The Multitude of Uses for Plant Growth Promoting Bacteria in Soil Remediation and Site Management

Elizabeth Murray, Michael Quesnel, Ben Poltorak, Bruce Greenberg, Kent Cryer, Adam Dunn, and Perry Gerwing

Elizabeth.Murray@Earthmaster.ab.ca

www.Earthmaster.ca

Earthmaster Environmental Strategies Inc.

A Canadian environmental technologies company:

- Founded in 1998 and based in Calgary, Alberta, Canada.
- Specializes in providing environmental services to the commercial/industrial and upstream oil and gas industry in Western Canada.
- In-house lab facilities for microbiological research and a growth facility for plant testing.
- Co-developed commercial phytoremediation systems (PEPSystems®) to treat contaminated soil in an eco-friendly and responsible manner.

Earthmaster uses a combination of plants and bacteria to remediate contaminants from soil in an eco-friendly way.

PEPSystems®

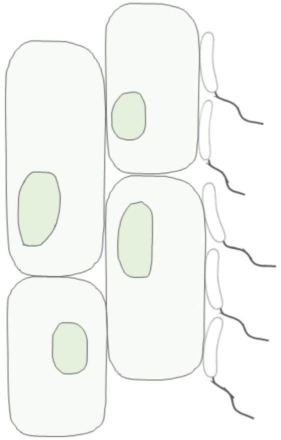
Plant Growth Promoting Rhizobacteria (PGPR) - Enhanced Phytoremediation Systems

Getting Plants to Grow in Challenging Conditions

Use bacteria to help the plants grow in stressful conditions.

Plant seeds: Coated with natural soil bacteria

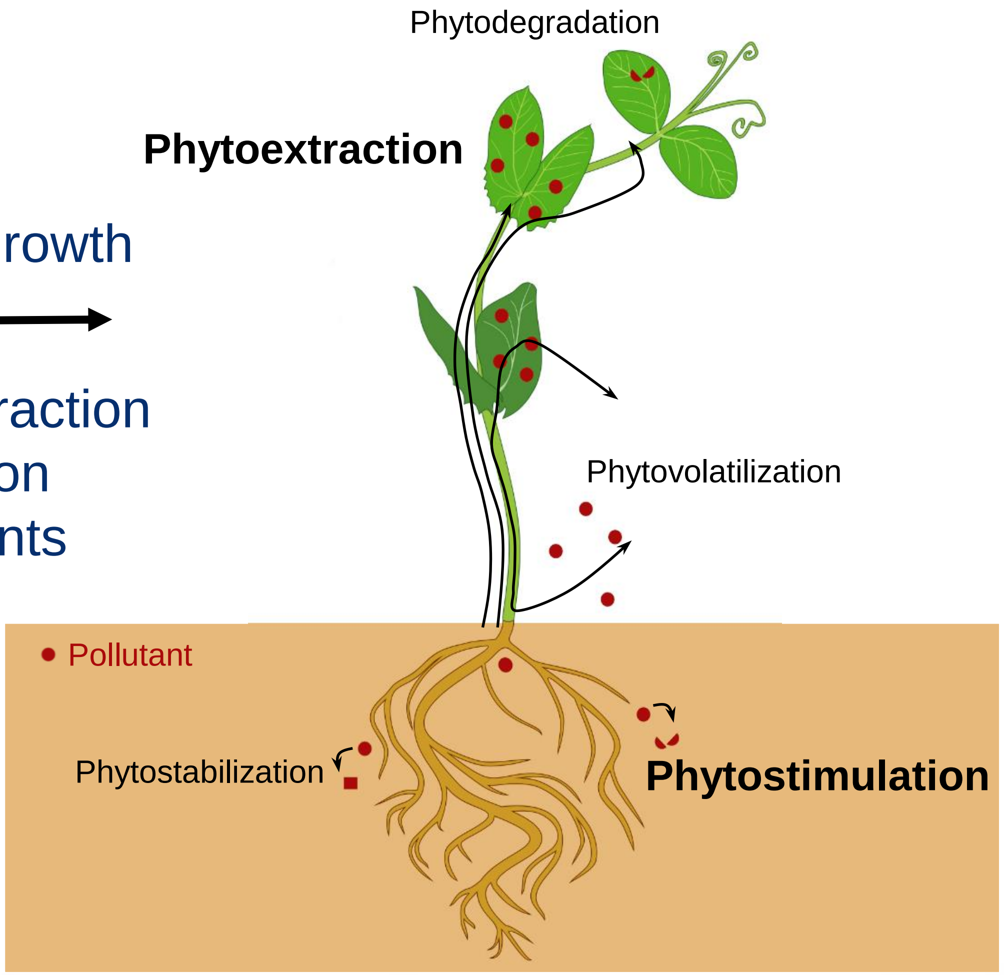
Active rhizosphere: Bacteria co-localize with developing roots



Plant cell: Bacteria interact with root cells –
↑ hormones
↓ stress response

Facilitate plant growth

Exploit phytoextraction & phytostimulation properties of plants



Remediation - Hydrocarbons & Salts

Treatment Pad Construction

Site preparation

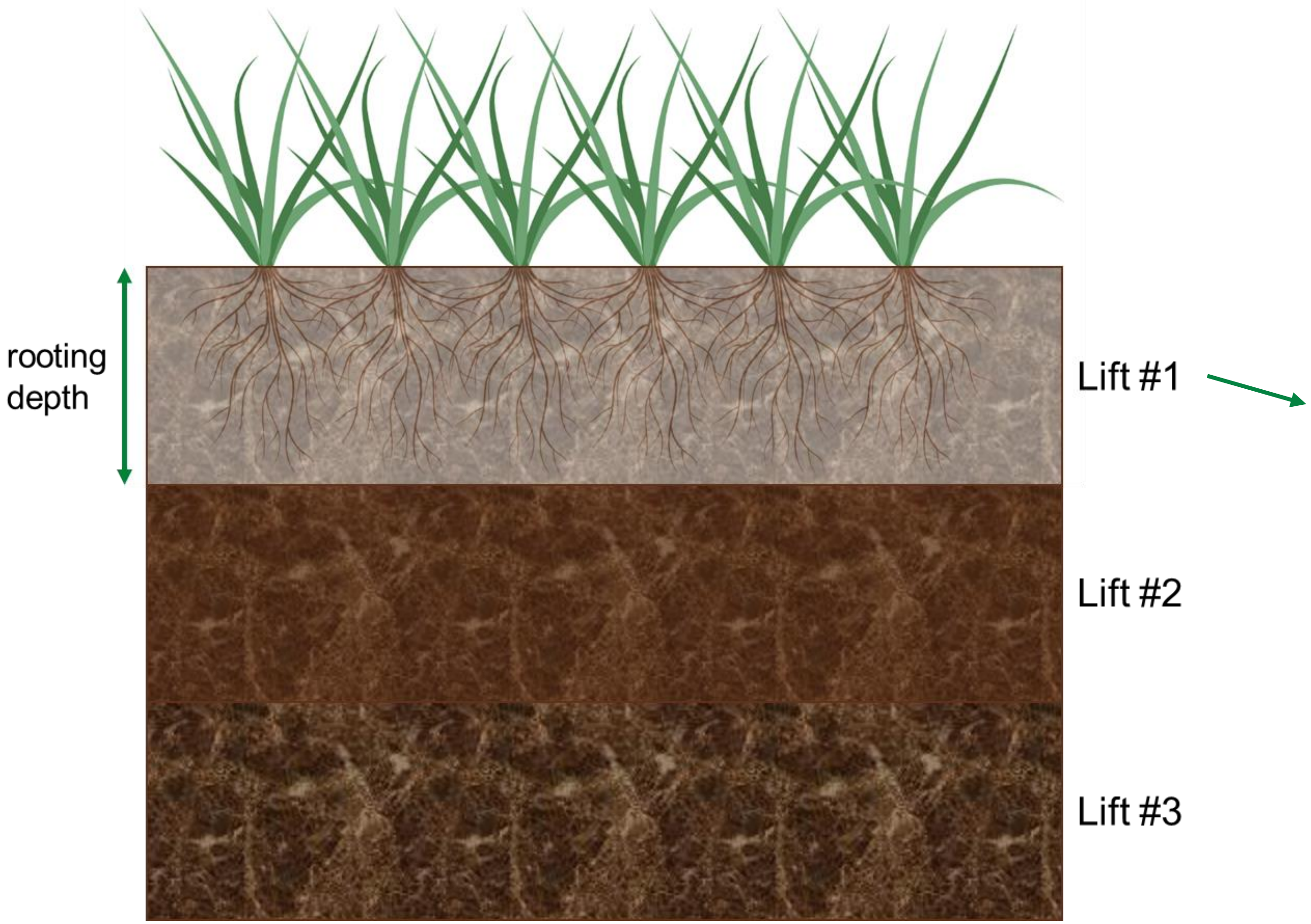
Treatment Pad Construction

Finished treatment pad

Soil Placement

Seed Bed Preparation

Phytoremediating Subsoil in Lifts



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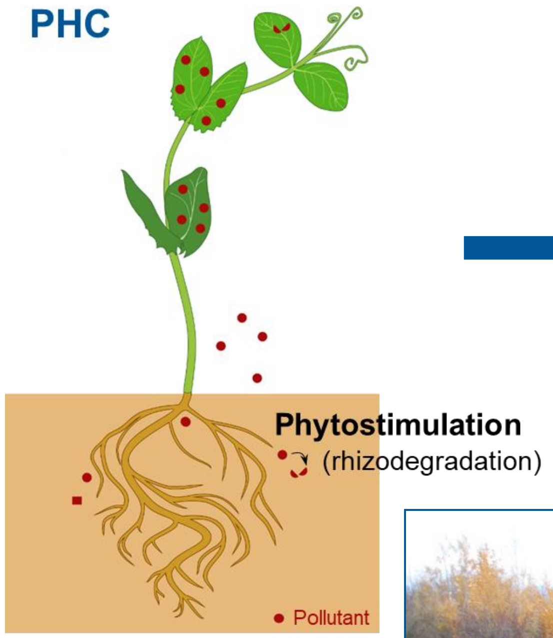
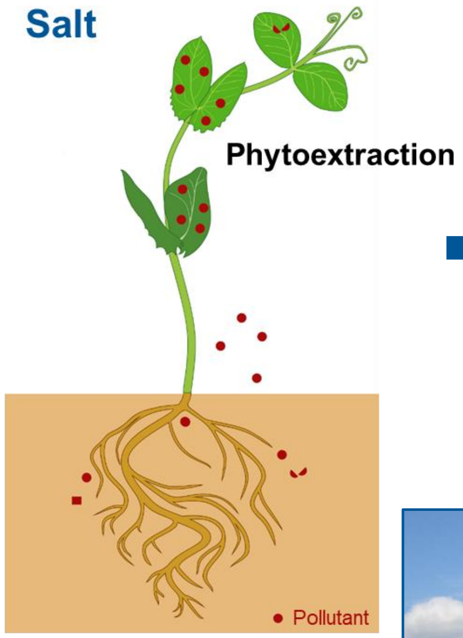
Hydrocarbon vs. Salt Phytoremediation



incorporate
plants into
the soil



harvest
above ground
biomass

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Edson 14-19

Site contained impacted material in former DWDAs, wellbore area, and disturbed area:

- Land use – natural
- Soil texture – fine
- AB remediation guideline values F2:
  - surface soil – 150 mg/kg
  - subsoil – 300 or 1,000 mg/kg
- Two wellbores were present onsite
- Topsoil was stripped and stockpiled onsite
- Treatment pad was constructed in 2013
- Impacted material was excavated and placed on the treatment pad

