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

Bruce Greenberg, Xiao-Dong Huang, Karen Gerhardt, Xiao-Ming Yu, Scott Liddycoat, Xiabo Lu, Julie Nykamp, Brianne McCallum, Greg MacNeill, Peter Mosley, Jolanta Gurska, Nicole Knezevich, Han Zhong & Perry Gerwing

WATERLOO ENVIRONMENTAL BIOTECHNOLOGY INC.

EARTHMASTER ENVIRONMENTAL STRATEGIES INC.

University of Waterloo

Partners: Talisman; PennWest; Canadian Forest Oil; Devon; Baytex; ConocoPhillips; Solaction; Shell; Seaway Energy Services; Questerre Beaver River; Maxxam; 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**

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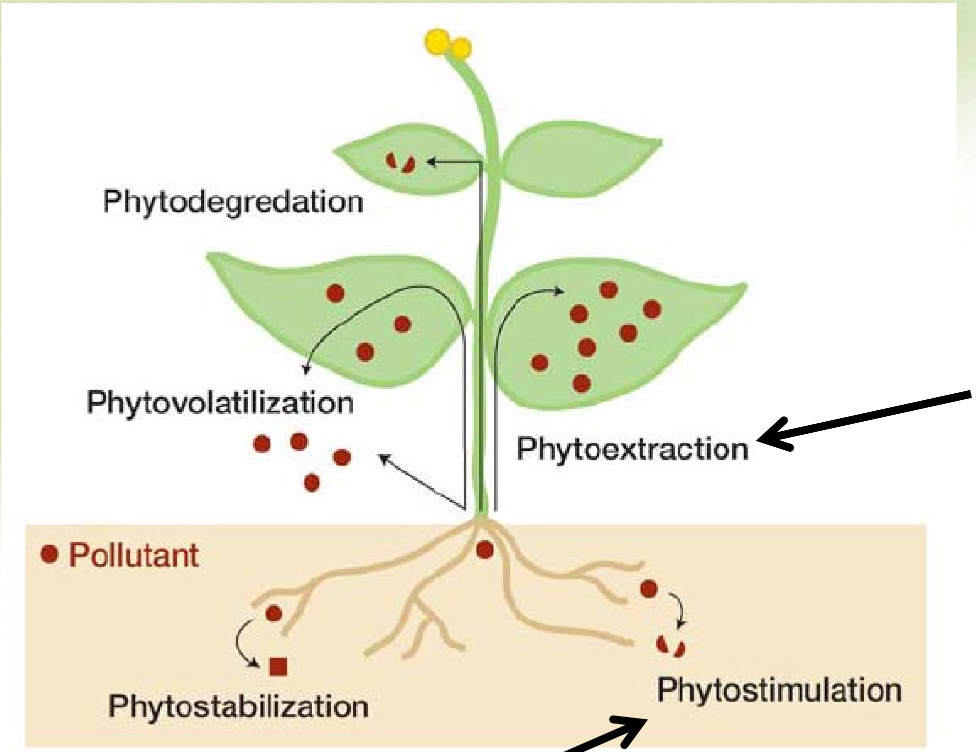
Process of Phytoremediation



- Volatilization
- Phytodegradation
- Chelation/compartment in leaves

Salt

- Translocation: root symplast→ xylem
- Chelation/compartment in roots
- Plant uptake soil→root
- Rhizosphere Processes
- Bioavailability particle→water

Rhizodegradation - PHC

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

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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)

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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**

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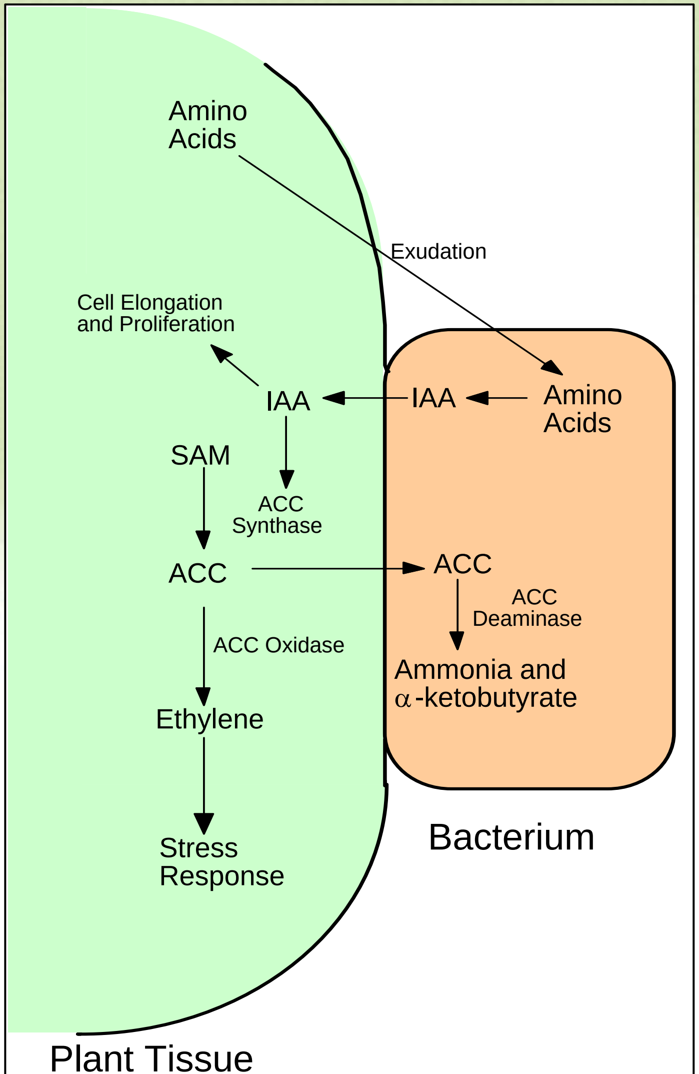
Interaction of a PGPR Containing ACC Deaminase with a Plant Seed or Root



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

- 100% increase in plant biomass due to PGPR, root growth to 50 cm below ground level
- 30 to 40% remediation per year with PEPS; 100% faster than plants without PGPR
- Rhizosphere microbes (esp. PHC degraders) elevated 10 to 100 fold with the PEPS - microbes and plants consume PHC
- Very low ^{14}C detected in soil microbial fatty acids – Carbon came from PHC metabolism (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 detected in plant tissue as it disappears from the soil
- CCME PHC analytical method used effectively to show extent of remediation

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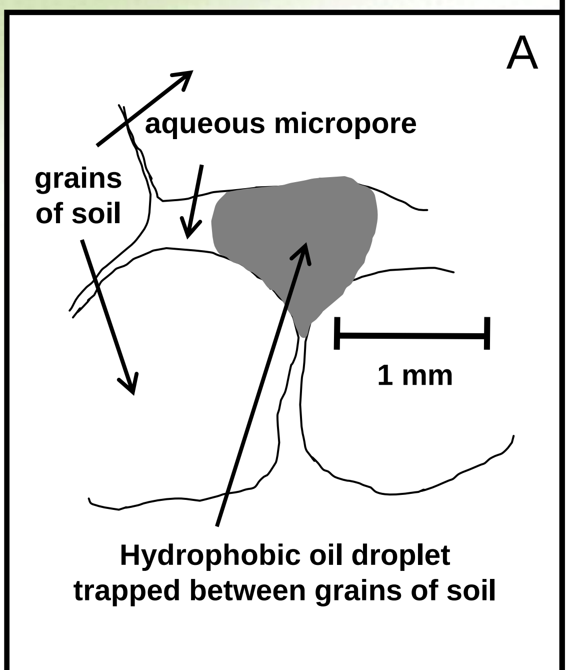
Phytoremediation of PHC

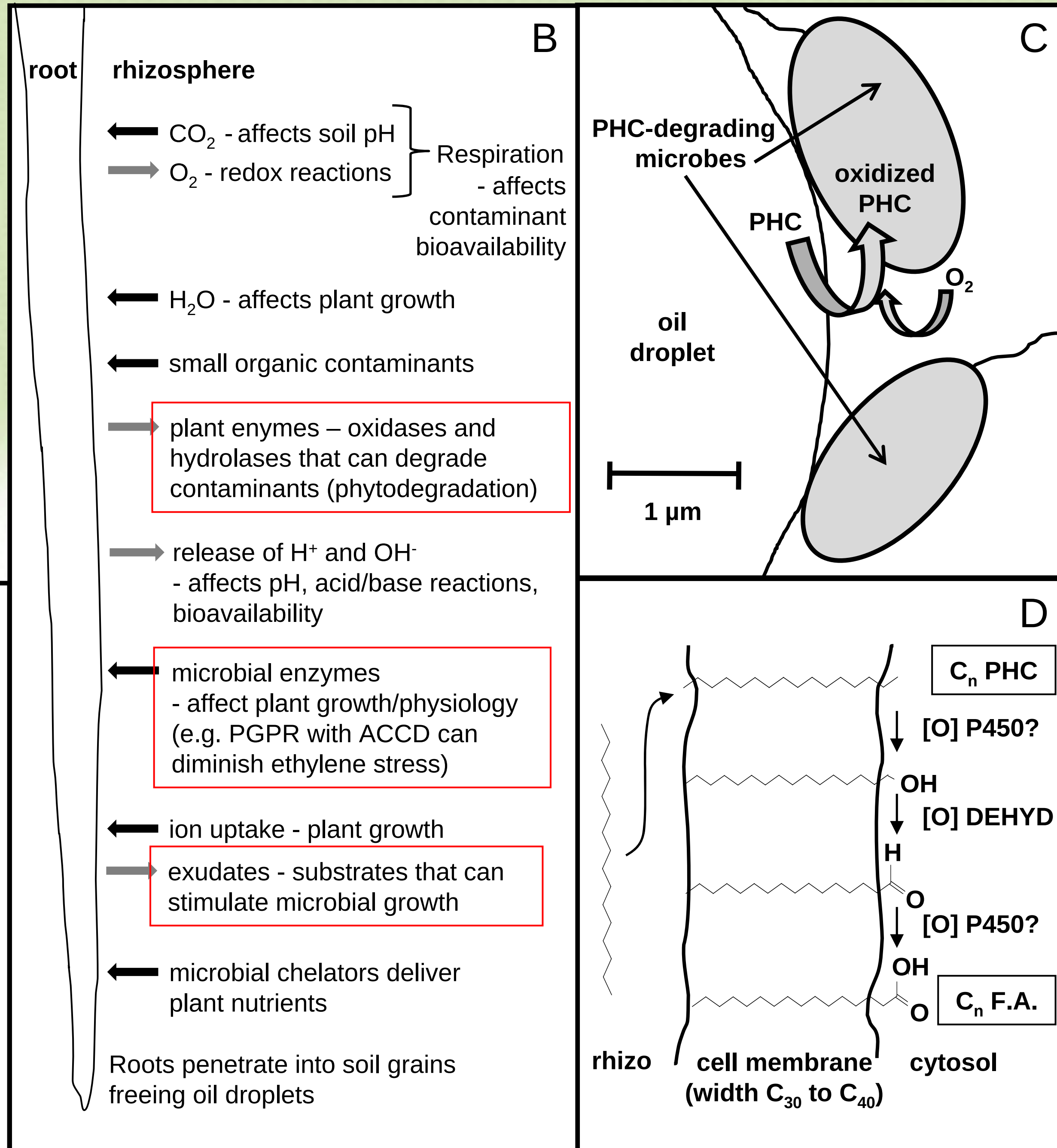
(A) Bioavailability of PHC

(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 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

