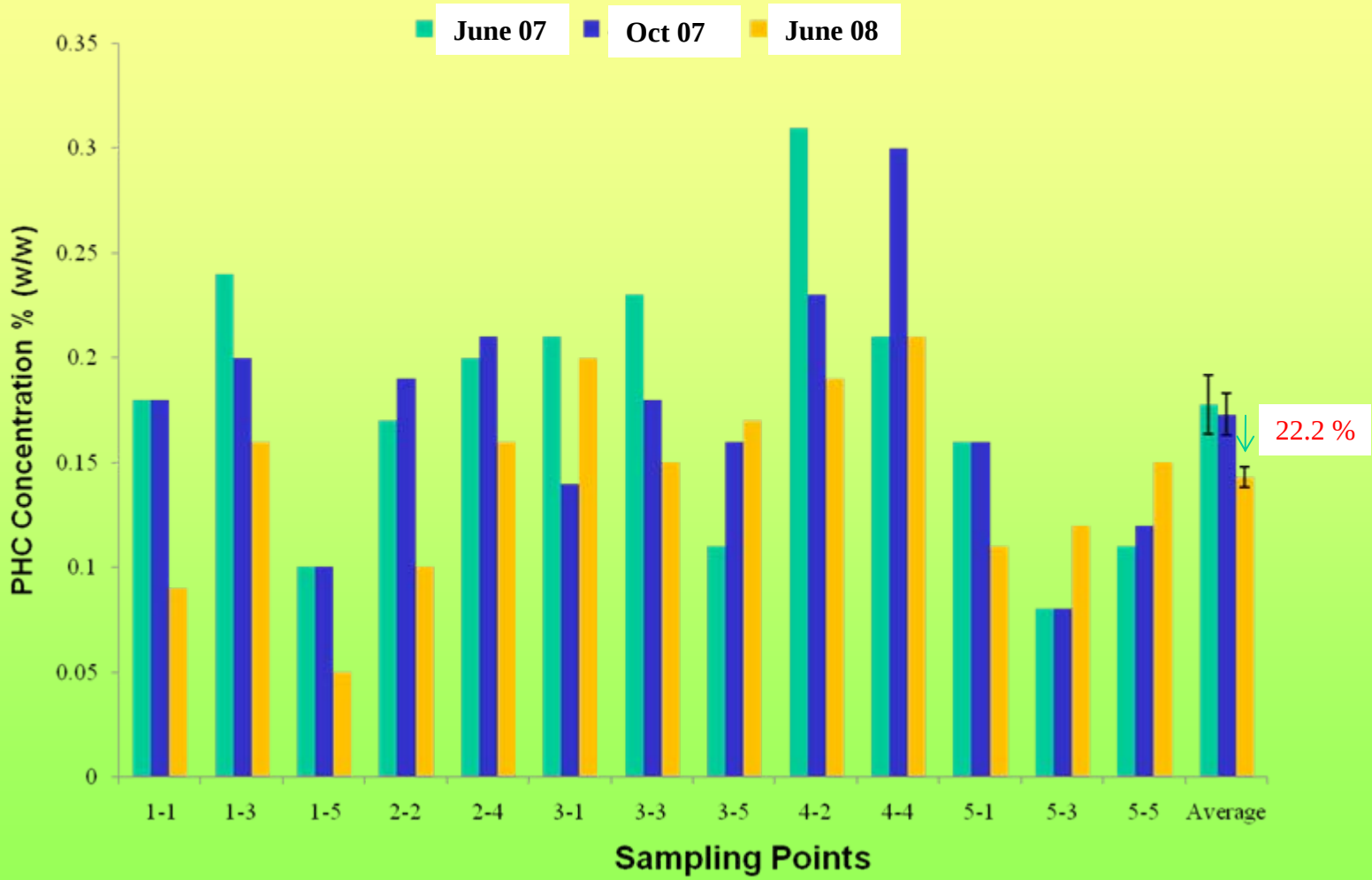
Edson site, AB

Contaminant: TPH

Area: 120 x 100 = 12000 m^2= 3 acre

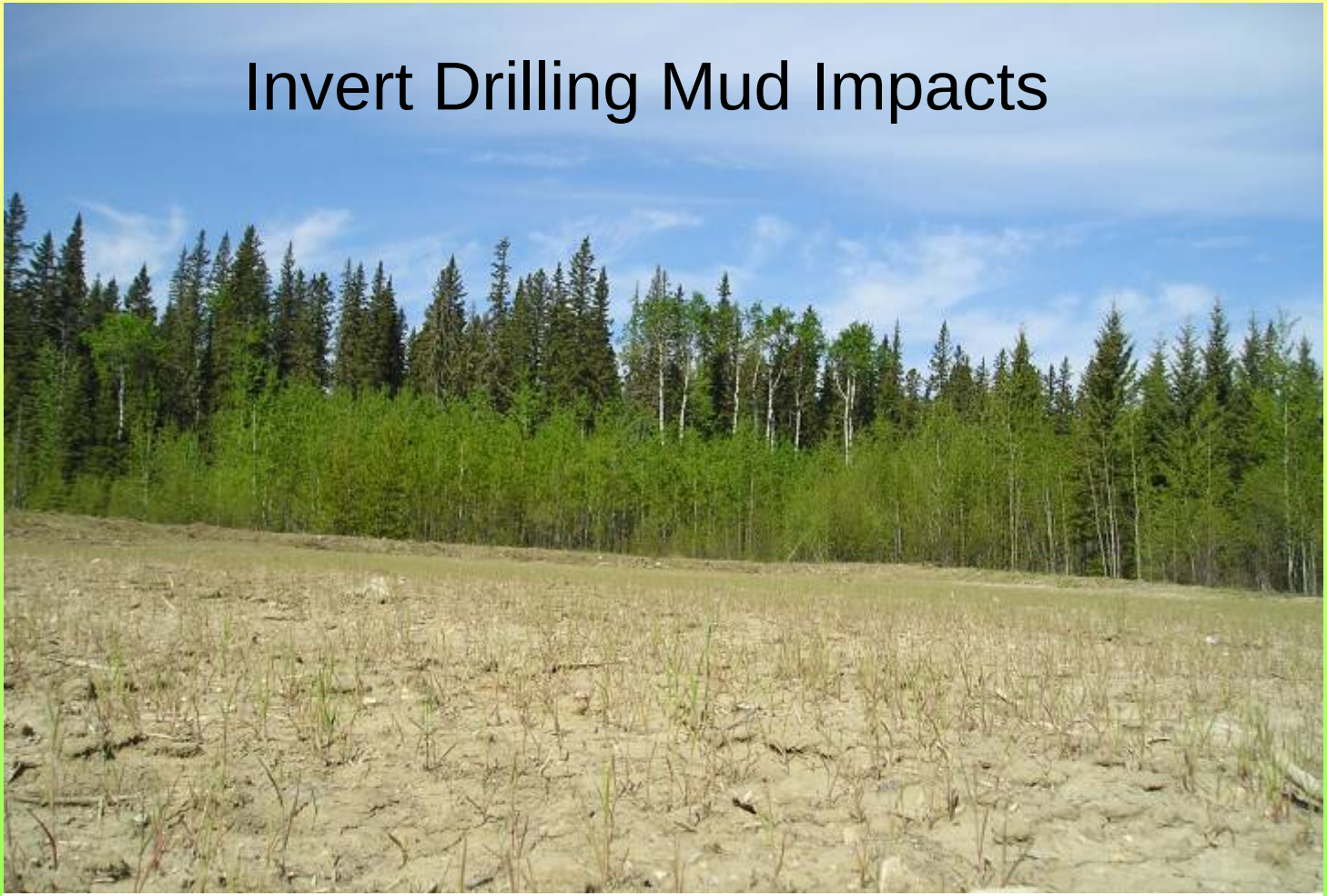
○ Sampling points

Edson Site PHC Remediation



~ 70 % F3. Site may be fully remediated at end of the season.