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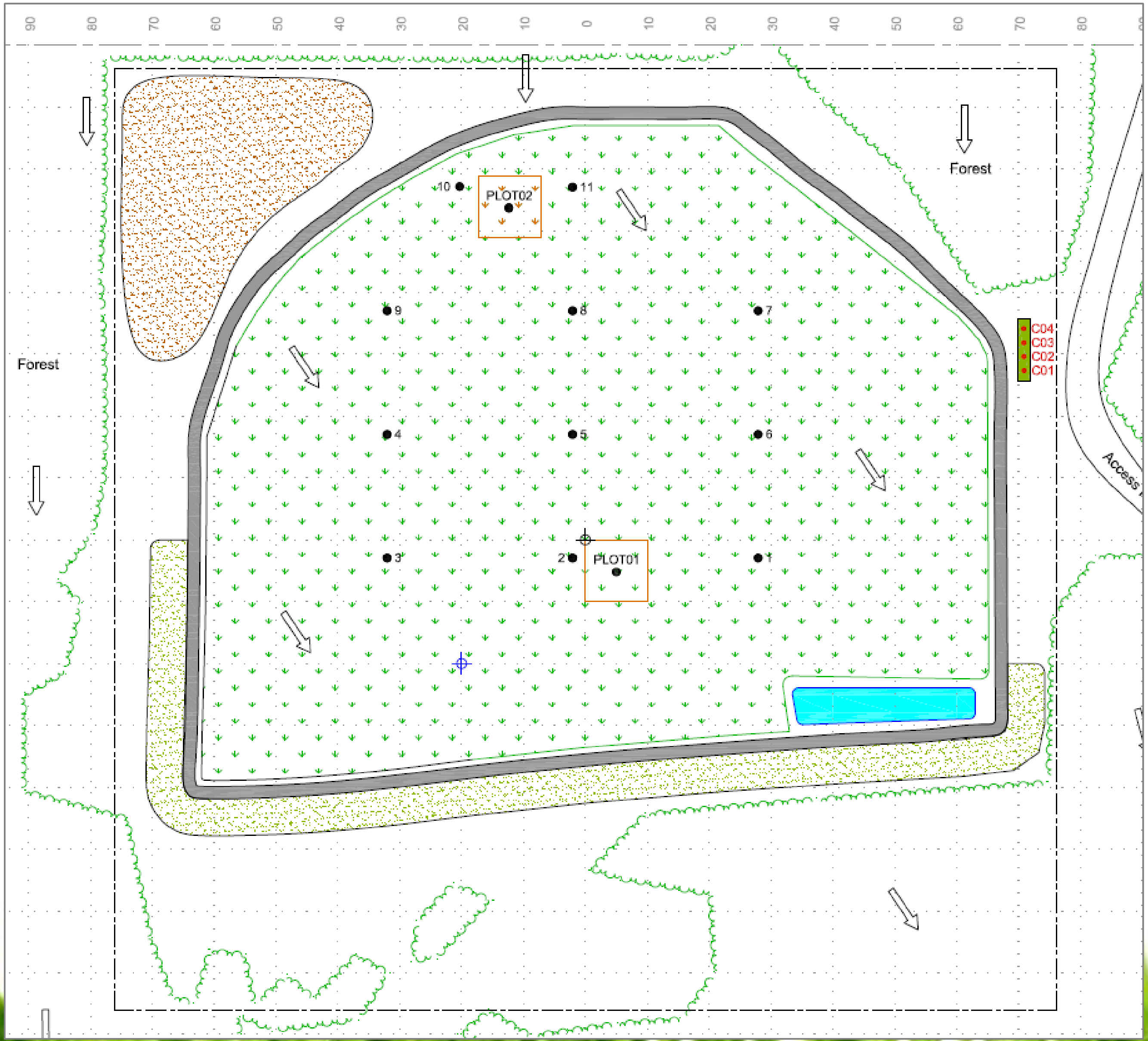
Edson 14-19



15,000 m³ of impacted material excavated from 2 former DWDAs, wellbore area, and a disturbed area was spread to a depth of 1.5 m:

- Treatment pad construction
  - Bermed with surface water collection sump
- Permanent assessment points
- Seed – ARG, PRG, TF
- PGPR – *Pseudomonas sp.*
- Lift #1 T=0 October 2013
  - Initiation of phytoremediation
  - Avg. PHC F2 concentration = 320 mg/kg
  - 28/33 samples failed criteria

Edson 14-19

Lift #1: October 2013 – November 2015

- 4,000 m³ remediated
- PHC – F2 decreased from 320 to 95 mg/kg (70%)

Lift #2: October 2016 – July 2017

- 3,000 m³ remediated
- PHC – F2 decreased from 310 to 163 mg/kg (47%)

Lift #3: December 2017 – October 2018

- 1,600 m³ remediated
- PHC – F2 decreased from 285 to 190 mg/kg (35%)

Lift #4: December 2018 – October 2019

- 2,000 m³ remediated
- PHC – F2 decreased from 200 to 99 mg/kg (50%)

Edson 14-19

Lift #5: December 2019 – December 2021

- 2,000 m³ remediated
- PHC – F2 decreased from 231 to 40 mg/kg (82%)

Lift #6: December 2019 – present

- 2,200 m³ remaining to be remediated
- Average PHC F2 concentration = 360 mg/kg

To date 12,950 m³ of mostly F2 contaminated soil has successfully been remediated.

Site should be completed in the fall of 2023.

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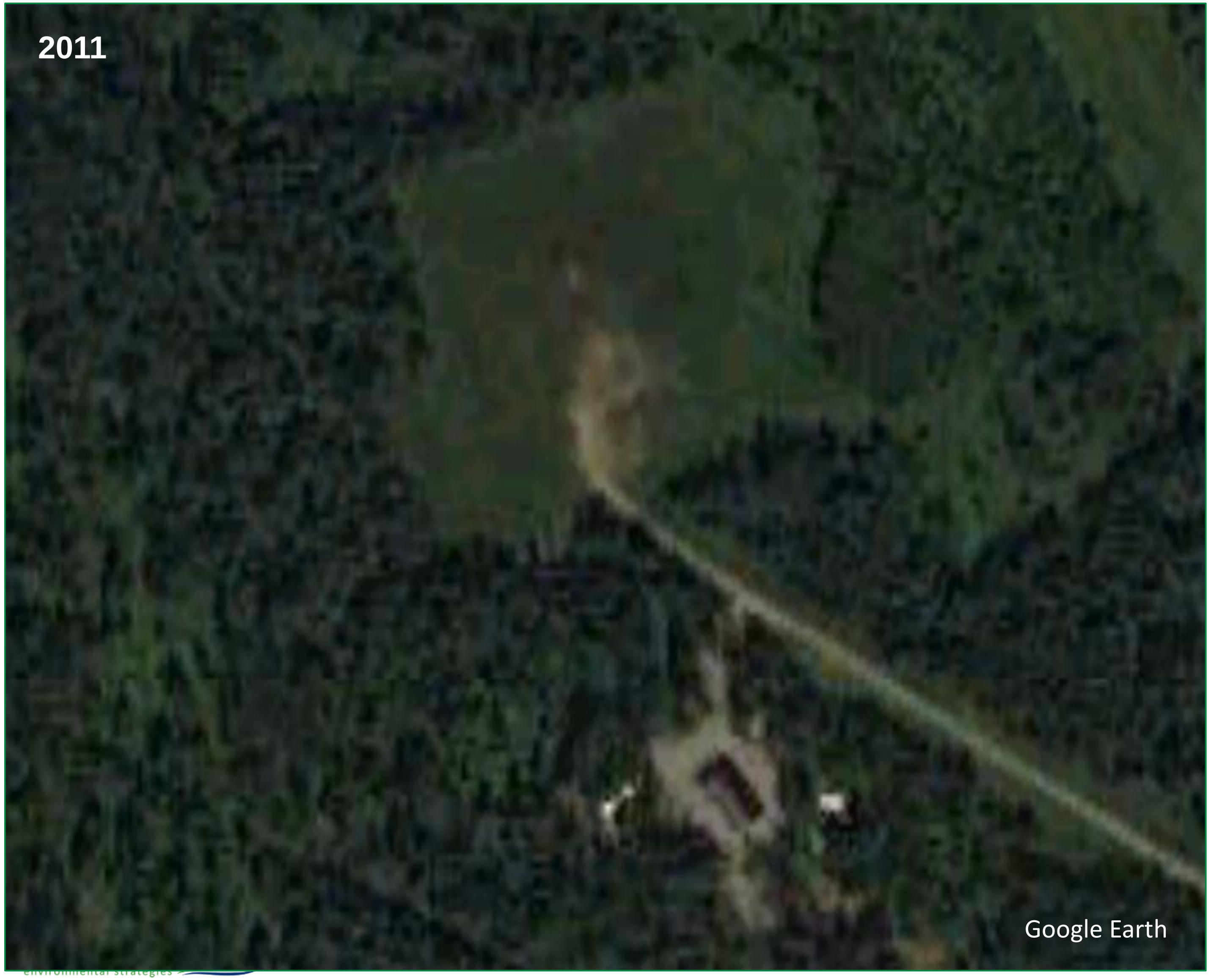
Sundre 06-08



Site contained impacted material in the former wellbore area, former DWDA, and had a spill onsite:

- Land use – natural
- Soil texture – coarse
- AB remediation guideline values F2:
  - surface soil – 150 mg/kg
  - subsoil –1,000 mg/kg
- AB remediation guideline values F3:
  - surface soil – 300 mg/kg
  - subsoil – 2,500 mg/kg
- Topsoil and clean overburden were stripped and stockpiled onsite
- Treatment pad was constructed in 2017

