Phytotechnologies for Remediation & Reclamation of High Salinity Soil

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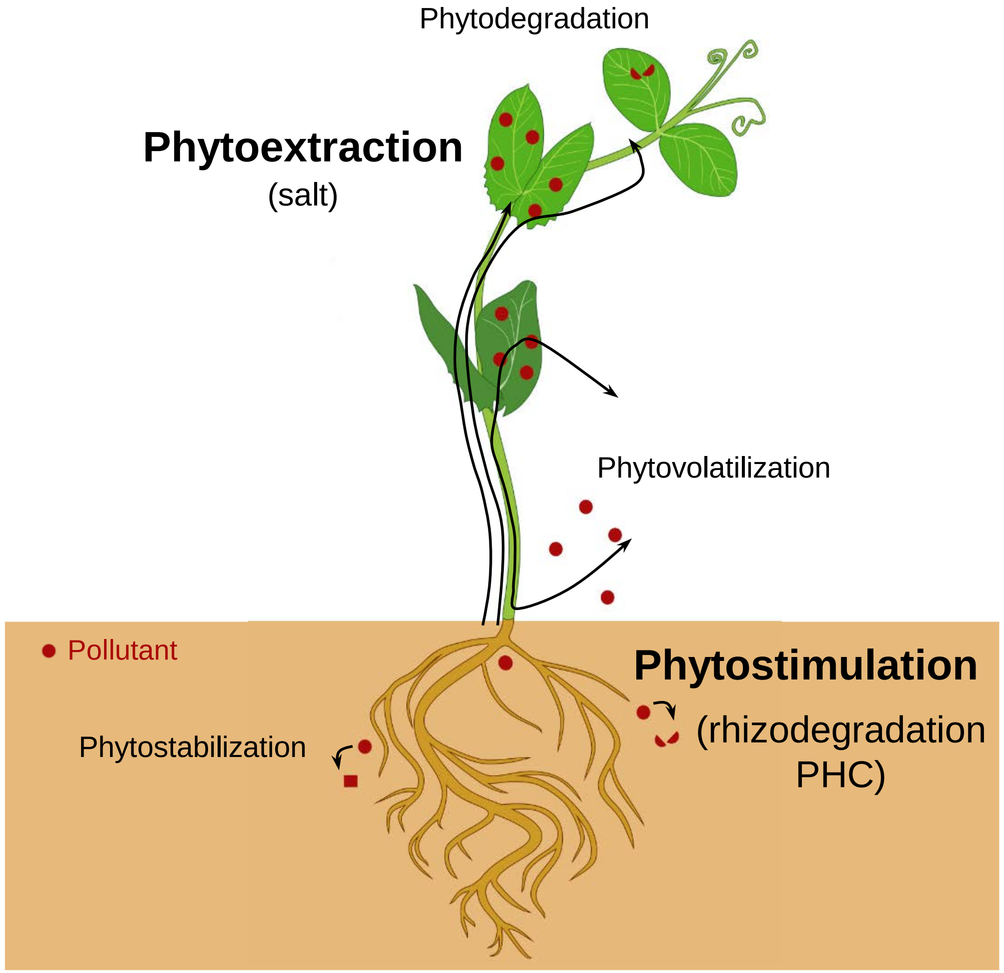
Earthmaster Environmental Strategies Inc.

A Canadian environmental technologies company:

- Based in Calgary, Alberta.
- Founded in 1998.
- Specializes in providing environmental services to the commercial/industrial and upstream oil and gas industries in Western Canada.
- Team of environmental consultants consisting of professional agrologists, biologists, chemists, ecologists, engineers, geoscientists, soil scientists, plant scientists, aquatic specialists, and foresters.
- Co-developed commercial phytoremediation systems (PEPSystems®) to treat contaminated soil in an eco-friendly and responsible manner.

Phytotechnology – How it Works



- Improved rhizosphere
  - Soil
  - Organic matter
  - Bacteria
  - Water
  - Roots
  - Contaminants
- Phytostimulation

  soil →bacteria degradation
  - Petroleum Hydrocarbons
- Phytoextraction

  soil→root→foliage
  - Salts
  - Metals

Challenge – getting the plants to grow.

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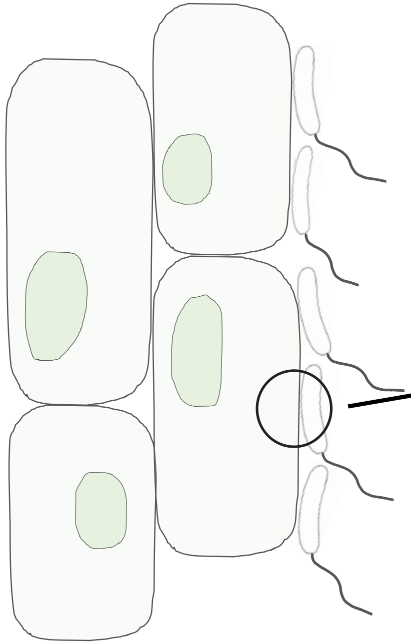
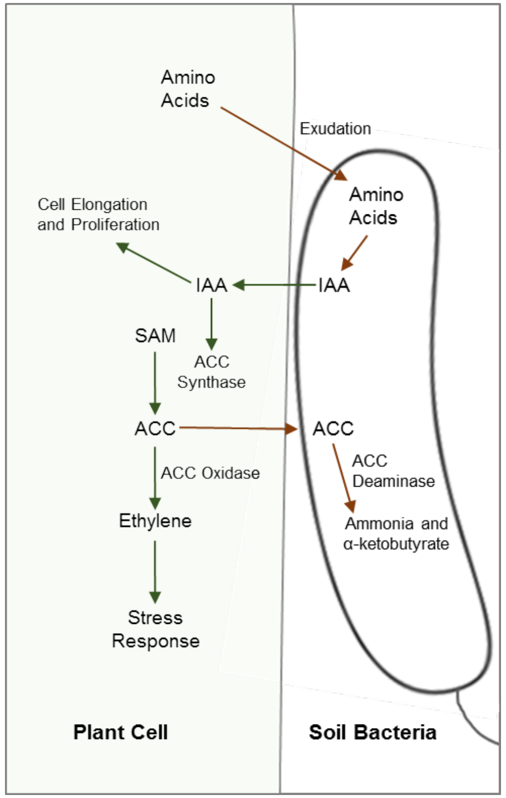
Plant Growth Promoting Rhizobacteria (PGPR) - Enhanced Phytoremediation Systems

PGPR – Facilitating Plant Growth in Challenging Conditions

Plant seeds:
Coated with PGPR

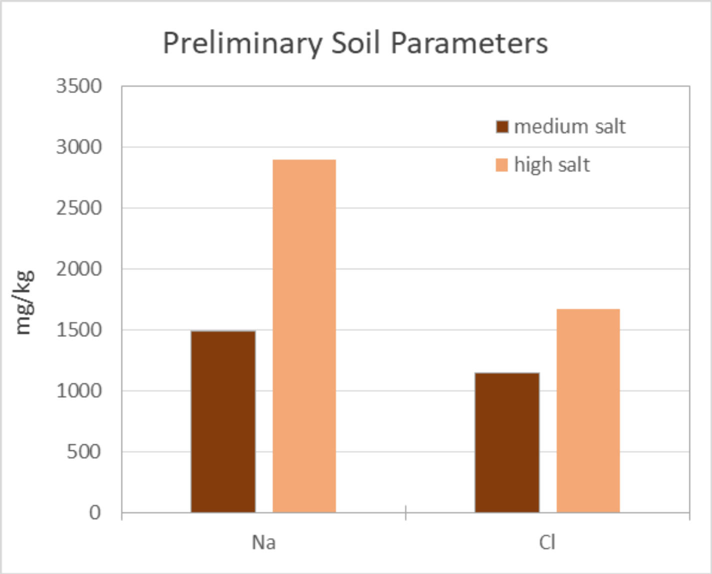
Active rhizosphere:
PGPR co-localize with developing roots