Peace River Full Scale Use of the MPPS, 2007



June 2007 before planting and t_0 sampling

With P Gerwing at Earthmaster and M Lansing at TerraLogix

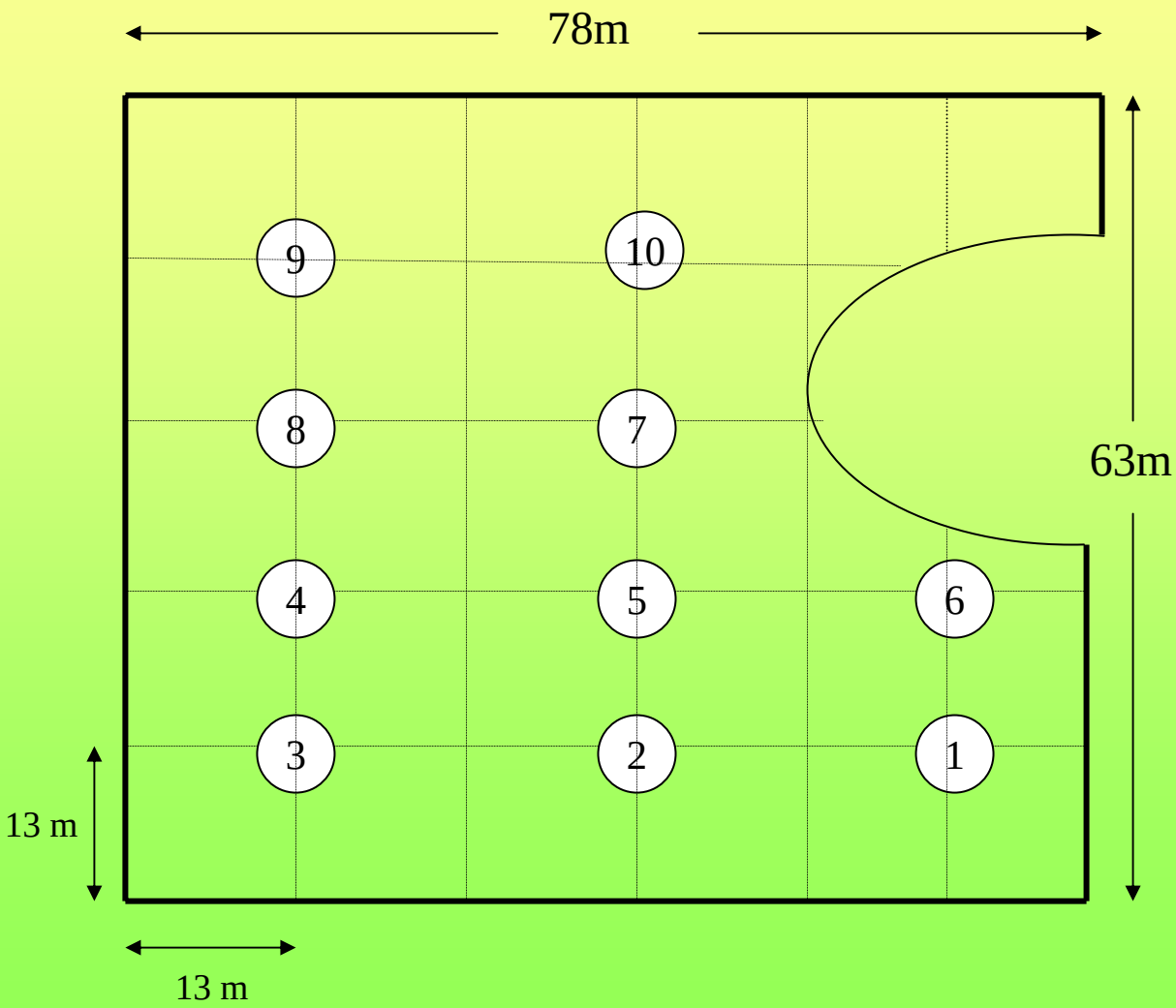
Peace river site

June 19, 2008

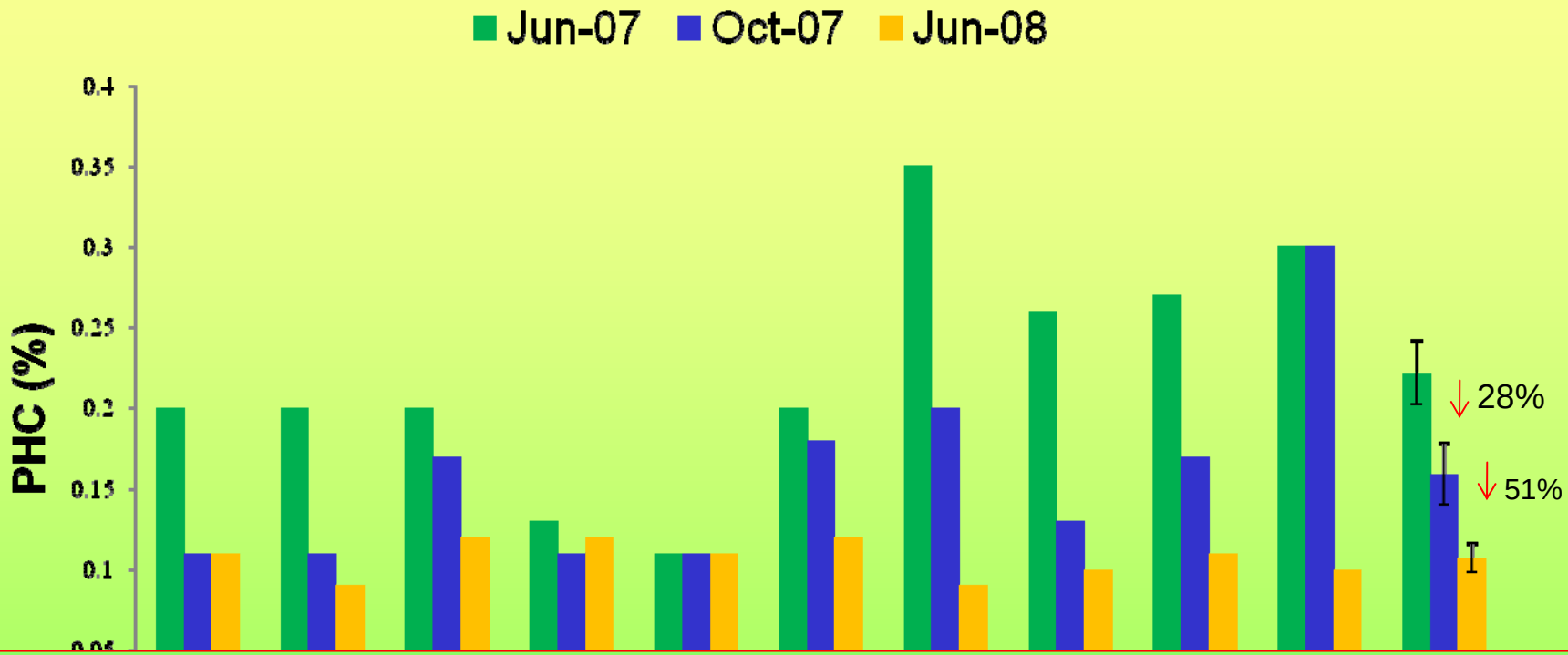
Oct 1, 2008

Peace River Full Scale Use of the MPPS, 2007

Plants: Barley (ACR) + Mix (InfernoTF + Annual RG)



Peace River PHC Remediation



In June: F2: < 150 mg/kg except one point (180 mg/kg)
F3 was at 1500 to 2500 mg/kg in 2006
F3 at all Points < 1000 mg/kg in 2008
Remediation successful

Bottom Line from Application of the PEPS

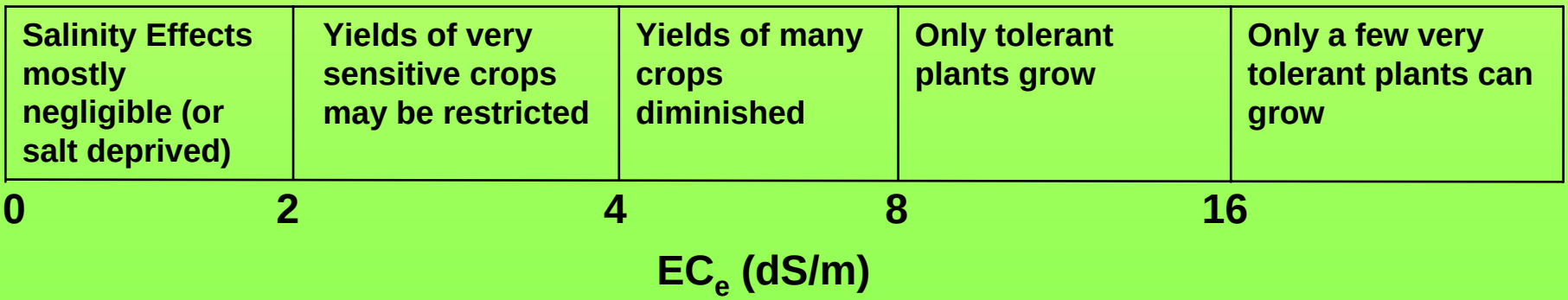
- Fine grain soils with F3 from 2000 to 10,000 mg/kg
  - Site can be phytoremediated in 2 to 4 years
  - Tier I standards can be met using CCME methods
- Fine grain soils with F3 above 10,000 mg/kg
  - Site can be phytoremediated in 3 to 6 years
  - Tier II approach may be required to differentiate petrogenic hydrocarbons from phytogenic hydrocarbons
- Coarse grain soils with F3 above 3000 mg/kg
  - Phytoremediation will bring petroleum hydrocarbons down significantly
  - However, a Tier II approach may be required because remediation targets are very low and phytogenic hydrocarbons could interfere with analyses