Impacted material was excavated and placed on the treatment pad

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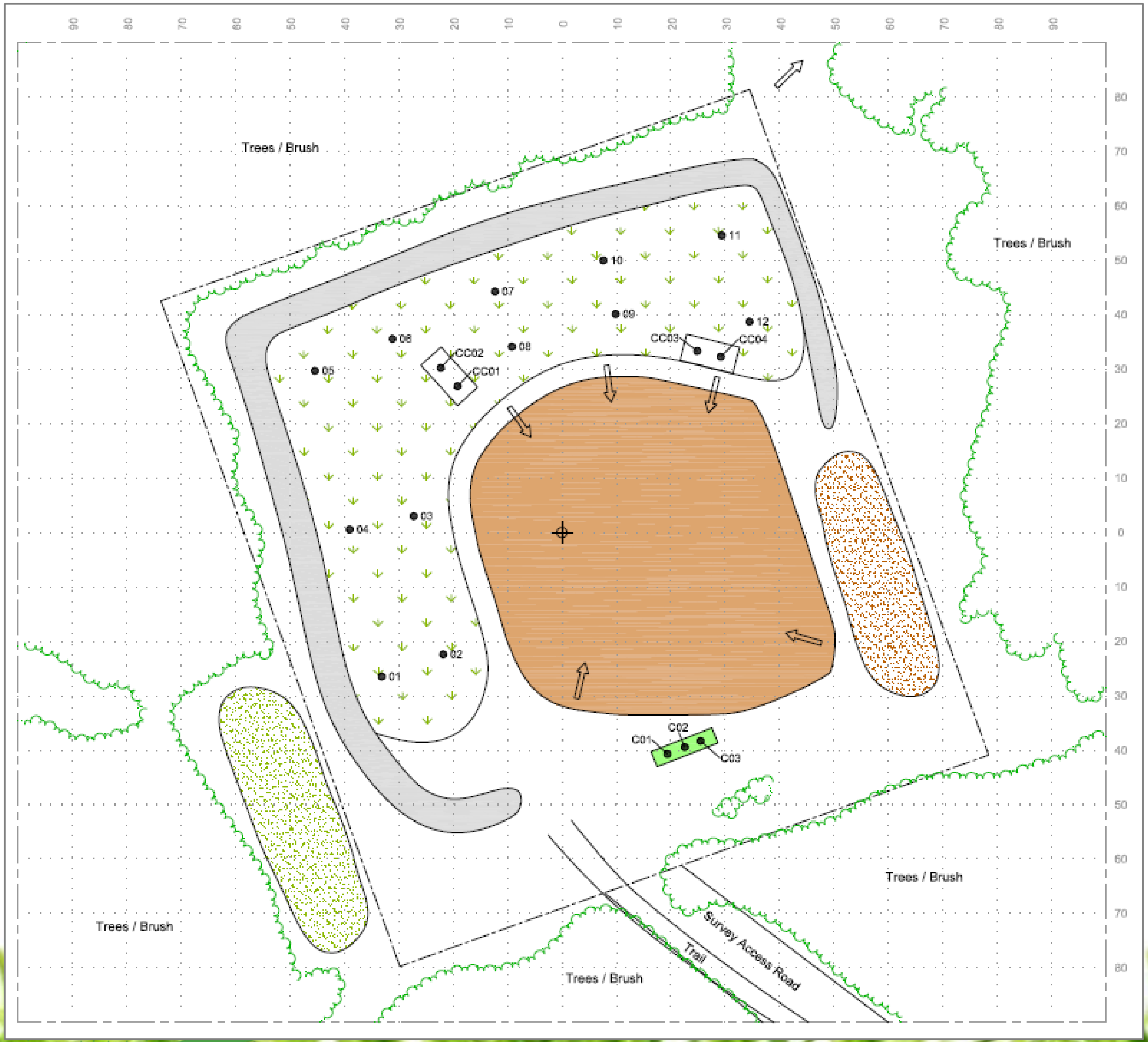
Sundre 06-08



7,300 m³ of material excavated from a former DWDA, the wellbore area, and a spill area was spread to a depth of 1.85 m:

- Seed – ARG, PRG, TF
- PGPR – *Pseudomonas sp.*
- Lift #1 T=0 May 2017
    - Initiation of phytoremediation
- This site experiences a lot of heavy grazing
- A residence was located south of the site

Sundre 06-08

Lift #1: May 2017– June 2018

- 1,200 m³ remediated
- PHC – F2 decreased from 825 to 661 mg/kg (20%)

Lift #2: June 2018– October 2018

- 1,600 m³ remediated
- PHC – F2 decreased from 823 to 514 mg/kg (38%)
- PHC – F3 decreased from 535 to 493 mg/kg (9%)

Lift #3: July 2019– July 2020

- 1,500 m³ remediated
- PHC – F2 decreased from 691 to 461 mg/kg (33%)
- PHC – F3 decreased from 420 to 331 mg/kg (21%)

Sundre 06-08

Lift #4: July 2020– July 2021

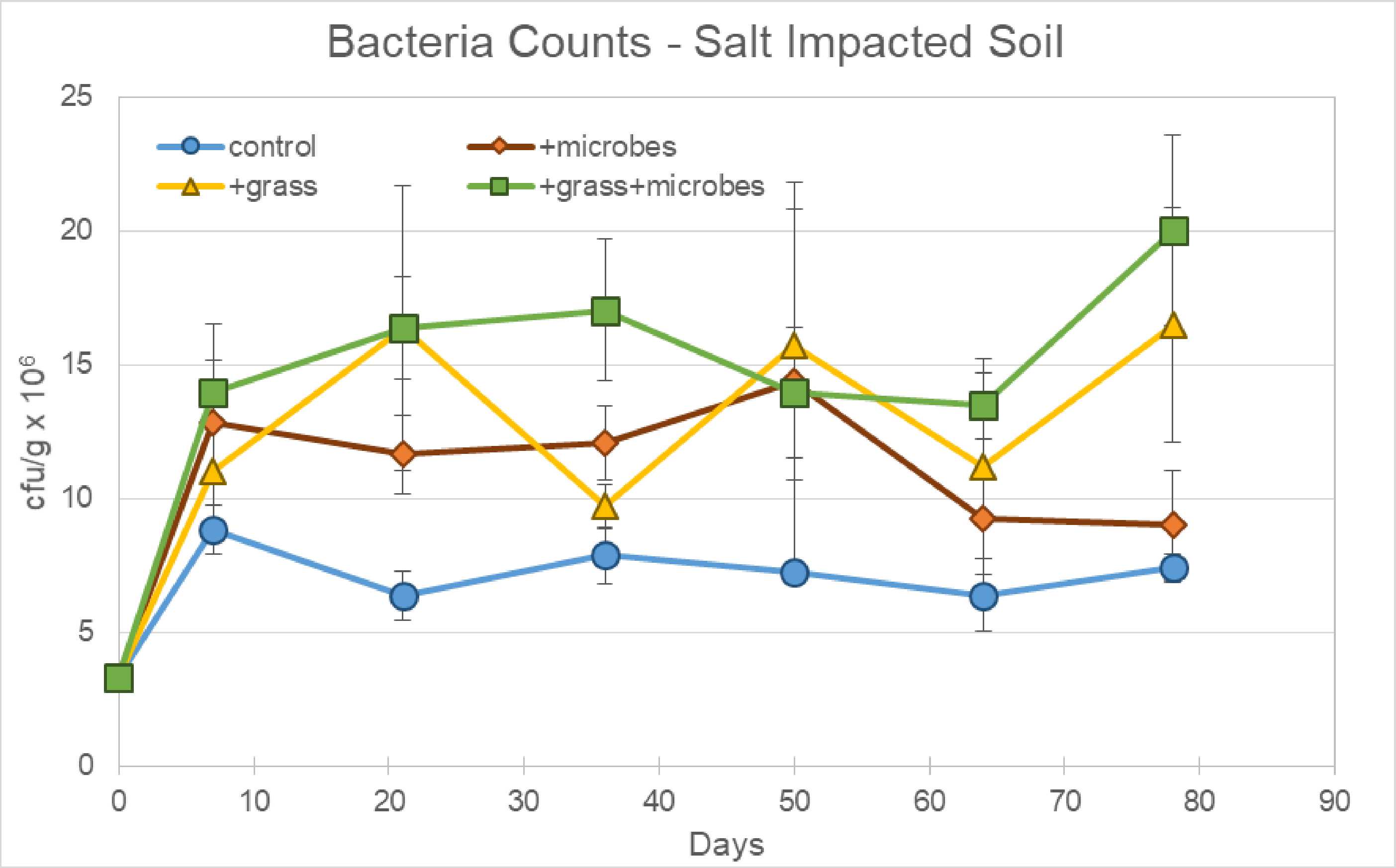
- 3,000 m³ stripped and stockpiled of which 400 m³ was remediated
- PHC – F2 decreased from 504 to 128 mg/kg (75%)
- PHC – F3 decreased from 319 to 145 mg/kg (55%)

Lift #5: May 2022– present

- 2,600 m³

Why Include the Plants?

- Lab studies suggest that culturable bacteria levels are higher and more sustained with plants.
- More favourable for clients and landowners to have the soil piles vegetated.
- Remediation begins again quickly in the spring with germination.
- Plants add organics to poor quality soil which facilitates remediation.



Predicting PHC Remediation Times

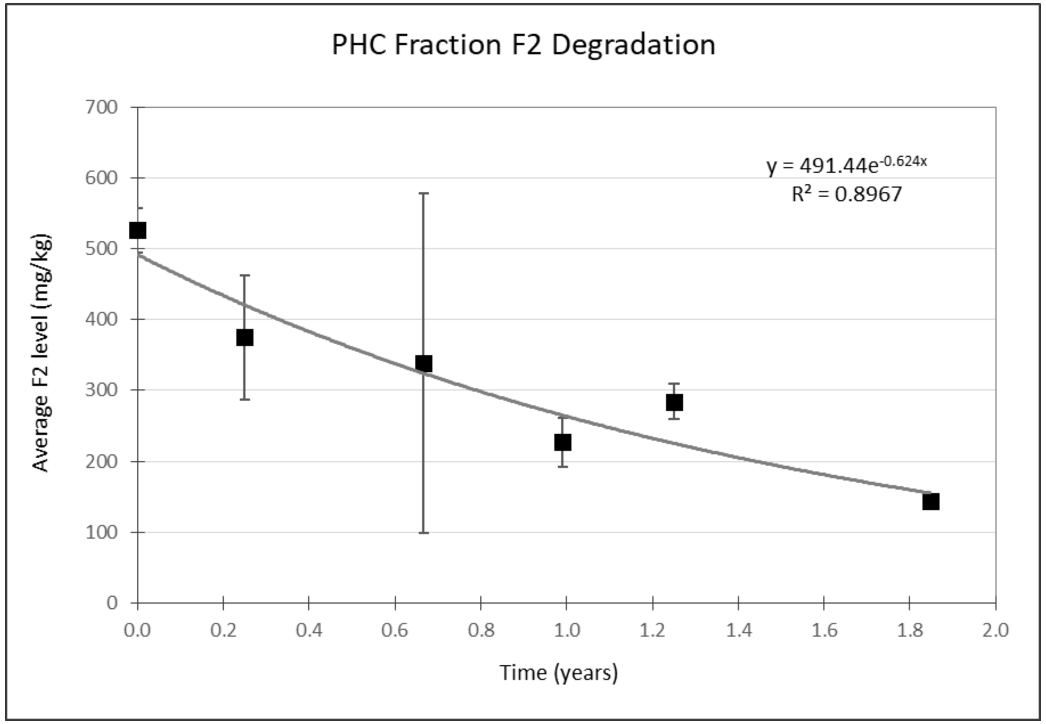
Fractions F2 and F3

time

$$y/C_o = e^{-0.624x}$$

final concentration mg/kg

initial concentration mg/kg



INTERNATIONAL JOURNAL OF PHYTOREMEDIATION
2019, VOL. 21, NO. 1, 27–33
https://doi.org/10.1080/15226514.2018.1523870
Taylor & Francis
Taylor & Francis Group

Check for updates

Kinetics of phytoremediation of petroleum hydrocarbon contaminated soil

Elizabeth W. Murray[a], Bruce M. Greenberg[b], Kent Cryer[a], Ben Poltorak[a], Justin McKeown[a], Jess Spies[a], and Perry D. Gerwing[a]

[a]Earthmaster Environmental Strategies Inc, Calgary, Alberta, Canada; [b]University of Waterloo, Waterloo, Ontario, Canada

