Phytoremediation of Impacted Soil

Field Research Trials with New Applications, Species, and Challenges.

Elizabeth Murray, Bruce Greenberg, Ben Poltorak, Kent Cryer, Perry Gerwing.

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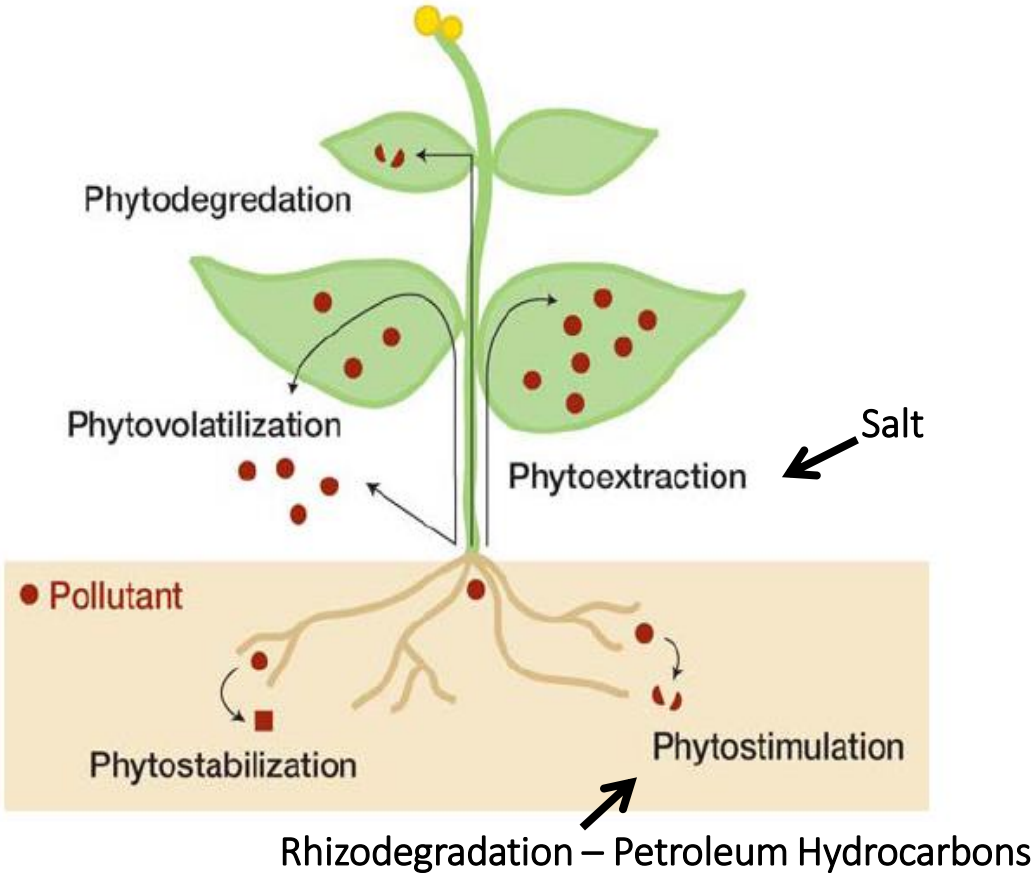
PGPR

PEPS – Plant Growth Promoting Rhizobacteria (PGPR)-Enhanced Phytoremediation Systems.

- Developed through collaboration with Dr. Bruce Greenberg of the University of Waterloo and Earthmaster Environmental.
- Earthmaster has assumed control of the PEPS technology and continues to collaborate with Dr. Greenberg.
- Earthmaster now manages all PGPR testing, selection and seed treating in Calgary.
- Earthmaster is conducting new research into how PGPR can be used in other applications such as enhanced reclamation.

Phytoremediation – How it Works



- Improved rhizosphere
  - Soil
  - Organic matter
  - Bacteria
  - Water
  - Roots
  - Contaminants
- Phytostimulation
  - Petroleum Hydrocarbons
- Phytoextraction
  soil→root→foliage
  - Salts
  - Metals

Challenge – getting the plants to grow.