Development of the PEPS for Salt Impacted Sites

Plant responses to salinity

- **Inhibited germination**
- **Decreased water uptake →**

  Low water potential (drought)
- **Unbalanced sodium/potassium ratios**
- **Inhibition of photosynthesis**
- **Increased reactive oxygen species (ROS)**
- **Increased ethylene production**



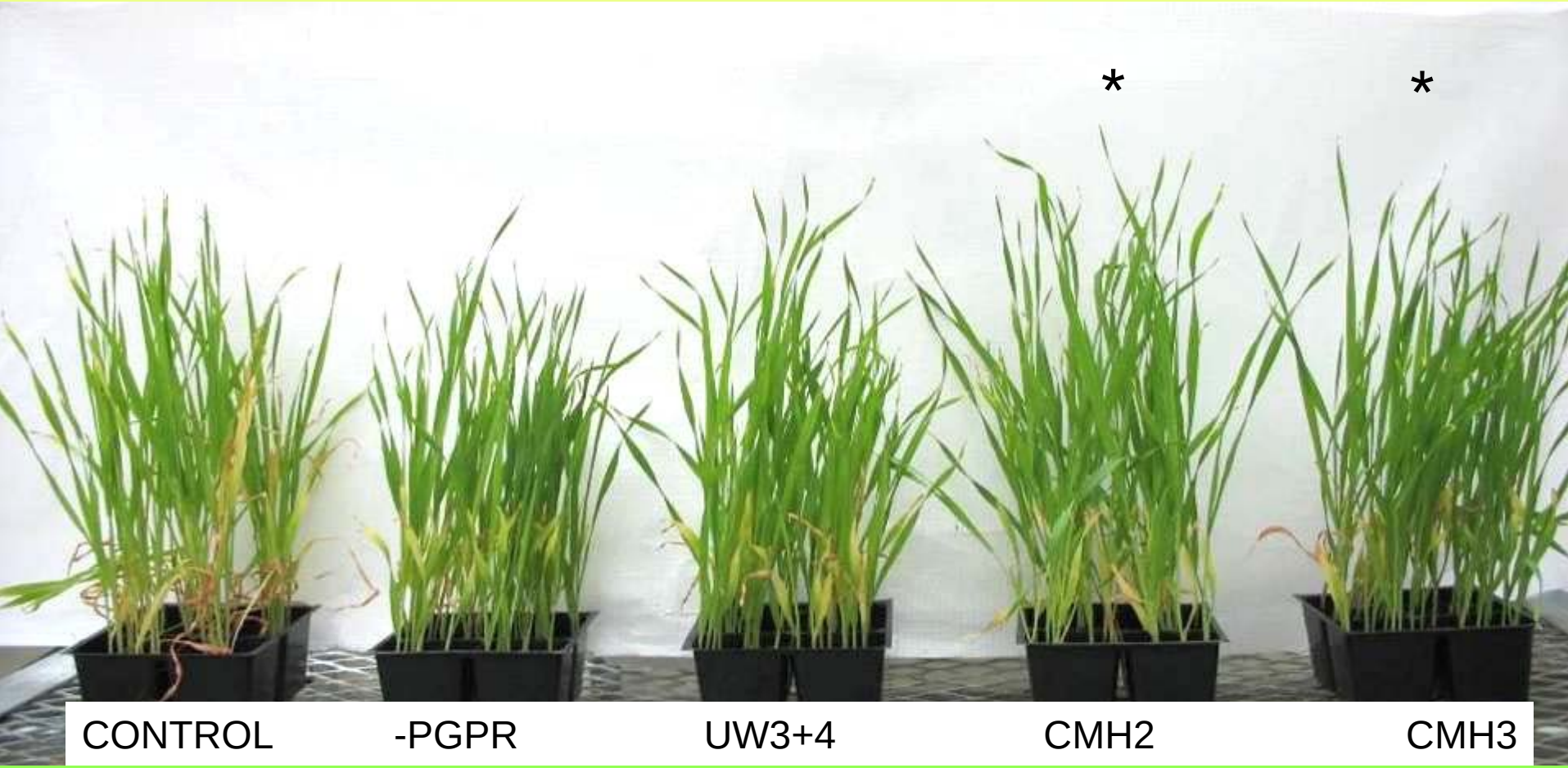
Sites for Development of the PEPS for Salt Remediation