Site Management – Salts

Seed Germination Studies – Produced Water

Seeds

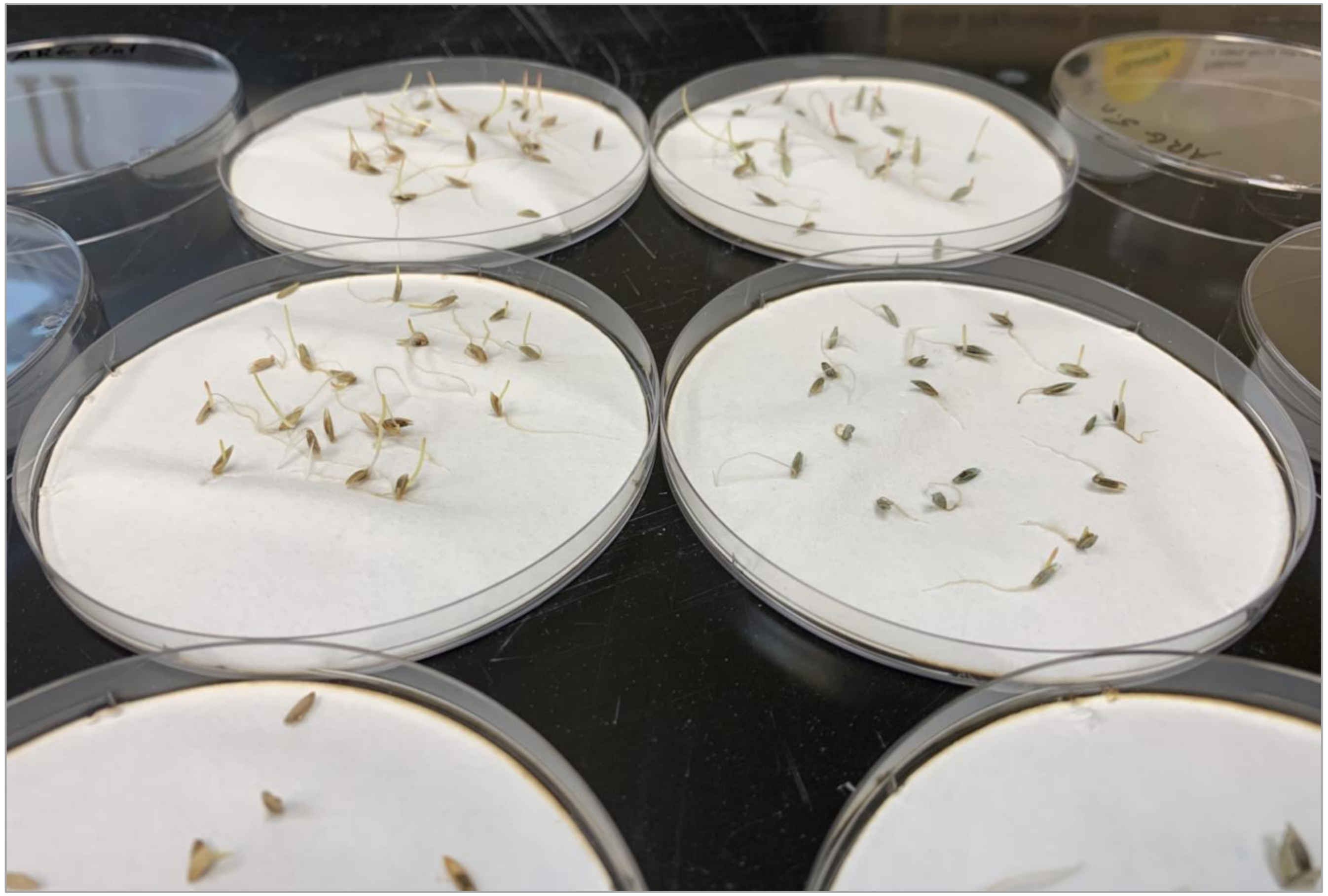
- Different species
- +/- PGPR
- +/- other additives

Contaminant

- Produced water 0-100%

Growth conditions

- 25°C for 14 days



Sample Description	Routine Chemistry								
Sample Location	Chloride (mg/L)	Calcium (mg/L)	Potassium (mg/L)	Magnesium (mg/L)	Sodium (mg/L)	Sulphate (mg/L)	pH	EC (dS/m)	SAR
Alberta Tier 1 Groundwater Remediation Guidelines Agricultural Land Use: Fine Soil	100	-	-	-	200	429	6.5-8.5	1	5
AEP EQGASW - Protection of Aquatic Life	120	-	-	-	-	429	6.5-9.0	-	-
Water samples									
Produced Water 1	36775	4223	406	498	18430	1372	7.1	73	71
Produced Water 2	78870	9264	4054	1839	32970	997	6.5	125	82

LEGEND

[grey box] Denotes values that exceed Alberta Tier 1 Soil and Groundwater Remediation Guidelines and/or Surface Water Quality Guidelines for Use in Alberta as described in the text of the letter/report.

Environmental Quality Guidelines for Alberta Surface Waters (EQGASW) - Alberta Environment and Parks (AEP). July 2014.

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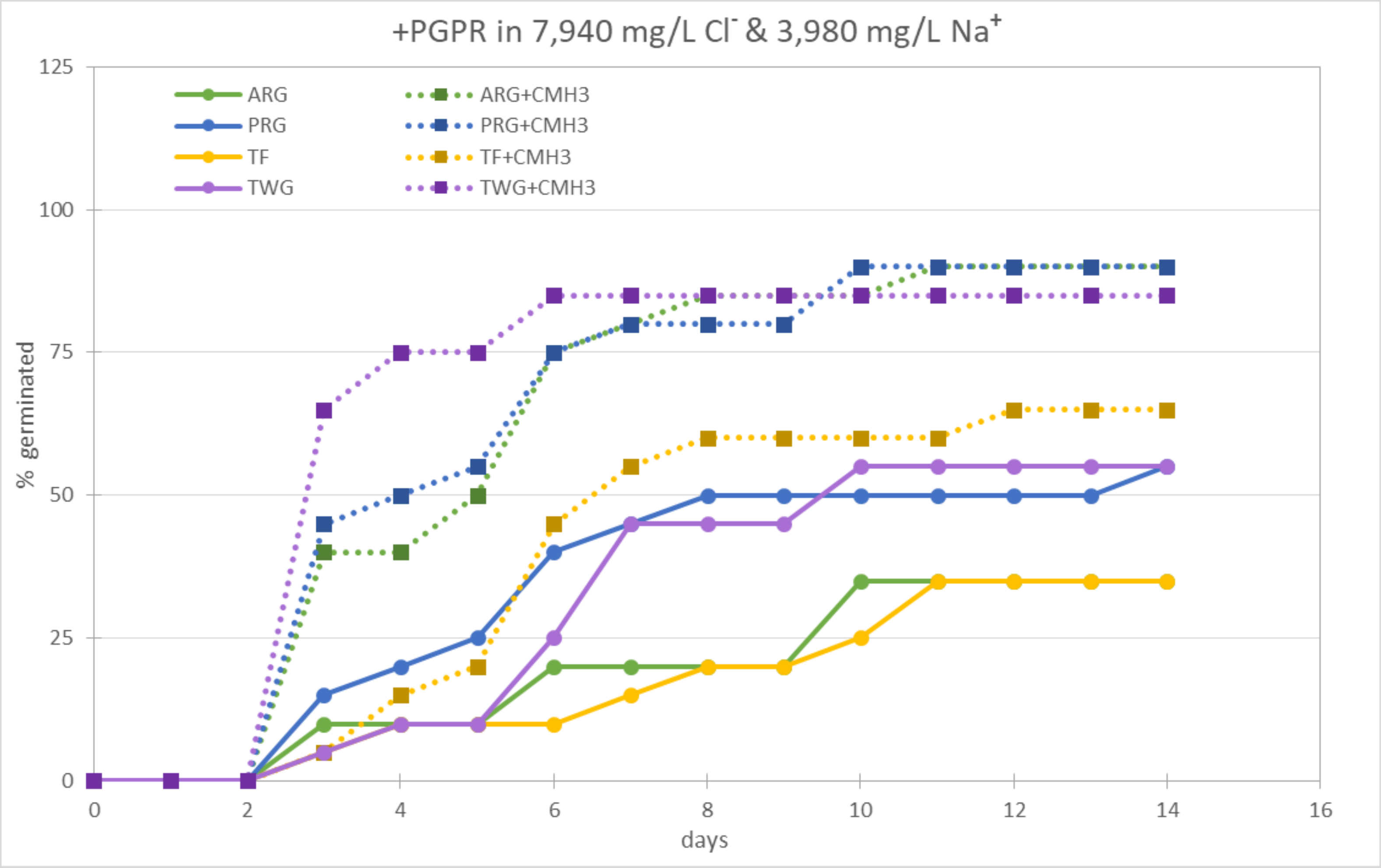
The Effects of PGPR on Seed

Seed Germination

Addition of PGPR increases the % germination with increasing amounts of produced water.

Generate the LC50 values from these curves.



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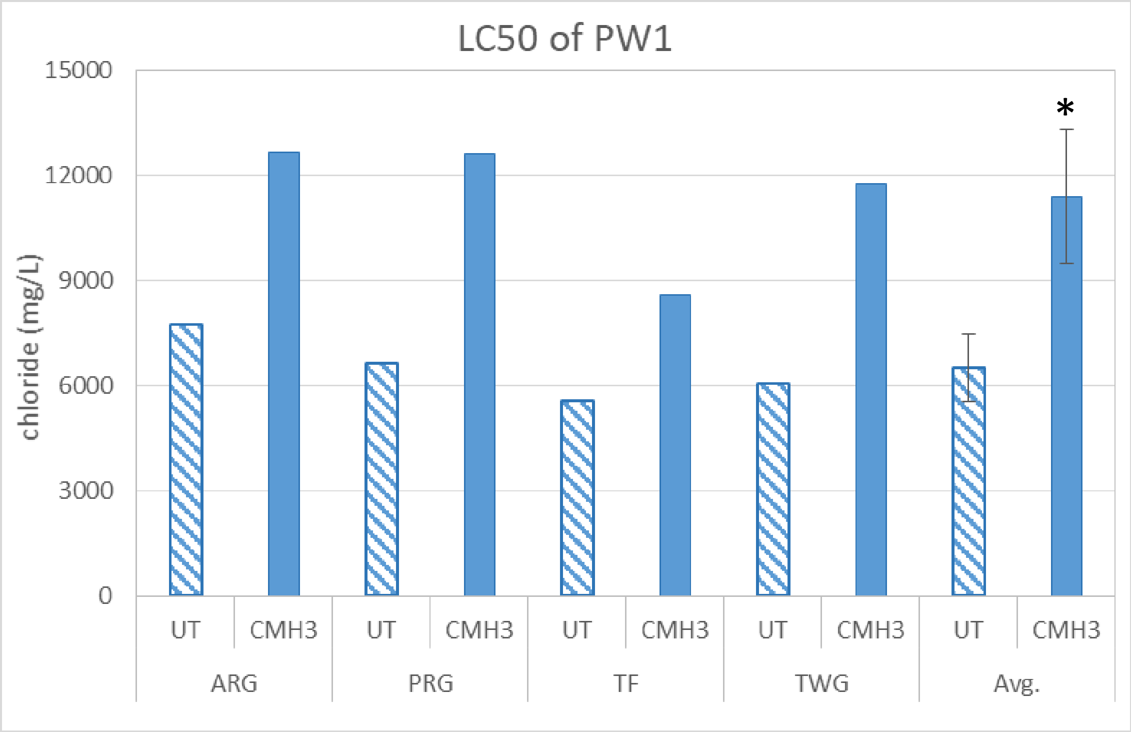
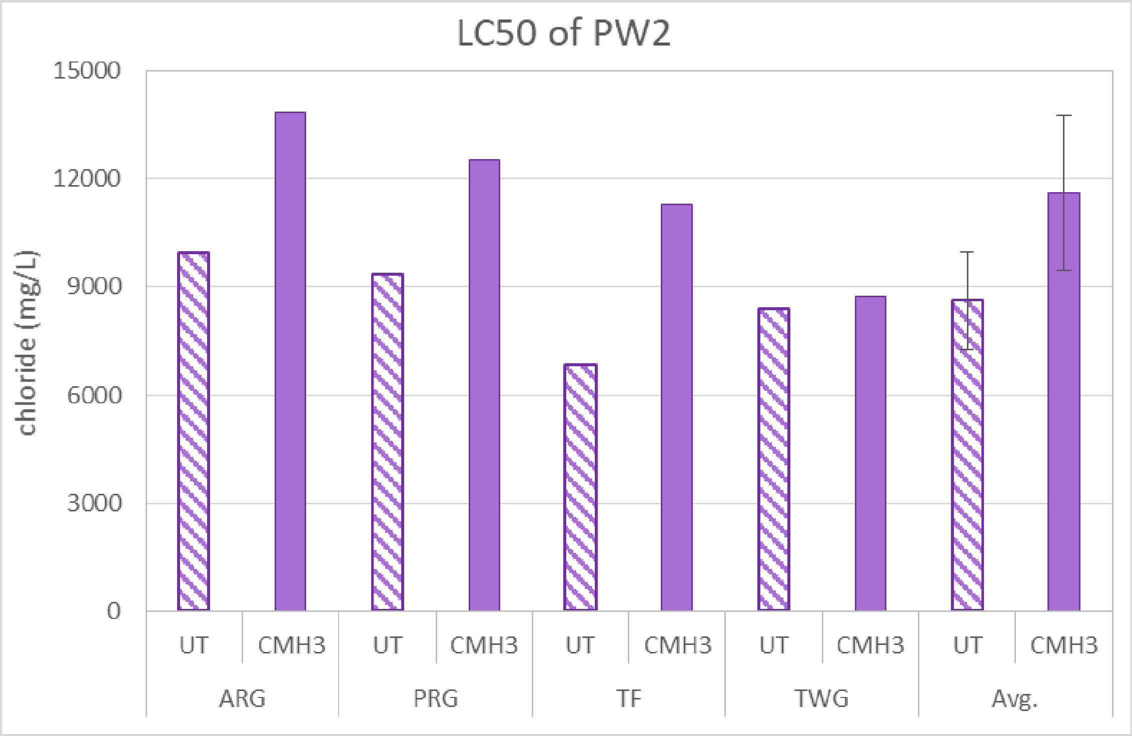
Quantifying the Effects of PGPR - LC50 (Tolerance)