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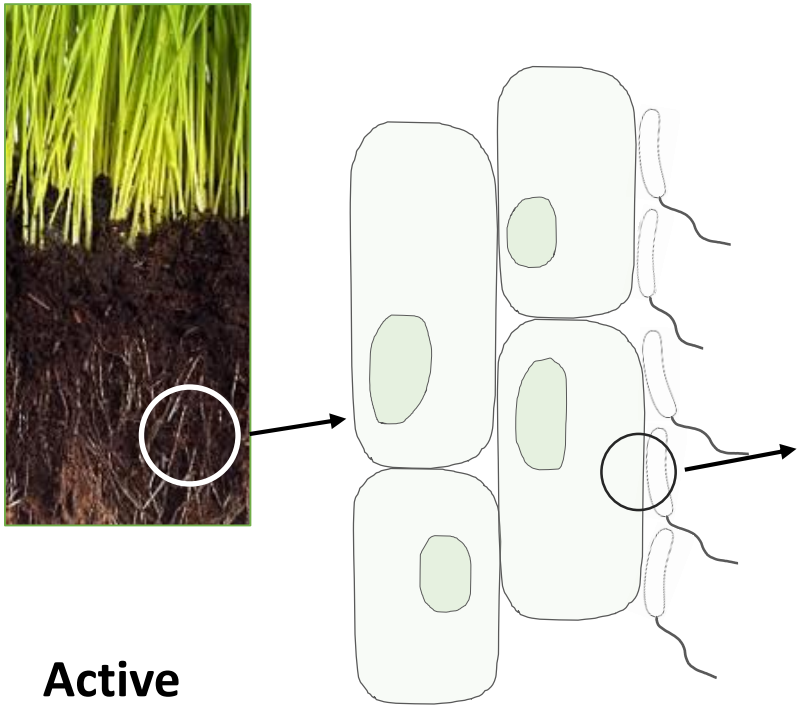
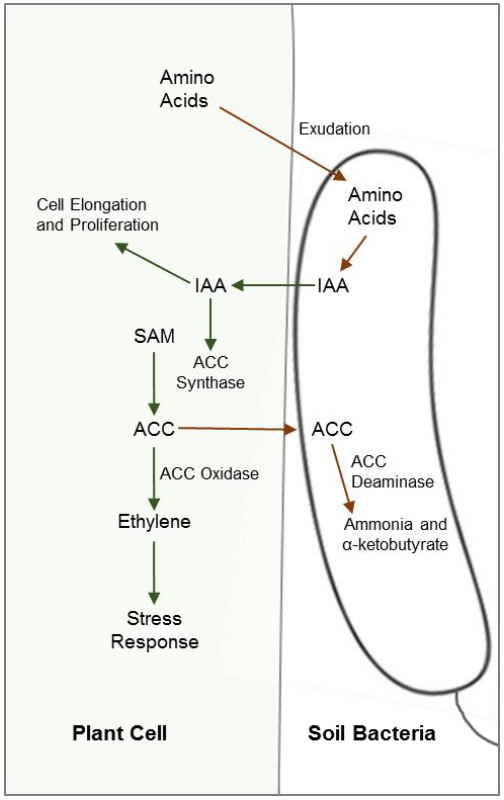
PGPR – Facilitating Plant Growth in Challenging Conditions





Active rhizosphere:
PGPR co-localize with developing roots

- Stress ethylene
- Plant vigor
- Root development
- Rhizobacteria
- Leaves
- Salt and metals uptake
- Degradation of PHC

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Bacteria / Seed Selection for Remediation & Enhanced Reclamation

Bacteria (*Pseudomonas sp.)* are isolated from area soil:

- Naturally occurring soil/water bacteria.
- Ubiquitous, geographically relevant, and frequently associated with plants.
- Not genetically modified.
- In general, they are classified as biosafety level 1 – no threat to humans, wildlife, or the environment.

PGPR are cultured in the lab:

- Tested and selected for ACC deaminase and auxin (IAA) levels.
- Cultured and tested in greenhouse trials as individual species.

Grasses are selected based on surrounding area:

- Suitable for the area – not prohibited.
- Must produce high biomass.

Development and Full Scale Application of PEPS

Joint activities by the University of Waterloo and Earthmaster Environmental:

- PEPS is based on peer reviewed research published in scientific journals by many groups world wide.
- 15+ years of research (Dr. Greenberg) and 10+ years of full-scale commercial field remediation at >30 sites across Canada.
- Successful remediation of both PHC and salt impacted sites in 7 Canadian provinces and territories since 2004.

Phytoremediation of PHC – Northern Alberta

Baytex Red Earth Creek oilfield emulsion spills

- 9,200 m³ of PHC and salt impacted soil
- Soil from historical spill sites - numerous treatment methods had been attempted previously
- Earthmaster constructed 3 soil treatment facilities:
  - Engineered clay pad minimum 0.60 m thick
  - Perimeter clay berm to contain material
  - Surface water run off collection system
    - Channels
    - Collection sumps with poly liners
  - AER compliant
- Impacted soil was spread across the clay liners
  - ~0.45 m thick

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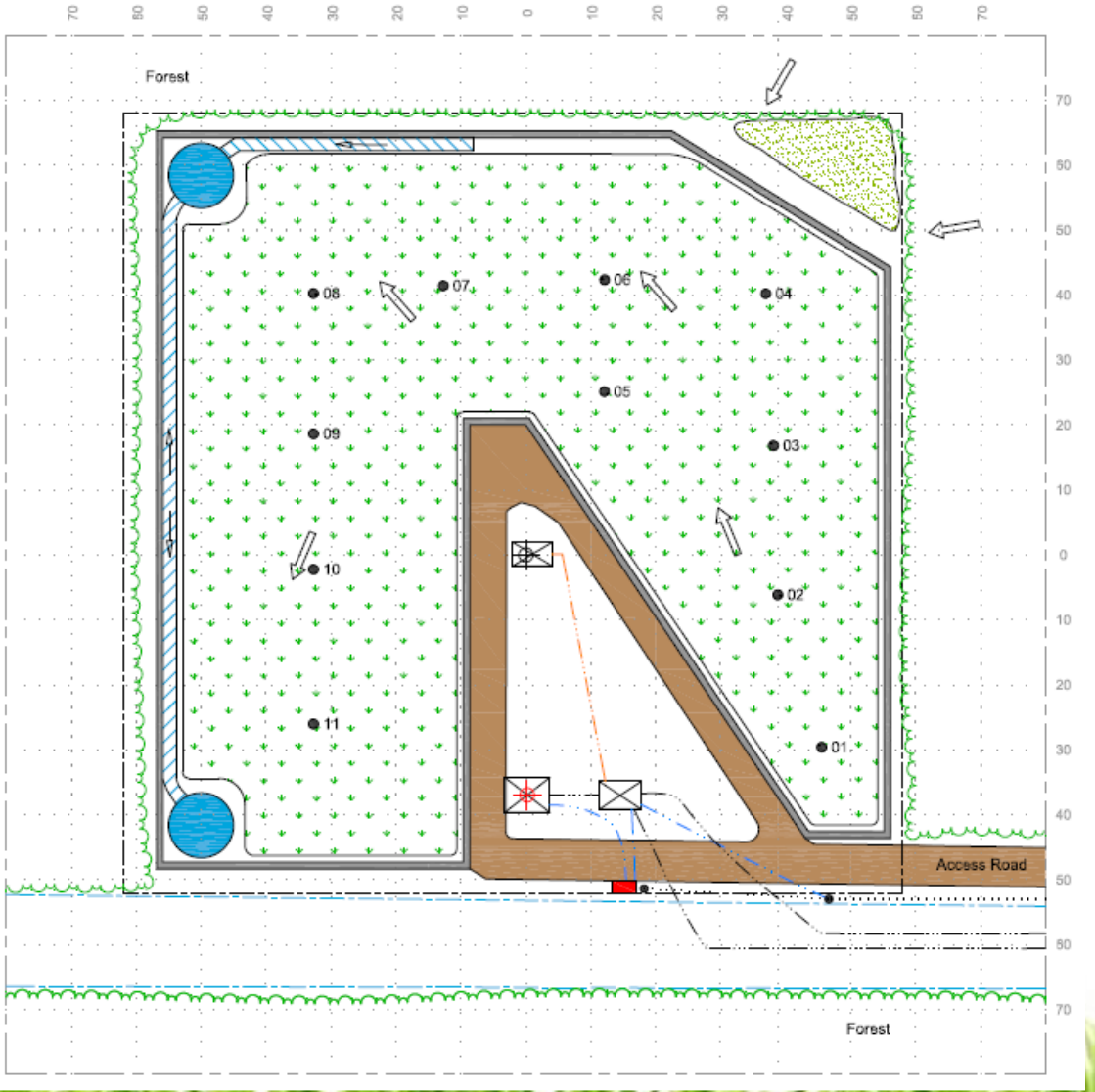
Starting Material