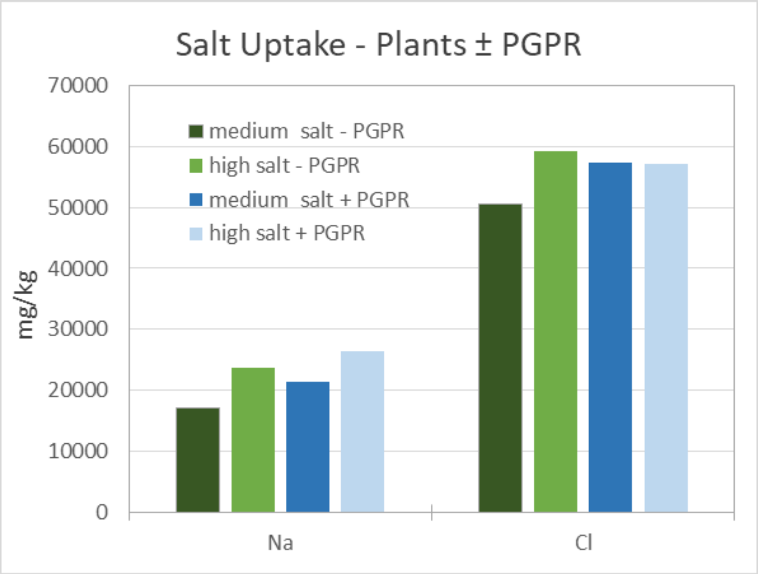


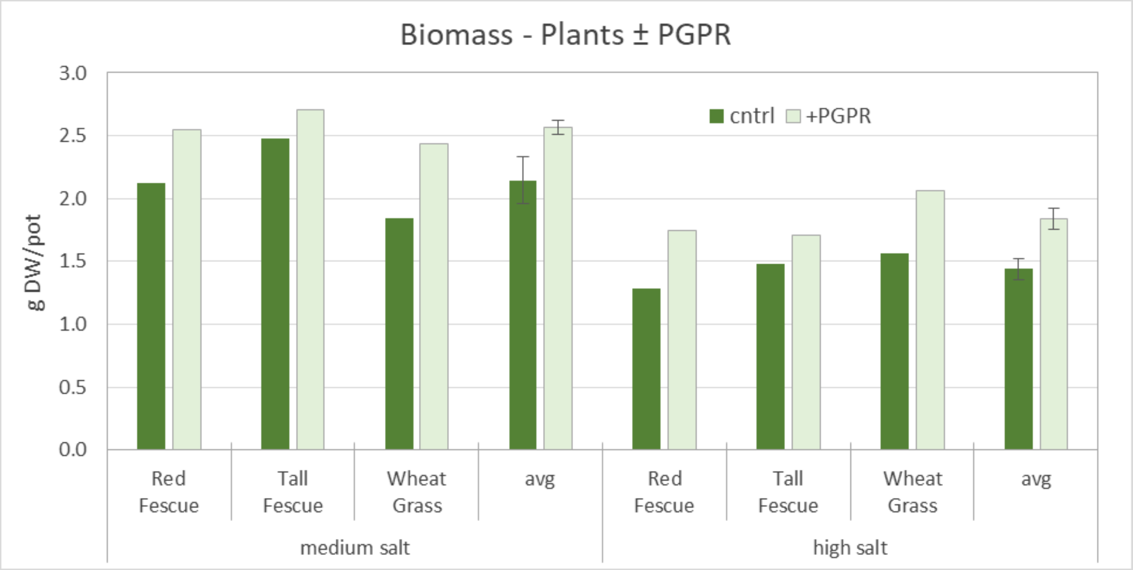


- ↓ Stress ethylene
- ↑ Plant vigor
- ↑ Root development
- ↑ Rhizobacteria
- ↑ Leaves
- ↑ Salt and metals uptake into plant tissue
- ↑ Degradation of PHC in soil

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.

Seed Germination Studies - Produced Water Details

Laboratory studies using produced water – goal is to increase plant biomass.

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 -
Groundwater Guidelines									
Alberta Tier 1 Groundwater Remediation Guidelines Agricultural Land Use: Fine Soil	100	-	-	-	200	429	6.5-8.5	1	5
Surface Water Guidelines									
AEP EQGASW - Protection of Aquatic Life (PAL)	120	-	-	-	-	429	6.5-9.0	-	-
Water samples									
Produced Water 1	36775	4223	406	498	18430	1372	7.1	72750	71.5
Produced Water 2	78870	9264	4054	1839	32970	997	6.5	125000	81.9

Seed Germination Studies

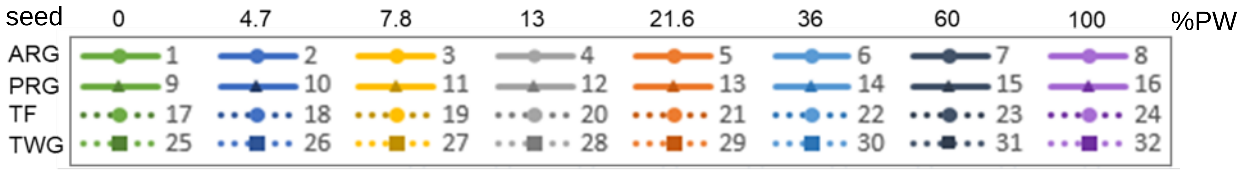
Seeds
- 4 individual species
- 20 per plate
- +/- PGPR
- +/- other additives

Growing surface
- Whatman filter paper

Contaminant
- Produced water
- Range of 0-100%

Growth conditions
- 25°C for 14 days

Sample data generated:



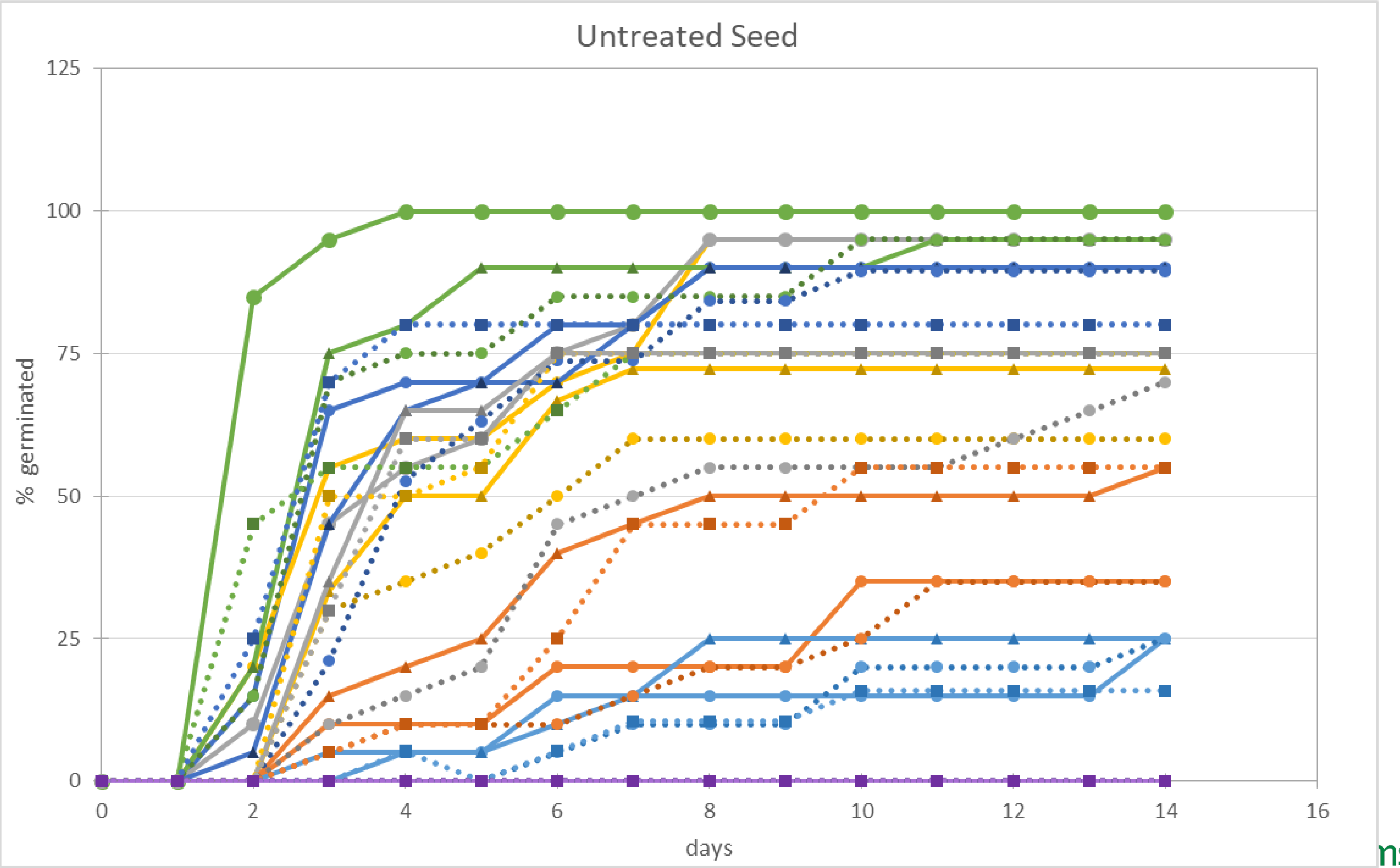
Analysis:

