



The Potential for Using Native Plants in In Situ Remediation

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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

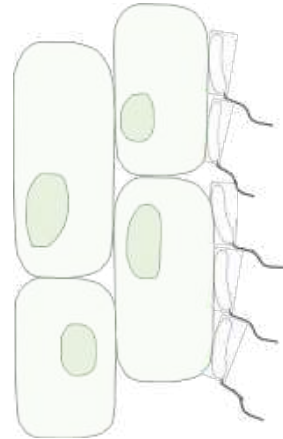
Use bacteria to help the plants grow in stressful conditions.



Plant seeds:
Coated with natural soil bacteria



Active rhizosphere:
Bacteria co-localize with developing roots

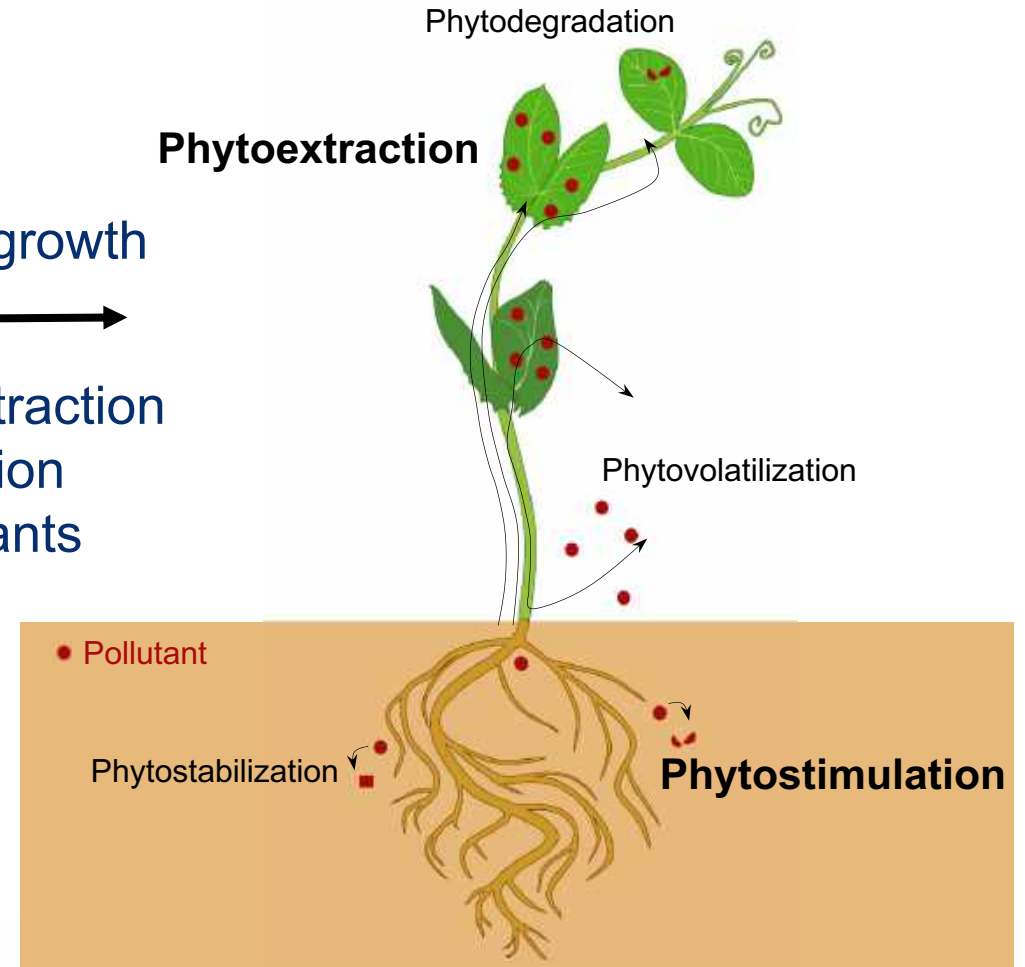


Plant cell:
Bacteria interact with root cells –
↑ hormones
↓ stress response

Facilitate plant growth



Exploit phytoextraction & phytostimulation properties of plants



Current PEPSystems Plants

In phytoremediation - biomass is everything!

Currently use agronomic plants, not native species.

- Annual ryegrass (ARG)
- Perennial ryegrass (PRG)
- Tall fescue (TF)
- Relatively easy to grow in poor quality soil with PGPR
- Lots of root biomass – branching vs taproot
 - depth of roots/remediation about 0.5 m
- Lots of aboveground biomass – salt uptake
- Cost effective
- Large quantities readily available

But – may need to be removed at the end of phytoremediation.

Contamination Depth - Regulations

Depth of phytoremediation will be limited by the depth of the roots of the plants. If the contamination goes deeper – will need to dig up the soil.

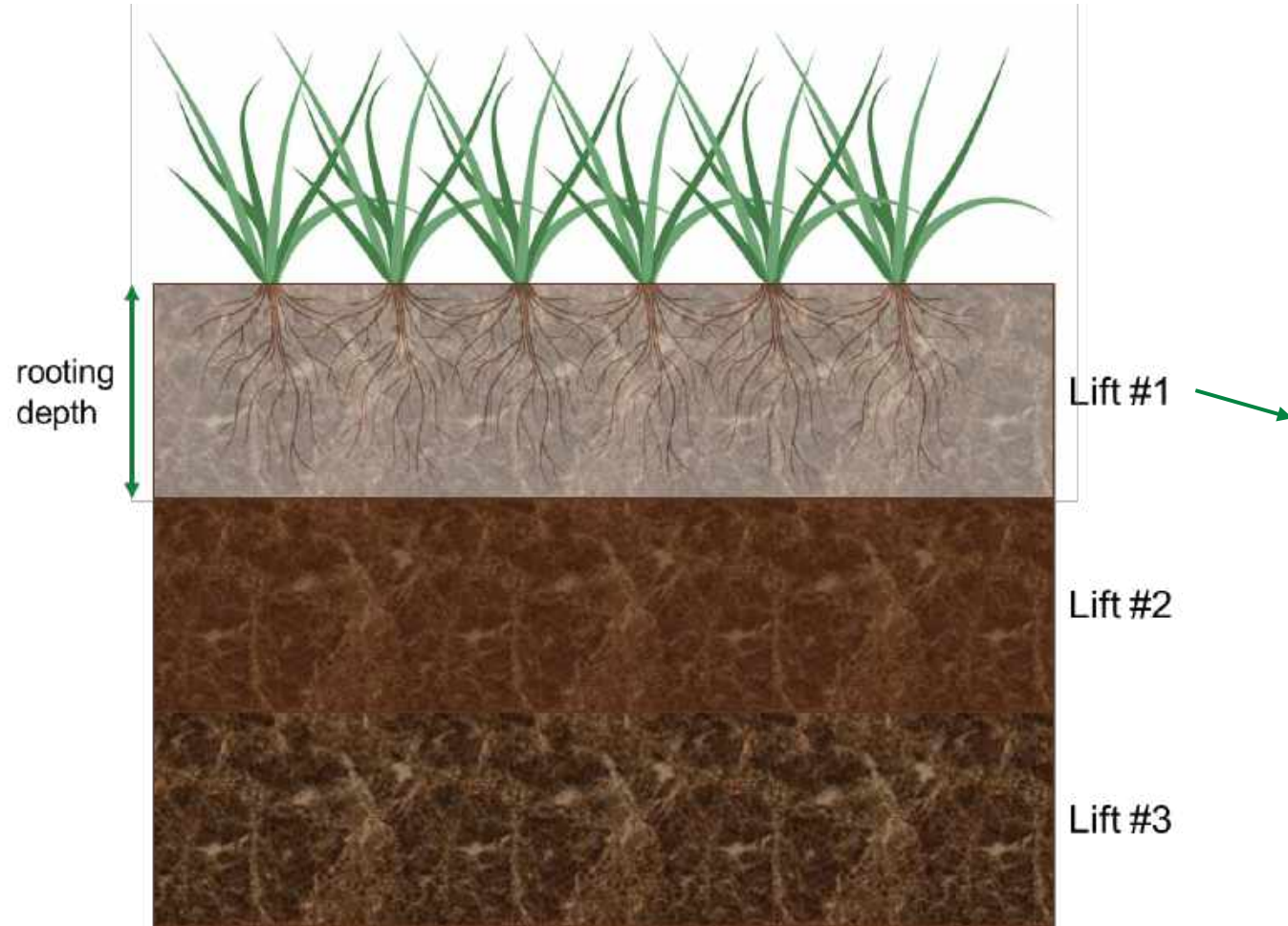
- In AB - if contaminated soil is excavated, it needs to be put in a containment cell to prevent contaminants from spreading.
- In BC – a containment cell is required for bioremediation facilities.

Containment facilities are not required if the soil is left in place and treated.

Plants can be left in place at the end if suitable native species are used.

Can effective phytoremediation be achieved using native grasses?