Impacted soil guideline value exceedances (surface soil comparison):

- F1: 310 to 1,100 mg/kg
- F2: 170 to 3,000 mg/kg
- F3: 1,500 to 7,500 mg/kg
- F4: complied
- Benzene: 0.062 to 0.880 mg/kg
- Ethylbenzene: 0.190 to 1.200 mg/kg
- Toluene: 0.63 mg/kg
- Xylenes: complied
- Salts: complied with AER agreed to levels

Site / Phytoremediation Details



- Topsoil stockpiles
- Bermed clay treatment pads
- Collection channels and sumps
- Active lease sites
- Permanent assessment points
- End point – meet remediation guideline values for natural land use fine textured soil
- Seed – ARG, PRG, TF
- PGPR – UW3, UW4
- First seeding – fall 2011

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Stockpiled Impacted Soil

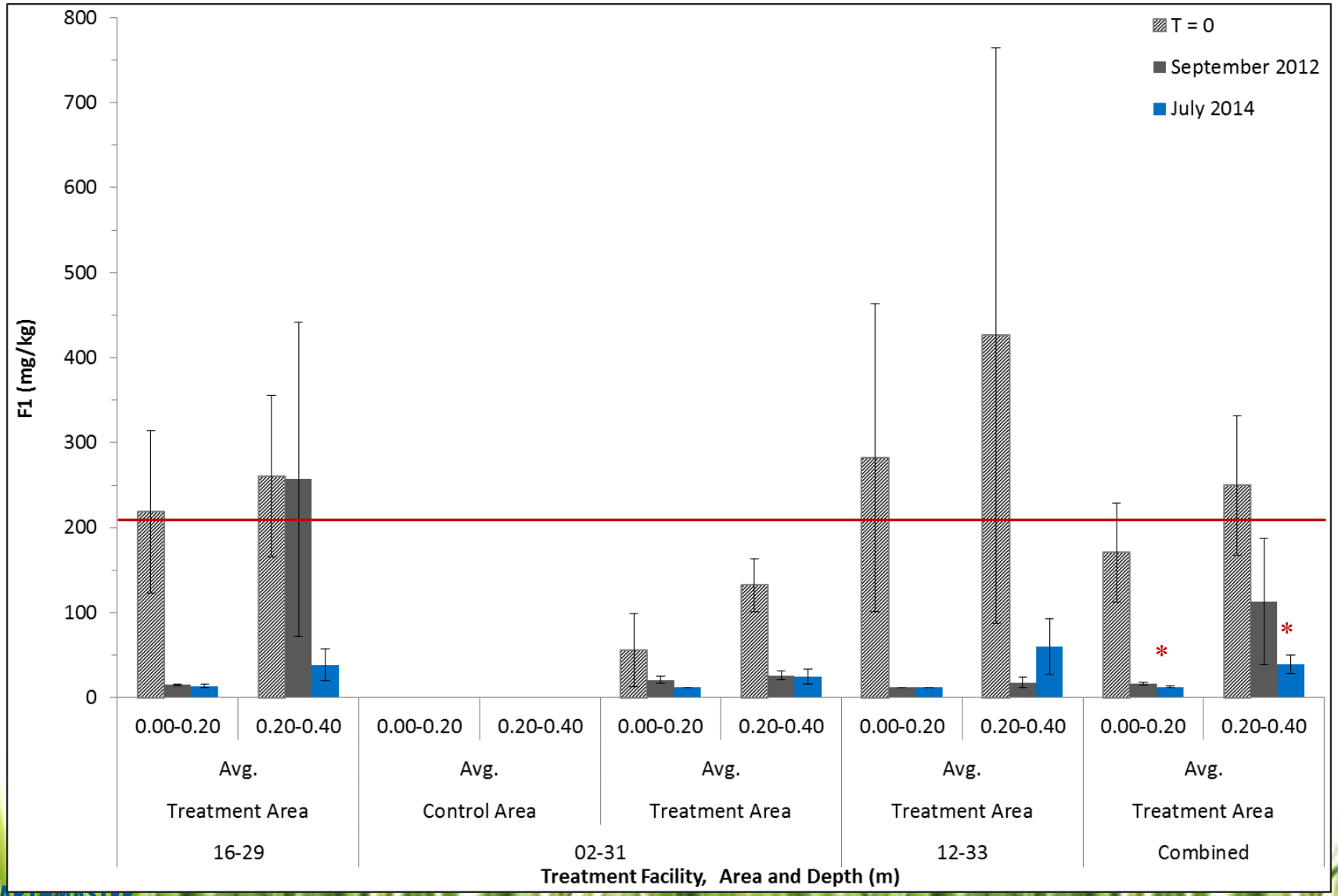
Clay Pad Construction

First Year Growth

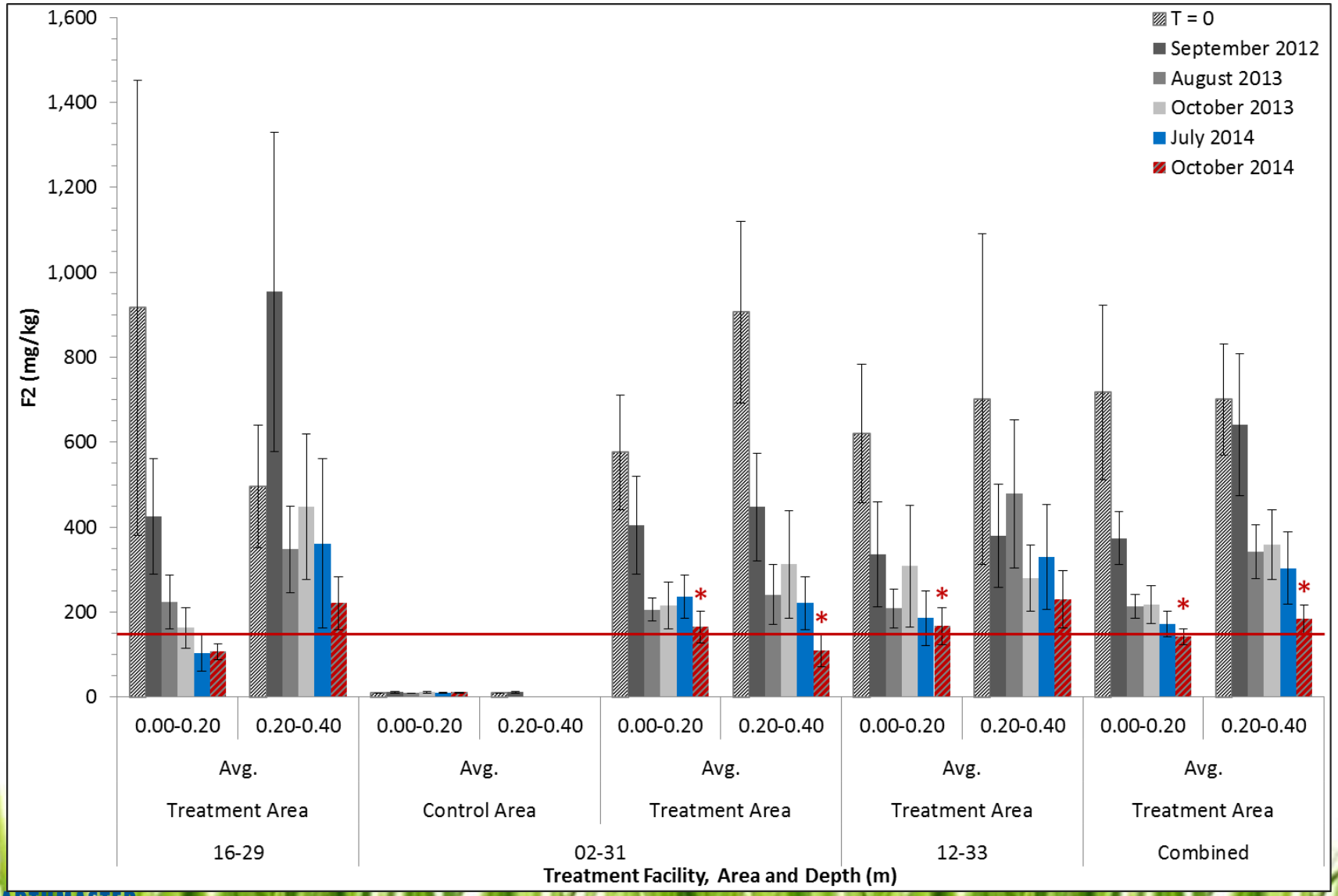
October 2012

Second Year Growth

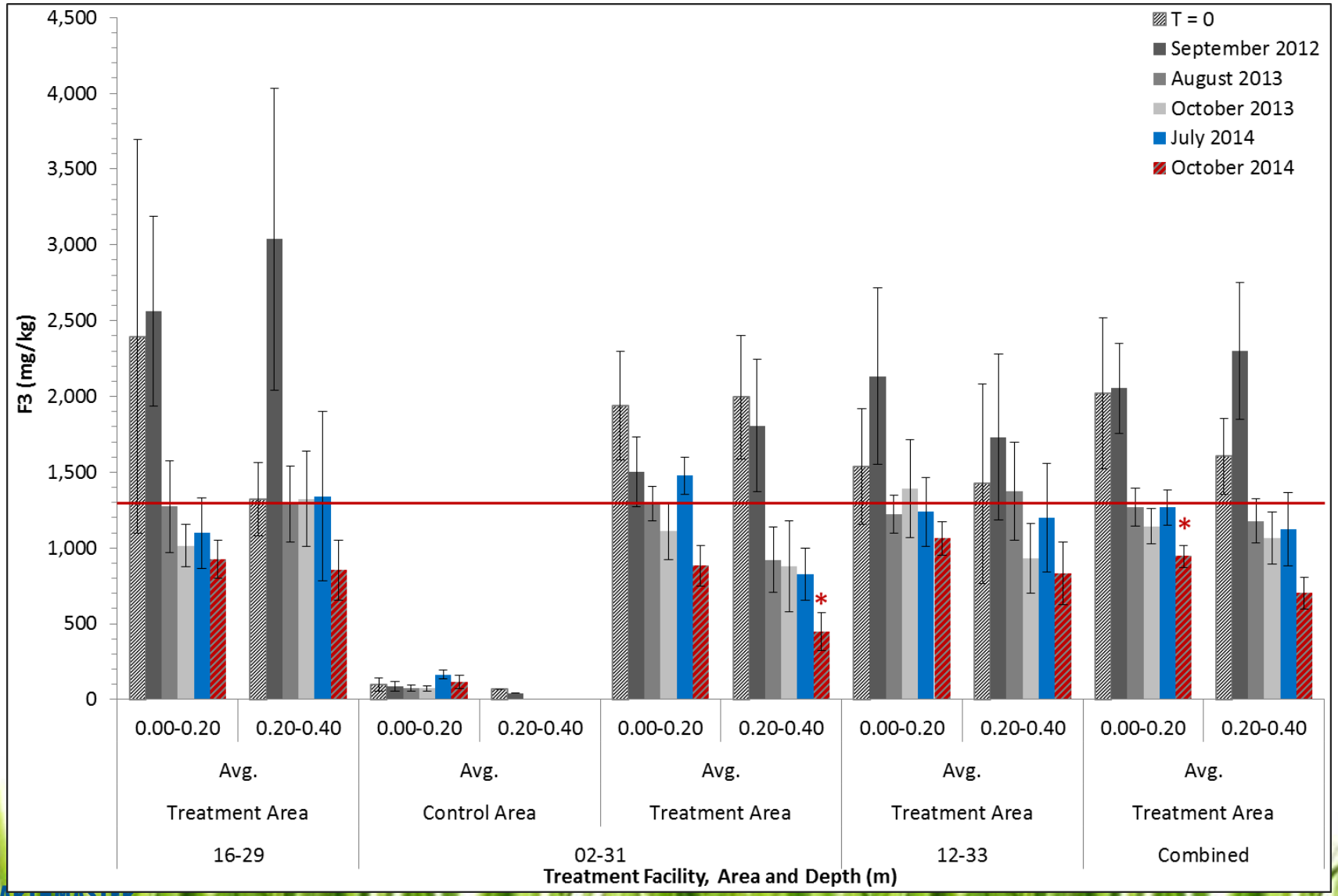
Phytoremediation Mid-Point – F1



Phytoremediation Mid-Point – F2



Phytoremediation Mid-Point – F3



Soil Stripping

Phytoremediation of PHC – Northern Alberta

Soil stockpiles arising from 2015 stripping:

- If they complied with surface and/or subsoil remediation guideline values, they were designated for the corresponding use.
- If they did not comply they were re-spread for additional phytoremediation.

Remaining treatment areas:

- As of August 2016, 23 of 26 assessment points complied with surface soil F2 criteria (3 pts between 160-230 mg/kg).
- Impacted soil has met all remediation goals.
- Site will be completed in the spring of 2017.
- Soil piles will remain onsite for future use.

Enhanced Reclamation – Research Goals

New research – using PGPR to improve efficacy of re-vegetation of marginal and disturbed soils:

- Previous studies have shown that PGPR can increase the tolerance of plants to stressed conditions:
  - Hydrocarbons and salts
  - Poor soil conditions
  - Used in combination with fertilizer and specific seeding densities
- Can PGPR be used in a more traditional way to assist in reclamation or revegetation of “clean” soil:
  - Use lower density seeding rates with native grass mixes
  - Increase plant emergence, growth, and survival of plants on marginal soils
- IRAP funded project

Enhanced Reclamation – Central Alberta Site

Former gas plant located east of Drumheller (traditional phytoremediation site):

- 8,000 m³ of excavated PHC impacted soil.
- Earthmaster constructed a soil treatment facility:
  - AER compliant
- Impacted soil was spread across the clay liner.
- Clean topsoil was placed in the NW corner – used for **test plot #1** (not an ideal location).
- Several lifts of soil have been treated.
- Treated soil was placed back in the excavation – used for **test plot #2.**

This is a preliminary study.