Growth curves over a range of PW concentrations

Lethal concentration (LC50) – concentration required to kill 50% of the population (prevent germination)

Mean germination time (MGT) – time to emergence

% germinated - out of 20 seeds



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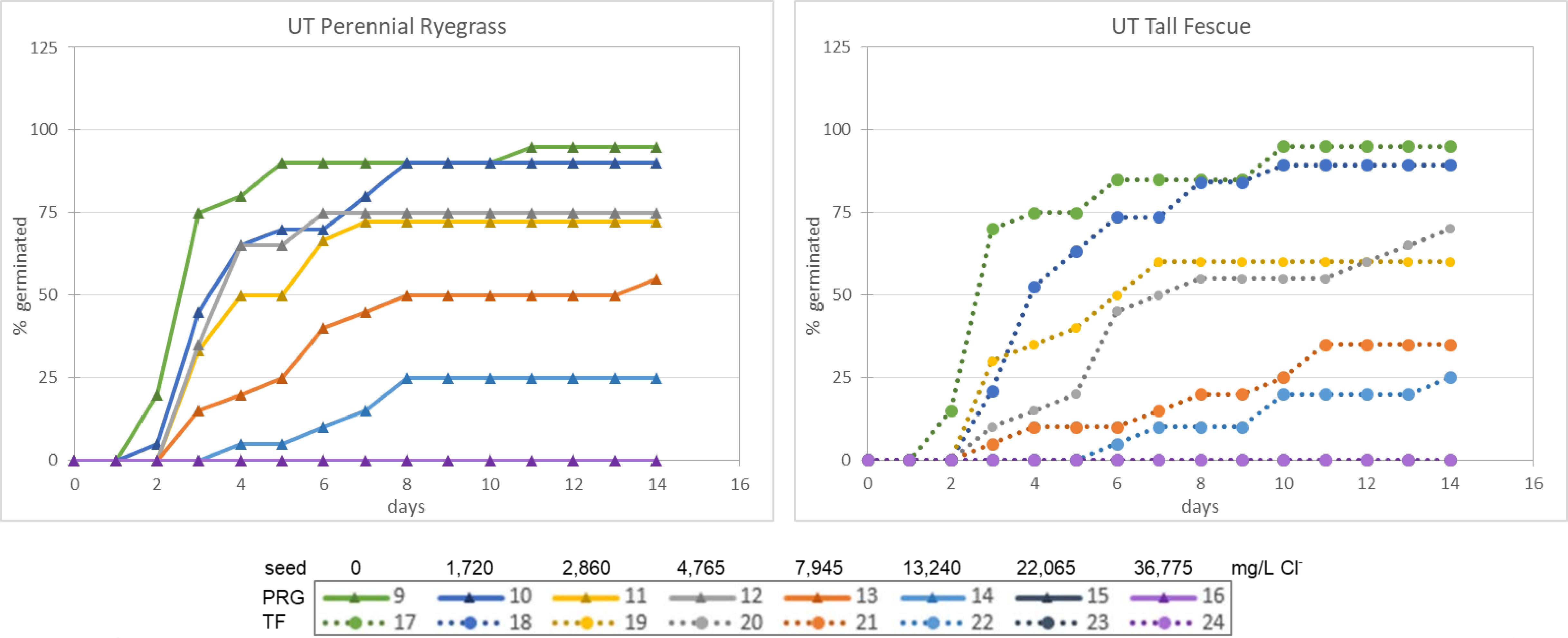
Effect of Produced Water

Untreated seed:



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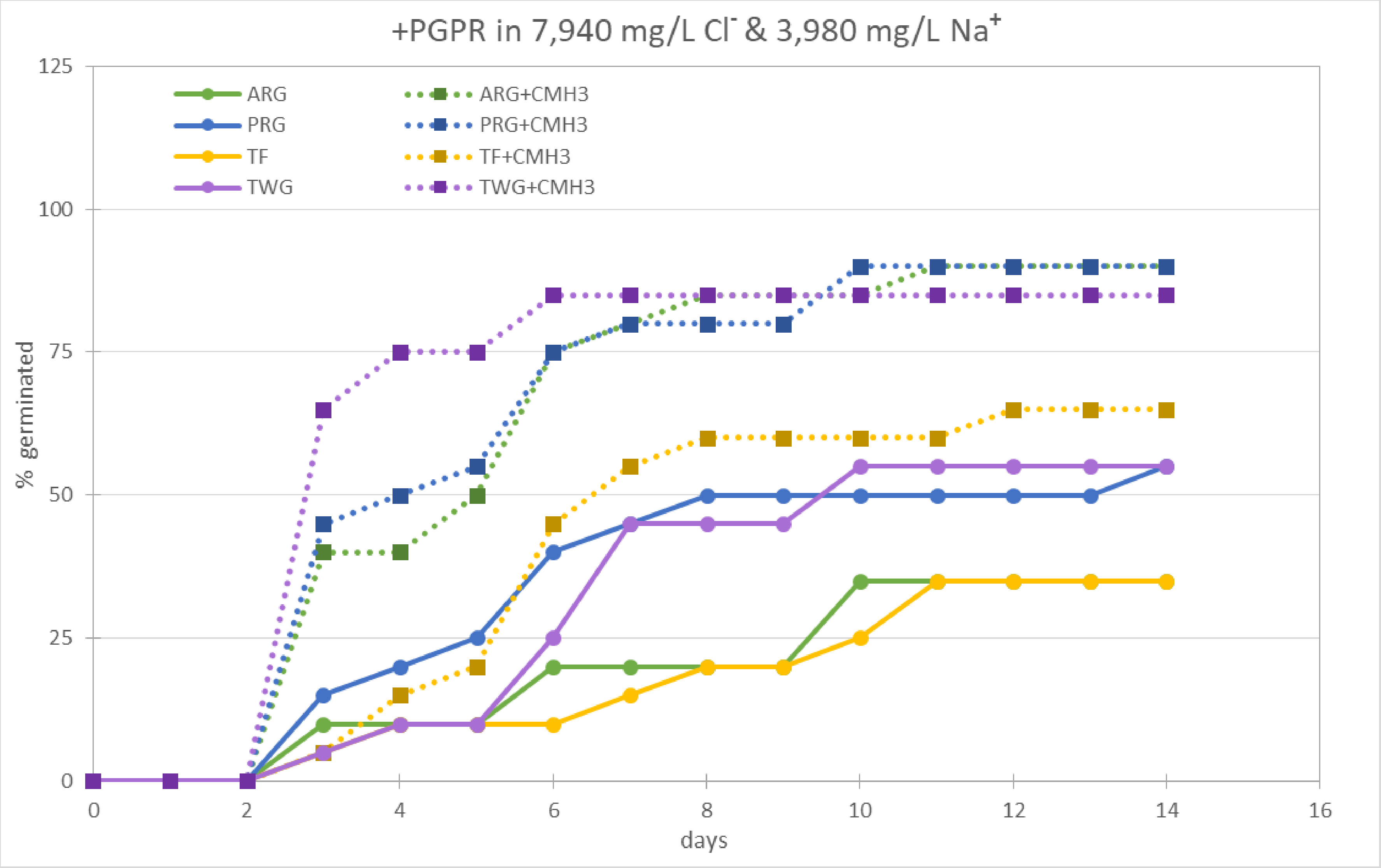
The Effects of PGPR

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

Generate the LC50 values from these curves.