Phytoremediating Subsoil in Lifts



Containment/Soil Placement



Contaminated soil

Seed Bed Preparation



Fertilize & seed

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%)

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



Hydrocarbon vs. Salt Phytoremediation

PHC

Phytostimulation
(rhizodegradation)

incorporate plants into the soil

DEERE

Pollutant

Salt

Phytoextraction

harvest above ground biomass

Pollutant

Phytoremediation with Native Plants

These plants can be left in place, additional reclamation may not be required.

No removal of soil if roots can penetrate deep enough.

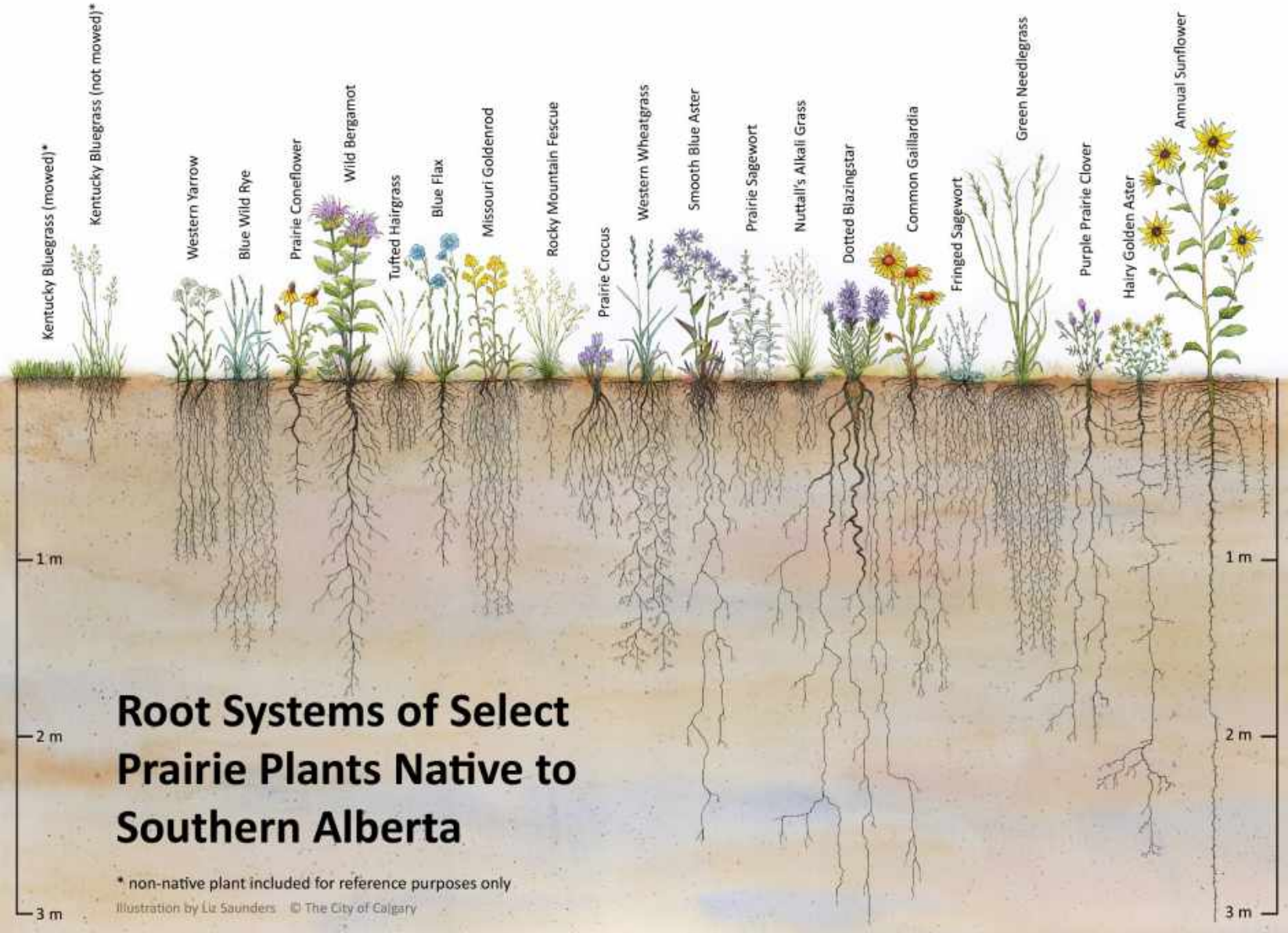
Problem – native species may not tolerate the conditions:

- Contaminated subsoil will affect establishment and growth
- Use PGPR?

Other limitations:

- Seed availability and cost
- Slow establishment rates – will take more time
- Less biomass?

The Native Plant Advantage



Seed Germination Studies – Produced Water

Seeds

- Different species
- +/- PGPR

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	408	498	18430	1372	7.1	73	71
Produced Water 2	78870	9264	4054	1839	32970	997	6.5	125	82

LEGEND

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.

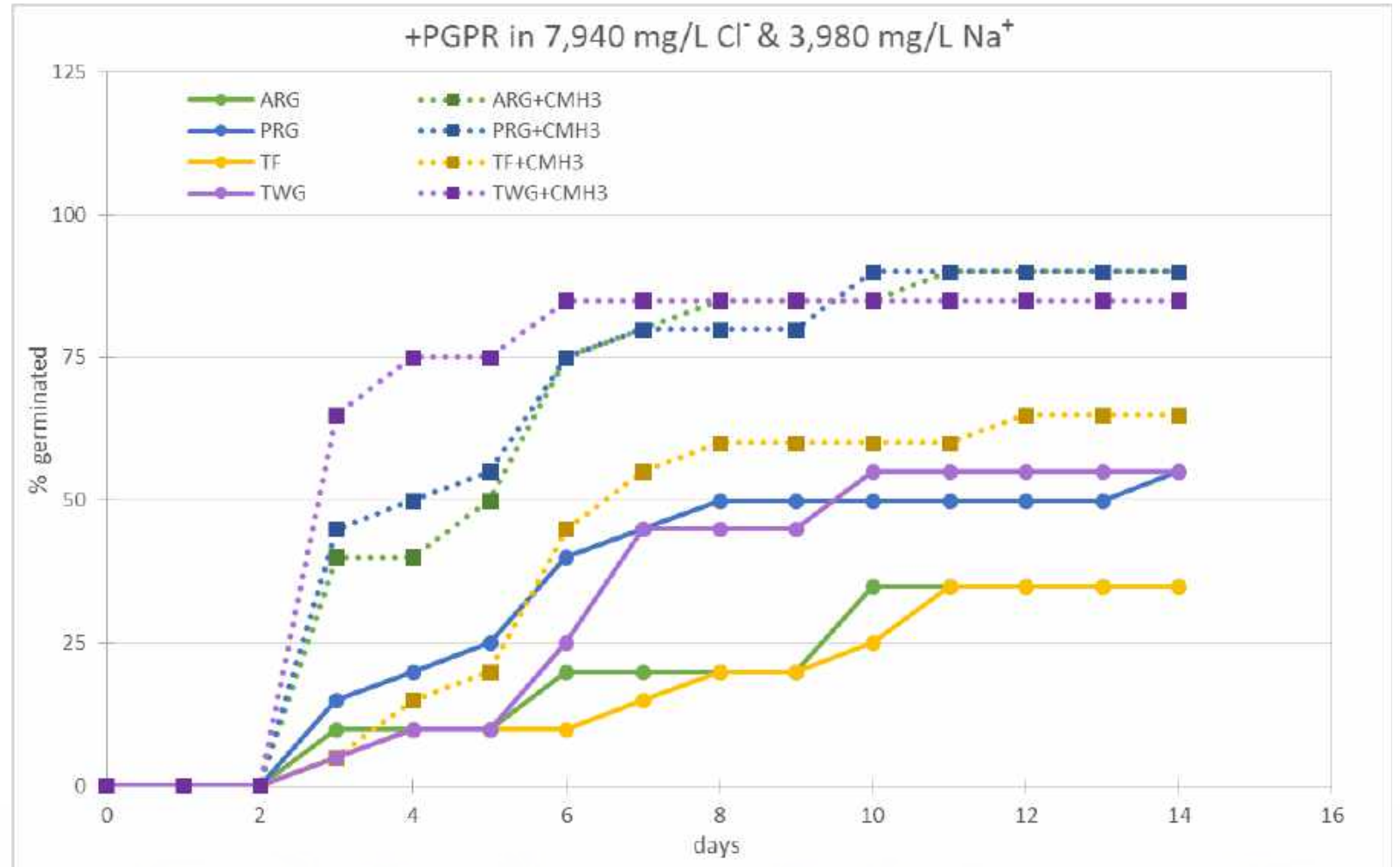
Environmental Quality Guidelines for Alberta Surface Waters (EQGASW) - Alberta Environment and Parks (AEP), July 2014.