1. Cannington Manor, SK
2. Alameda, SK
3. Kindersley, SK
4. Brezeau, AB
5. Norman Wells, NWT

Barley – Lab Work Example

Saskatchewan High Salt Soil

EC = 18 dS/m , SAR = 11, Cl = 2000 mg/kg

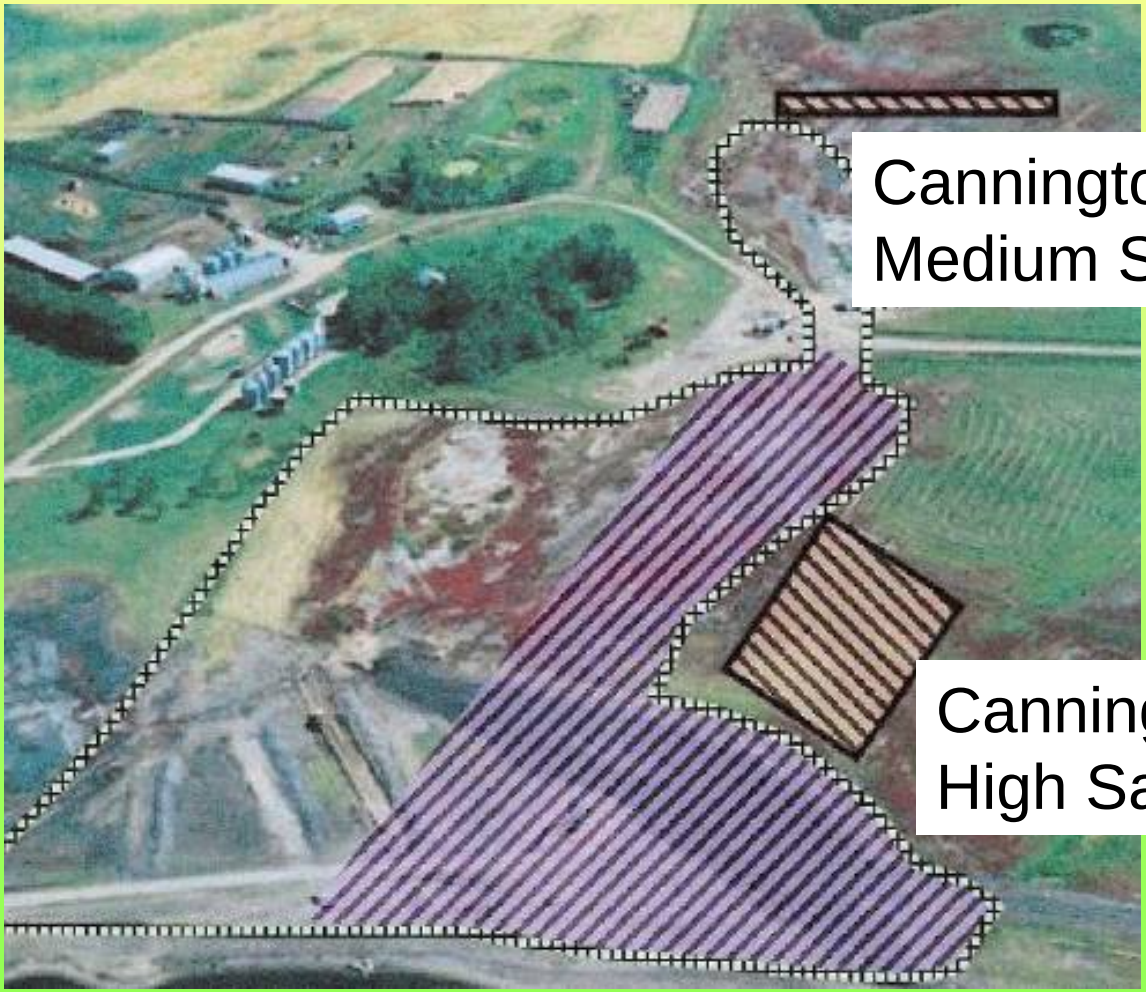


Lab Research Summary of the PEPS for Salt Impacted Soils

- 50 to 100 % increases in plant growth due to PGPR
- Plants can grown on soils with ECe ~ 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: 60 to 80 g NaCl per kg dry weight
- Phytoremediation is feasable: For soils with ECe of 15 to 20 dS/m in about 5 yrs