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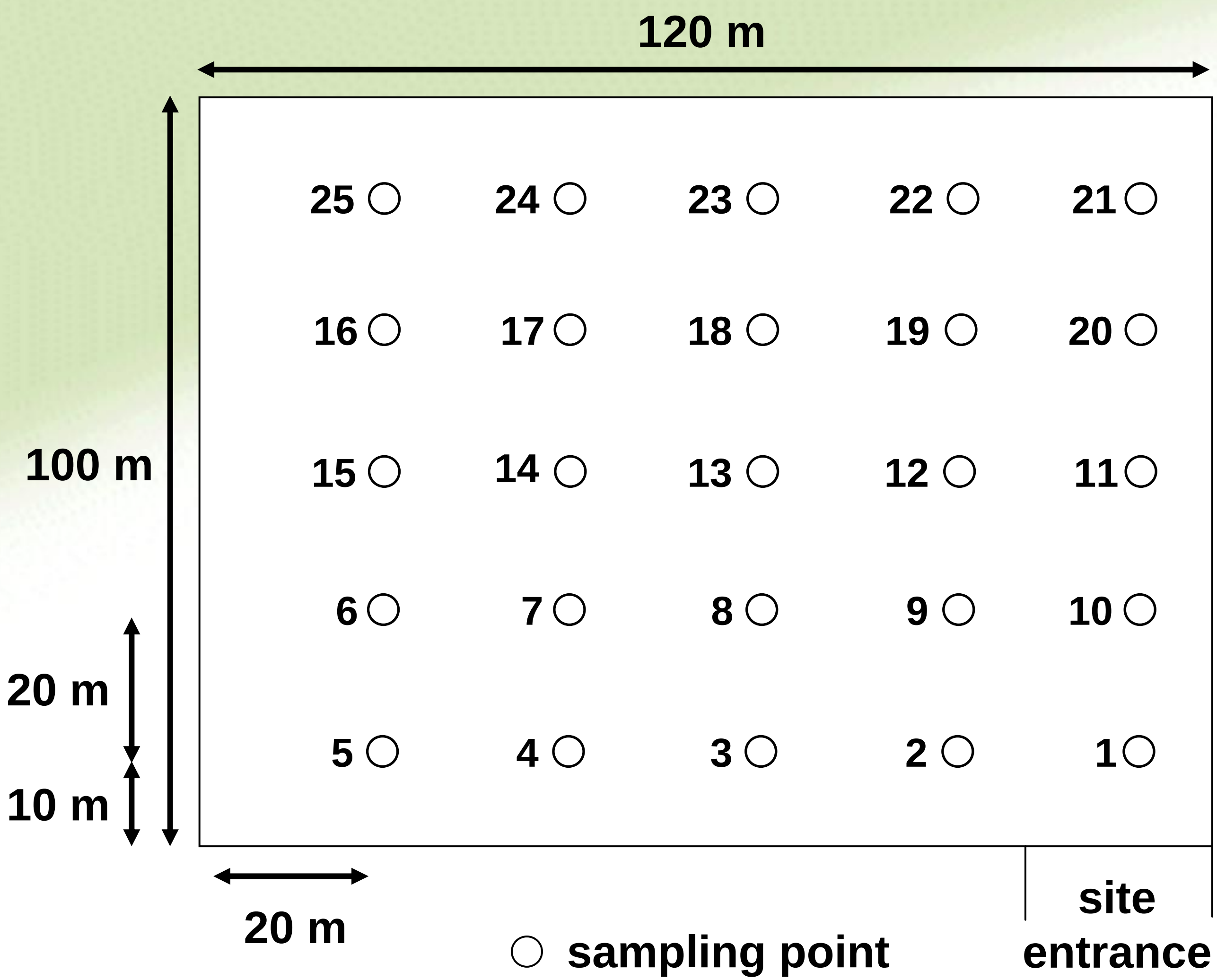
Edson, AB – Site and Sampling Map (2008)

Soil Impact – PHC (Diesel Invert; 85% F3)

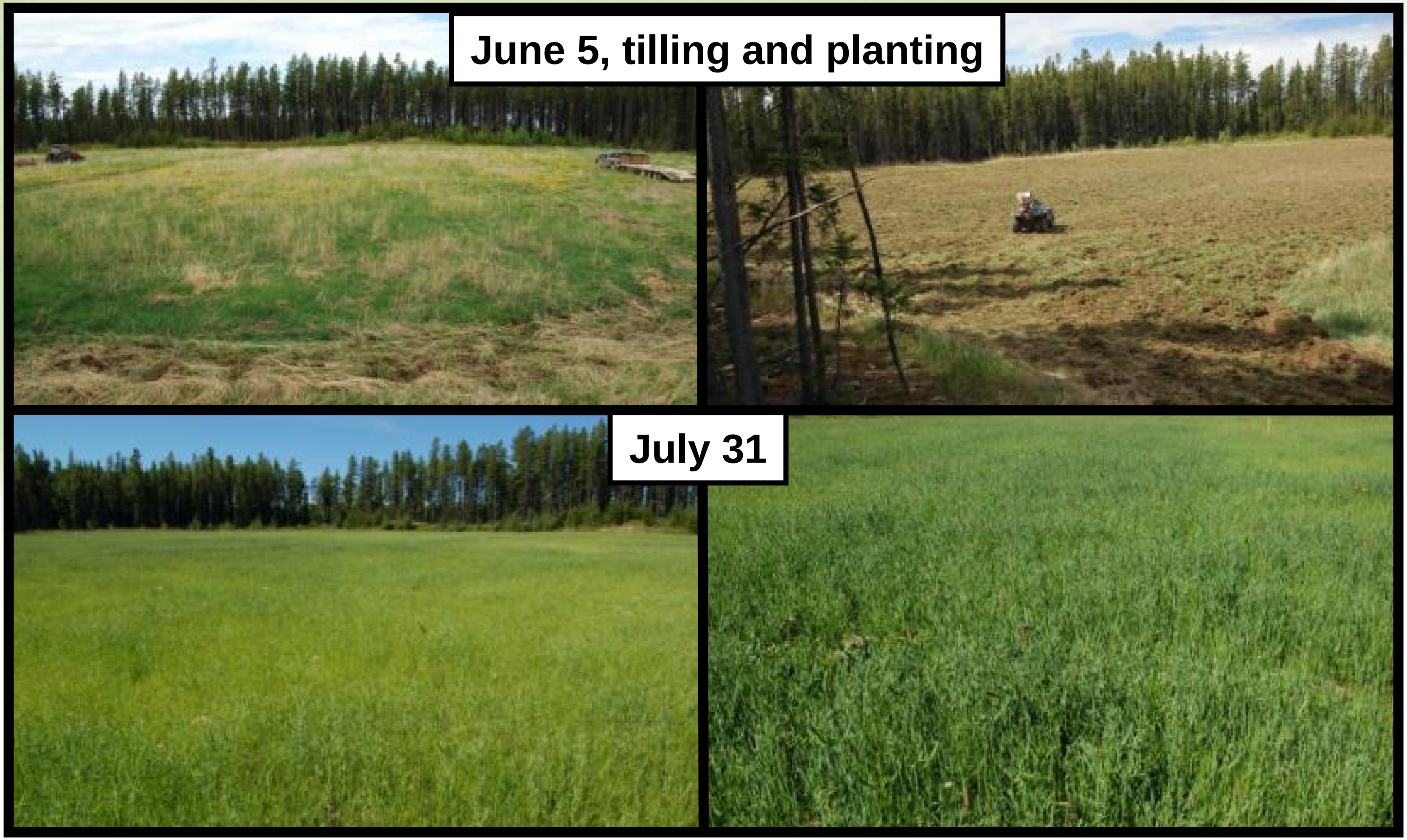


Area:
120 m x 100 m
= 12,000 m^2
= 3 acres

N

Edson, AB – Beginning and Mid-Season (2008)

Soil Impact – PHC (Diesel Invert; 85% F3)



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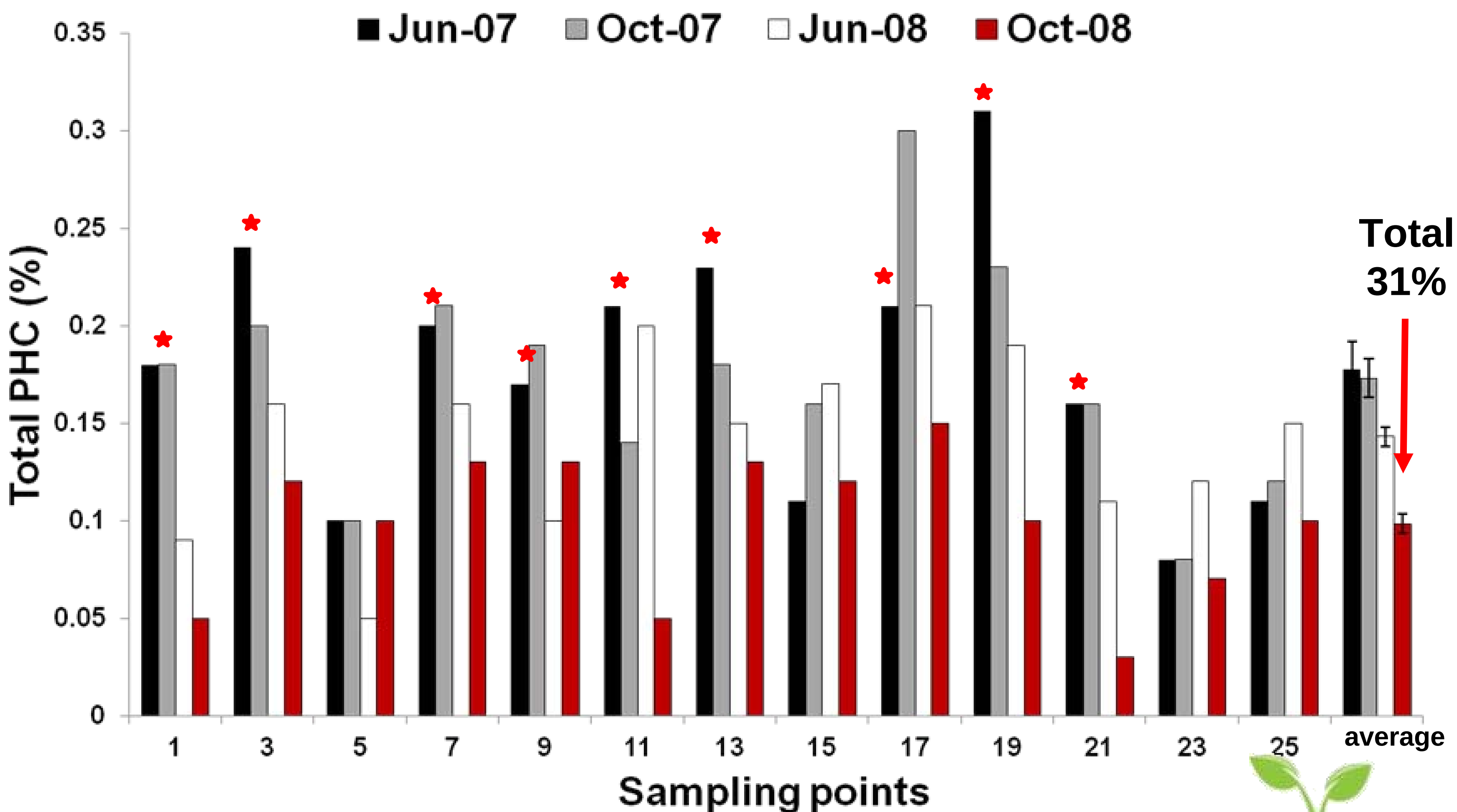
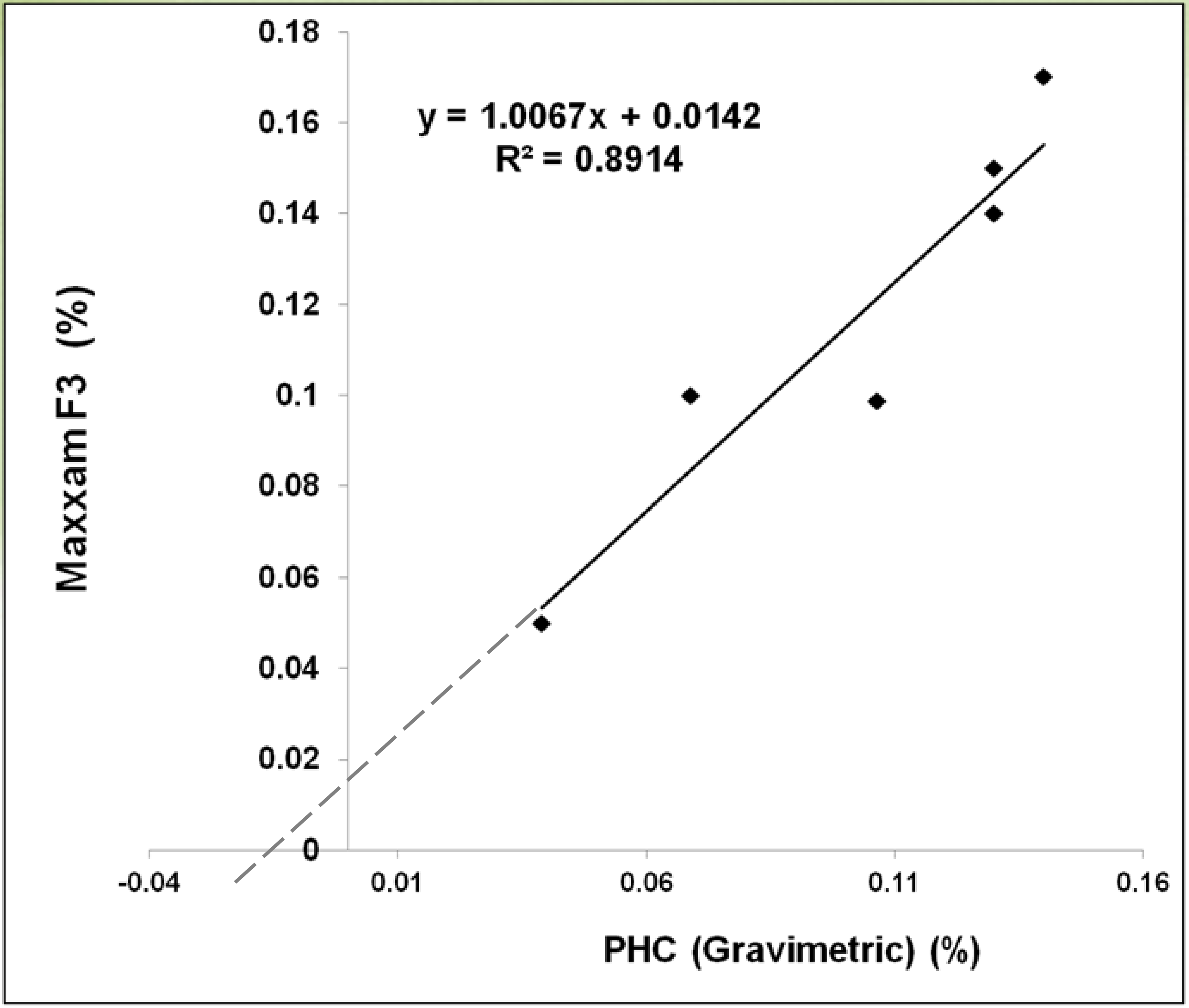
Edson, AB – PHC Remediation (2007- 08)