The Effects of PGPR on Seed – Agronomic Species

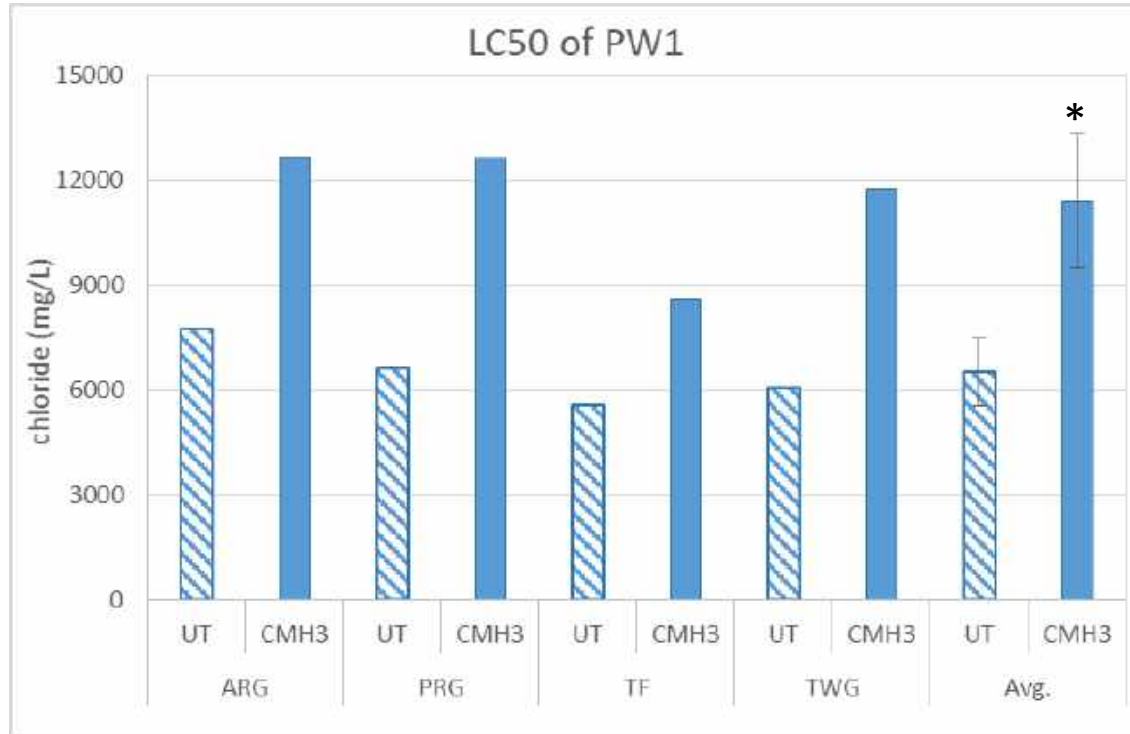
Seed Germination

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

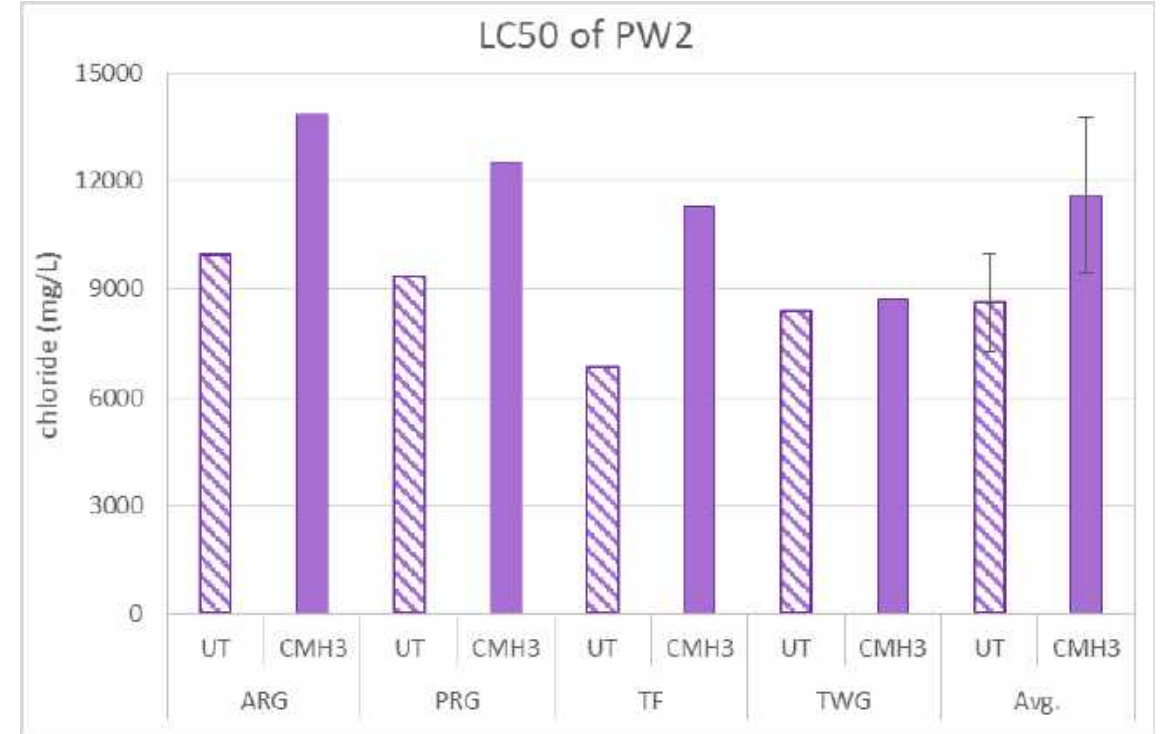
Generate the LC50 values from these curves.



Quantifying the Effects of PGPR - LC50 (Tolerance)

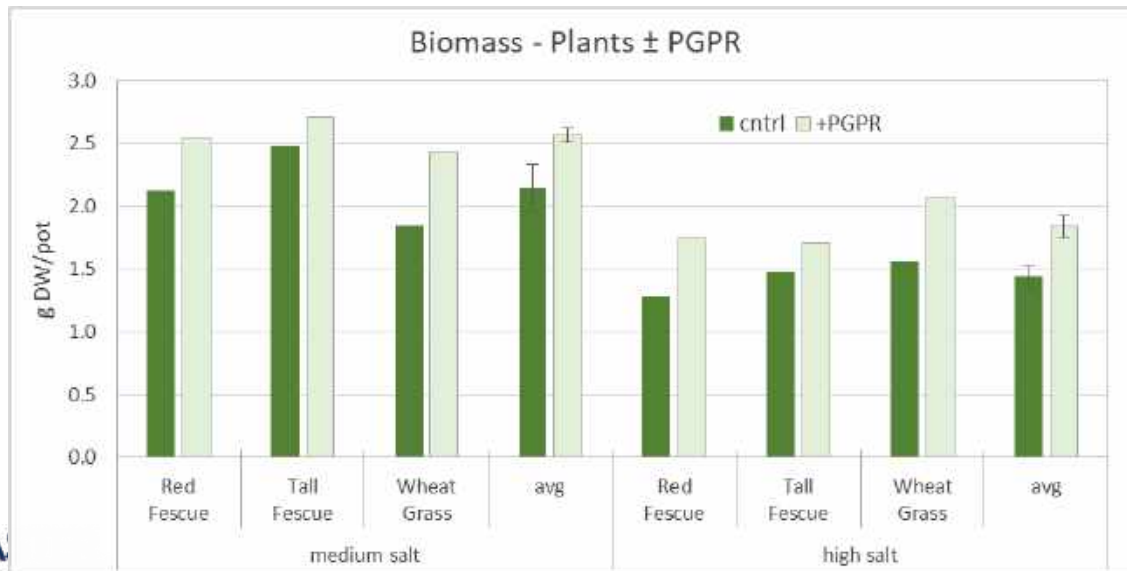
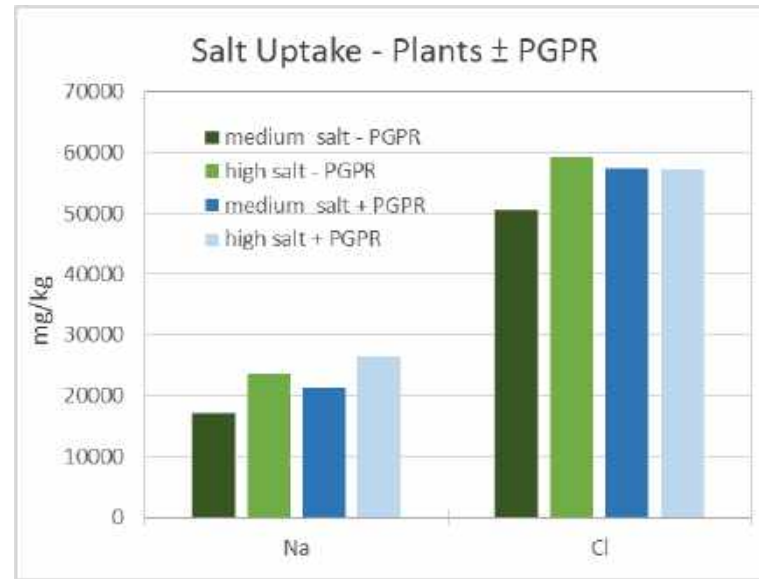
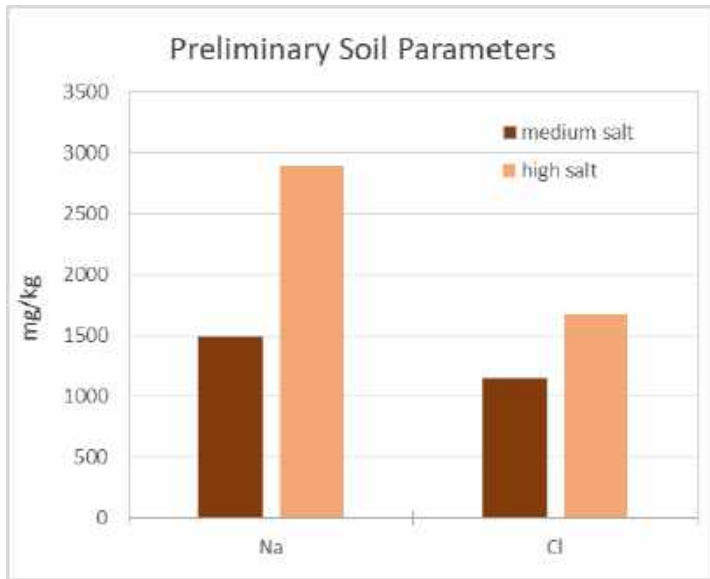