Field Work

Cannington Manor sites



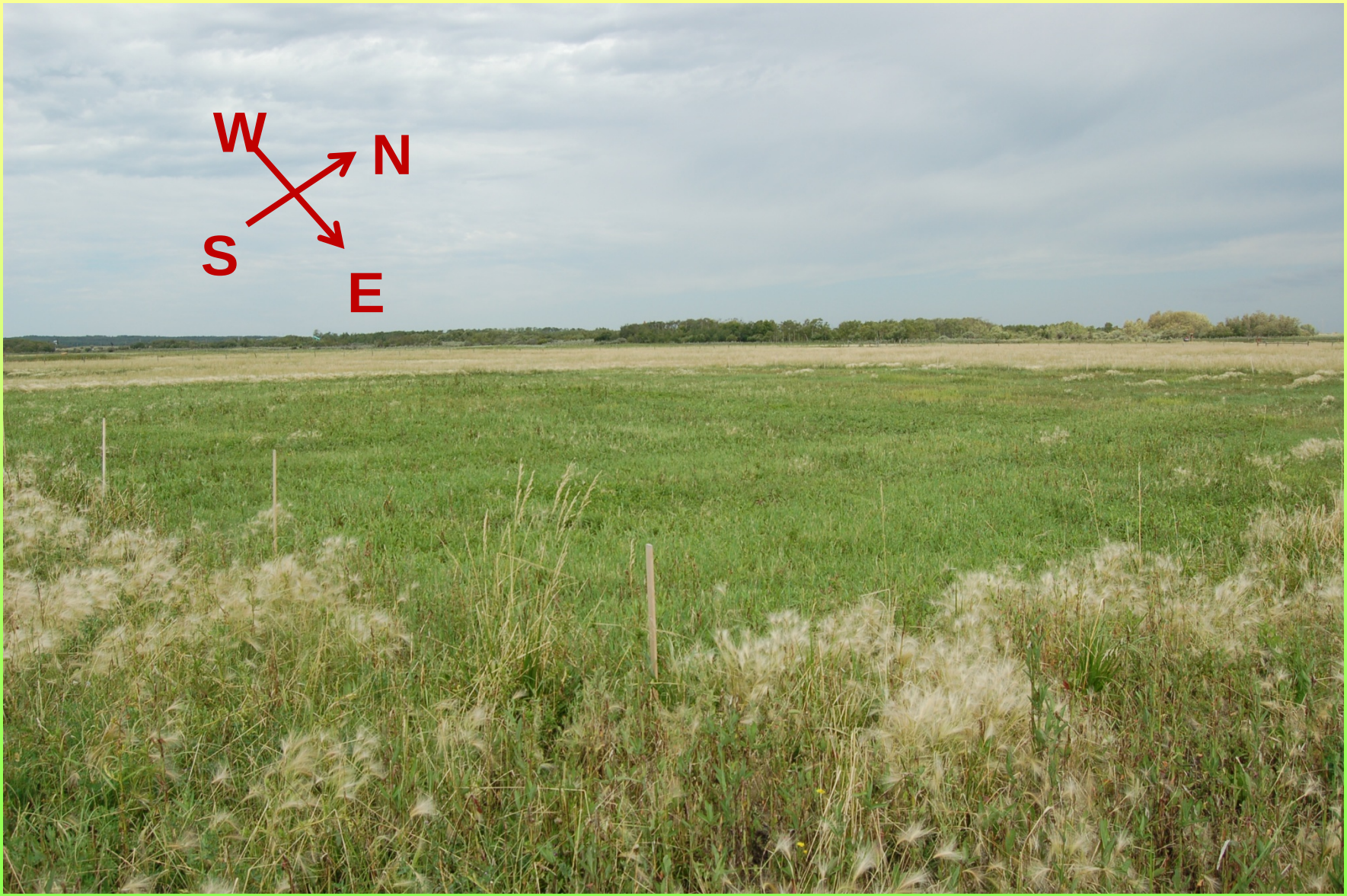
Cannington Manor (North)
Medium Salt

Cannington Manor (South)
High Salt

Characteristics of soils

Parameters\Sites	CMN	CMS	AL
pH	7.9	7.9	7.8
Organic matter (%)	14.1	10.2	7.9
Texture	Silt loam	Loam	Loam
EC_e(dS/m) Avg	7.1	14.5	27.0
Highest EC_e(dS/m)	16.6	32.2	45.3
SAR	17	12	18
Na (mg/kg)	2200	4350	2800
Cl (mg/kg)	1900	6500	5700
Mg (mg/kg)	19800	18700	6700
Ca (mg/kg)	7400	116000	18000
K (mg/kg)	1440	1260	2250
B (mg/kg)	36	47	15

Cannington Manor North – Med. Salt (CMN) – Aug 08



Plant growth on Cannington Manor North (CMN) – 3 months

Tall wheatgrass

No PGPR

EC_e = 9 dS/m

$DW(g)/0.25m^2$ = 23 g

Salt (NaCl)/DW = 18.1 g/kg

CMH3

EC_e = 8 dS/m

$DW(g)/0.25m^2$ = 58 g

Salt (NaCl)/DW = 21.5 g/kg

EC_e of Cannington Manor North (CMN) site

2007

Site Avg EC_e

6.5±0.6

50 m

				EC_e Avg
1	4	15	8	9
2	4	5	5	5
3	14	6	5	8
4	24	3	5	11
5	5	2	5	4
6	4	4	5	4
7	3	4	6	4
EC_e Avg	8	6	6	

50 m

2008

Site Avg EC_e

May - 7.1±0.6

Aug - 3.8±0.4

	1	2	3	4	5	6	7	8	9	10	11	12	EC_e Avg May	EC_e Avg Aug
A	5 2	3 1	4 2	3 1	3 2	2 1	4 1	4 2	6 2	7 3	10 8	10 7	5	3
B	8 3	7 7	11 3	6 2	9 2	7 2	6 4	7 4	6 2	3 3	7 4	9 4	7	3
C	3 8	2 7	7 2	9 13	6 6	8 3	13 1	7 3	12 5	16 4	13 7	16 4	9	5
May	5	4	7	6	6	6	7	6	8	9	10	12		
Aug	4	2	2	5	3	2	2	6	3	3	6	5		

EC_e Avg

Cannington Manor South - High Salt (CMS) – Aug 08

Plant growth on Cannington Manor South (CMS) – 3 months

Oats + Inferno tall fescue + Tall wheatgrass

No PGPR
EC_e = 3 dS/m
DW(g)/0.25m^2 = 40 g
Salt(NaCl)/DW =20.0 g/kg

UW4+UW3
EC_e = 5 dS/m
DW(g)/0.25m^2 = 55 g
Salt(NaCl)/DW =16.0 g/kg

CMH3
EC_e = 5 dS/m
DW(g)/0.25m^2 = 40 g
Salt(NaCl)/DW =23.6 g/kg

EC_e of Cannington Manor South (CMS) site

2007

Site Avg EC_e

17.6±1.4

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	13	6	6	6	6	16	23	26	22	28	26	23	35	23	22	18
	6	7	5	7	8	14	20	16	23	25	24	36	38	14	25	15
	8	8	6	6	10	15	13	17	16	12	17	36	36	13	18	31
EC_e Avg	9	7	6	6	8	15	19	20	20	22	22	32	36	17	22	21