Soil Impact – PHC (Diesel Invert; 85% F3)

*** In June 2007, 9 of 13 sampling points above Tier 1 criteria (F3 > 1300 mg/kg)**



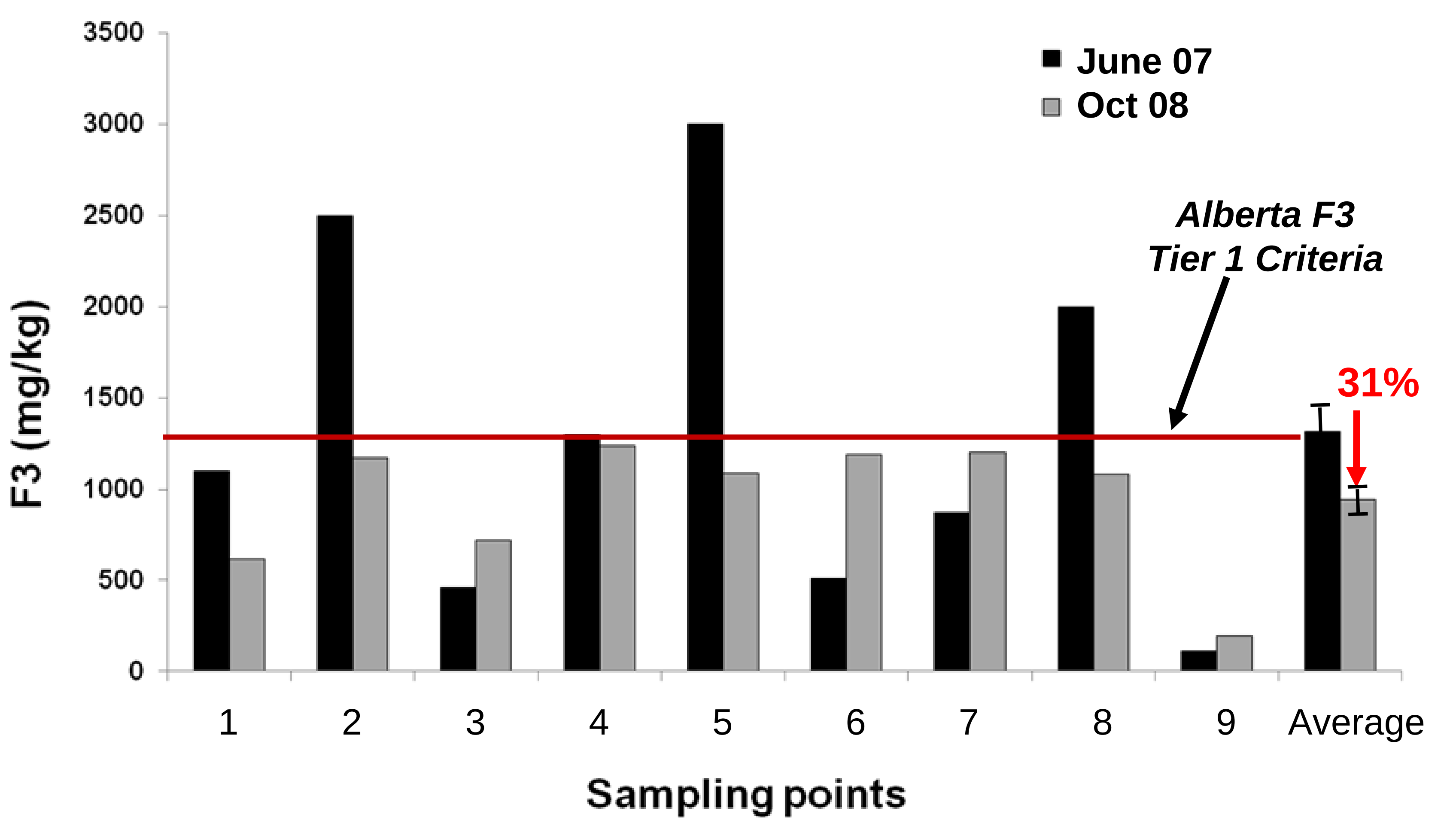
Gravimetric Total PHC vs Analytical Lab F3



- 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**

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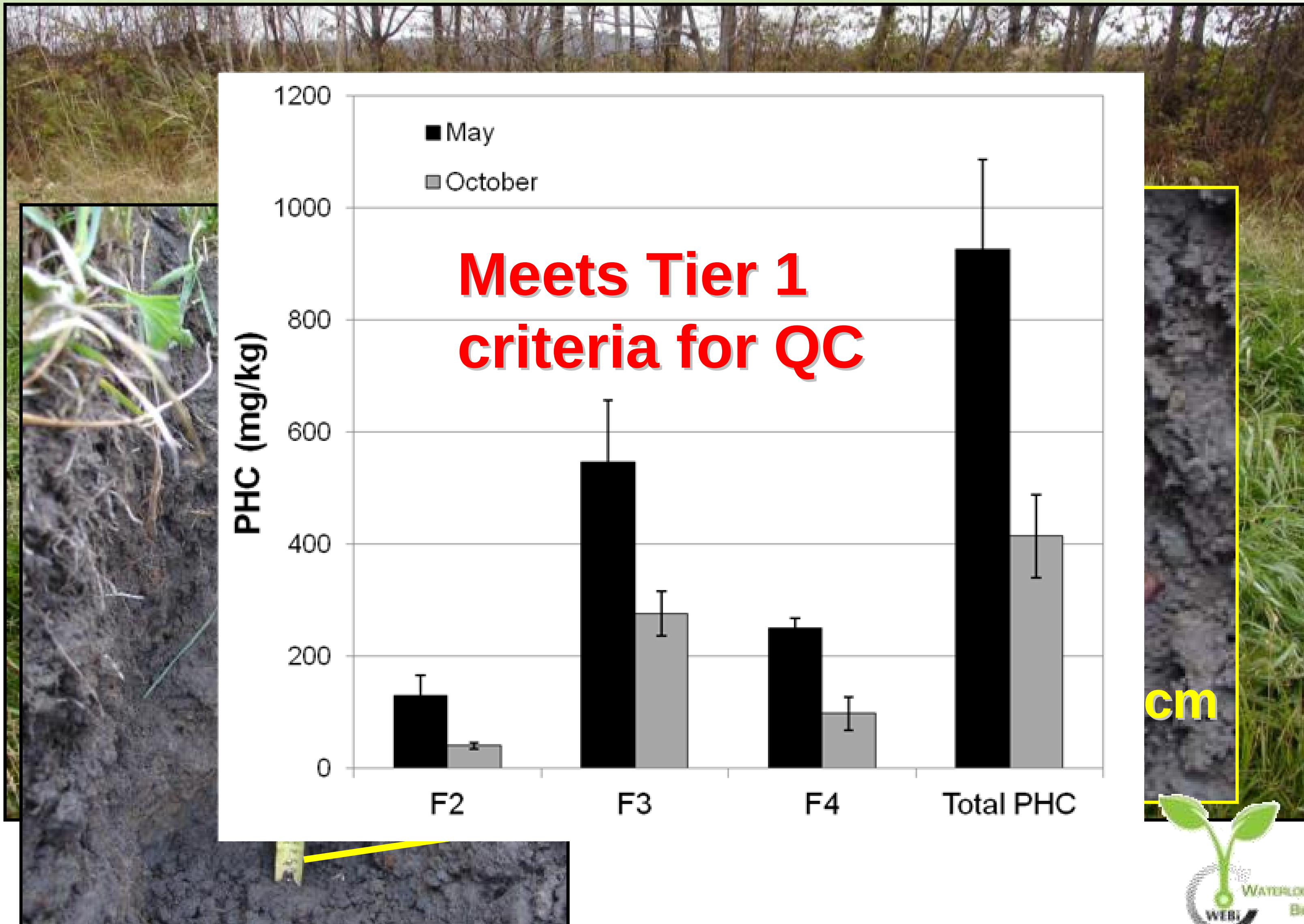
Quebec City, QC – End of Season (2009)

Soil Impact – PHC



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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)