seed	UT	CMH3	% change
ARG	7760	12651	63
PRG	6649	12614	90
TF	5553	8583	55
TWG	6064	11731	93
Avg.	6506	11395	75



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

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.

Germination Studies – Native Species

JG - June grass

GNG - Green needlegrass

NWG - Northern wheatgrass

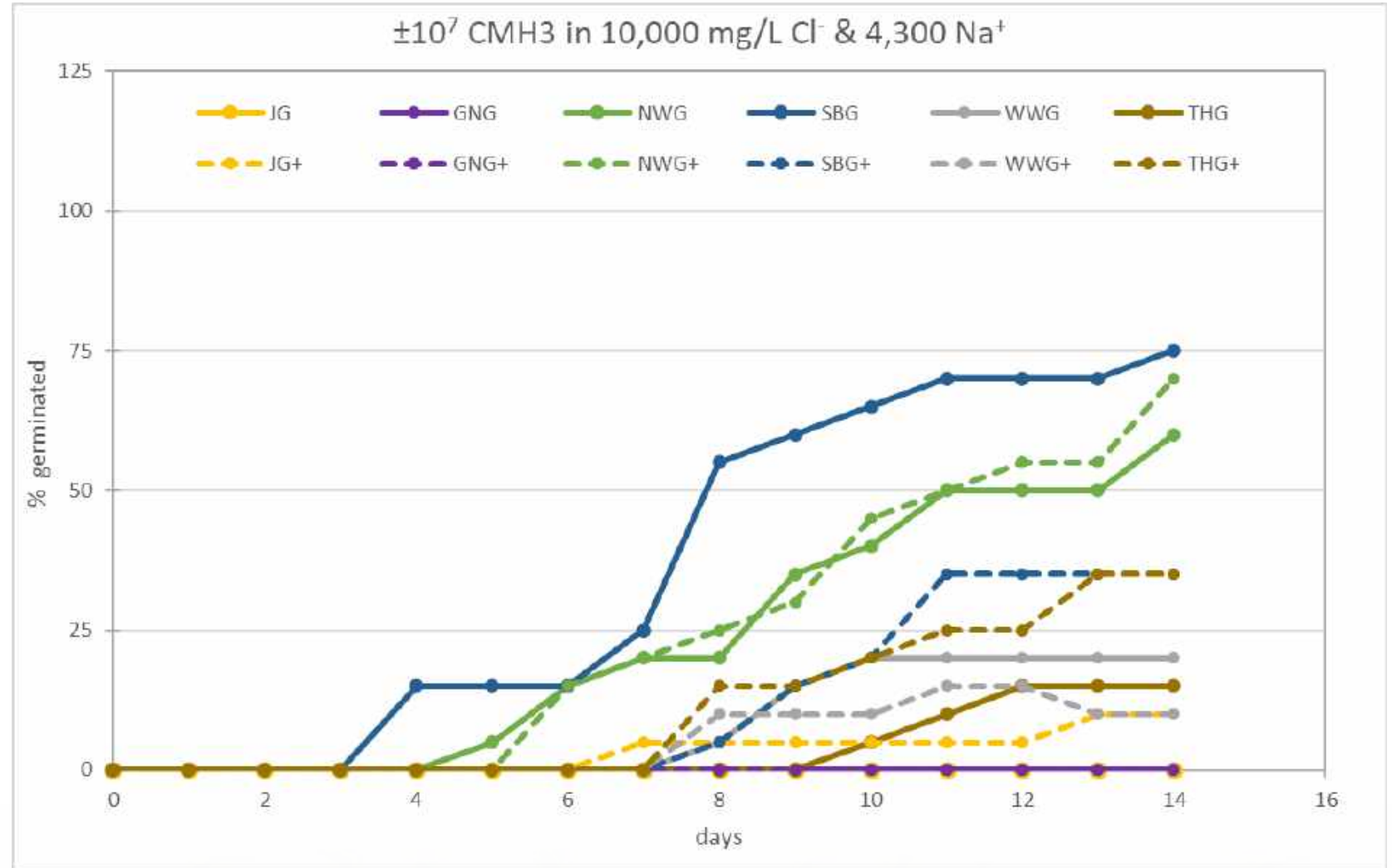
SBG - Sandberg bluegrass

WWG - Western wheatgrass

THG - Tufted hair grass

It's complicated –
no universal benefit

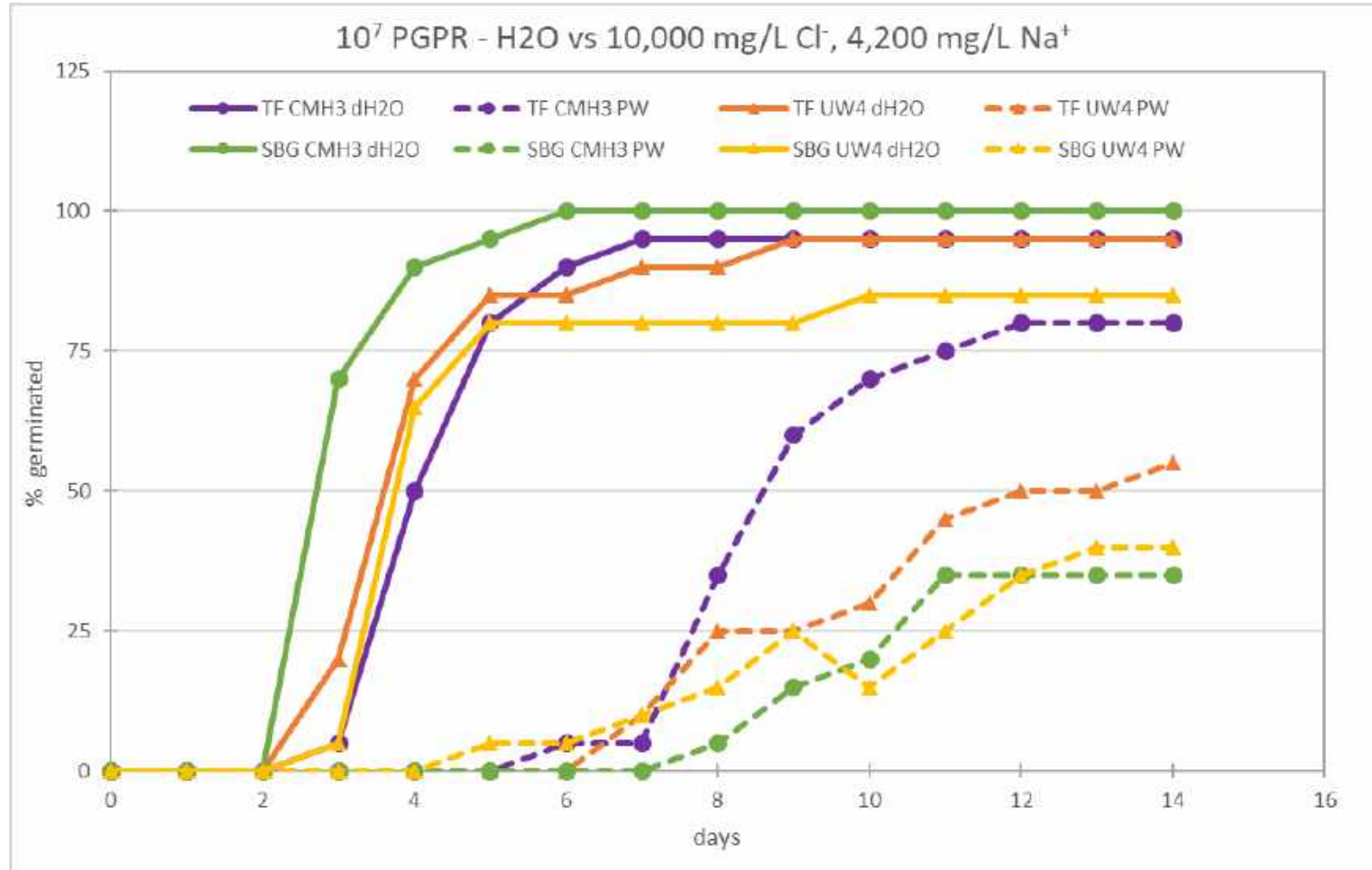
Note the low germination
rate compared to
agronomics.