15 m

90 m

2008

Site Avg EC_e

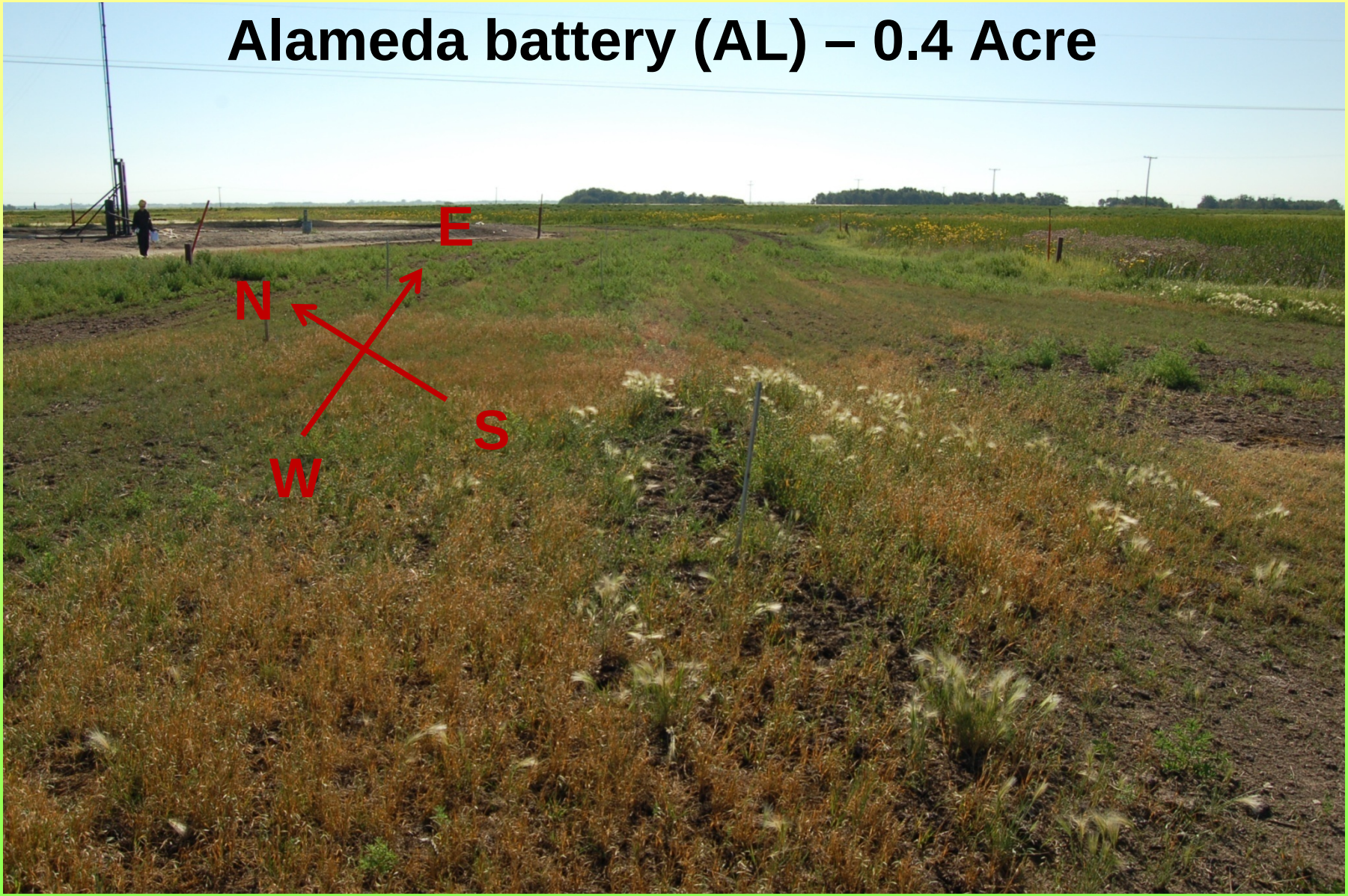
May - 14.5±1.6

Aug - 15.2±1.6

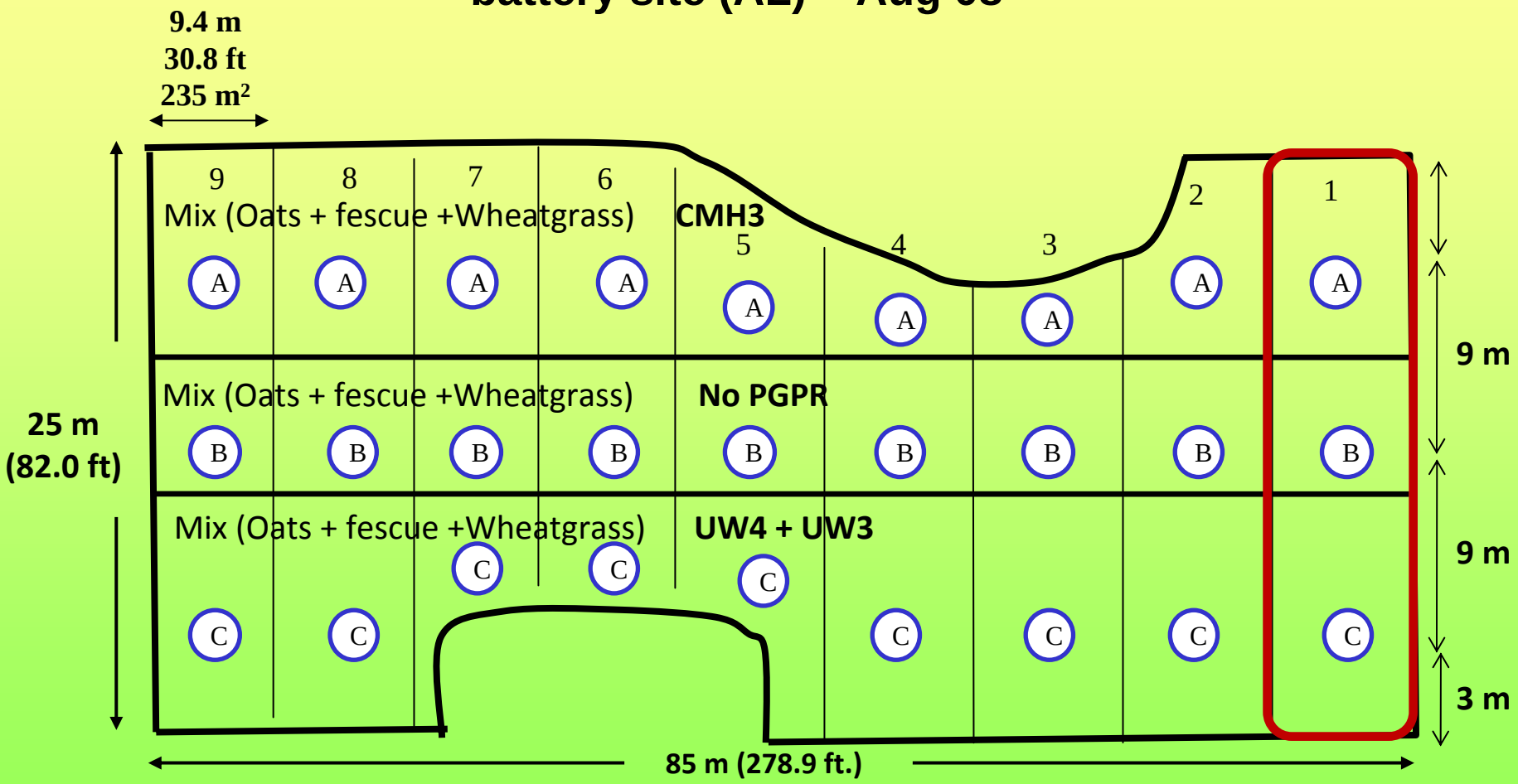
	1	2	3	4	5	6	7	8	9	10	11	12
A	5 5	3 3	5 5	2 2	11 11	26 25	24 19	25 29	18 26	17 19	29 33	14 23
B	4 7	4 5	5 6	7 2	14 11	12 17	31 18	20 18	12 17	32 31	20 19	15 20
C	5 4	2 2	5 10	3 3	9 8	16 23	18 12	13 15	17 17	32 37	27 20	232 4
EC_e Avg May	5	3	5	4	11	18	24	19	16	27	25	17
EC_e Avg Aug	5	3	7	2	10	20	16	21	20	29	24	22