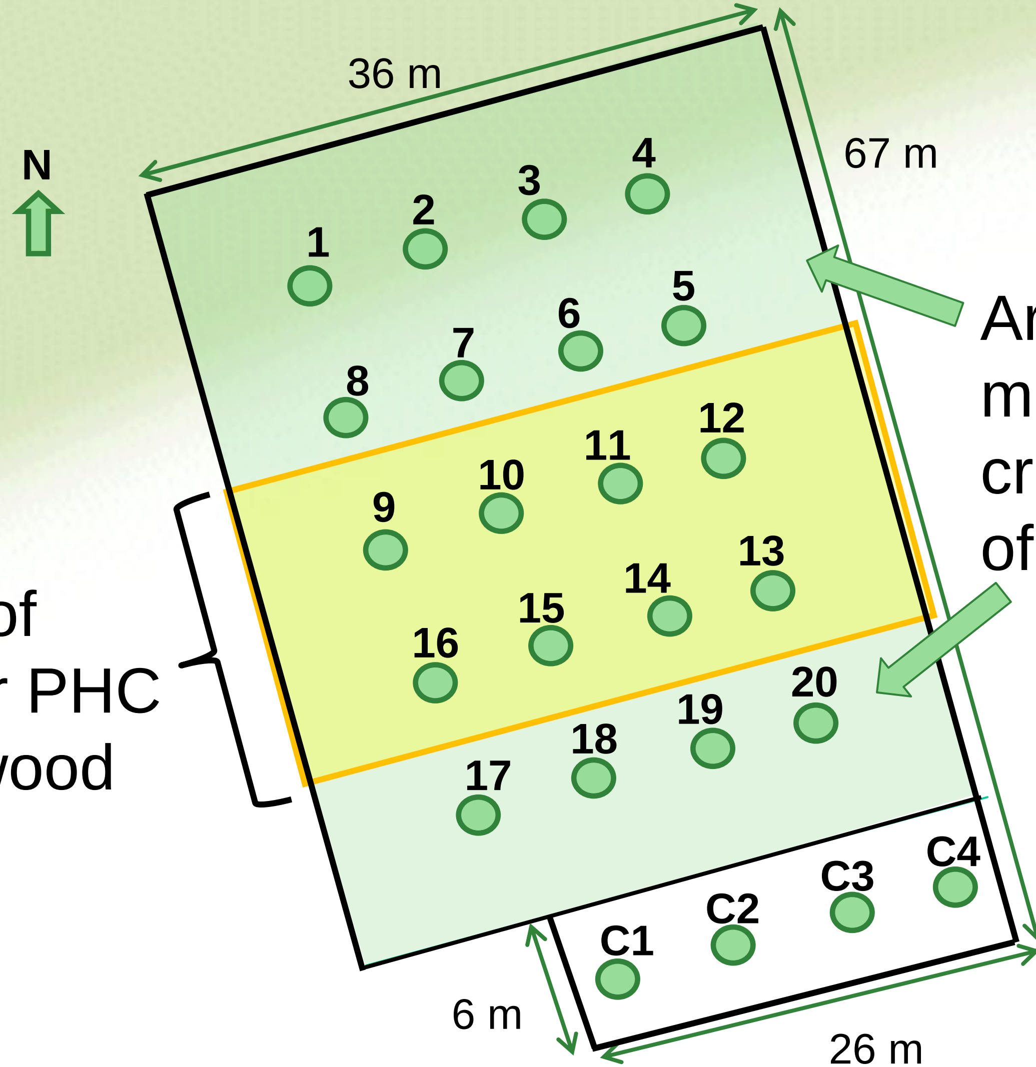
Soil Impact – PHC (Diesel Invert)

Mid-Season

End of Season

Northern BC near NWT Border – Site Map (2010)

Soil Impact – PHC (Diesel Invert)

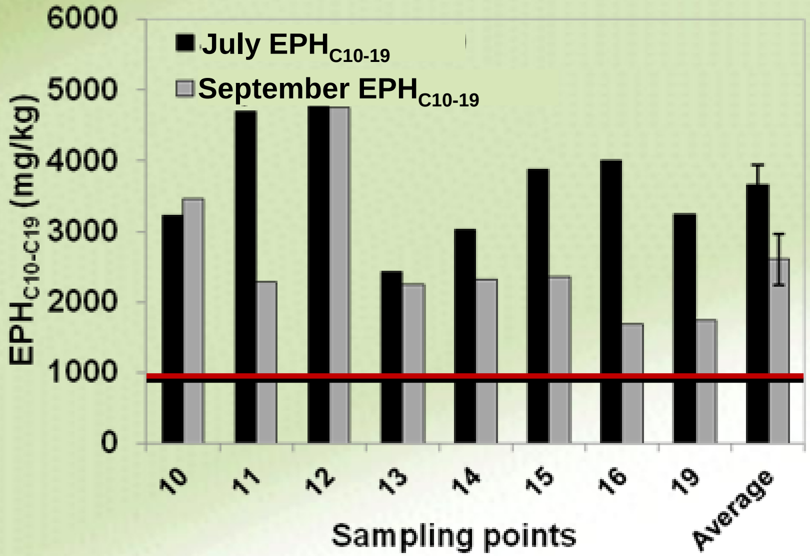


Site Area:
- 2400 m^2
- 0.6 acres

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

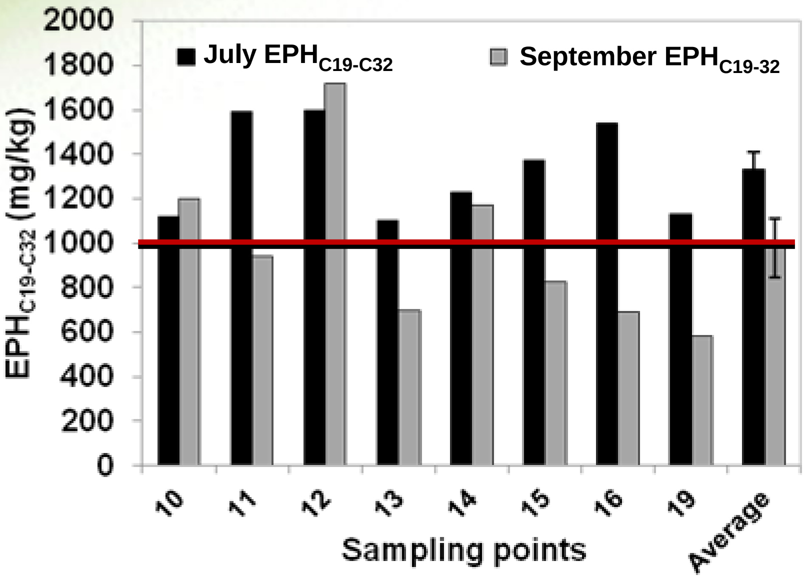
Area of higher PHC with wood chips

Northern BC – EPH Remediation (2010)



$EPH_{C10\text{-}C19}$ Remediation (Maxxam)

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



$EPH_{C19\text{-}C32}$ Remediation (Maxxam)

- 6 out of 8 sampling points showed a decrease in $EPH_{C19\text{-}C32}$ levels over two months
- Only 3 out of 9 sampling points had $EPH_{C19\text{-}C32}$ level above 1000 mg/kg criteria in September
- At the end of the season the average **$EPH_{C19\text{-}C32}$ level decreased by 27%**, from 1335 mg/kg in July to 979 mg/kg in September

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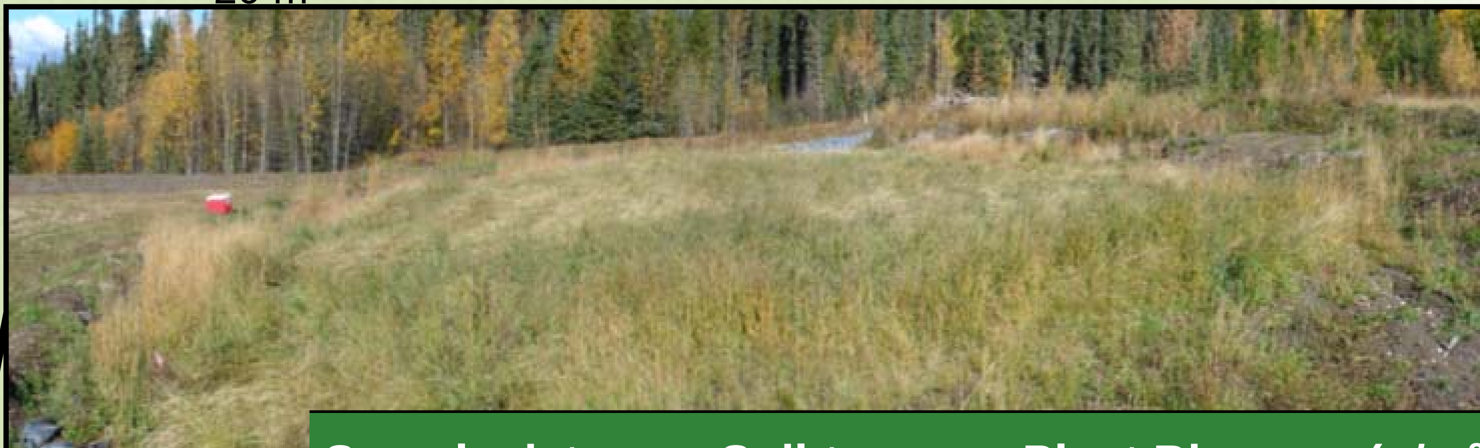
Swan Hills, AB – End of Season (2009 – 10)

Soil Impact – PHC

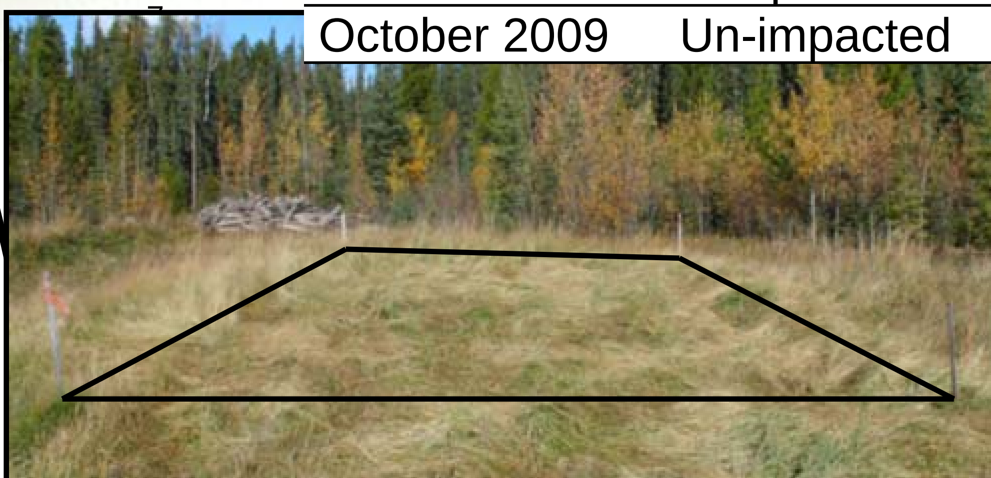
20 m



Impacted Plo

Sample date	Soil-type	Plant Biomass (g/m²) ± SD
October 2009	Impacted	427.7 ± 88.06
October 2009	Un-impacted	438.1 ± 53.56

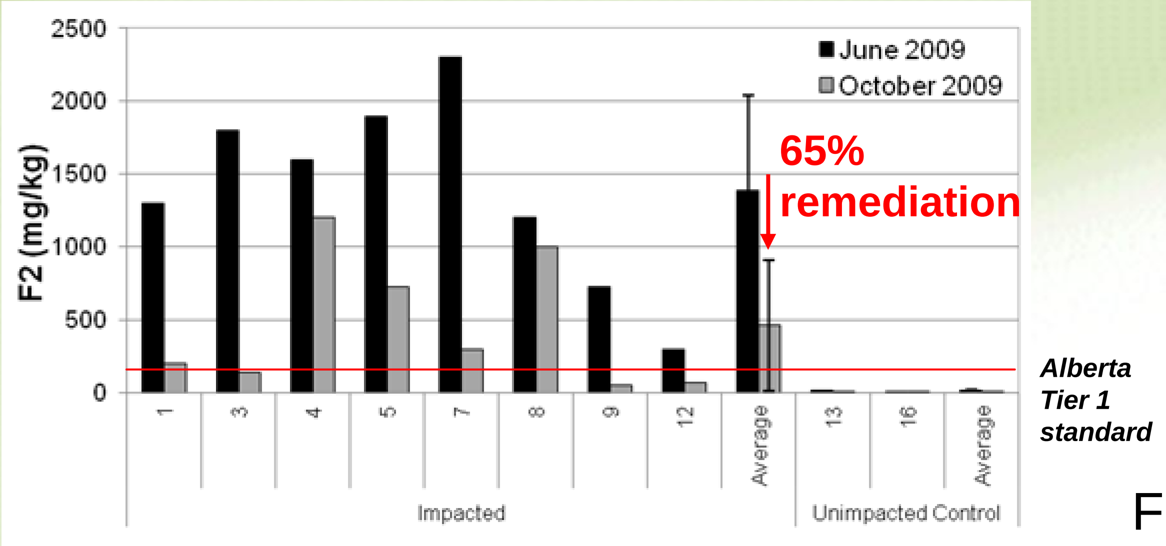
g point

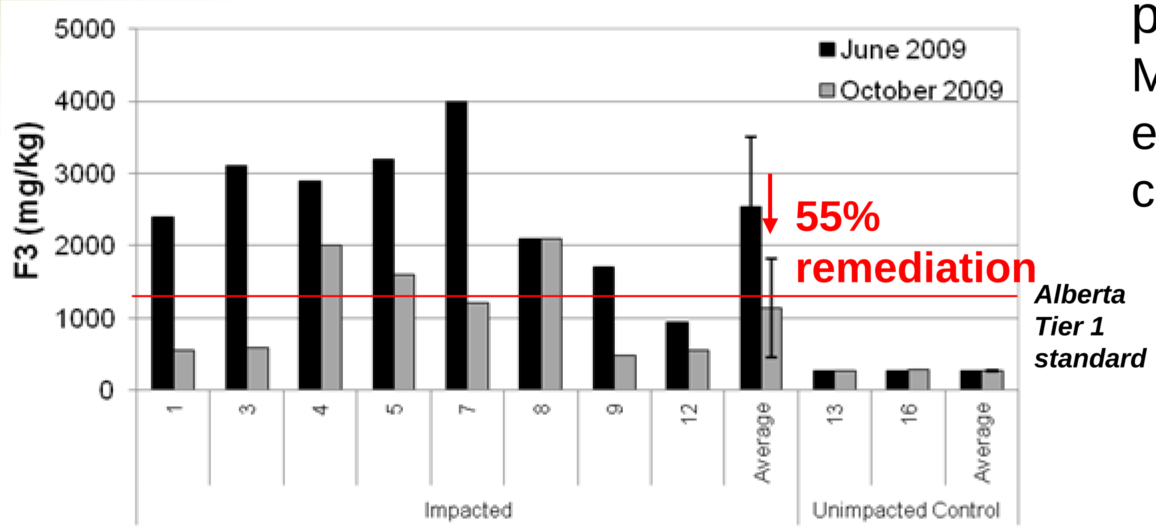


Un-impacted Plot

sump

Swan Hills, AB – F2/F3 Results





F2 & F3 analysis performed by Maxxam.