Site / Enhanced Reclamation Test Plot Details

- Clean disturbed soil
- Seed – commercial native grass mix
- PGPR – CMH3
- No fertilizer
- Lower density seeding rate

Topsoil test plot: 2 x 3.5 m

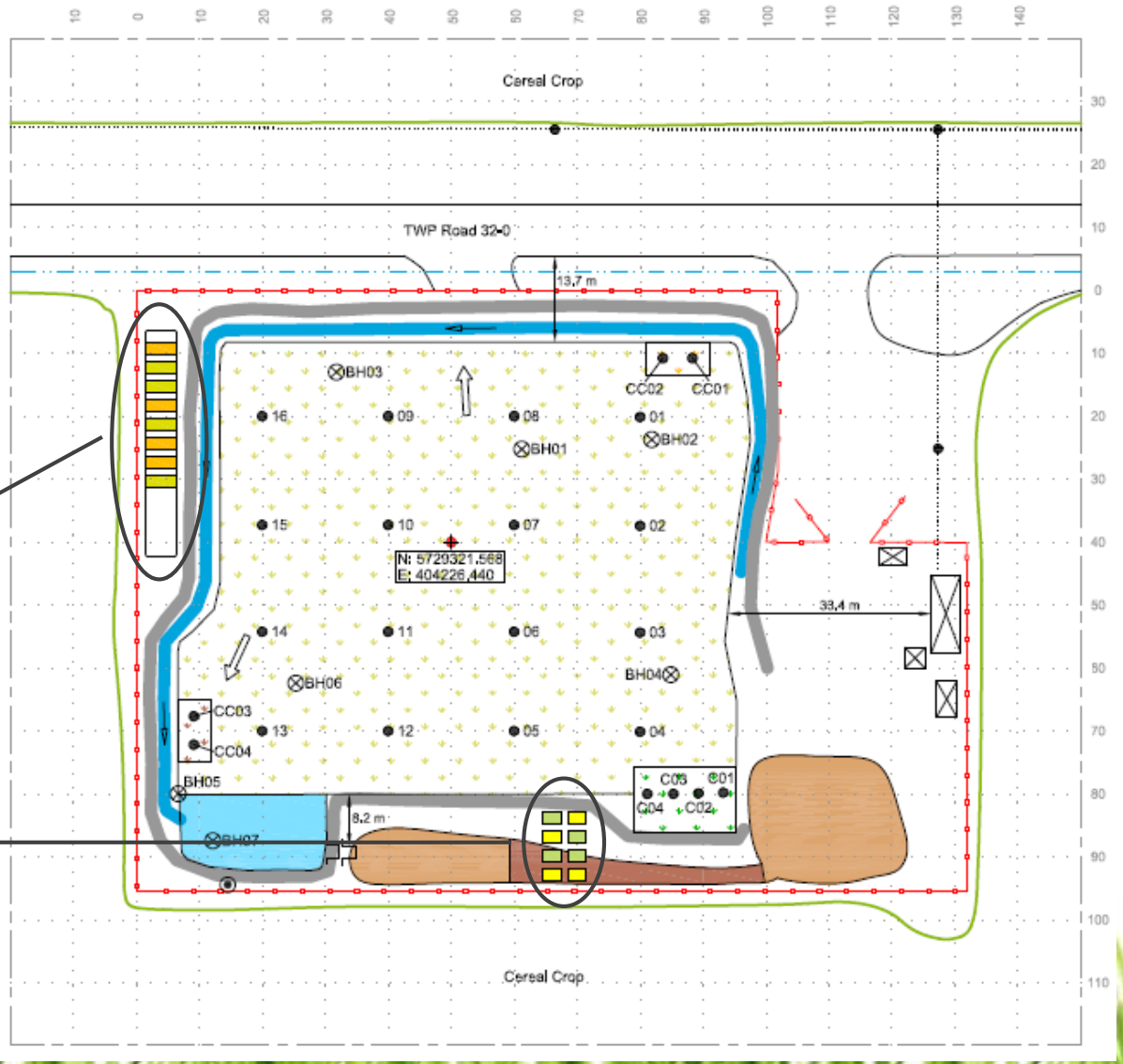
+PGPR

-PGPR

Subsoil test plot: 2 x 2 m

+PGPR

-PGPR



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Topsoil Test Plot

July 11, 2016 – 3 weeks

Subsoil Test Plot

July 11, 2016 – 3 weeks

Treatment Area

July 11, 2016 – 3 weeks

Topsoil Test Plot

Subsoil Test Plot

Topsoil Plot Results

Block #	Seed Status	16Aug2016		15Sep2016	
		% cover	Height (cm)	% cover	Height (cm)
IRAP Plot #1					
1	treated	15	22	30	43
2	untreated	5	29	20	36
3	untreated	1	14	15	19
4	treated	25	36	30	45
5	untreated	30	27	25	51
6	treated	5	31	10	55
7	treated	1	43	25	28
8	untreated	40	26	30	28
average	**untreated**	**19**	**24**	**23**	**34**
	treated	**12**	**33**	**24**	**43**

Subsoil Plot Results

Block #	Seed Status	16Aug2016		15Sep2016	
		% cover	Height (cm)	% cover	Height (cm)
IRAP Plot #2					
1	untreated	0	-	0	-
2	treated	1	5	1	6
3	untreated	0	-	0	-
4	treated	1	4	1	14
5	treated	2	7	5	11
6	untreated	5	5	5	10
7	untreated	5	7	5	8
8	treated	1	9	5	12
average	**untreated**	**2.5**	**6.0**	**2.5**	**9.0**
	treated	**1.3**	**6.3**	**3.0**	**10.8**

Results

What we learned:

- Plots were too small so edge effects were very pronounced.
- They cannot be compared to the treatment area as they were different plant species.
- Slight advantage to PGPR treated seed.
- Further studies are required using different seed types and different PGPR on larger plots so edge effects can be eliminated and efficacy of PGPR fairly evaluated.