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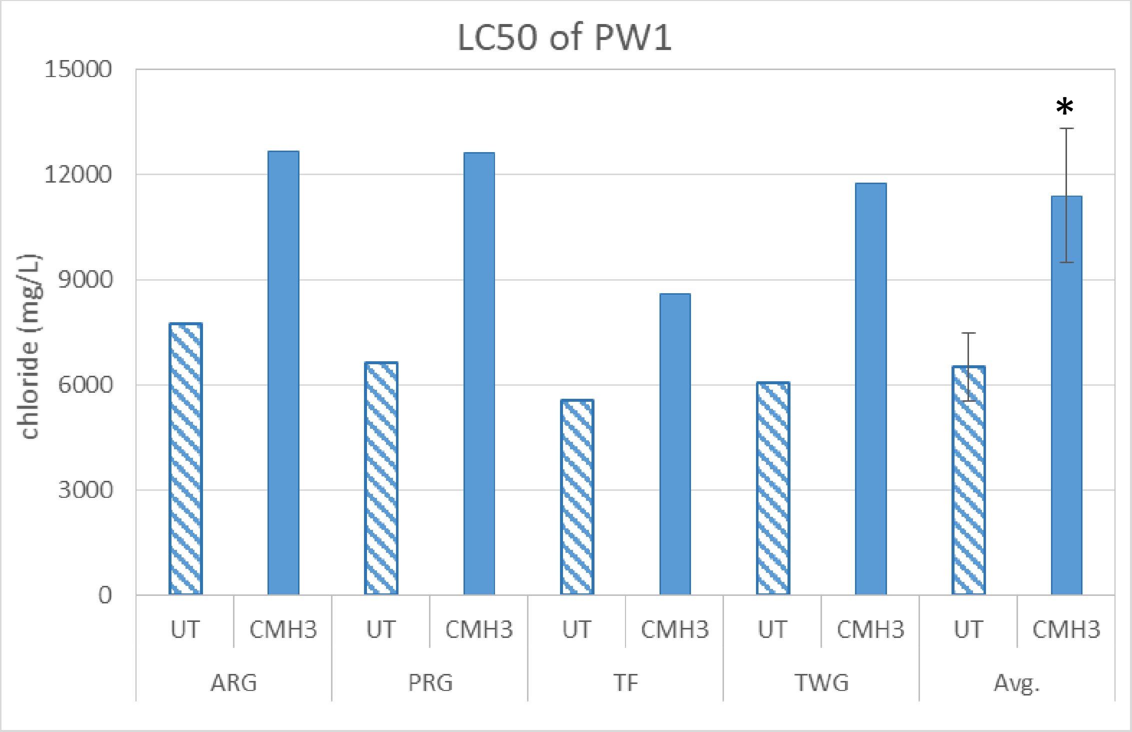
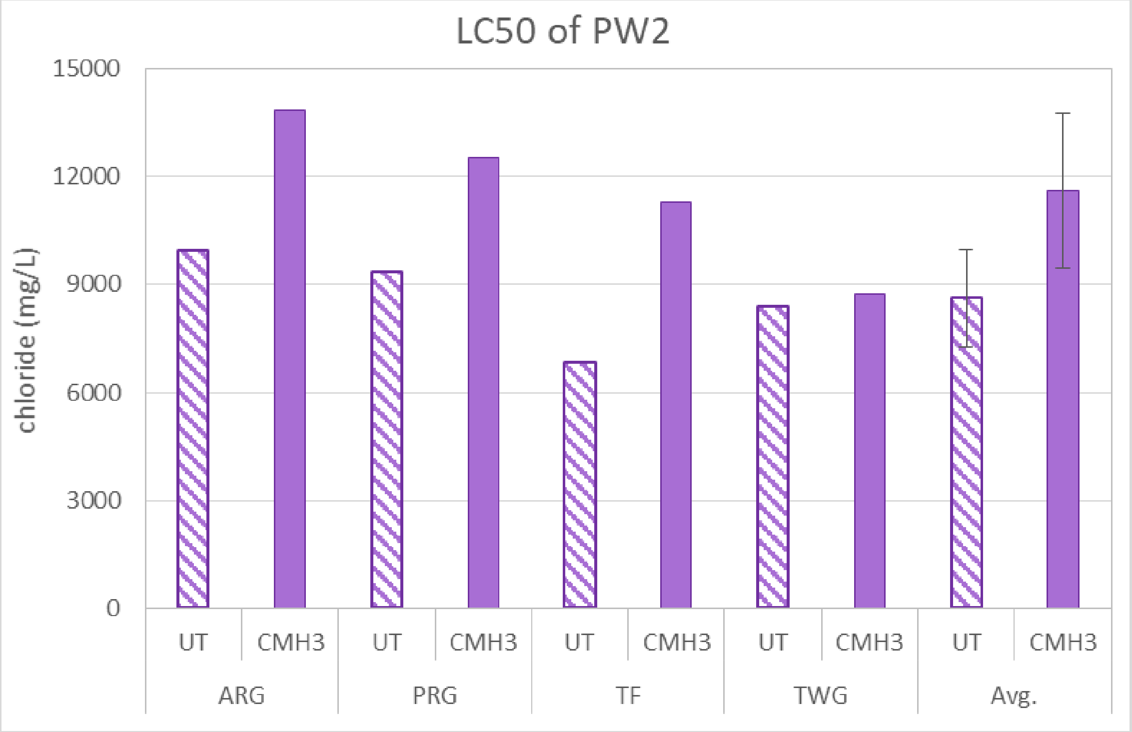
Quantifying the Effects of PGPR - LC50





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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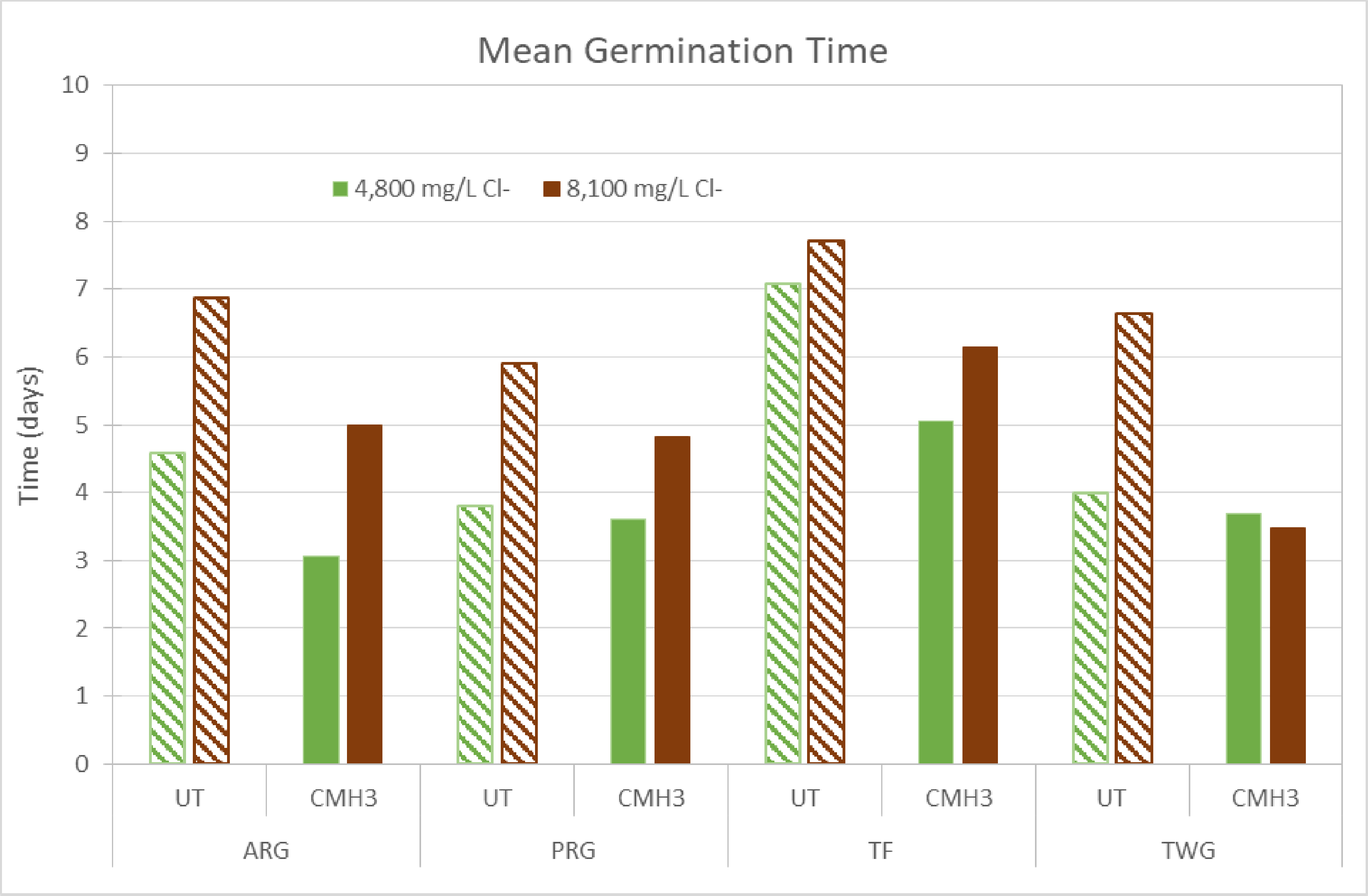
Quantifying the Effects of PGPR - MGT