PGPR Type

CMH3 (salt) vs UW4 (PHC)

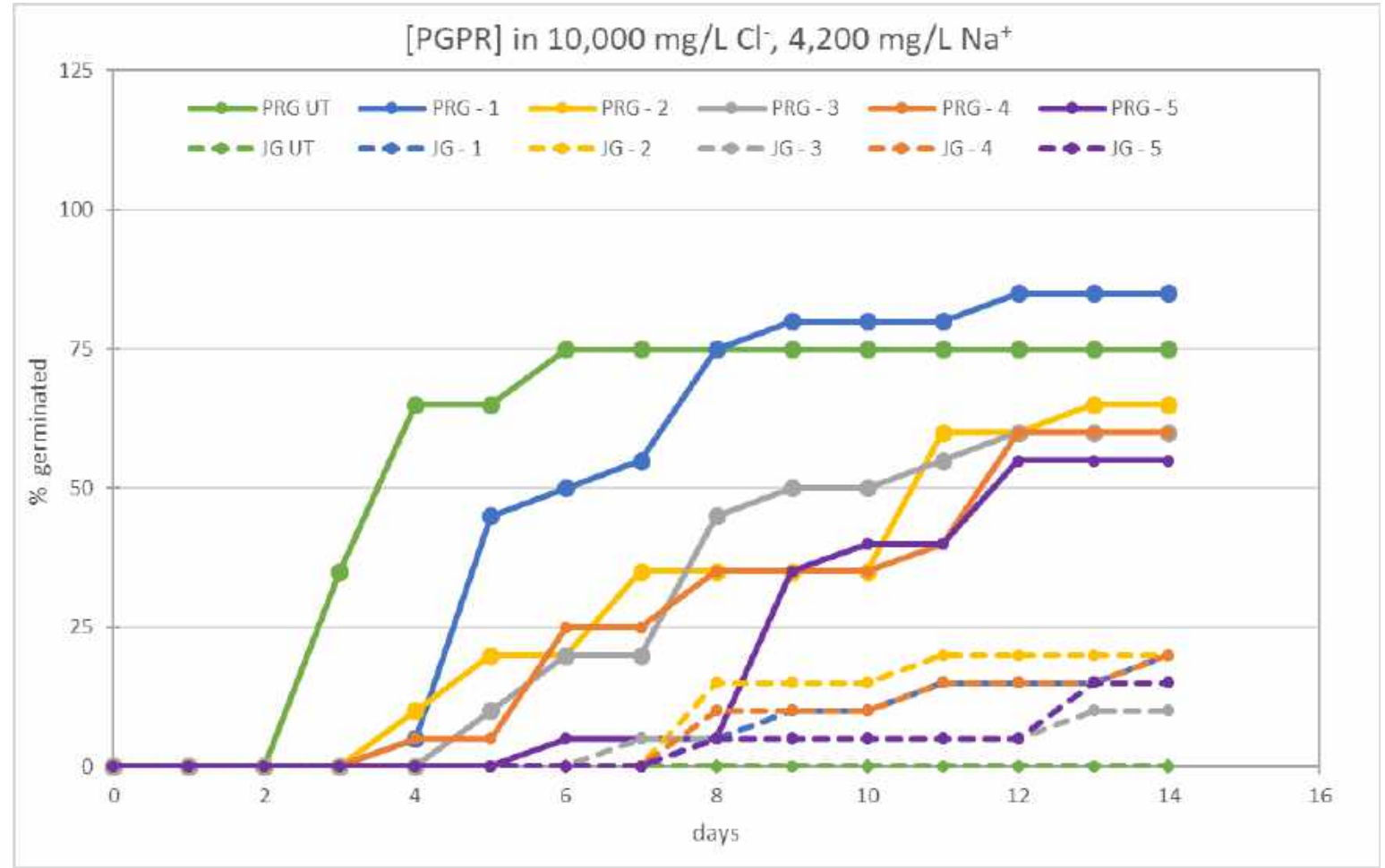
- Compared solid line vs dashed (dH2O vs PW)
 - CMH3 improves SBG germination in dH2O (85% UT and UW4)
 - TF better in PW than SBG
- Compared circles vs triangles (CMH3 vs UW4)
 - TF – difference in PW
 - SBG – no difference in PW
- Compared agronomic (TF) vs native species (SBG)
 - Germination much better for agronomic



PGPR Amounts

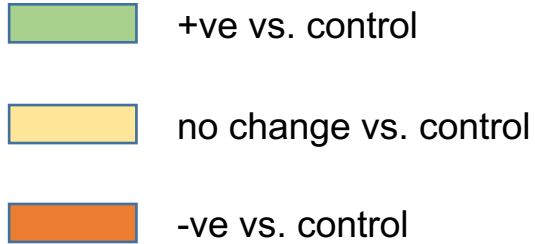
PGPR concentrations matter:

- PRG UT is fairly salt tolerant
- JG UT has no salt tolerance
- [PGPR] has a bigger influence on PRG than JG



Heat map

Combine germination time with maximum % germination



Trend – PGPR often only provide a benefit when there is stress, can be detrimental when there isn't.

Reclamation vs. remediation

dH2O

ARG
PRG
TF
TWG
JG
NWG
SBG
THG
WWG
GNG

	CMH3					UW4					PGPR concentration
	1	2	3	4	5	1	2	3	4	5	
ARG	0	-1	-1	-2	-2	0	0	0	0	0	
PRG	-1	-1	-1	-1	-1	-1	0	0	-1	-1	
TF	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	
TWG	0	0	0	-1	0	-1	-1	-2	-1	-1	
JG	-1	-1	0	-1	-2	0	0	-1	-2	-2	
NWG	0	-2	-1	-2	0	1	1	1	1	1	
SBG	1	2	2	1	1	0	0	-2	0	0	
THG	0	2	0	2	0						
WWG	0	-1	0	-1	0	0	1	-1	-1	-1	
GNG	2	0	0	0	N/A	-1	-1	0	0	N/A	

13% PW

ARG
PRG
TF
TWG
JG
NWG
SBG
THG
WWG
GNG

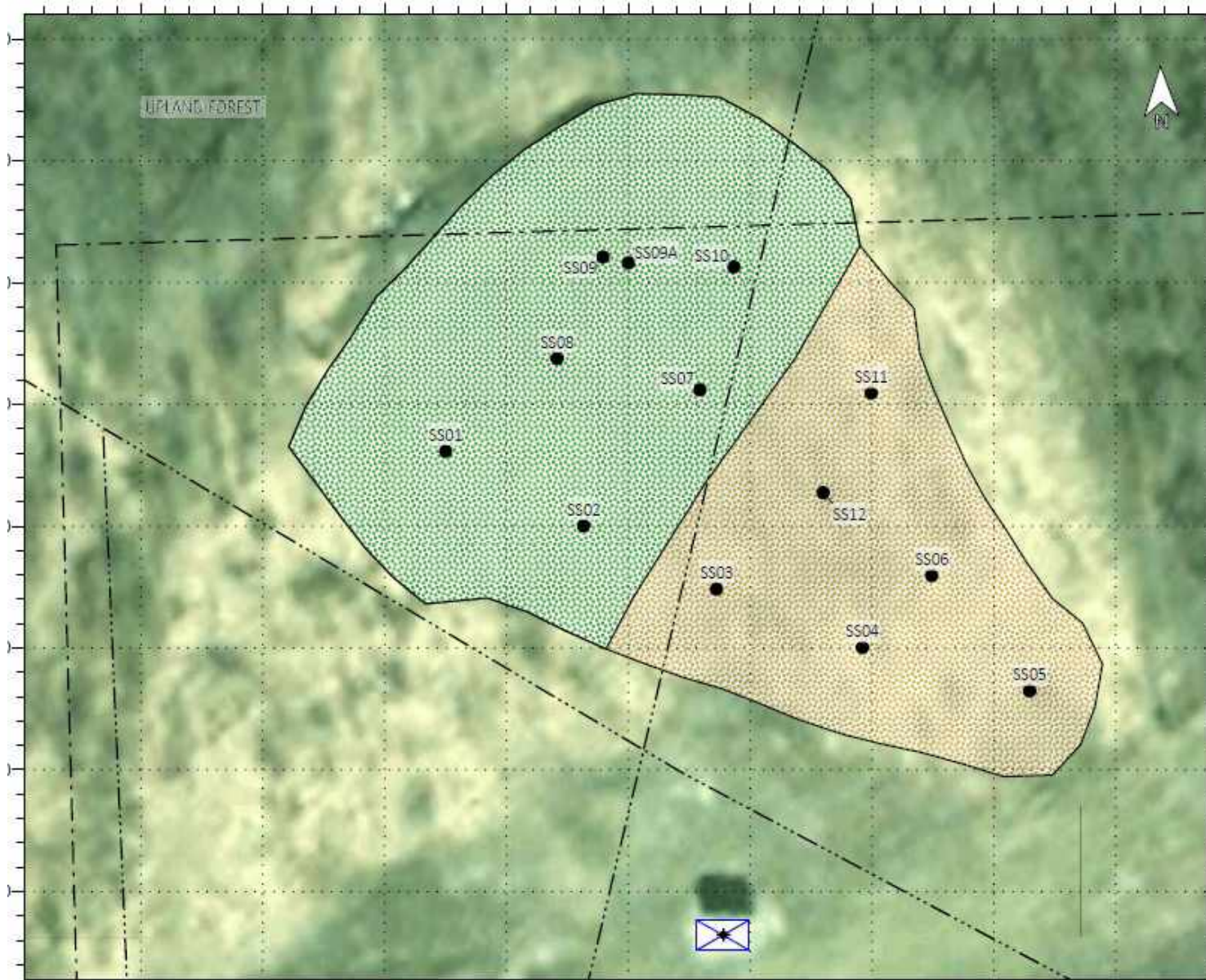
	CMH3					UW4					PGPR concentration
	1	2	3	4	5	1	2	3	4	5	
ARG	1	1	1	0	0	0	1	1	0	1	
PRG	-1	1	-1	0	-2	0	0	-1	-2	-1	
TF	0	-1	0	-1	-2	0	-1	-2	0	0	
TWG	1	1	1	0	1	1	1	0	1	N/A	
JG	1	2	1	1	0	1	0	0	1	1	
NWG	-1	-1	-1	0	0	1	2	2	2	2	
SBG	-1	-1	-2	-1	-1	-1	-1	0	-1	1	
THG	0	NA	1	2	2						
WWG	0	NA	NA	-1	-2	-1	-2	-2	-2	0	
GNG						NA	NA	NA	NA	NA	