15 m

Alameda battery (AL) – 0.4 Acre



Plant biomass (dry weight) per 0.25m² of Alameda battery site (AL) – Aug 08



		9	8	7	6	5	4	3	2	1
EC_e Avg	May	33	33	29	25	26	27	24	23	23
	Aug	27	28	26	27	24	27	29	30	17

Mix: Oats (common oats)/Inferno tall fescue/Tall Wheatgrass (1:1:1)

Plant growth on Alameda battery- 3 months

Oats + Inferno tall fescue + Tall wheatgrass

No PGPR

EC_e = 11 dS/m

DW(g)/0.25m^2 = 49 g

Salt(NaCl)/DW = 20.4 g/kg

UW4+UW3

EC_e = 24 dS/m

DW(g)/0.25m^2 = 30 g

Salt(NaCl)/DW = 26.2

CMH3

EC_e = 34 dS/m

DW(g)/0.25m^2 = 45 g

Salt(NaCl)/DW = 40.6 g/kg

Kindersley – May 29/July 29, 2008

EC_e Values

May 29
Ec_e = 5.6
dS/m

1	2	3	4	5	6	7	8	9	10	11	12
4.61	2.36	3.22	3.58	4.80	4.08	3.00	4.09	0.33	4.31	1.31	6.07
3.90	10.37	9.69	6.07	8.33	7.47	10.14	4.80	6.63	8.16	7.49	4.48
0.96	4.68	5.70	16.09	8.96	8.97	0.67	14.40	7.50	1.36	1.10	2.05

July 29
Ec_e = 3.98
dS/m

3.17	2.00	2.95	3.44	3.05	2.75	1.42	3.43	1.32	3.51	3.81	4.86
2.31	5.16	5.46	5.60	6.29	4.53	5.55	4.04	3.61	6.11	4.74	5.00
1.54	2.46	5.84	8.30	5.53	4.95	1.29	8.37	5.07	2.57	1.31	1.85

All plots planted with Oats, Tall Fescue and Tall Wheatgrass

← N

Kindersley – Before Planting
May 29, 2008

Kindersley – July 29, 2008

Plot 2: – PGPR
25 g DW/0.25 m^2

Plot 1: CMH3
48 g DW/0.25 m^2

Summary of Salt uptake – Oct 2007

Site	EC_e (dS/m)	Block	PGPR	Plant	Na	Cl	NaCl
CM South	19	7	-PGPR	INF	5660	27600	33260
	20	8	UW3+4	INF	5860	32400	38260
	15	6	CMH3	INF	4820	26400	31220
	20	9	-PGPR	OT	13900	43500	57400
	22	11	UW3+4	OT	17300	50000	67300
	22	10	CMH3	OT	13000	35900	48900
		Average					46057
CM North	9	6-2	-PGPR	BL	18801	36564	55365
	3	7-2	UW3+4	BL	18100	32200	50300
	21	6-1	-PGPR	BL	8530	22600	31130
	25	7-1	UW3+4	BL	17700	55900	73600
		Average					50892
Alameda	22	4	-PGPR	INF	4120	31900	36020
	18	3	UW3+4	INF	2430	35400	37830
	22	4	-PGPR	OT	18000	78700	96700
	18	3	UW3+4	OT	11000	50600	61600
		Average					59440

INF: Inferno tall fescue; OT: Baler oats; BL: Ranger barley. Units: mg/kg

Summary of salt uptake in plants Aug 2008 (mg/kg DW)

Site	Plot EC_e (dS/m)	Treatment	Plant	Na (mg/kg)	Cl (mg/kg)	NaCl (mg/kg)	Cl/Na ratio
CMN	4	No PGPR	O	8590	19900	28490	2.3
	5	CMH3	O	10100	20400	30500	2.0
	9	No PGPR	WG	3090	15000	18090	4.9
	8	CMH3	WG	3720	17800	21520	4.8
CMS	3	No PGPR	Mix (O+TF+WG)	7270	12700	19970	1.7
	5	CMH3	Mix (O+TF+WG)	8110	15500	23610	1.9
	5	UW3+UW4	Mix (O+TF+WG)	4370	11600	15970	2.7
AL	11	No PGPR	Mix (O+TF+WG)	4840	15600	20440	3.2
	34	CMH3	Mix (O+TF+WG)	7680	32900	40580	4.3
	24	UW3+UW4	Mix (O+TF+WG)	4120	22100	26220	5.4

O = Common oats

TF = Inferno tall fescue

WG = Tall wheatgrass

CONCLUSIONS

- **The PEPS has great potential for efficient remediation of organic, salt and metal contaminated sites**
- **PGPR is the key: healthy plants with vigorous roots in PAH, PHC, salt and metal contam. soils**
- **PGPR alleviate stress and promote growth: Low ethylene and high auxin**
- **5 years of fields tests successful: PEPS removed 20 % to 40 % of recalcitrant PHCs per year**
- **PHC metabolized and/or degraded**
- **50 to 100 % increases in plant growth on salt impacted sites**
- **Salt impacted sites can be remediated in about 5 years**
- **Great promise for restoration of oil and salt impacted sites as well as brownfields**

Colleagues and partners

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