% Change in MGT		
seed	**4,800 mg/L**	**8,100 mg/L**
ARG	-33	-27
PRG	-5	-18
TF	-29	-20
TWG	-8	-48



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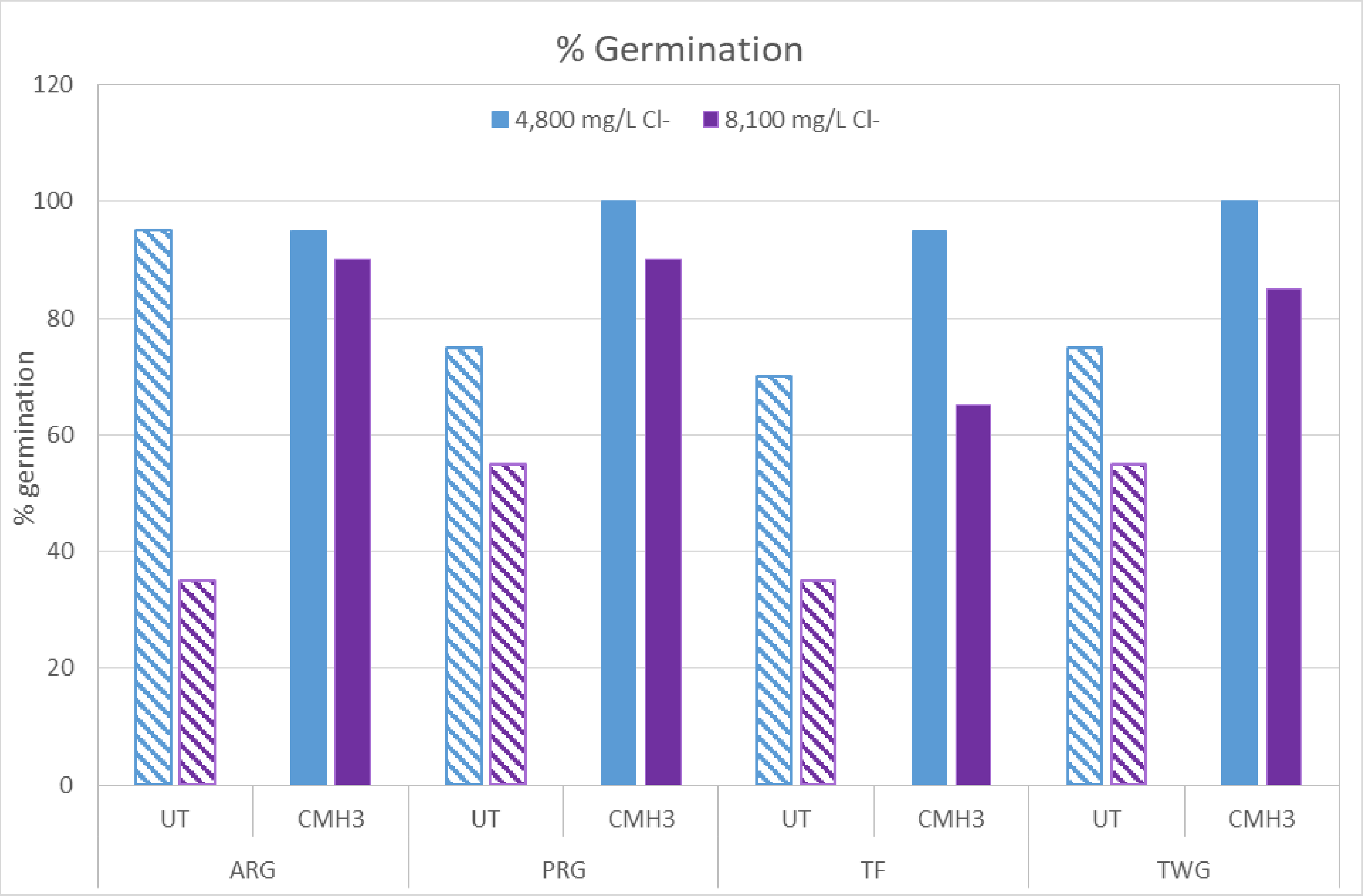
Quantifying the Effects of PGPR - % Germination

% Change in Germination		
seed	**4,800 mg/L**	**8,100 mg/L**
ARG	0	157
PRG	33	64
TF	36	86
TWG	33	55



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Germination Studies With Additives

Goal – Improve seed germination dynamics by including additional substances in the seed coating.

Salt remediation is done through plant uptake:

- Sodium (Na^+)
- Chloride (Cl^-)

Uptake amounts are generally fixed for PEPSystems grass mix:

- ~16 g/kg sodium
- ~40 g/kg chloride

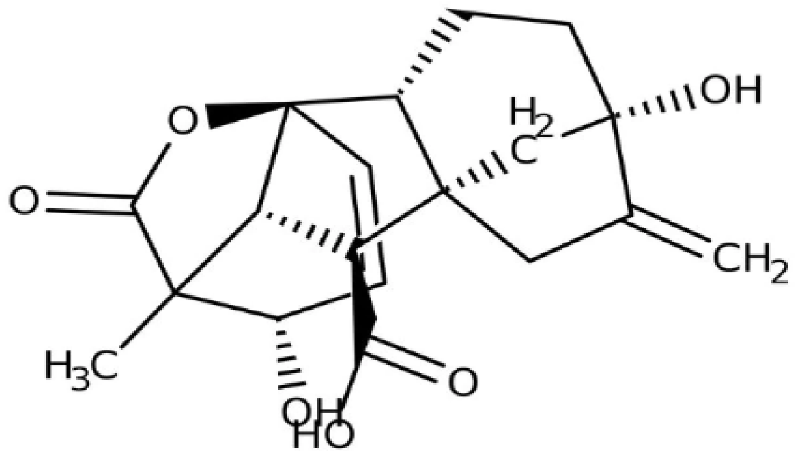
↑ remediation by ↑ plant biomass.

Improved germination should lead to better growth and more biomass in the field.