Method used: cold shake extraction, single silica-column clean-up

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Phytoremediation Cost analysis for the Edson Site

- 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 3rd party costs
  - Does not include work required this spring for final site prep, final sampling/analysis and reporting ~$10k
- Landfilling this material would have cost $70/$m^3$
  - Assumes a 2 h truck turnaround time
  - No backfill required – if backfill was required the cost would rise to $80/$m^3$

Generic cost comparison: Phytoremediation vs. Landfilling

Landfilling

ALL COSTS EXCEPT FOR EXCAVATION	8000 m3			35,000 m3		
	2 Hr TAT	6 Hr TAT	10 Hr TAT	2 Hr TAT	6 Hr TAT	10 Hr TAT
TOTAL COST	$621,450.00	$1,031,450.00	$1,441,450.00	$2,730,000.00	$4,480,000.00	$6,300,000.00
TOTAL COST (less 10%)	na	na	na	$2,457,000.00	$4,032,000.00	$5,670,000.00
TOTAL COST ($/M^3)	$77.68	$128.93	$180.18	$70.20	$115.20	$162.00
TOTAL COST ($/Tonne)	$45.69	$75.84	$105.99	$41.29	$67.76	$95.29

Assumptions: 1. 10% discount to landfill 35,000 m^3 pile; 2. Contaminated soil already stockpiled; 3. 20 trucks/day are available for hauling paid at an hourly rate; 4. Disturbed area reclamation included; 5. Bulk Density = 1.7 g/cm^3

Phytoremediation

ALL COSTS EXCEPT FOR EXCAVATION	Soil Volumes		
	8000 m^3	35,000 m^3	100,000 m^3
TOTAL COST ($/M^3)	$51.28	$32.16	$25.55
TOTAL COST ($/Tonne)	$30.16	$18.92	$15.03
TREATMENT COST - EXCLUDES TOPSOIL REMOVAL FROM TREATMENT AREA			
TOTAL COST ($/M^3)	$47.00	$28.25	$24.18
TOTAL COST ($/Tonne)	$27.65	$16.62	$14.22
TREATMENT COST - IN-SITU TREATMENT - MINIMAL TREATMENT AREA PREPARATION			
TOTAL COST ($/M^3)	$38.90	$20.90	$17.03
TOTAL COST ($/Tonne)	$22.88	$12.29	$10.02

Assumptions:

1. 5% PHC contaminated soil
2. Some bench scale testing
3. Contaminated soil already stockpiled
4. Time to remediate 1 treatment layer = 4 years
5. Tier II toxicity testing to confirm remedial endpoint
6. Disturbed area reclamation included
7. Bulk Density = 1.7 g/cm^3

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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

- Fine grain soils - F3 from 2000 to 10,000 mg/kg
  - In 2 to 4 years, will meet Alberta Tier 1 standards
- Fine grain soils - F3 above 10,000 mg/kg
  - In 3 to 6 years, will meet Alberta Tier 1 or 2 standards
- Coarse grain soils - F3 above 3000 mg/kg
  - Phytoremediation will significantly lower F3
  - Tier 2 approach may be required

COST

- Actual cost for the Edson site (3,400 m^3) was \$33/m^3
- Cost to landfill (landfill 1 h from site) would have been \$70/m^3

Development of PEPS for Salt Impacted Sites

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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**

Salinity effects mostly negligible (or salt deprived)	**Yields of very sensitive crops may be restricted**	**Yields of many crops diminished**	**Only tolerant plants grow**	**Only a few very tolerant plants can grow**

0 2 4 8 16

EC_e (dS/m)

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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

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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 ~ 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

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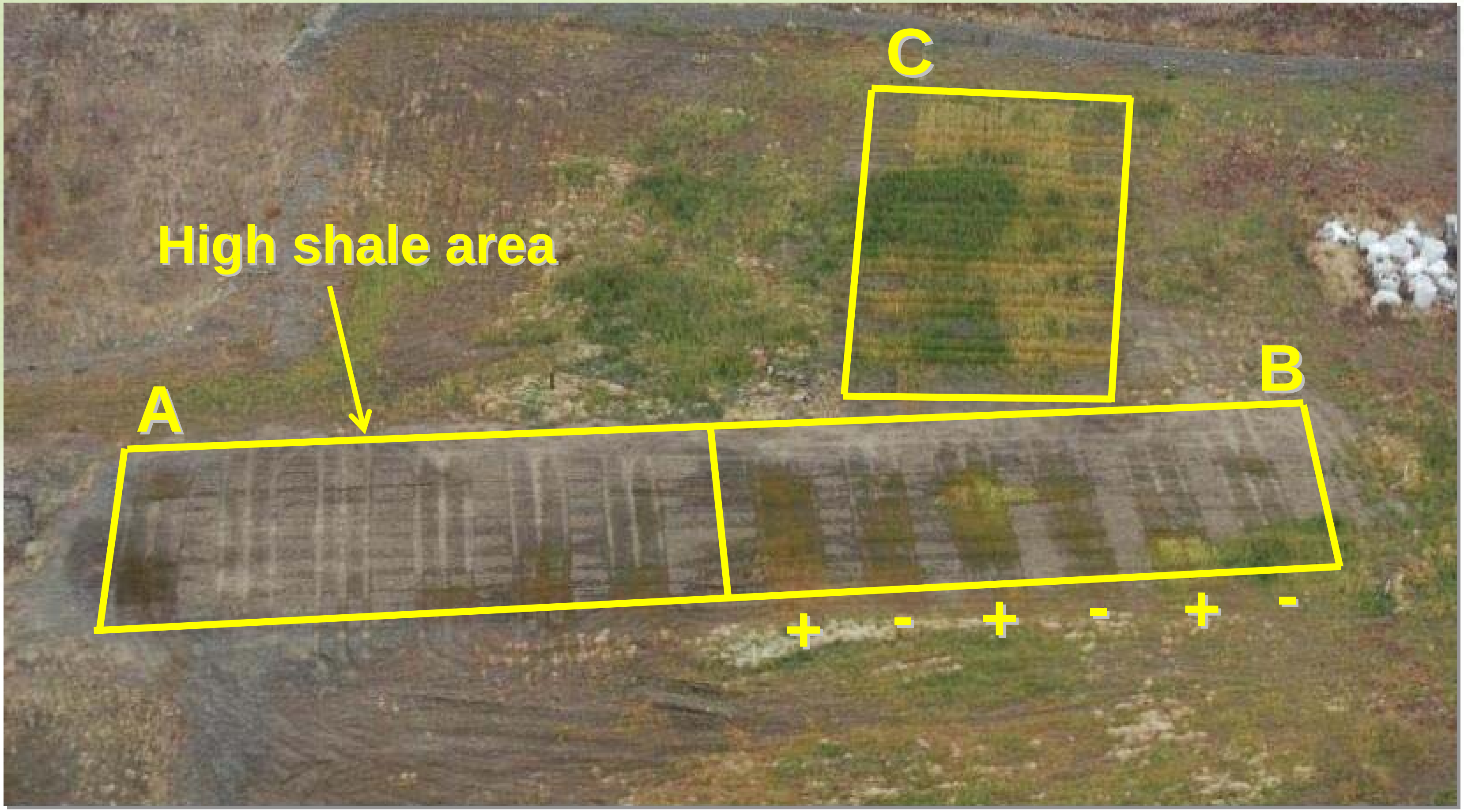
Norman Wells, NWT – End of Season (2010)

Soil Impact – Salt

Norman Wells, NWT – End of Season (2008)

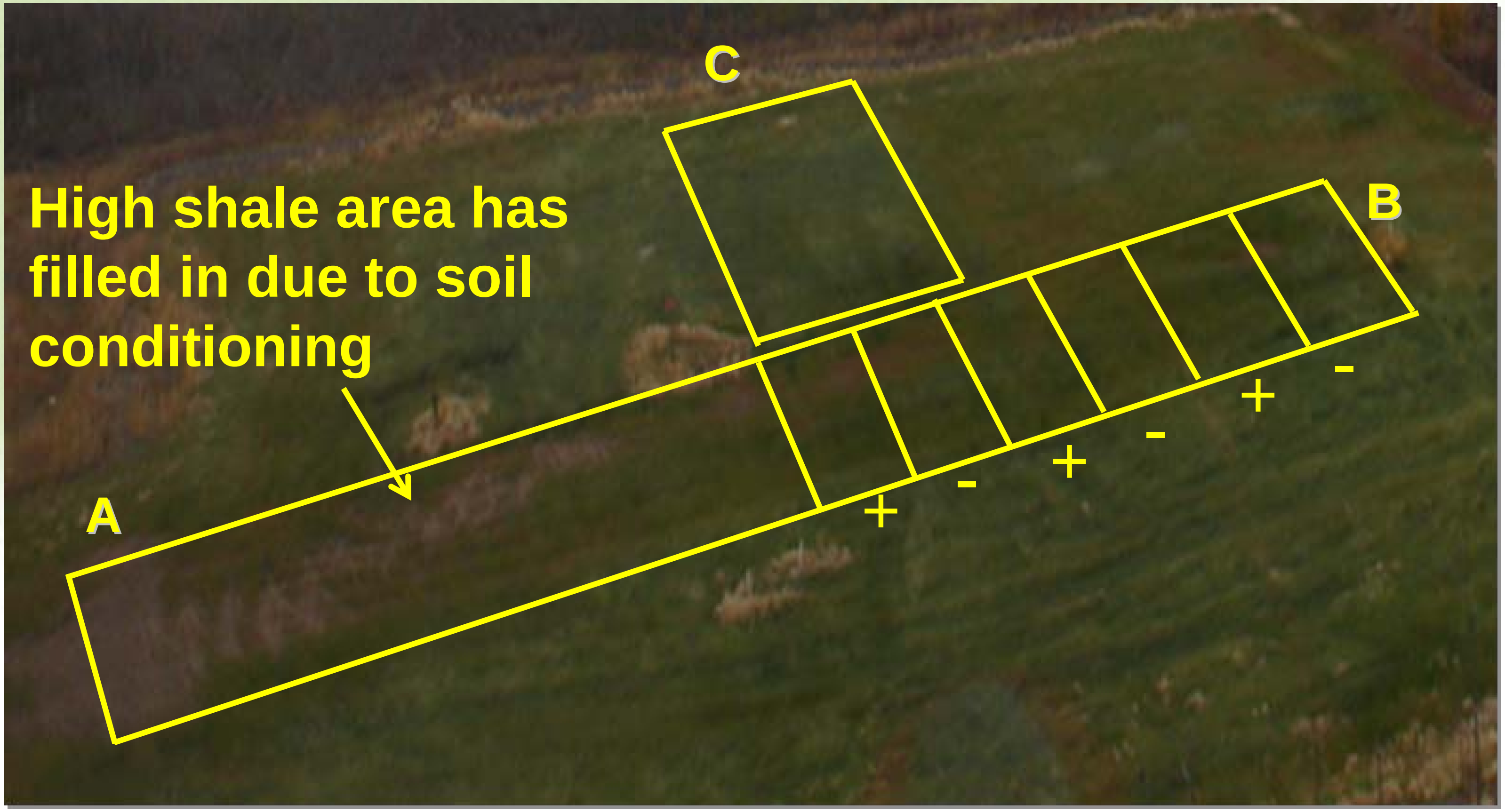
Soil Impact – Salt



Plants used: slender wheatgrass and red fescue
No soil conditioning

Norman Wells, NWT – End of Season (2009)