seed	UT	CMH3	%change
ARG	7760	12651	63
PRG	6649	12614	90
TF	5553	8583	55
TWG	6064	11731	93
Avg.	**6506**	**11395**	**75**

seed	UT	CMH3	%change
ARG	9953	13857	39
PRG	9346	12525	34
TF	6846	11302	65
TWG	8392	8755	4
Avg.	**8634**	**11610**	**34**

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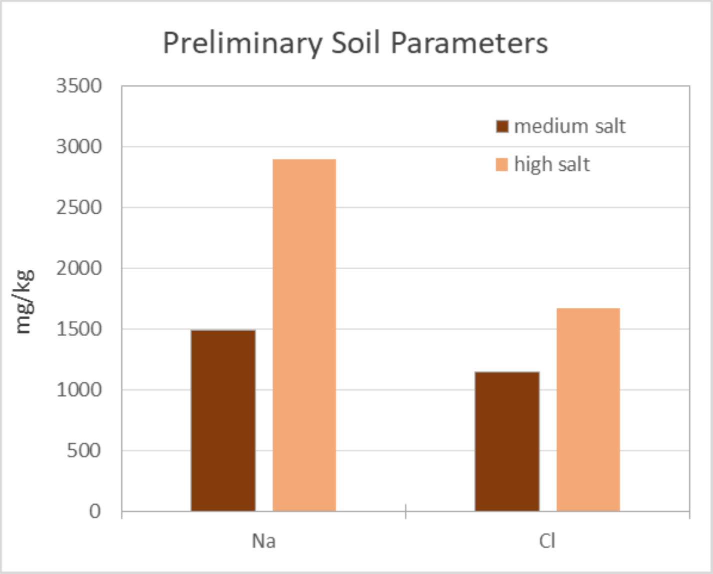
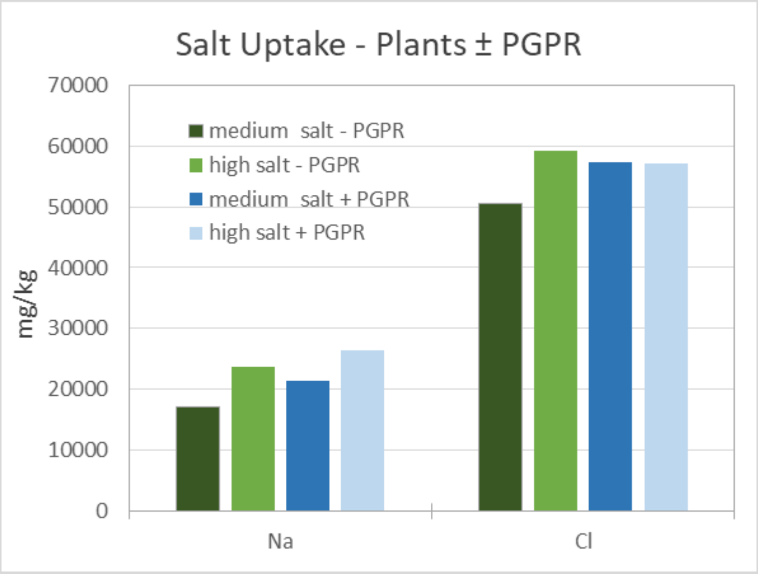
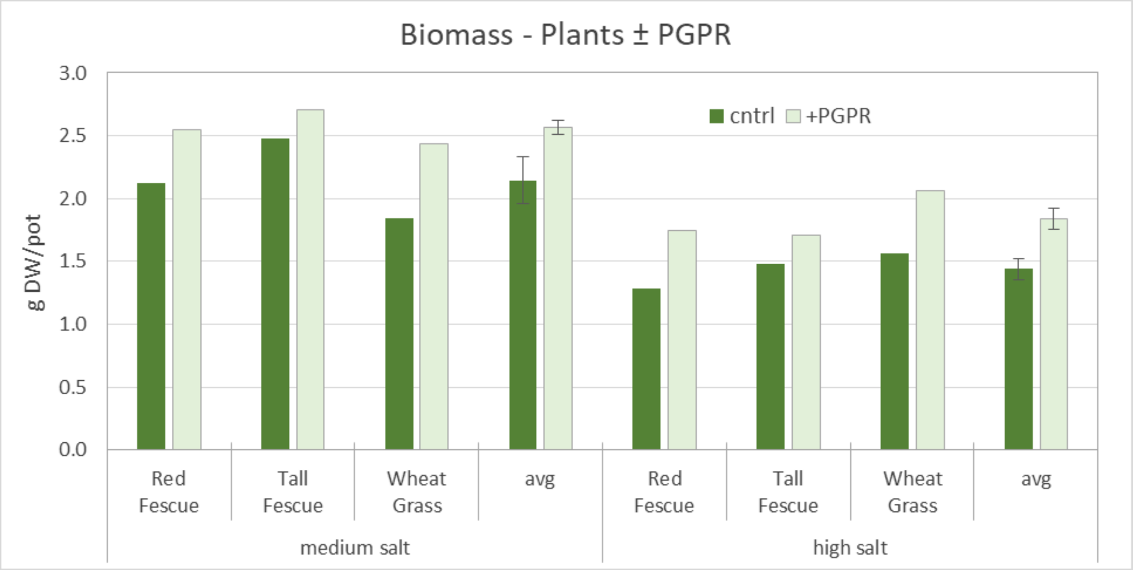
Initial Laboratory Experiments – Elevated Salinity







The advantages of PGPR:

- Regardless of soil salt content, plants take up approximately the same amount of Na^+ and Cl^-.
- PGPR has no effect on the ability of plants to take up Na^+ and Cl^-.
- PGPR significantly increases the biomass of the plants grown in higher salt conditions:
  - 19.5% ↑ in medium salt
  - 27.7% ↑ in high salt
- The increase is species dependent.
- Grasses are able to remove ~65 g NaCl per kg of dry plant material.

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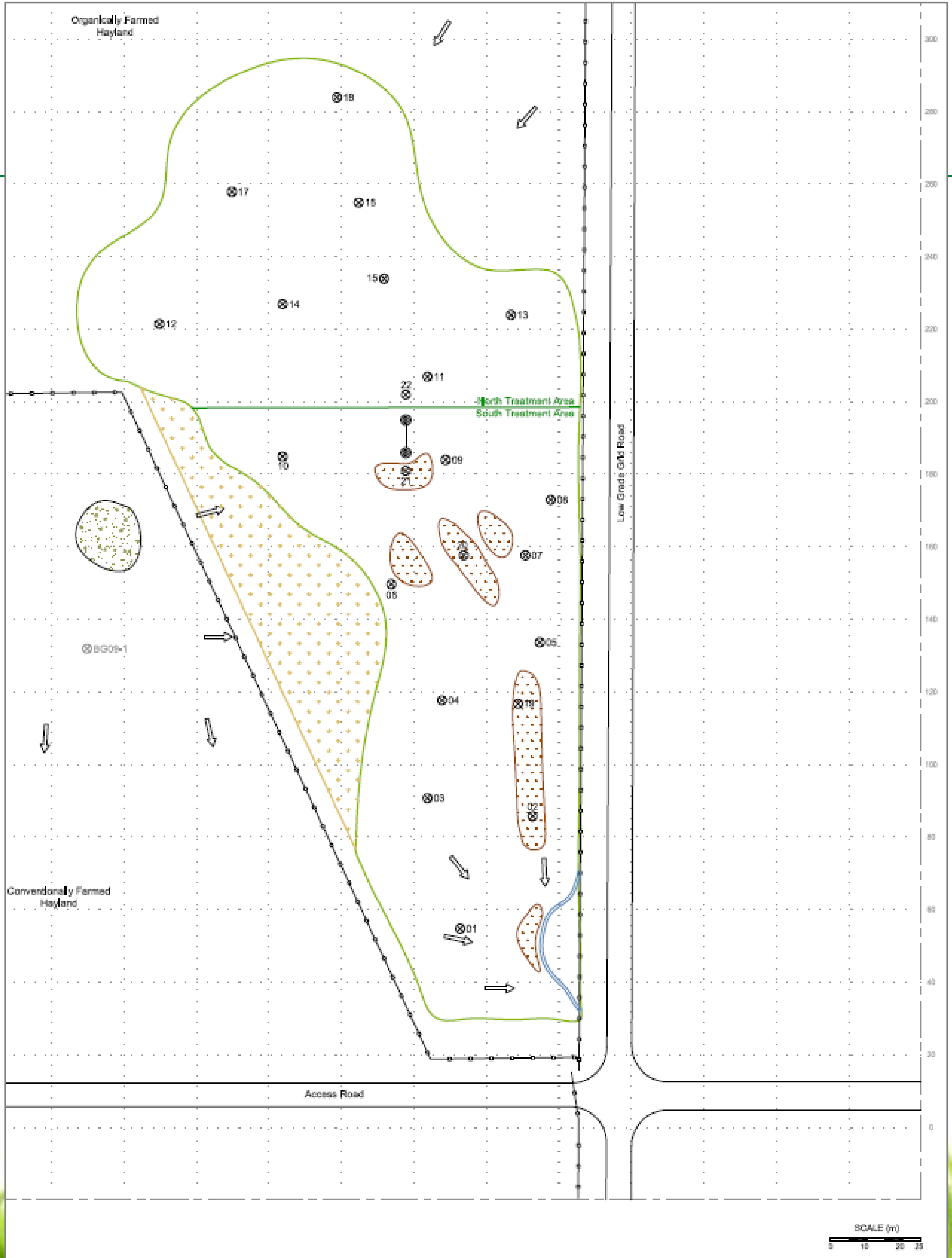
Site Management – Salt Soil

Project Goals Weyburn 08-31:

- Re-vegetate the grassland site to at least 70% of background levels.
- Reduce soil salt levels over time to allow for sustainable plant growth.
- EC levels?
- PEPSystems was deployed in the summer of 2010
  - Seed – ARG, PRG, Oats, TWG
  - PGPR – *Pseudomonas corrugata*
- Treatment area soils were managed over three growing seasons to re-vegetate the area.