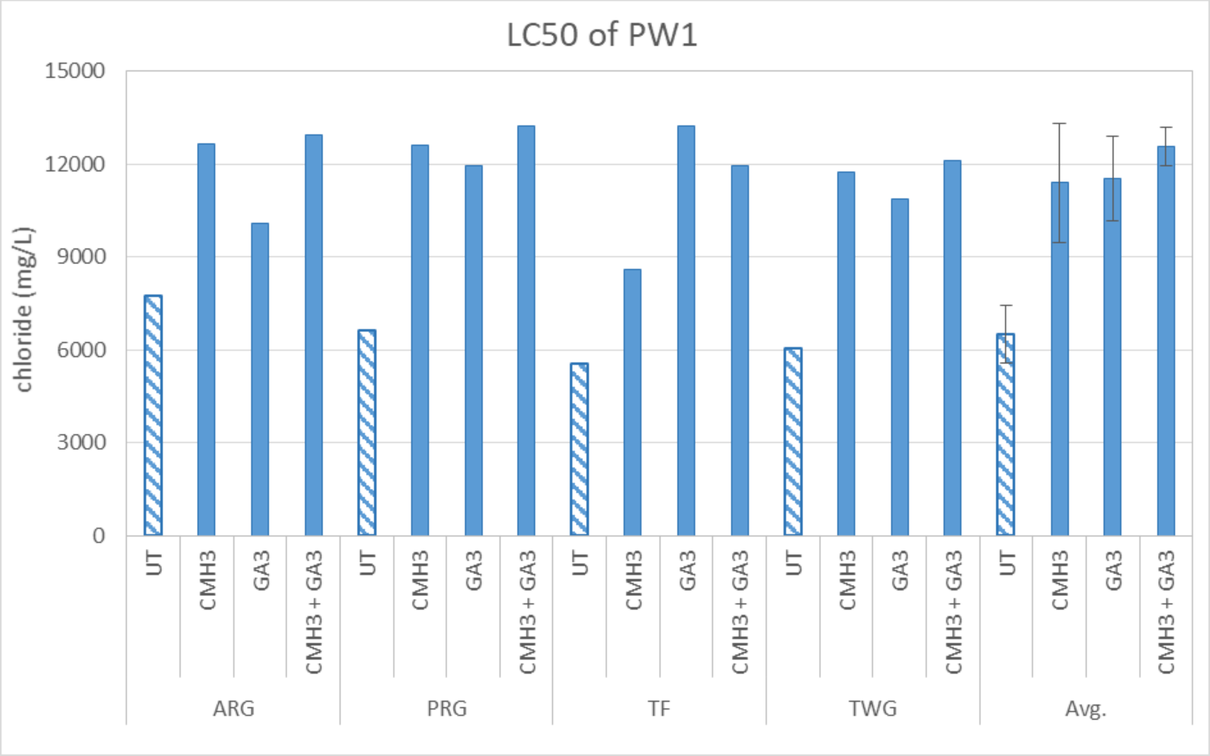
Gibberellin (GA3)

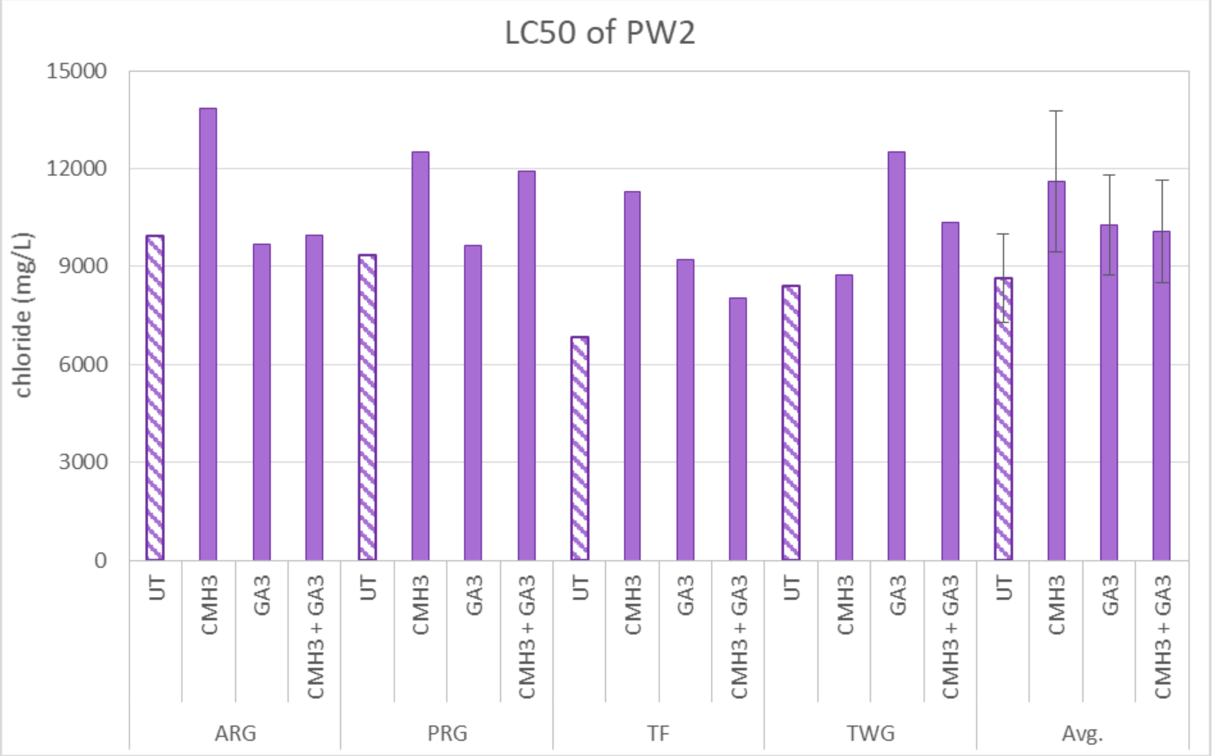
Plant hormone – regulates germination, stem elongation, etc.

- Chemical messenger that breaks seed dormancy
- Should function to promote germination and stem growth
- Stress ethylene can reduce the amount of bioactive GA
- Can treating the seed with GA3 should overcome this?