Soil Impact – Salt



Plants used: slender wheatgrass, ryegrass and red fescue
Soil conditioned

Norman Wells, NWT – End of Season (2010)

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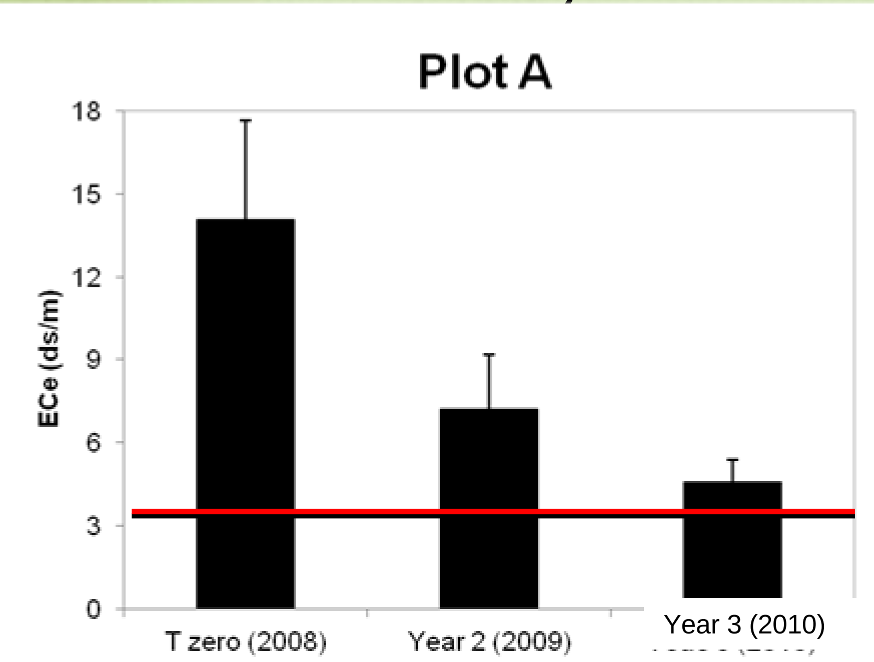
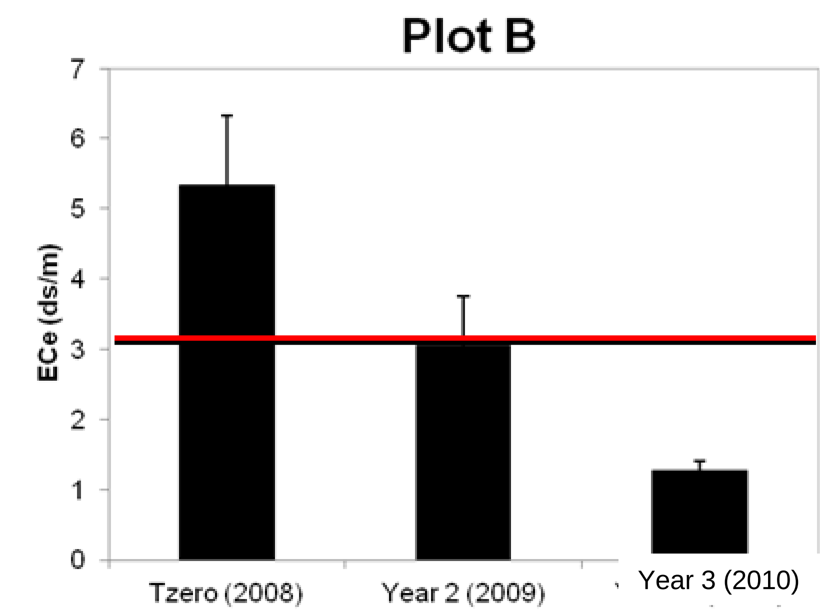
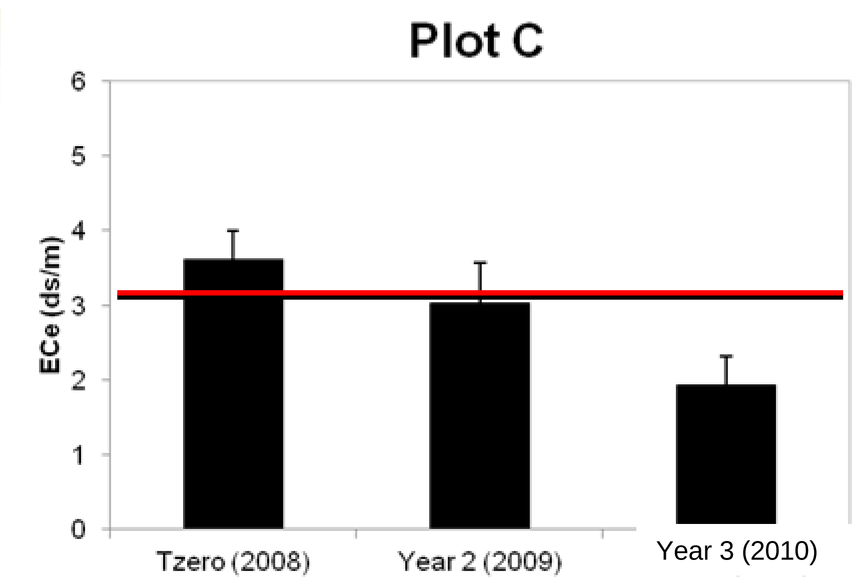
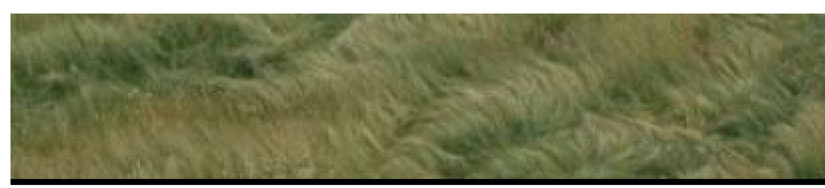
Salt Remediation









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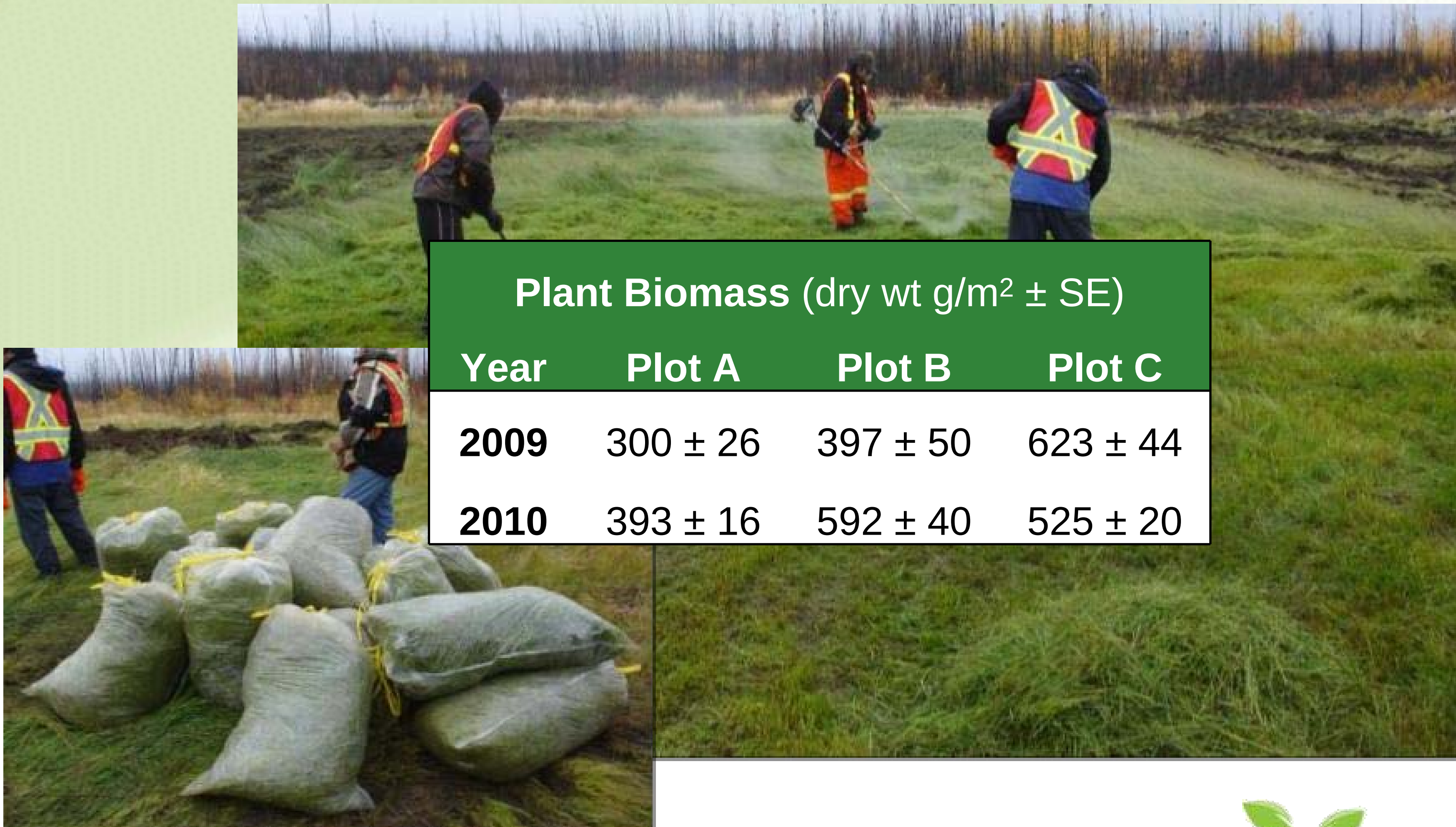
Norman Wells, NWT – End of Season (2009)