Parameter	Treatment Area	Test Plots
seed type	ARG, PRG, TF	native grass mix
PGPR	UW3, UW4	CMH3 or none
fertilizer	yes	no
seeding rate	higher rate	lower rate
seeding	seeder	seeder
edge effects	yes	yes

Historical Salt Impacts in Southern Saskatchewan

Plant Growth Three Months After Seeding

Plant Growth Year 3

Salt Remediation – Research Goals

New research - using PGPR with commercial seed treatments to improve efficacy/speed of salt remediation:

- Previous field projects have identified a seed/PGPR combination capable of remediating salt impacts in soil.
- Remediation currently takes ~4-10 years depending on the soil, groundwater, weather conditions, and salt levels.
- Can the speed of this process be increased using a commercial seed treatment in combination with PGPR?
  - Decrease time to meet remediation goals (<5 years)
  - Increase plant biomass, rooting depth, and salt uptake rates
- Worked with commercial suppliers to find a suitable treatment compatible with PGPR.
- IRAP funded project.

Salt Spill – Southern Saskatchewan Wetland

Produced water release in southeastern SK:

- 500 m^3 of produced water was released from a pipeline:
  - Flowed north into a non-agricultural wet-meadow
  - Impacted area ~30,000 m^2 in size
  - Area is prone to flooding for periods of time
  - Surrounding land use is cultivated farmland
- Impacted soil will be treated in situ:
  - ECe: 8 to 18 dS/m
  - SAR: 13 to 40
- Remediation goal:
  - Revegetate the affected area
  - Remove salt from the surface soil to allow for sustainable plant growth

Salt Spill – Southern Saskatchewan

Lowland Seed Selection

Seed mix requirements:

- Suitable for both flooded and dry conditions.
- Must be common to southern SK.
- Must not be on the SK invasive plant list.
- Must be able to take up and accumulate salt.
- Must be somewhat tolerant to saline conditions.
- Should be able to sow the seeds with a seed drill or broadcast spreader.
- Should rapidly accumulate biomass.
- Must be able to be harvested from the site to remove accumulated salt.
- Prefer perennials to avoid replanting.
- Prefer quick regrowth following harvest.

Seed Mixes

Uplands mix – based on previous field work:

- Annual ryegrass
- Perennial ryegrass
- Tall Fescue

Lowlands mix – based on criteria, availability, and price:

- Western wheatgrass
- American sloughgrass
- Perennial ryegrass
- Soft rush
- Faults alkali grass
- Fowl bluegrass
- Cattails