10-12 Reclamation Site

Site is in Slave Lake area

- Seed – Central Mixedwood

Sample location	2020			2021			2022		
	height (cm)	cover (%)	veg dry weight	height (cm)	cover (%)	veg dry weight	height (cm)	cover (%)	veg dry weight
Treated									
SS01	77	90	44.9	90	85	25.2	72	70	-
SS02	88	97	-	75	75	-	53	50	-
SS07	87	100	67.2	60	70	11.1	84	60	-
SS08	70	65	-	77	55	-	85	70	-
SS09	80	100	43.0	102	85	49.5	92	85	-
SS10	60	70	-	92	85	-	91	85	-
avg.	77	87	51.7	83	76	28.6	80	70	-
Untreated									
SS03	83	100	-	89	90	-	103	90	-
SS04	79	100	54.8	100	90	67.1	113	95	-
SS05	73	100	102.4	108	80	42.6	113	90	-
SS06	84	100	-	98	75	-	89	75	-
SS11	89	100	-	94	90	-	106	95	-
SS12	83	100	98.7			72.0	111	90	-
avg.	82	100	85.3	98	85	60.6	106	89	-



2021

34% Slender Wheatgrass
15% Rocky Mountain Fescue
35% Coated Fringed Bromegrass
3% Tufted Hairgrass
8% Coated Junegrass
3% Fowl Bluegrass
2% Tickelgrass

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.

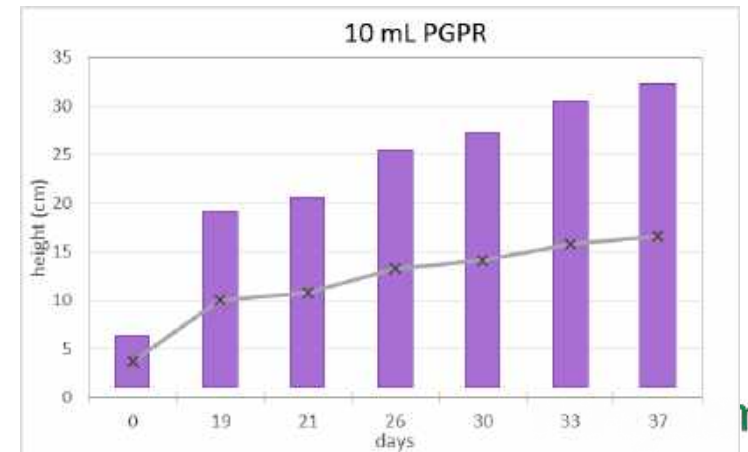
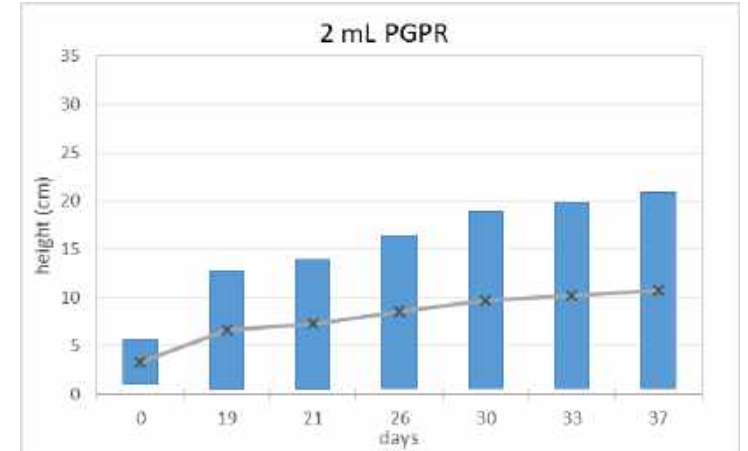
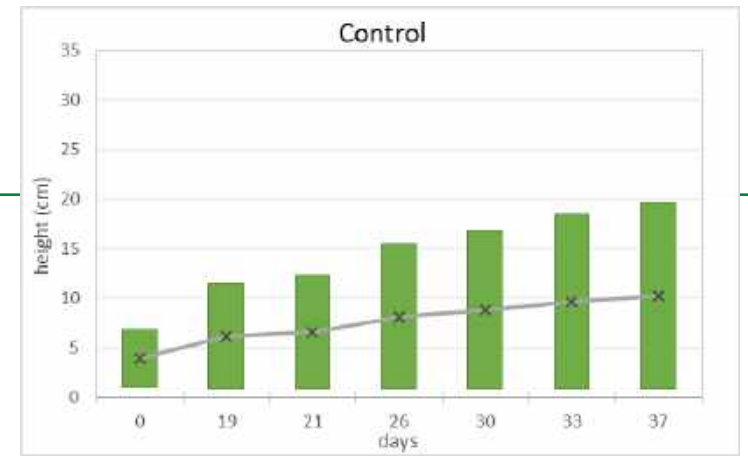
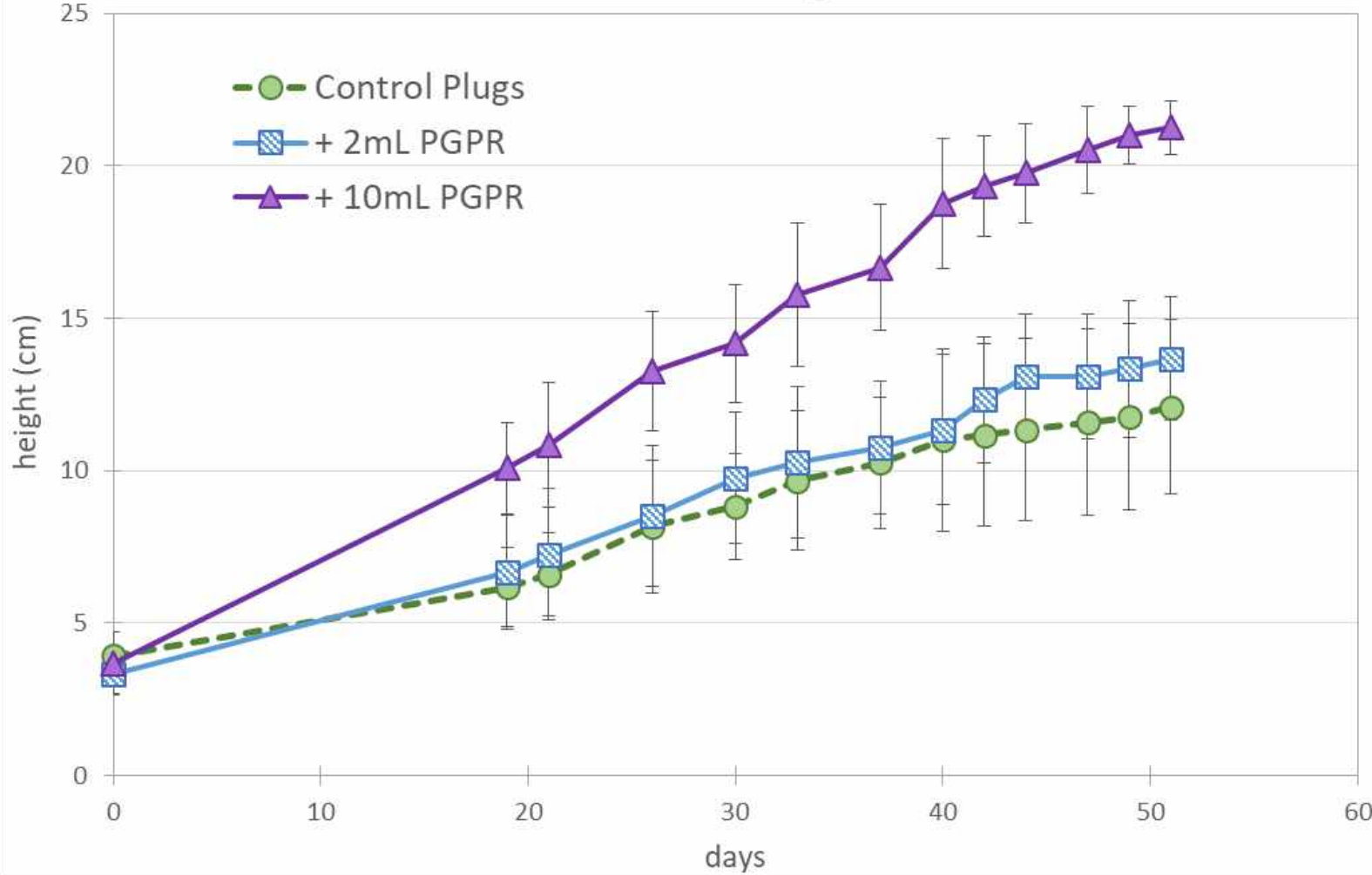
Pots were not fertilized.