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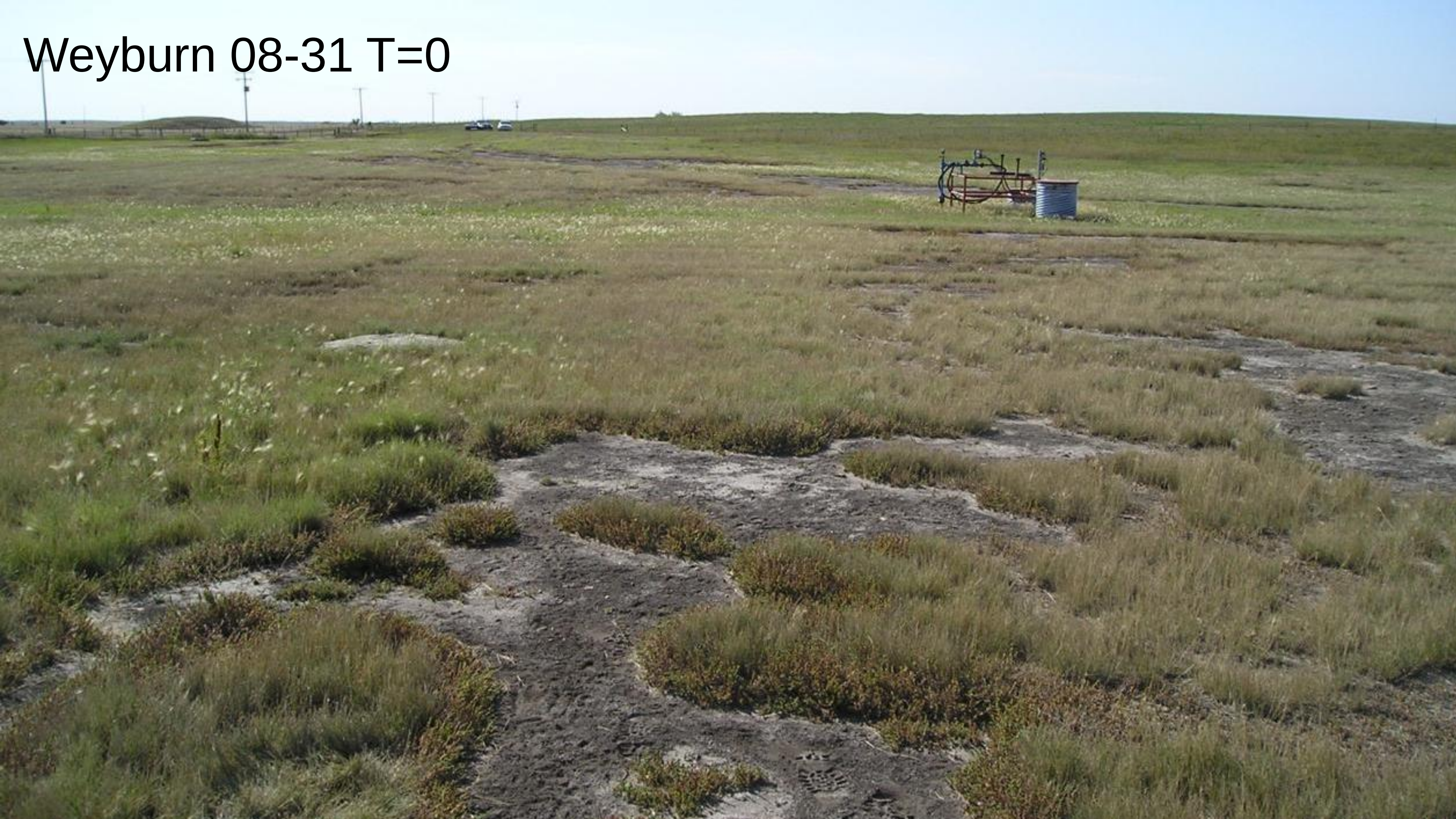
Weyburn 08-31 T=0



Weyburn 08-31 - Three Months After Seeding

Weyburn 08-31 - Year 7 (2019)

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Site Management – Salt Effluent and Compaction

Project Goals:

- Revegetate commercial site for use by owner.
  - Highly compacted, poor quality subsoil
- Brewery with on-site reverse osmosis process → produces effluent with elevated salinity.
  - Use effluent to irrigate the revegetated area.
  - Maintain vegetation.

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T=6 Weeks
2020


T=25 Weeks
2020


1 Year
2021

Site Management – Native Grasses

Native Prairie Grass Plugs – Day 0

Blue grama grass (*Bouteloua gracilis*)

control | 2 ml PGPR | 10 ml PGPR

Native grasses can be hard to get established.

PGPR seed treating slurry was added directly to the root portion of the plug when planted.

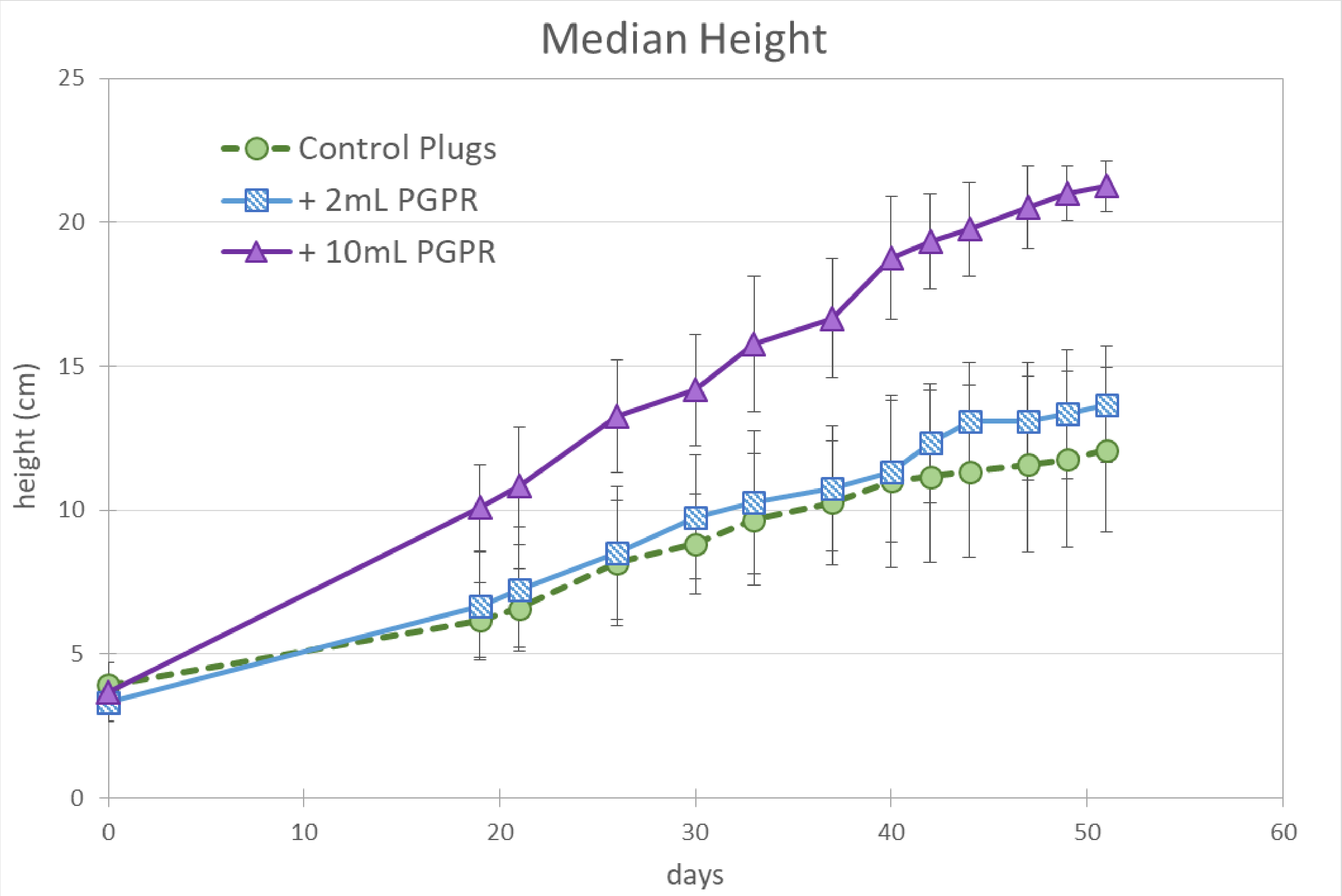
Pots contained salt contaminated soil to elicit same stress response as drought conditions.

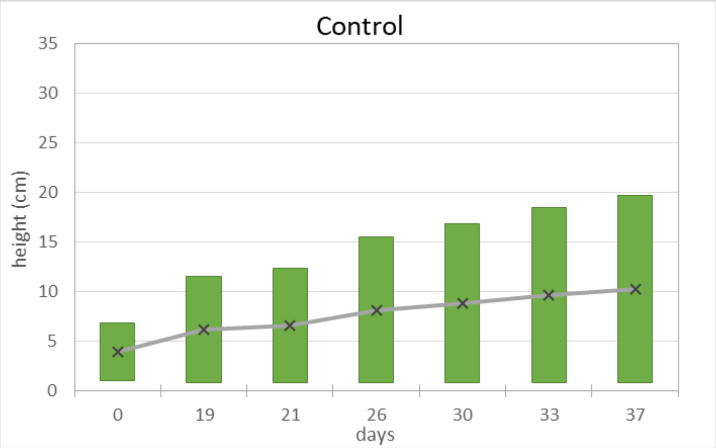
Pots were not fertilized.

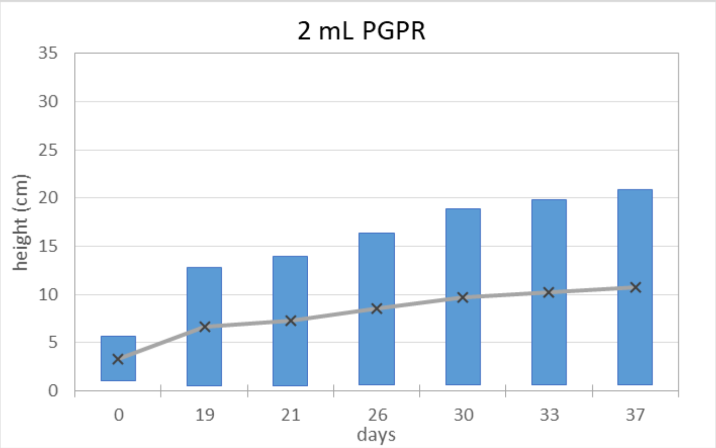
Soil moisture levels were maintained at 60% with regular watering.