Test Plot Set-up on Commercial Phytoremediation Site

Lowland test plot

Block 5	Block 6	Block 7	Block 8
5	6	7	8
8	5	6	7
7	8	5	6
6	7	8	5

Section 5: PGPR
Section 6: no treatment
Section 7: PT
Section 8: PGPR + PT

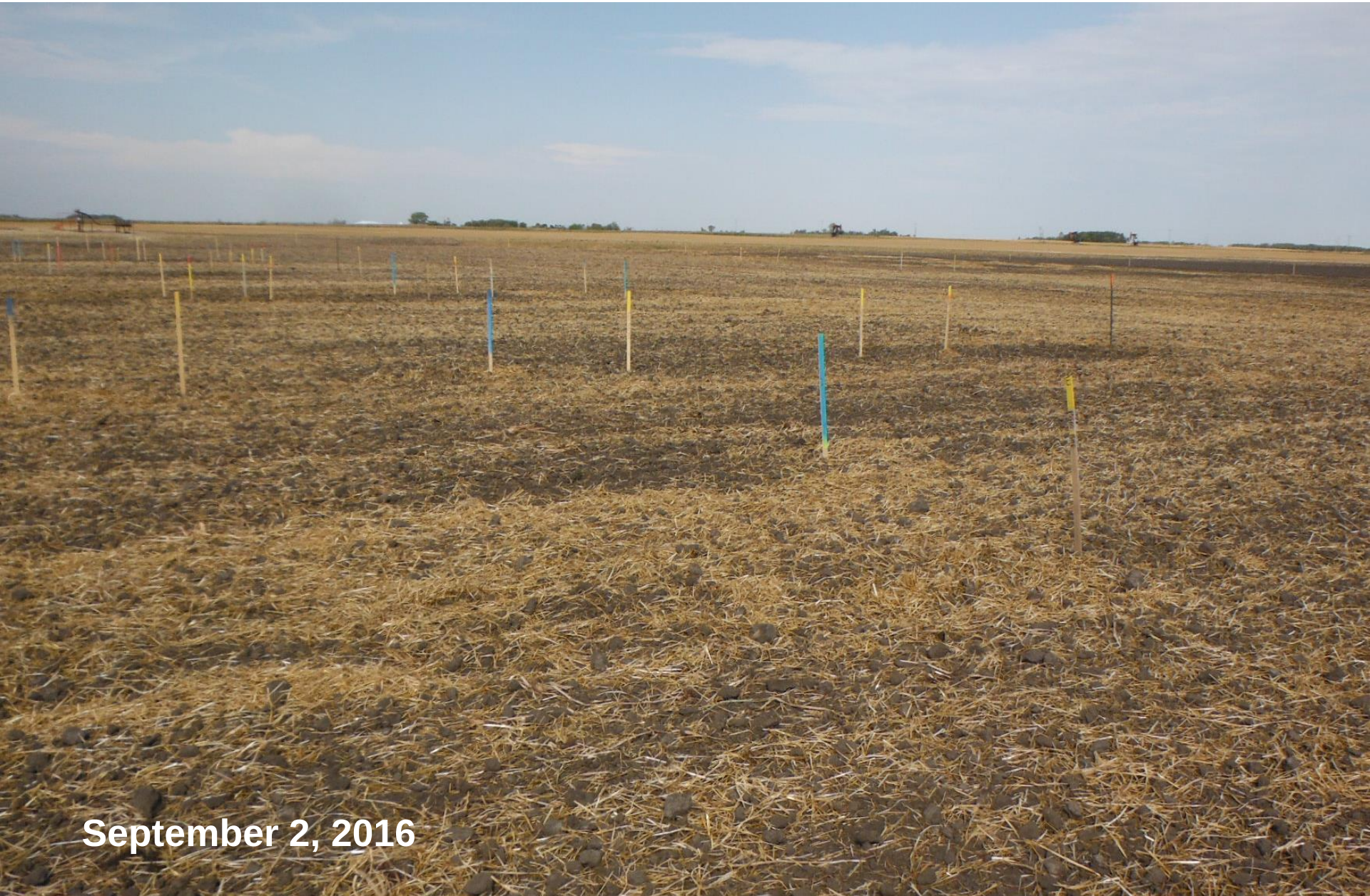
Upland test plot

1	2	3	4
4	1	2	3
3	4	1	2
2	3	4	1
Block 1	Block 2	Block 3	Block 4

Section 1: PGPR
Section 2: no treatment
Section 3: PT
Section 4: PGPR + PT

Sections are 4 x 16 m

Test Plots



Early Results – 1 Month

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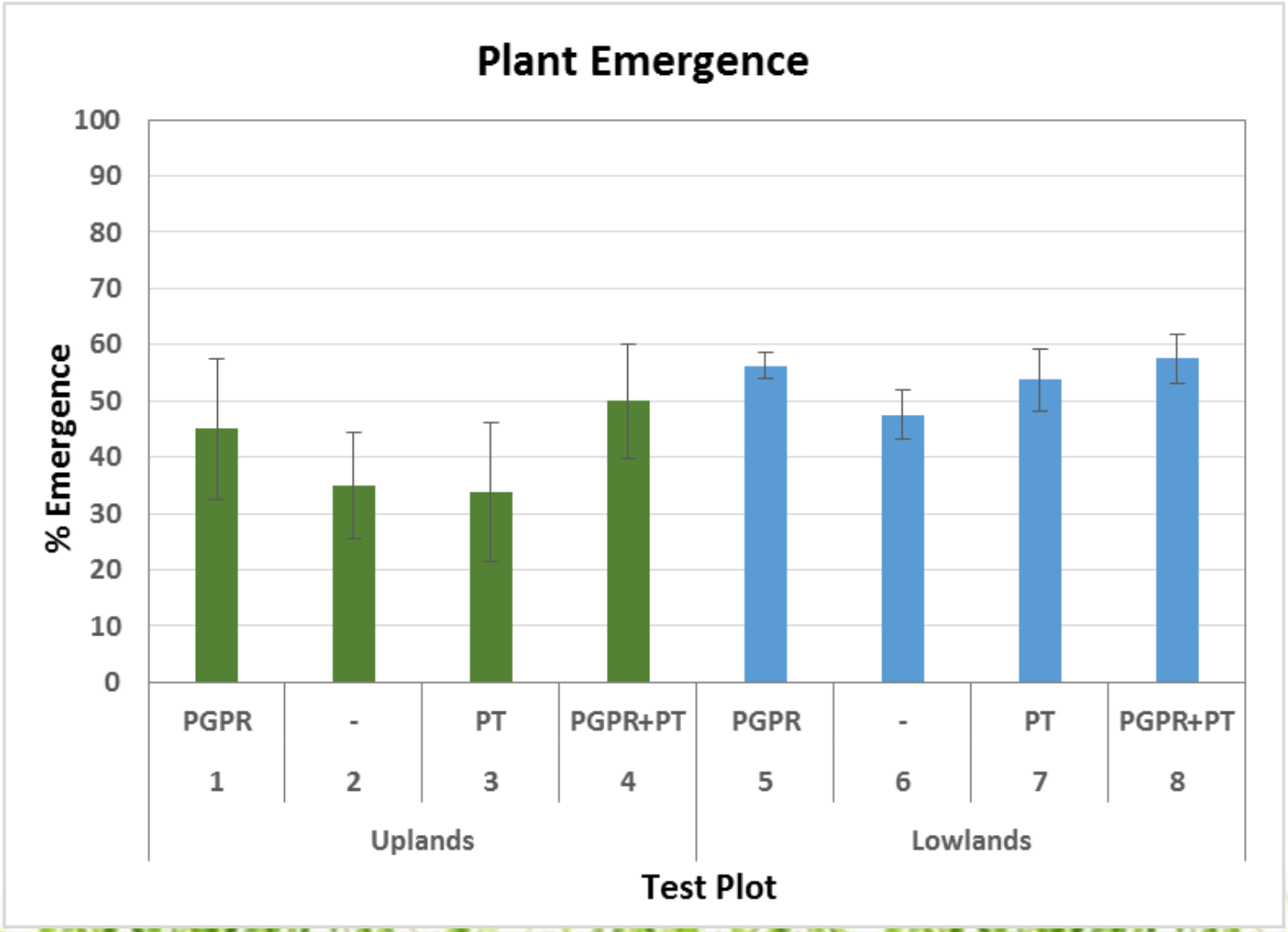
Early Results – 1 Month



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Hydroseeding – Proposed Research Project

Proposed PGPR/hydroseeding applications:

- Re-vegetation along roadways/highways
- Would involve disturbed soils
- Other challenges would be expected including:
  - High salt concentrations
  - Steep inclines

Unknowns to be tested:

- Will PGPR survive the hydroseeding process?
- Will the slurry mixture cause the PGPR to come off of the seed?
- What is the best seed/PGPR combination?

Acknowledgements

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Additional clients who have allowed Earthmaster to conduct field trials to advance the technology.

Thank You
Questions?