Quantifying the Effects of GA3 - LC50

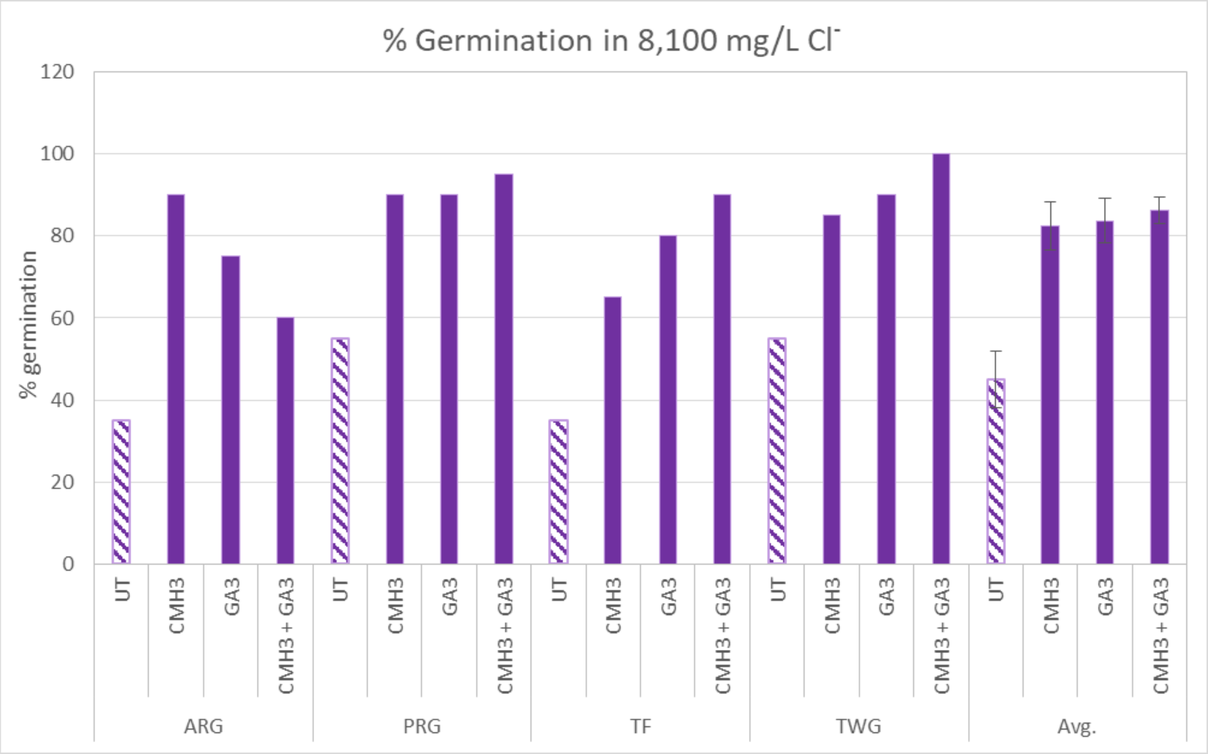


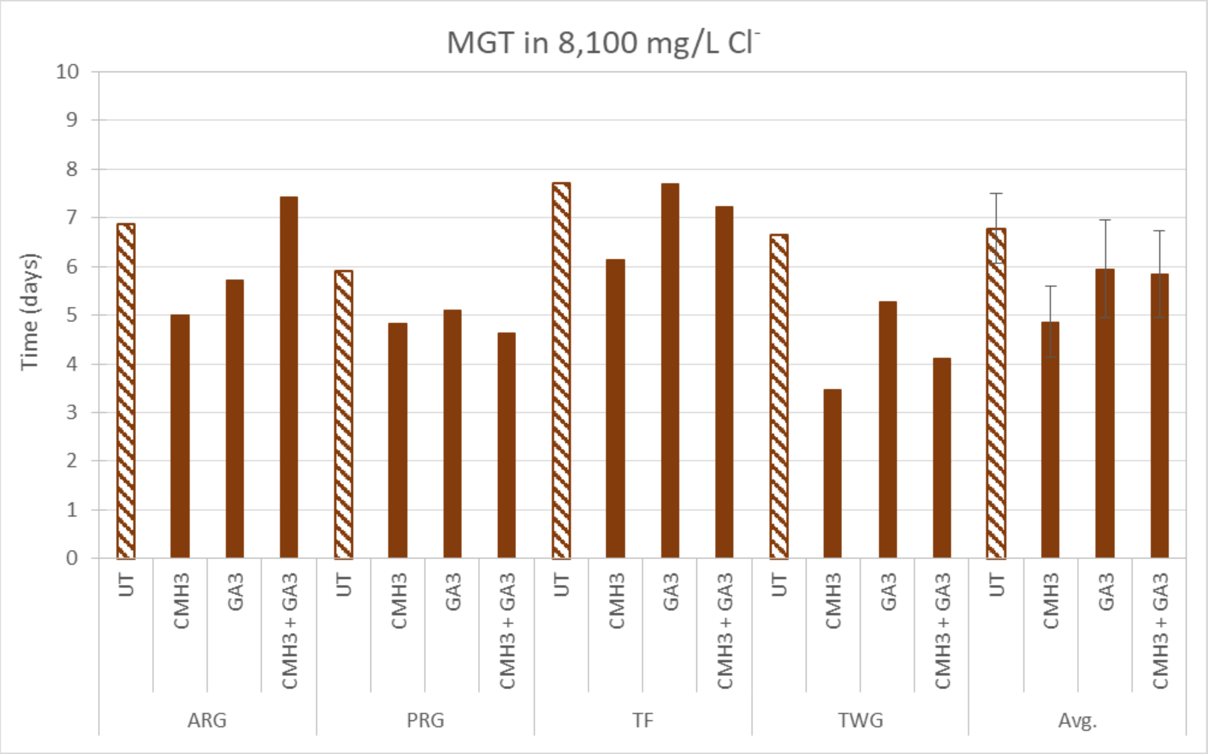


Want to see an increase in chloride concentration at 50% lethal concentration.

- GA3 appears to offer an advantage to TF seeds
- There doesn't appear to be an advantage overall over CMH3

Quantifying the Effects of GA3 - Germination





Want to see an increase in % germination and a decrease in MGT

- GA3 appears to offer a slight advantage in MGT to ARG and TF
- There doesn't appear to be an advantage overall over CMH3

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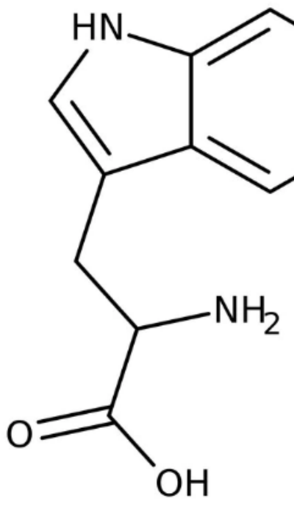
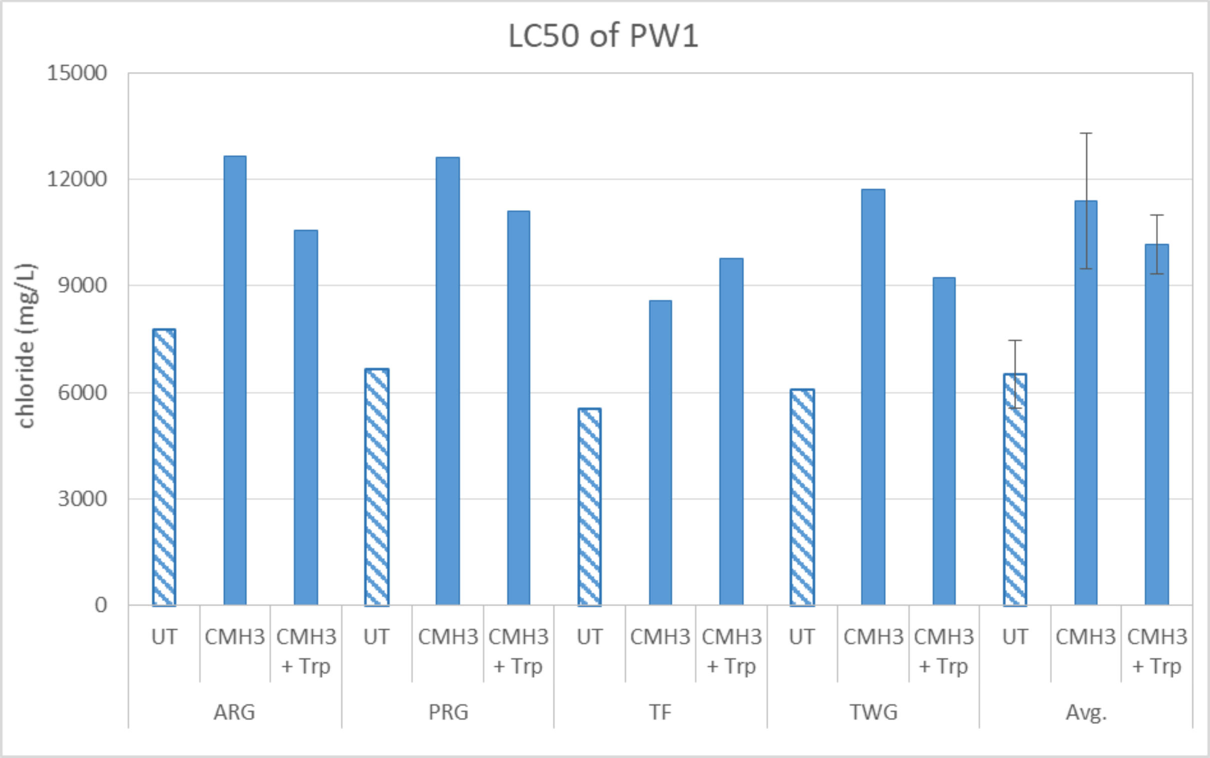
L-Tryptophan