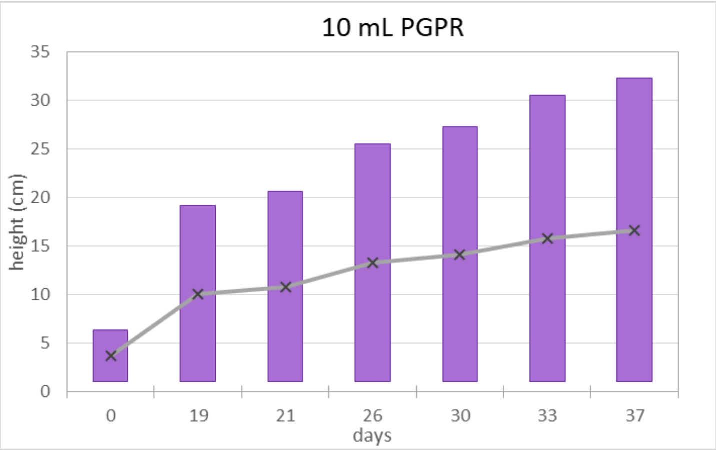
Growth was monitored for 7 weeks.

Plant Height









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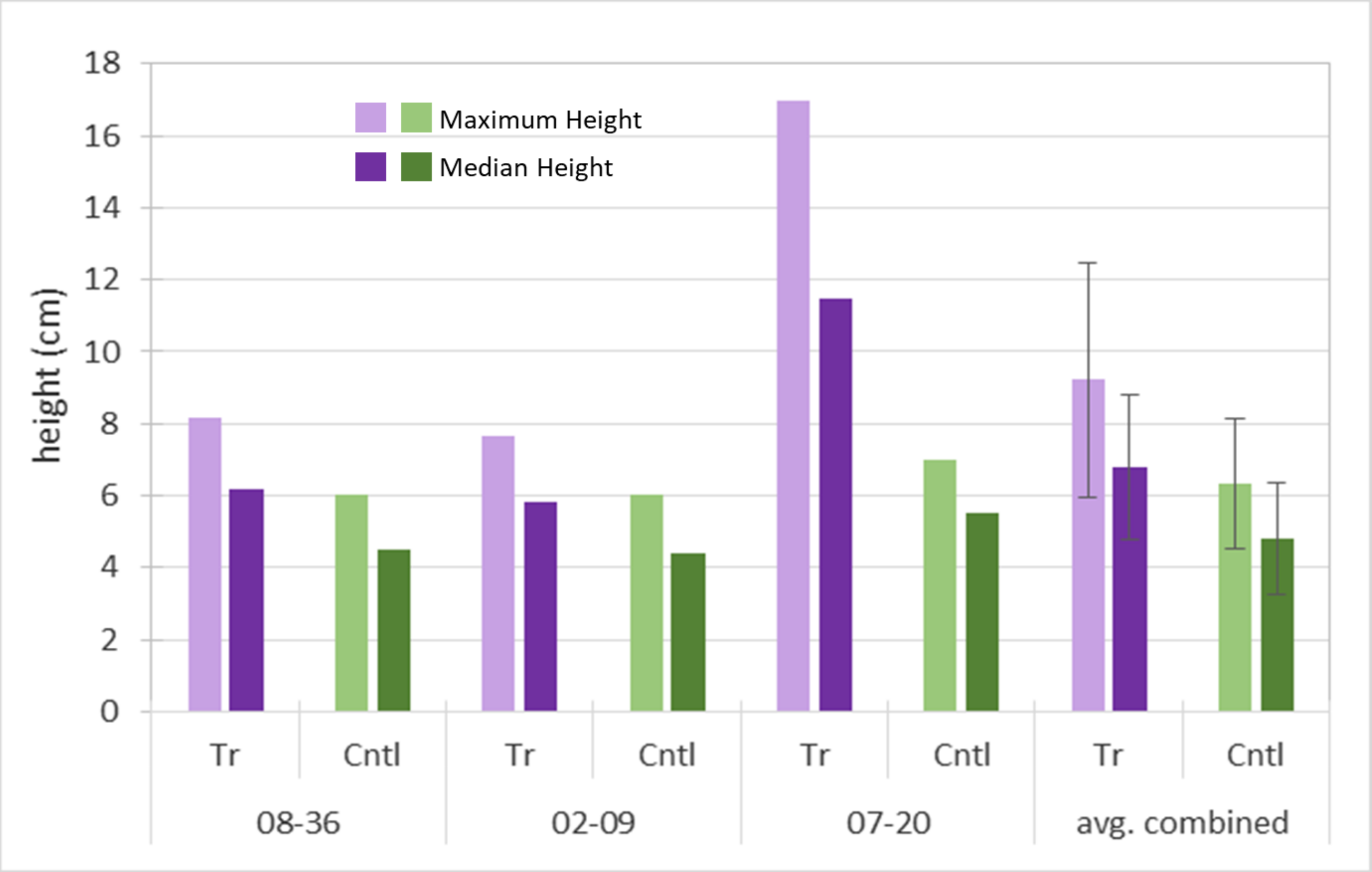
Reclamation Applications – Preliminary Field Trial



Sample Description			Nutrients		
Sample Location	Sampling Date	Sample Depth (m bgl)	Nitrogen (mg/kg)	Phosphorous (mg/kg)	Potassium (mg/kg)
Treatment Area					
08-36 Treated	Jun-24-20	0.00-0.25	4.3	24	401
08-36 Control	Jun-24-20	0.00-0.25	10.7	29	432
02-09 Treated	Jun-24-20	0.00-0.25	22.5	14	262
02-09 Control	Jun-24-20	0.00-0.25	4.2	32	355
07-20 Treated	Jun-24-20	0.00-0.25	5.2	20	400
07-20 Control	Jun-24-20	0.00-0.25	15.9	38	536

Native Grass Plug Field Trial - 2021

Study: 4 species, 4 sites in the Hanna AB area, hot dry conditions, 12 control and 12 treated plugs/ species/site:

- PGPR negatively affected NAT height and health.
- PGPR positively affected WWG height and NWG health (seed head development).
- JG no effect on height, positive effect on health

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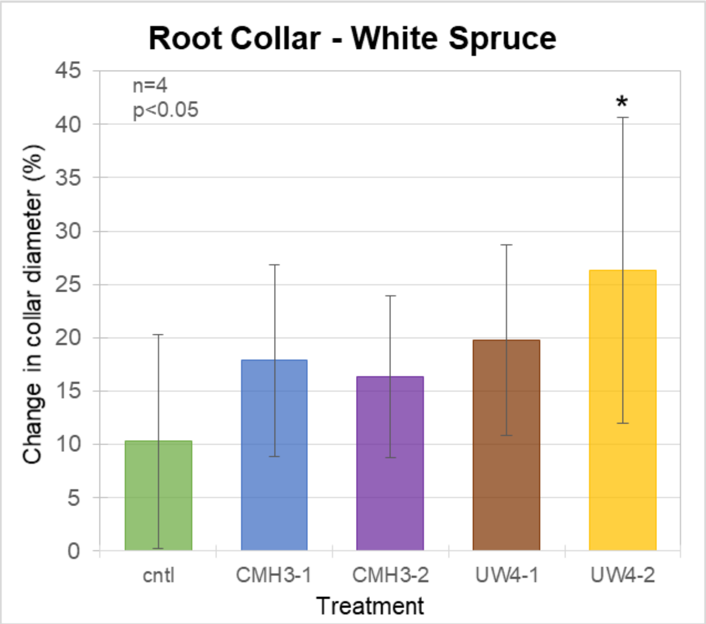
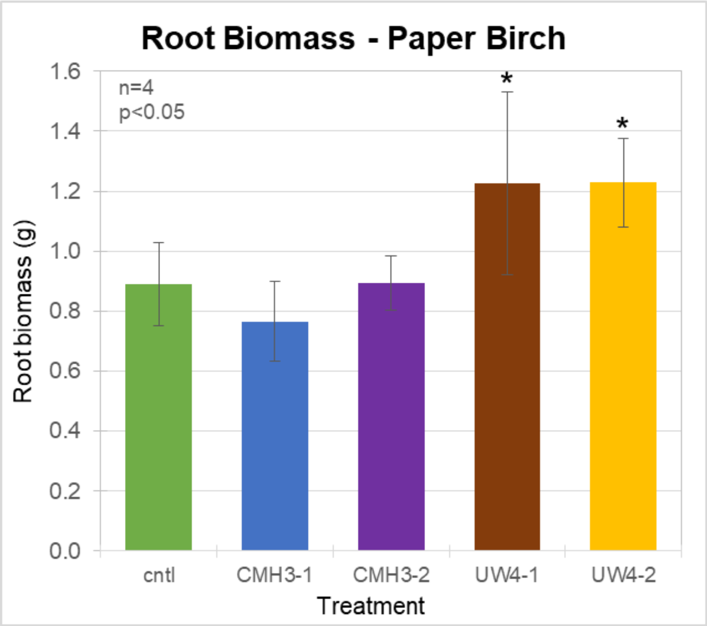
Site Management – Trees

Trees + PGPR

Reclamation challenge – re-treeing abandoned oil & gas sites

- Poor survival and growth due to:
  - Competition from non-native species
  - Poor quality soil
- Can PGPR enhance reclamation with trees?
  - Lab and field trials using paper birch and white spruce





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Trees and PGPR 2021

Urban Naturalization

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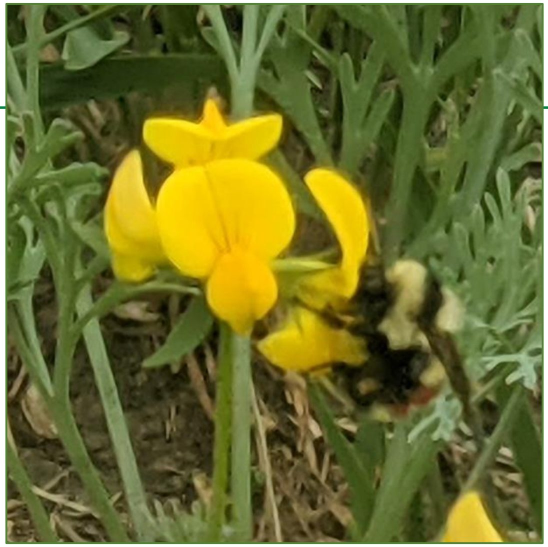
City of Calgary Pilot Project Goals



Change vegetation management practices to:

- Replace manicure turfgrass with:
  - Plants that are adaptable and can withstand extremes
    - Native grasses
    - Wildflowers
- Achieve environmental benefits including:
  - Increasing diversity
  - Reducing maintenance
    - Mowing, weed control
  - Increasing resilience to changing climate
  - Enhancing the pollinator population
  - Reduce fire risk

This is a three year project.

PEPSystems® Trial

Can PGPR facilitate establishment and survival of Mix B seed?