Soil Impact – Salt

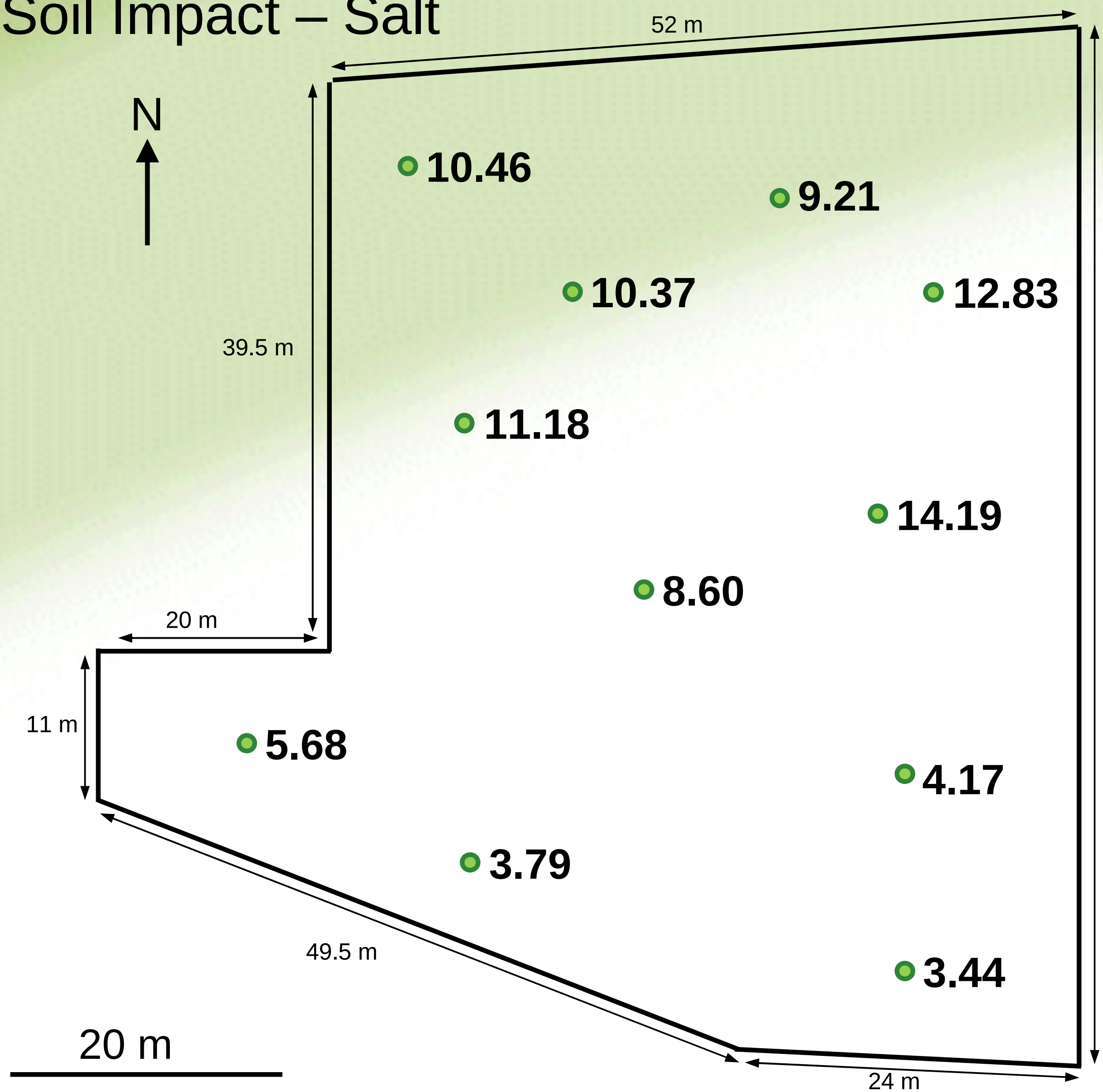


Plant Biomass (dry wt g/m² ± SE)			
Year	**Plot A**	**Plot B**	**Plot C**
2009	300 ± 26	397 ± 50	623 ± 44
2010	393 ± 16	592 ± 40	525 ± 20

High salt plant material was mowed and removed from the site

Weyburn, SK – 1 of 7 sites: Soil Salinity (EC_e) Map (2010, Year 1)

Soil Impact – Salt



Surface soil EC_e at each sample point

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 m^2
- 1.1 acres

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Weyburn, SK – 1: End of Season (2010, Year 1)

Soil Impact – Salt

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Weyburn, SK – 2 of 7 sites: Soil Salinity (EC_e) Map (2010, Year 1)

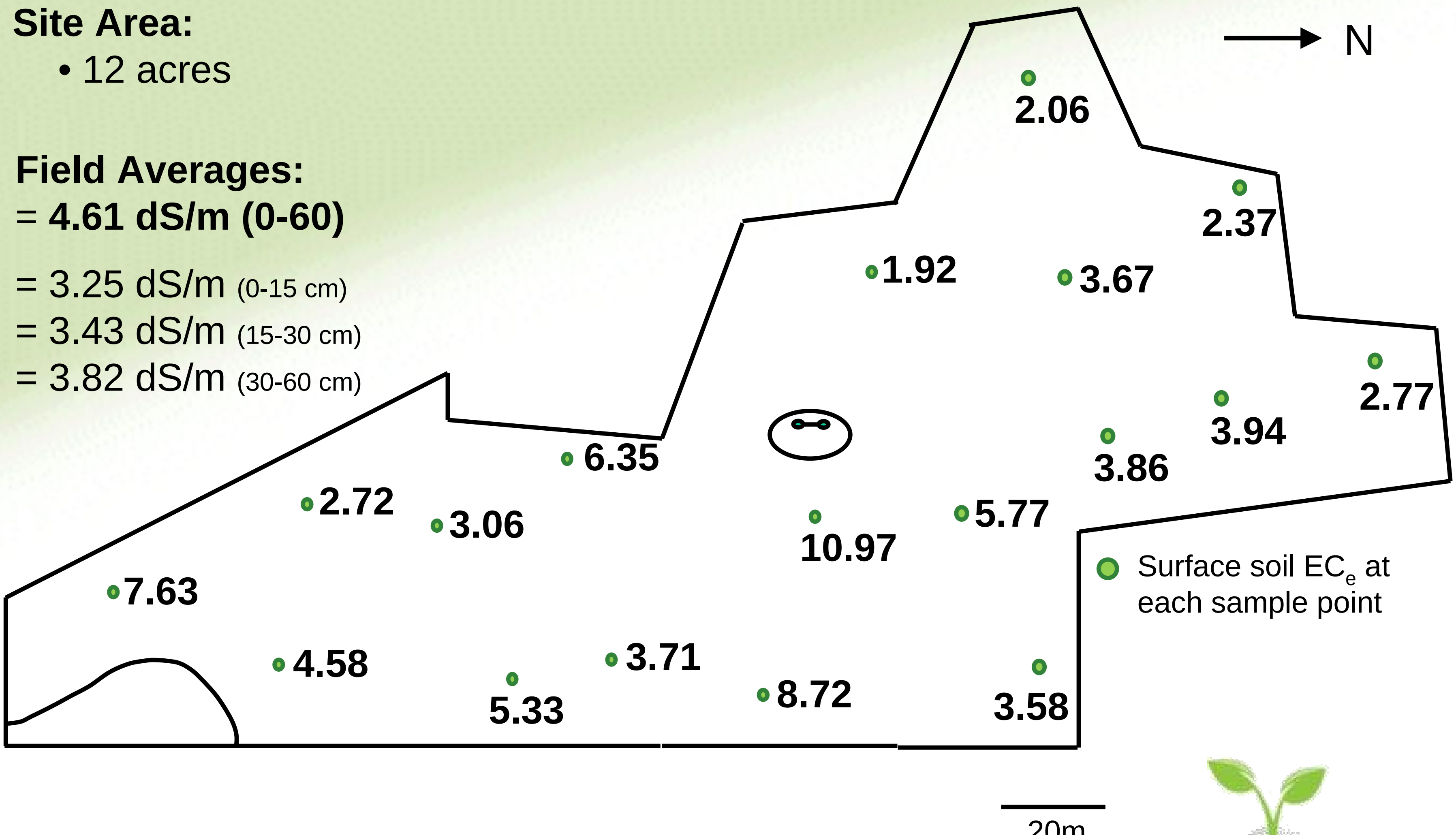
Soil Impact – Salt

Site Area:
- 12 acres

Field Averages:

= **4.61 dS/m (0-60)**

= 3.25 dS/m (0-15 cm)
= 3.43 dS/m (15-30 cm)
= 3.82 dS/m (30-60 cm)



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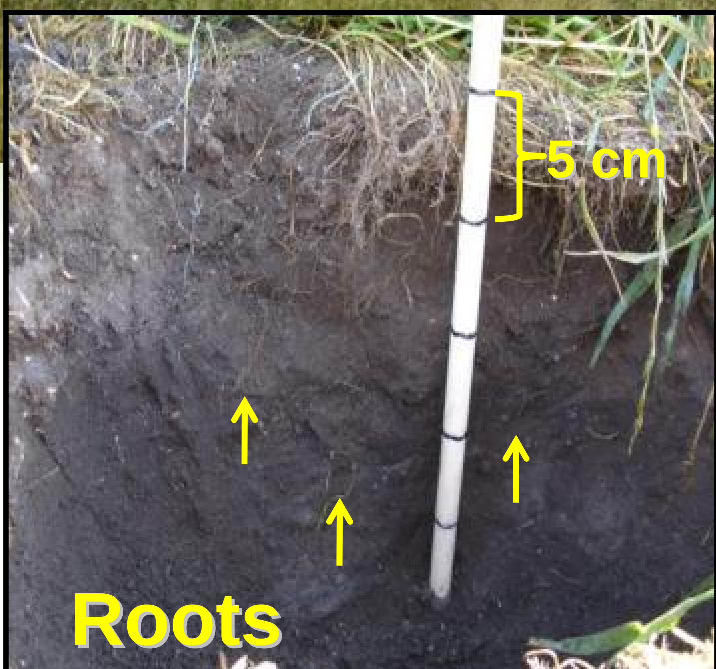
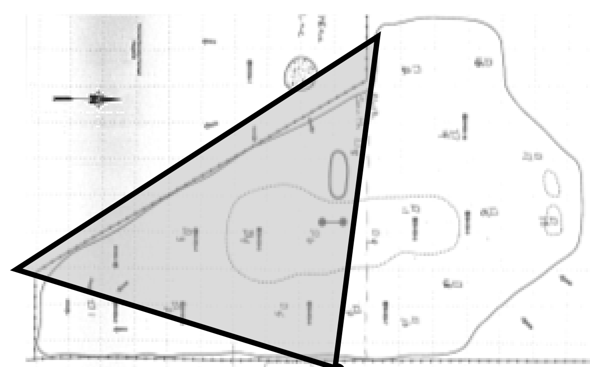
Weyburn – 2, SK: End of Season (2010, Year 1)

Soil Impact – Salt





Provost, AB: Mid-Season (2009, Year 1)

Soil Impact – Salt

High salt hot spots and poor soil from pipeline construction:
EC_e: 13-17 dS/m

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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**

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Red Earth, AB: 2009 - 2010

Soil Impact – Salt and Herbicide (Arsenal®)

October 6, 2009

Great improvement in plant growth, therefore, can remediate herbicides

September 29, 2010

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.

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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

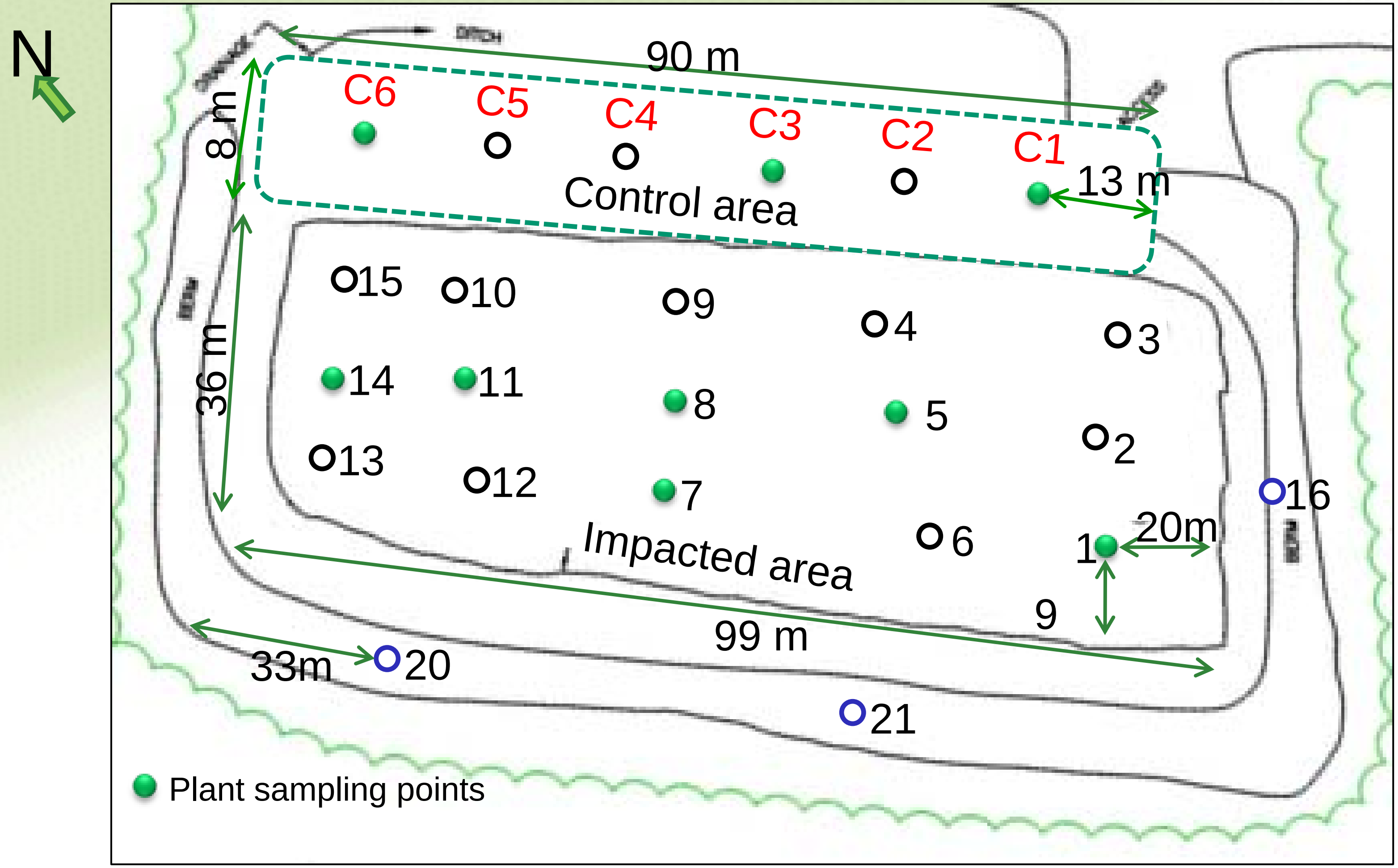
- **Collaborators**
  - Perry Gerwing, Earthmaster

- **Partners**
  - S Willets, O Mrklas, C Gordey, ConocoPhillips Canada
  - 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

WEBI Waterloo Environmental Biotechnology Inc.