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

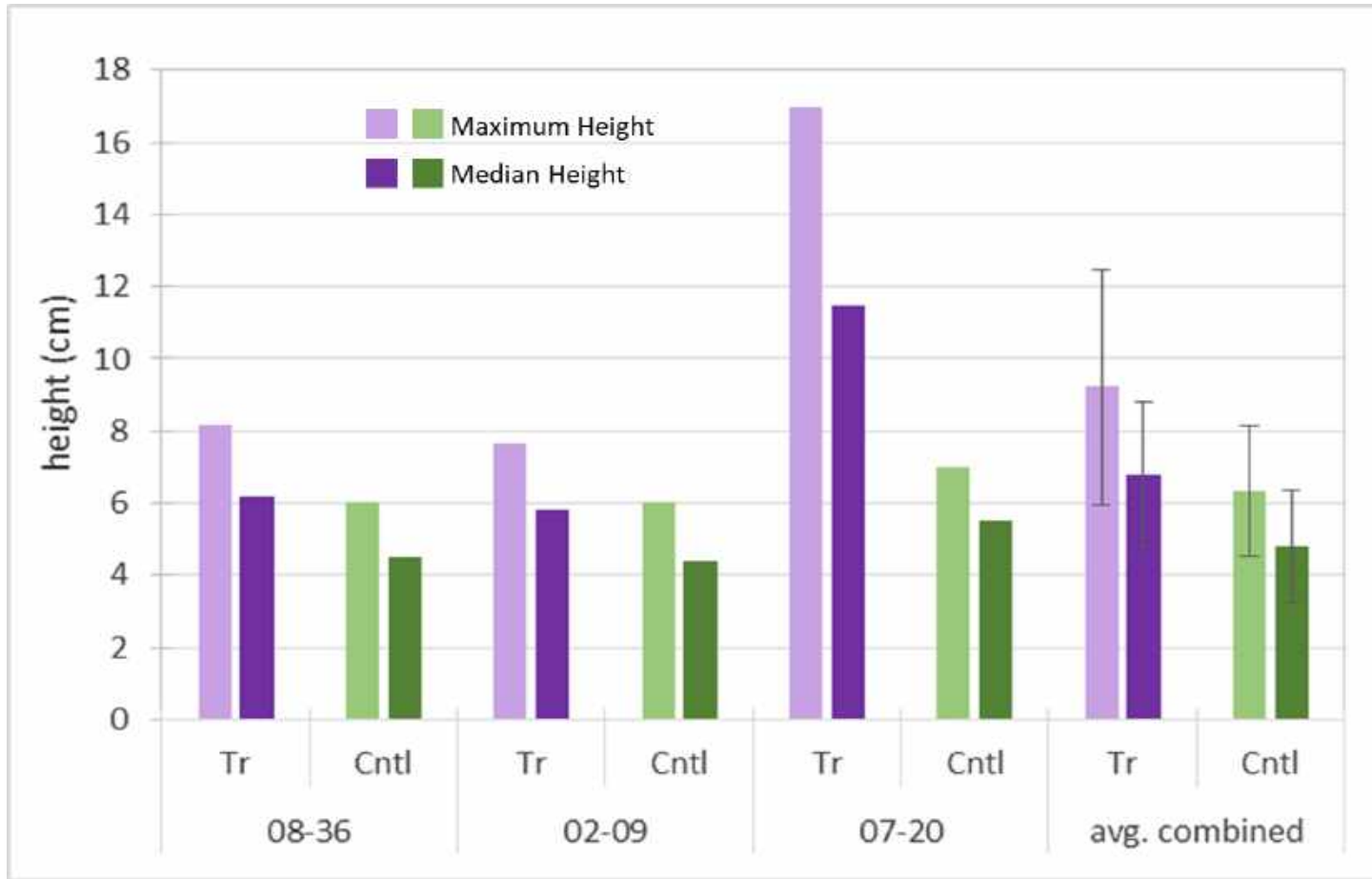
Growth was monitored for 7 weeks.

Plant Height

Median Height



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



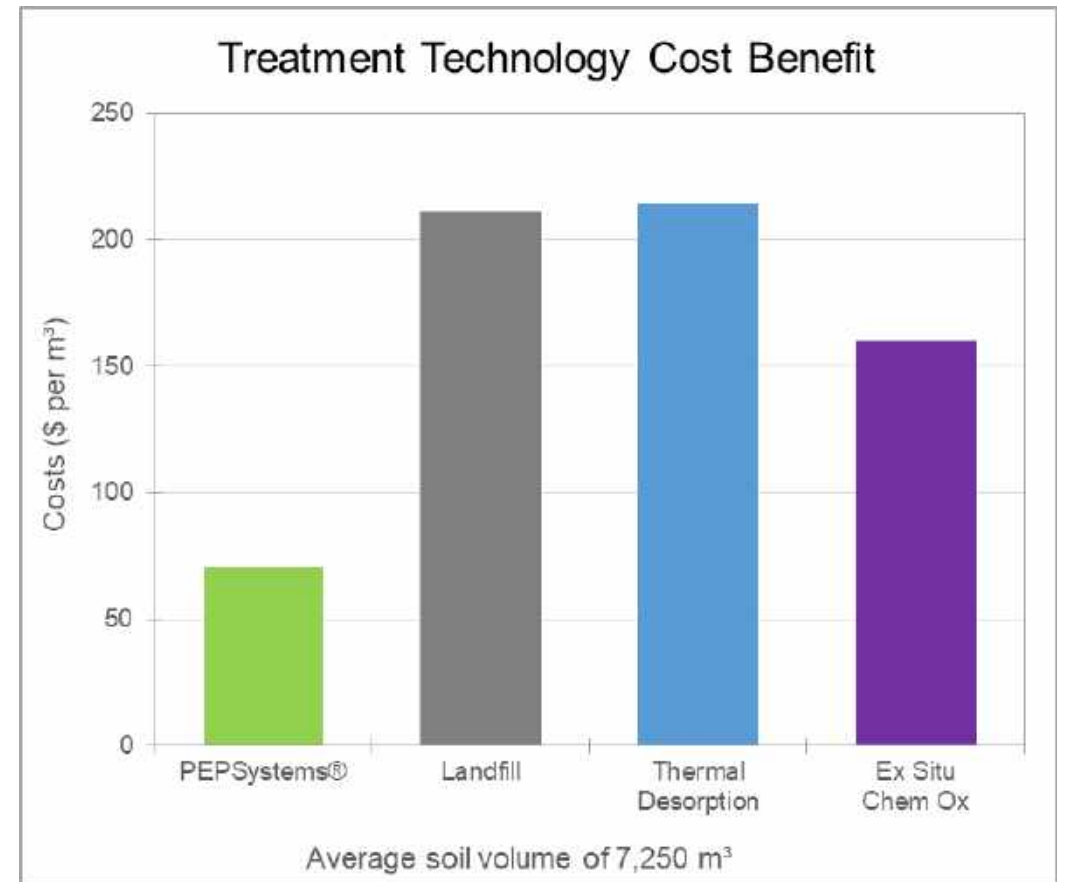
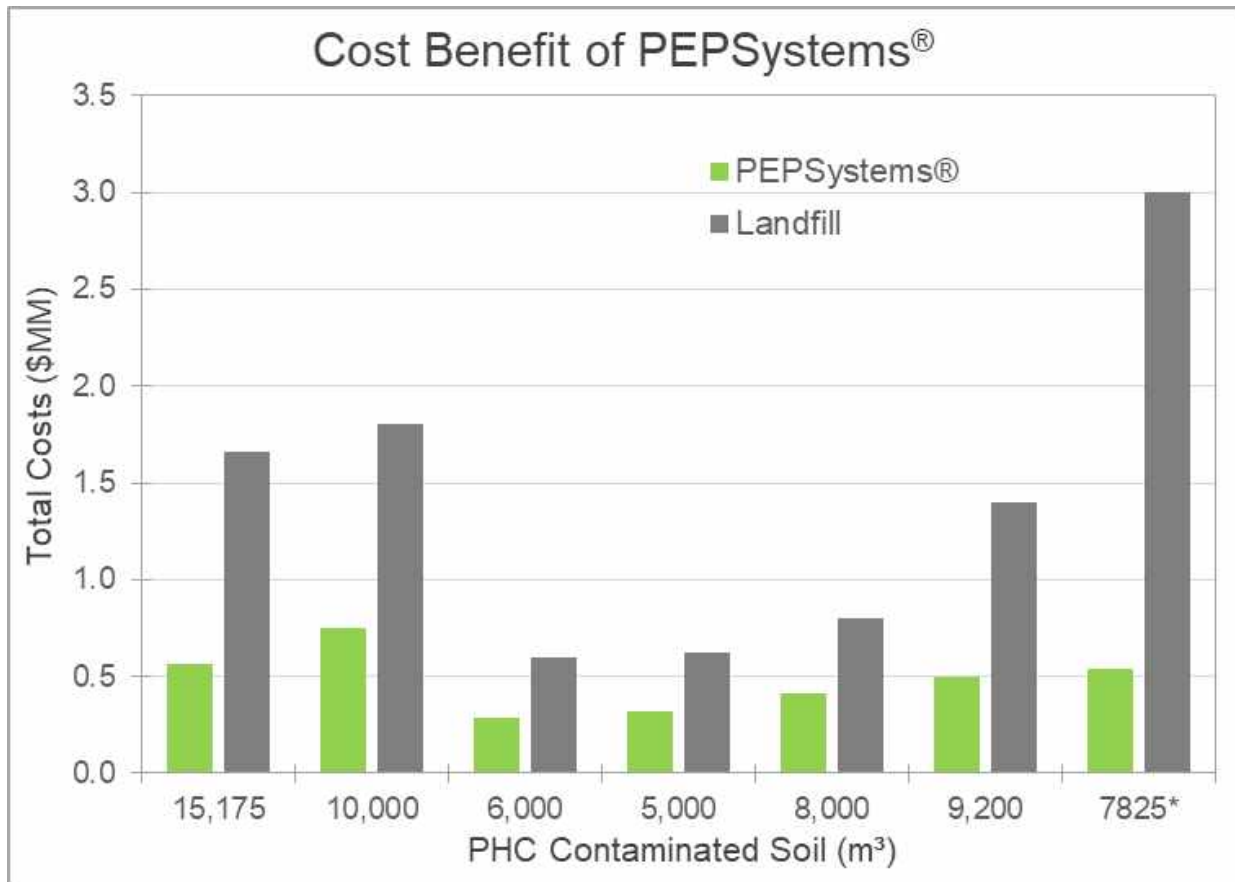
What's Next with Native Species