Results – PEPSystems Trial

North side of median (05 & 06):

- Mix B seed coated with PGPR

South side of median (07 & 08):

- Mix B seed untreated

Preliminary results:

- 05 and 08 have increased EC and SAR values
- Diversity was equivalent for both
- Plant size was equivalent for both
- +PGPR side had a larger # of plants for each species
- Winter survival will be telling

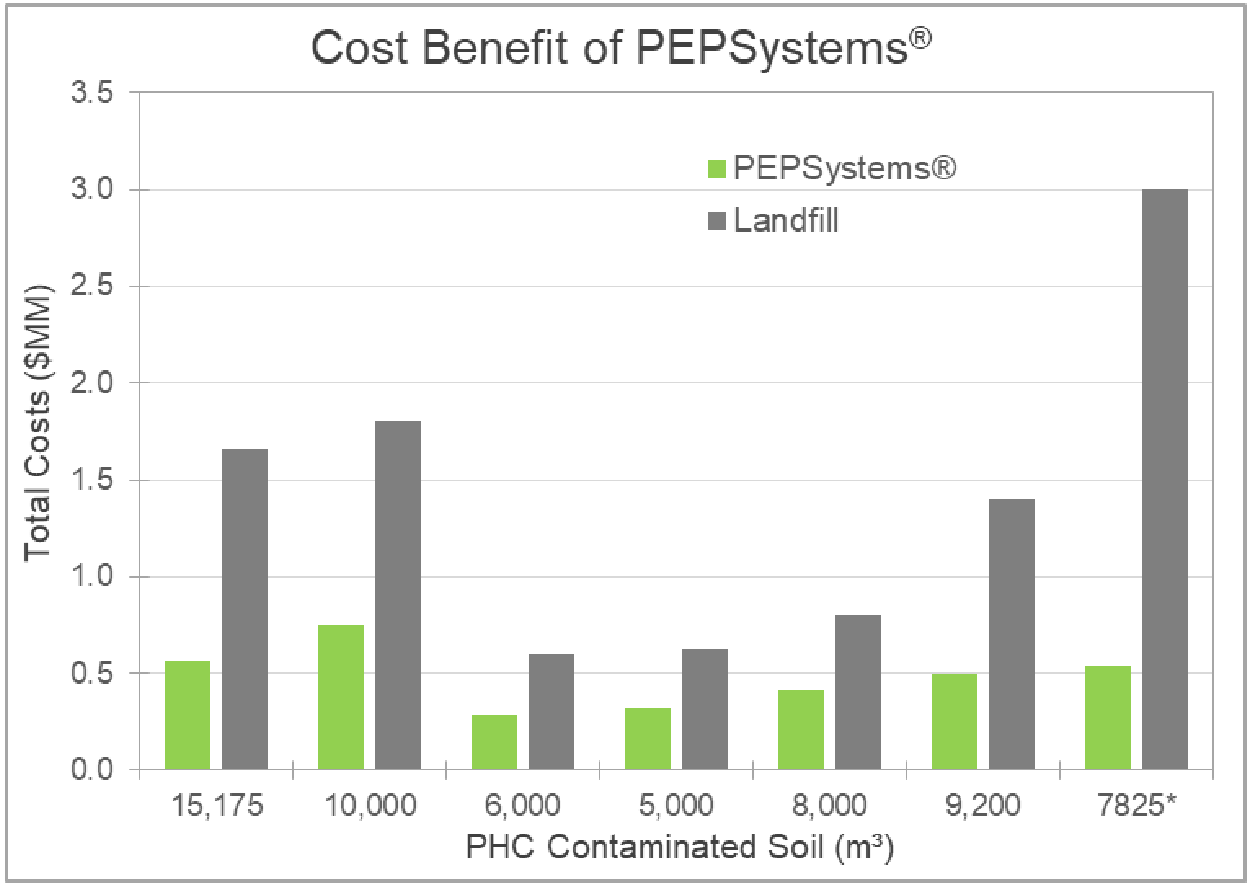
Flowers

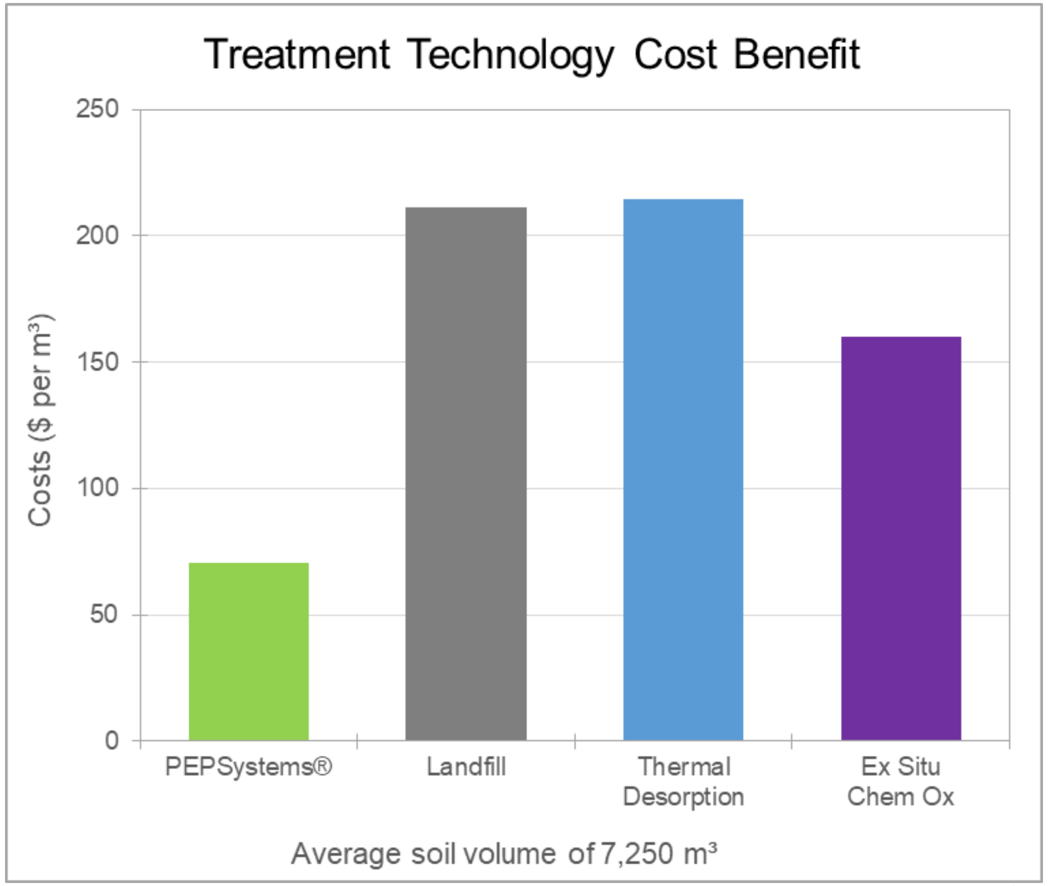
Sep 16, 2021

Benefits

The Economics of PEPSystems

Significant cost advantage to remediating onsite and using PEPSystems





The Carbon Benefits of PEPSystems

Average carbon sequestration for grasslands:

- 639 kg/ha/year

Compare carbon amounts emitted by:

- equipment in phytoremediation activities
- trucking to nearest landfill

Source of equipment emissions values:

- Published papers
- Industry information

Source of carbon sequestration values:

- Zirkle, et al. 2011. HortScience 46:808–814.
- Ginkel, et al. 1999. J. Environ. Qual., 28:1580-1584.
- Qian, et al. 2010. Soil Sci. Soc. Am. J. 74:366–371.
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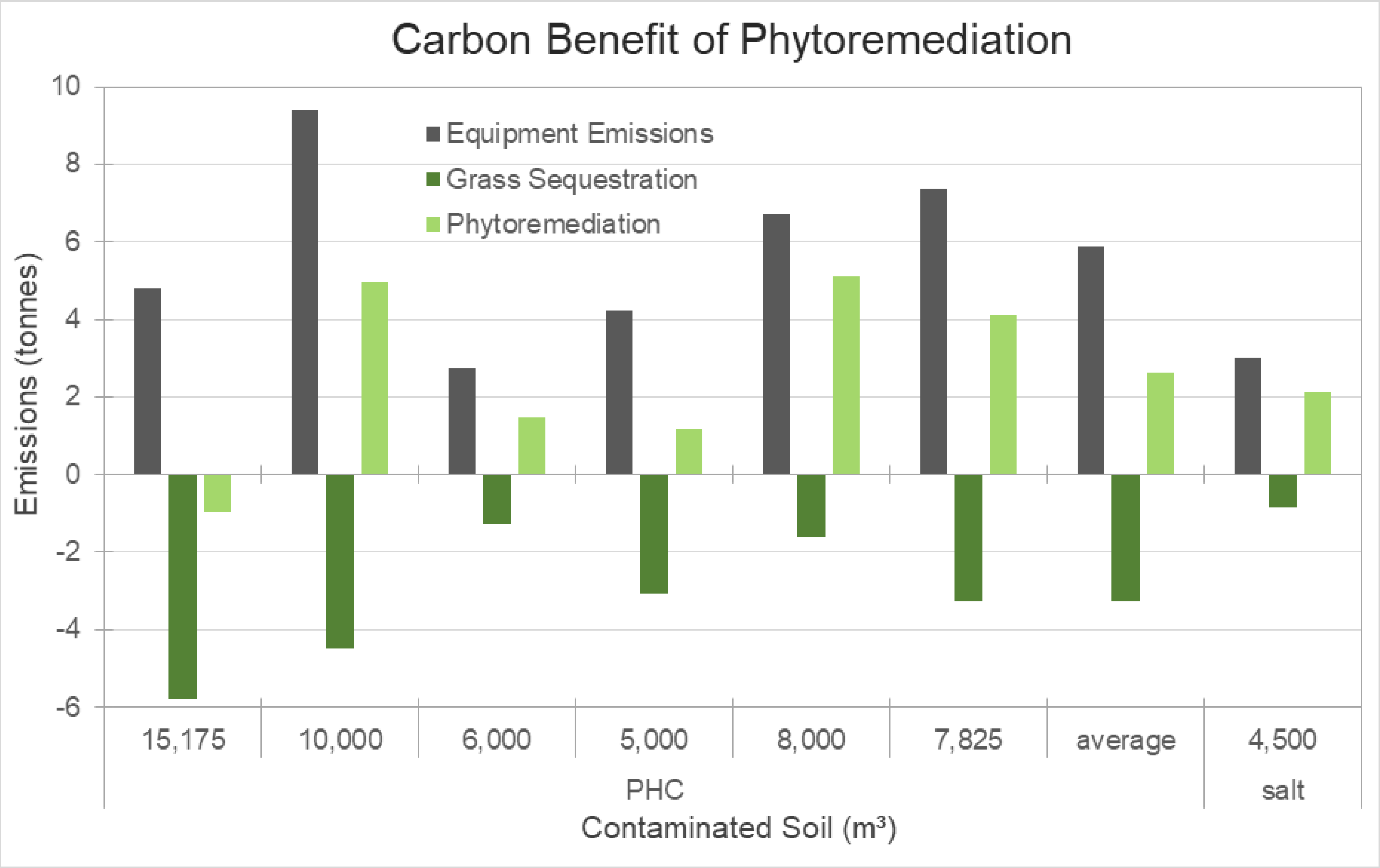

Advantages of PEPSystems

Environmentally responsible:

- Green technology, driven by solar energy.
- Soil is conserved and reused, quality is improved.
- Small carbon footprint (no offsite disposal; minimal heavy equipment usage).

Suitable for remote locations:

- Fly in seed and amendments, etc.
- No large scale equipment requirements or hauling requirements reducing truck traffic on roads.

Effective for challenging contaminants:

- PHC fractions F3 and F4.
- Salts and metals.

Effective for facilitating reclamation / revegetation in poor quality soil.

Economic advantages:

- Low cost as compared to other technologies.
- Overall remediation cost spread out over a number of years.

Acknowledgements

National Research Council – Industrial Research Assistance Program (IRAP).

Clients who have allowed Earthmaster to conduct field trials to advance the PEPSystems technology.

Thank You

Questions?

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REMEDIATION & RECLAMATION
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