Dawson Creek - 1, BC – End of Season (2009)

Soil Impact – PHC



Dawson Creek - 1, BC – End of Season (2009)

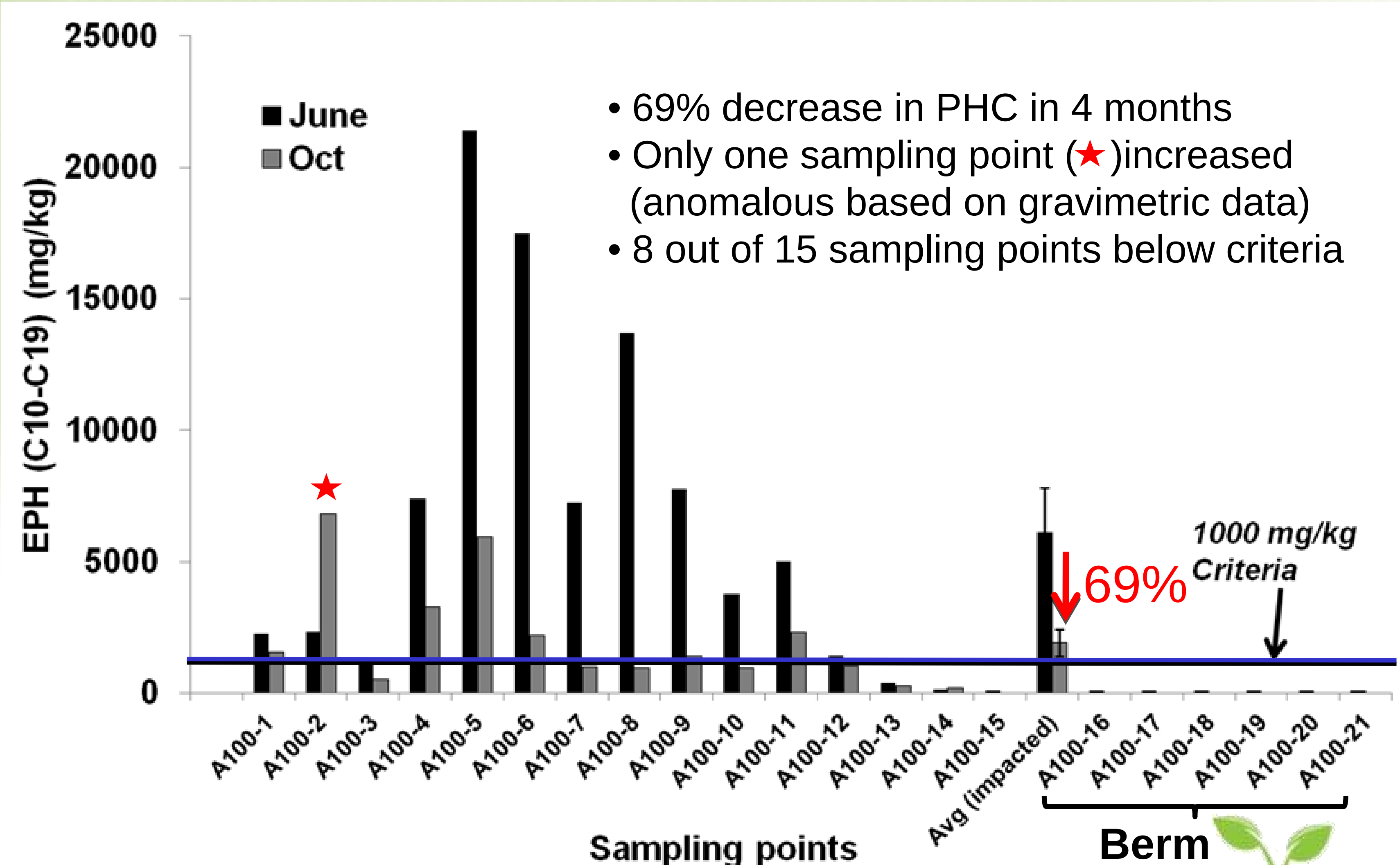
Soil Impact – PHC

Impacted area

Control

WATERLOO ENVIRONMENTAL BIOTECHNOLOGY INC.

Dawson Creek - 1, BC – EPH (C10-C19) Remediation (2009)



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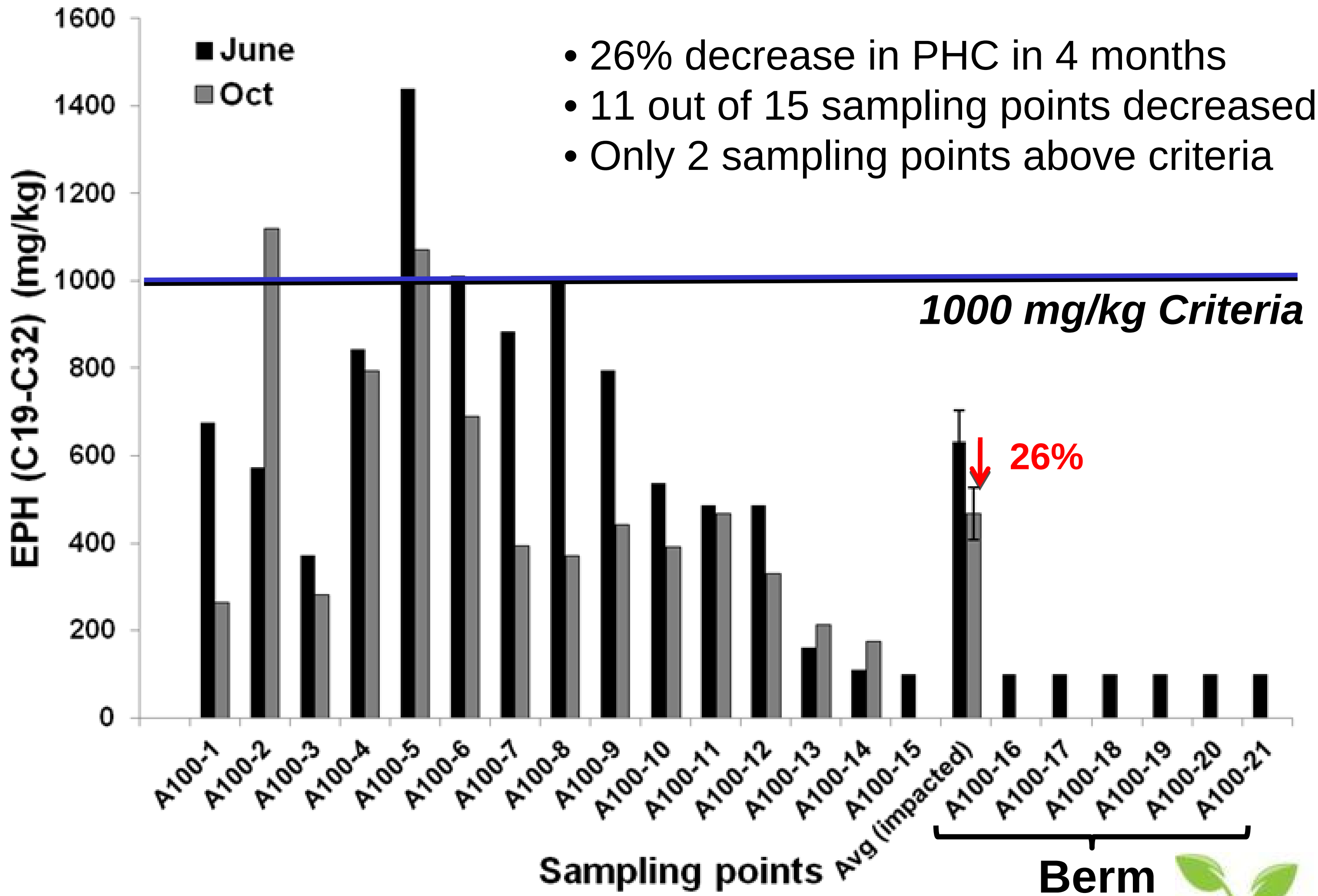
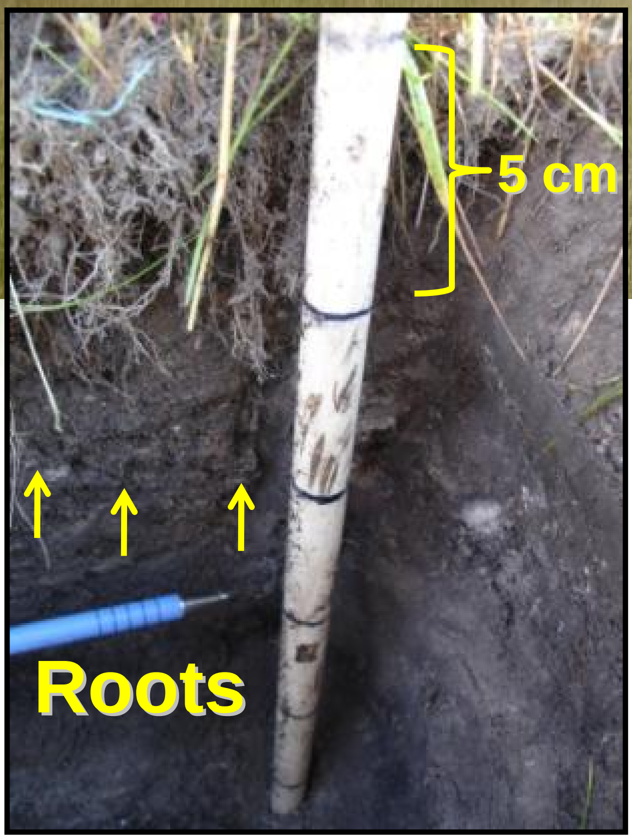
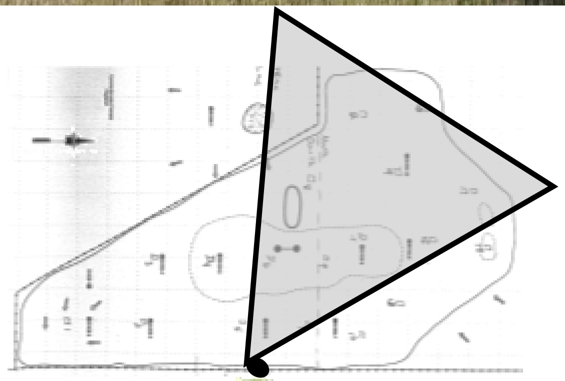
Dawson Creek - 1, BC – EPH (C19-C32) Remediation (2009)



Weyburn, SK – 2: End of Season (2010, Year 1)

Soil Impact – Salt





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