The potential is there but more work is needed:

- Seed is more expensive and harder to get in large volumes.
- Will take more time to get native species established.
- Might be a hard sell for PHC – speed matters/site closure.
- Salt remediation is likely a better fit.
- Less \$\$ for equipment/soil manipulation onsite.
- Better option for site where disturbance is not wanted.
- Biomass? Therefore efficiency?

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:

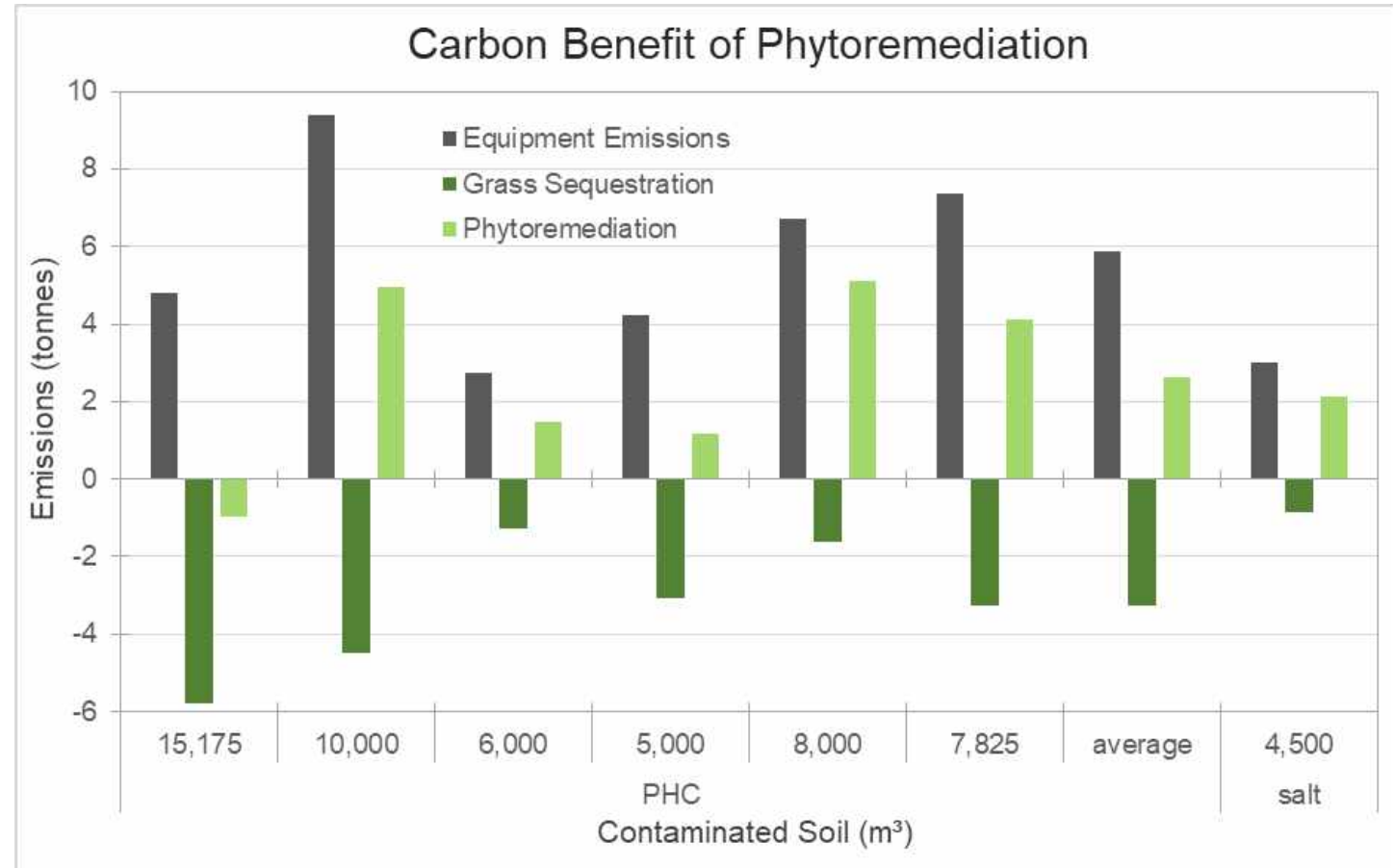
- equipment in phytoremediation activities
- trucking to nearest landfill

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.
- Jones and Donnelly. 2004. New Phytologist 164:423–439.
- Hungate et al. 1997. Nature 388:576-579.
- Integrated Crop Management Volume 11-2010.



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

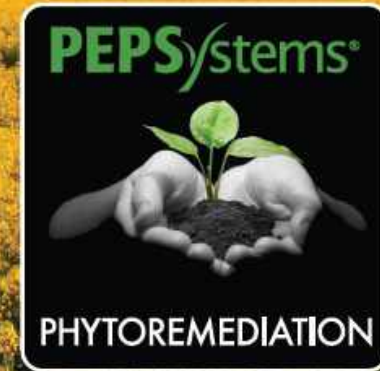
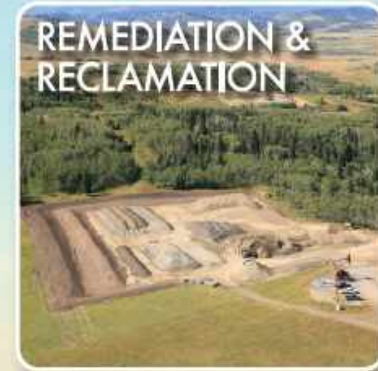
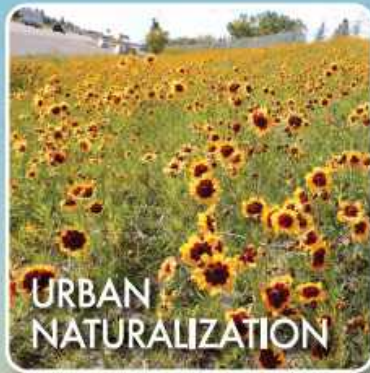
National Research Council – Industrial Research Assistance Program (IRAP).

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

Thank You
Questions?

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