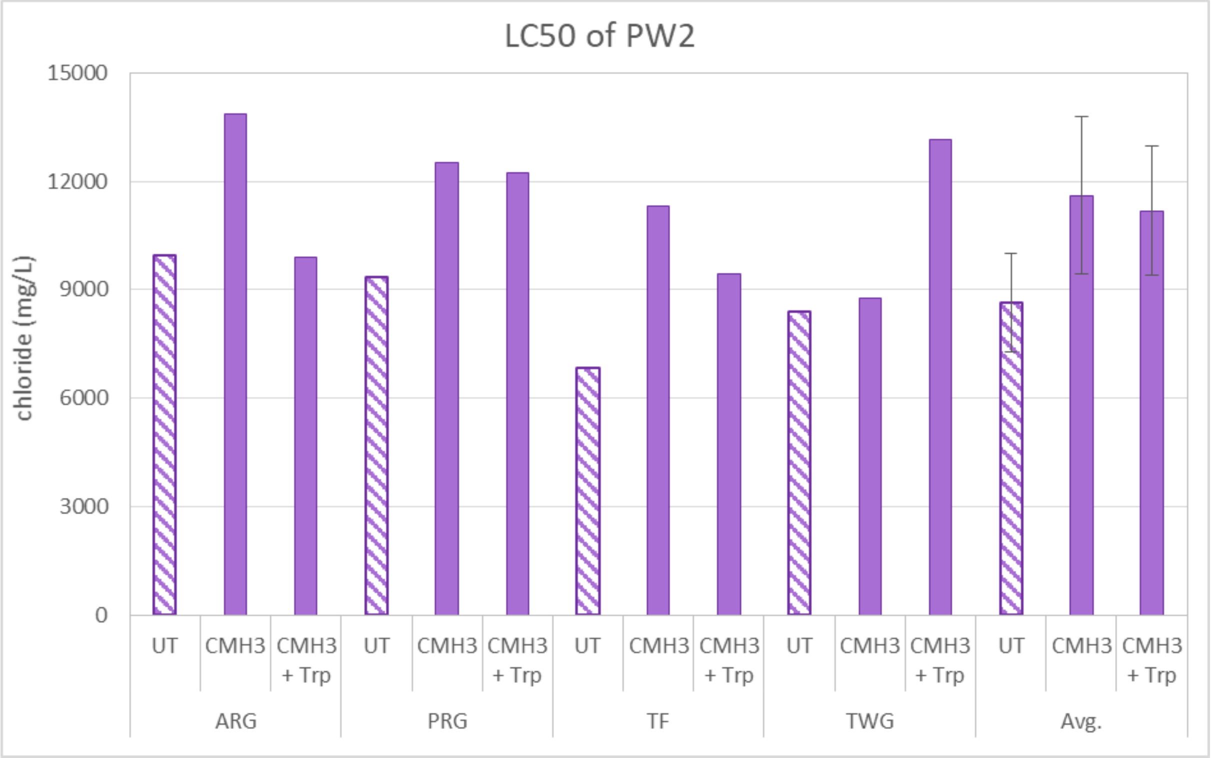
Precursor of IAA plant hormone – naturally present in root exudate

- Converted to IAA by PGPR
- Addition of L-tryptophan to soil can increase plant growth



Quantifying the Effects of Tryptophan – LC50

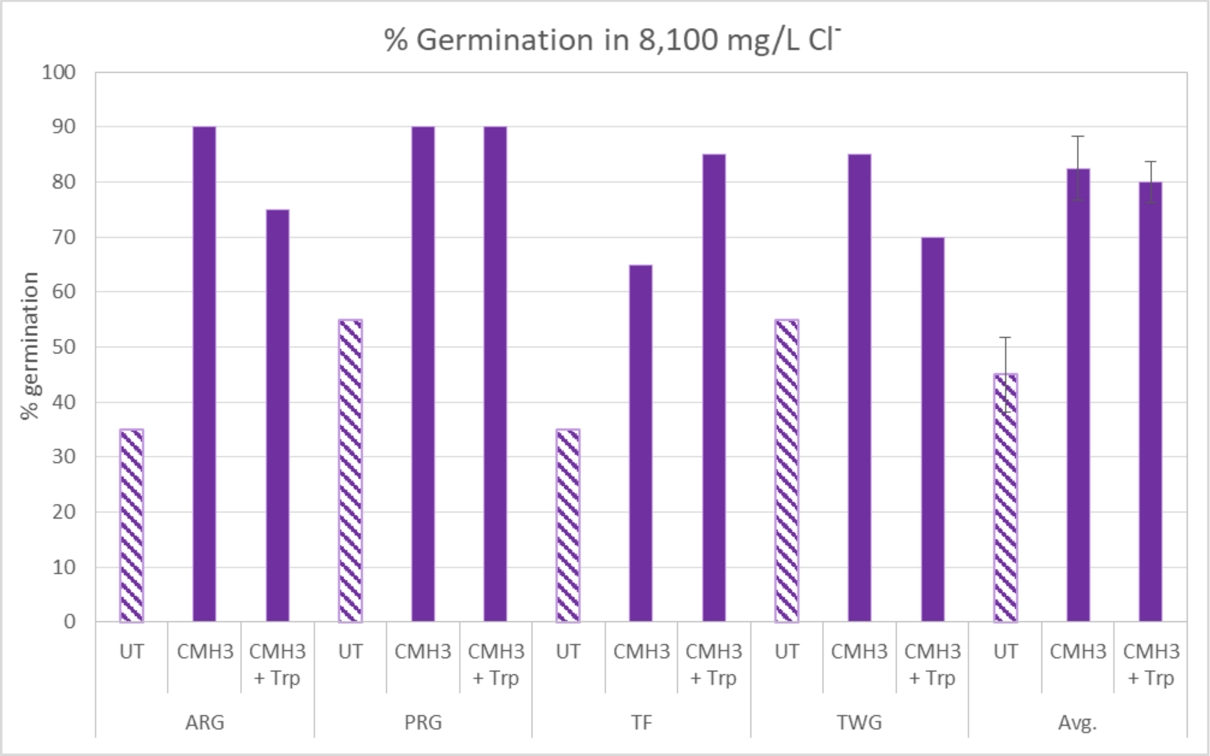


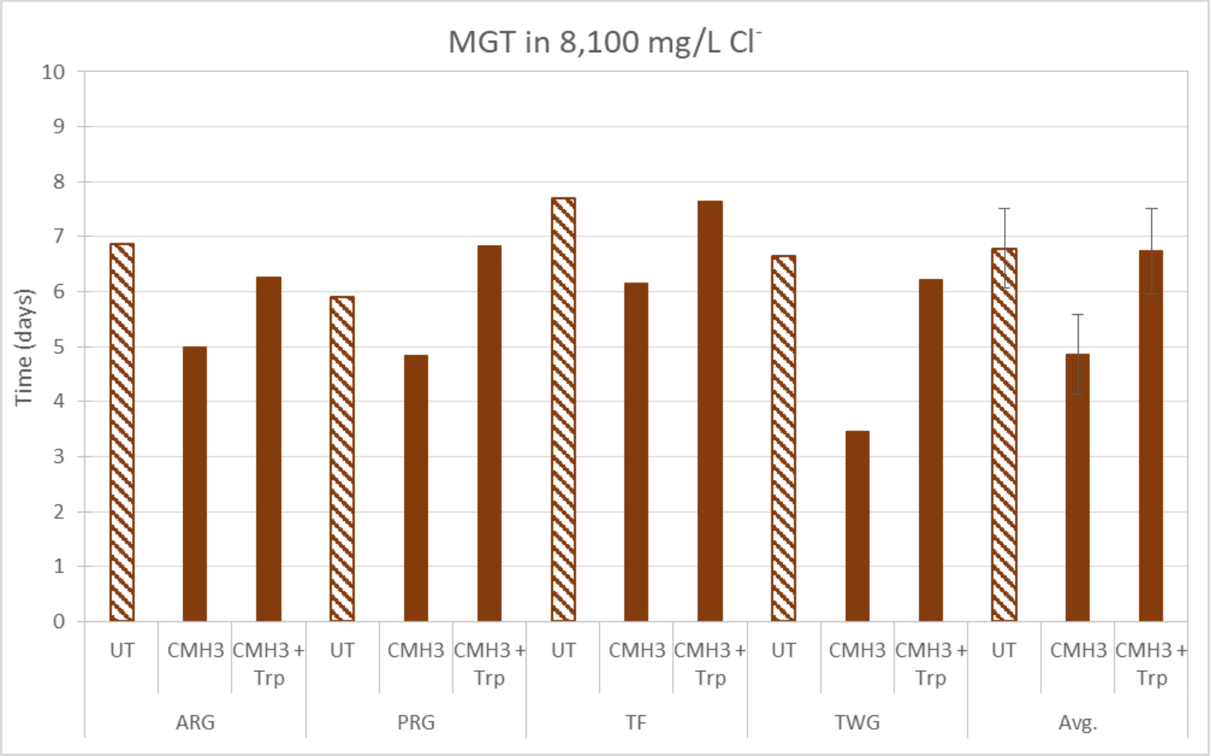


Want to see an increase in chloride concentration at 50% lethal concentration.

- There doesn't appear to be an advantage overall over CMH3

Quantifying the Effects of Tryptophan - Germination





Want to see an increase in % germination and a decrease in MGT

- Tryptophan appears to offer a slight advantage for TF in % germination
- There is no advantage overall over CMH3

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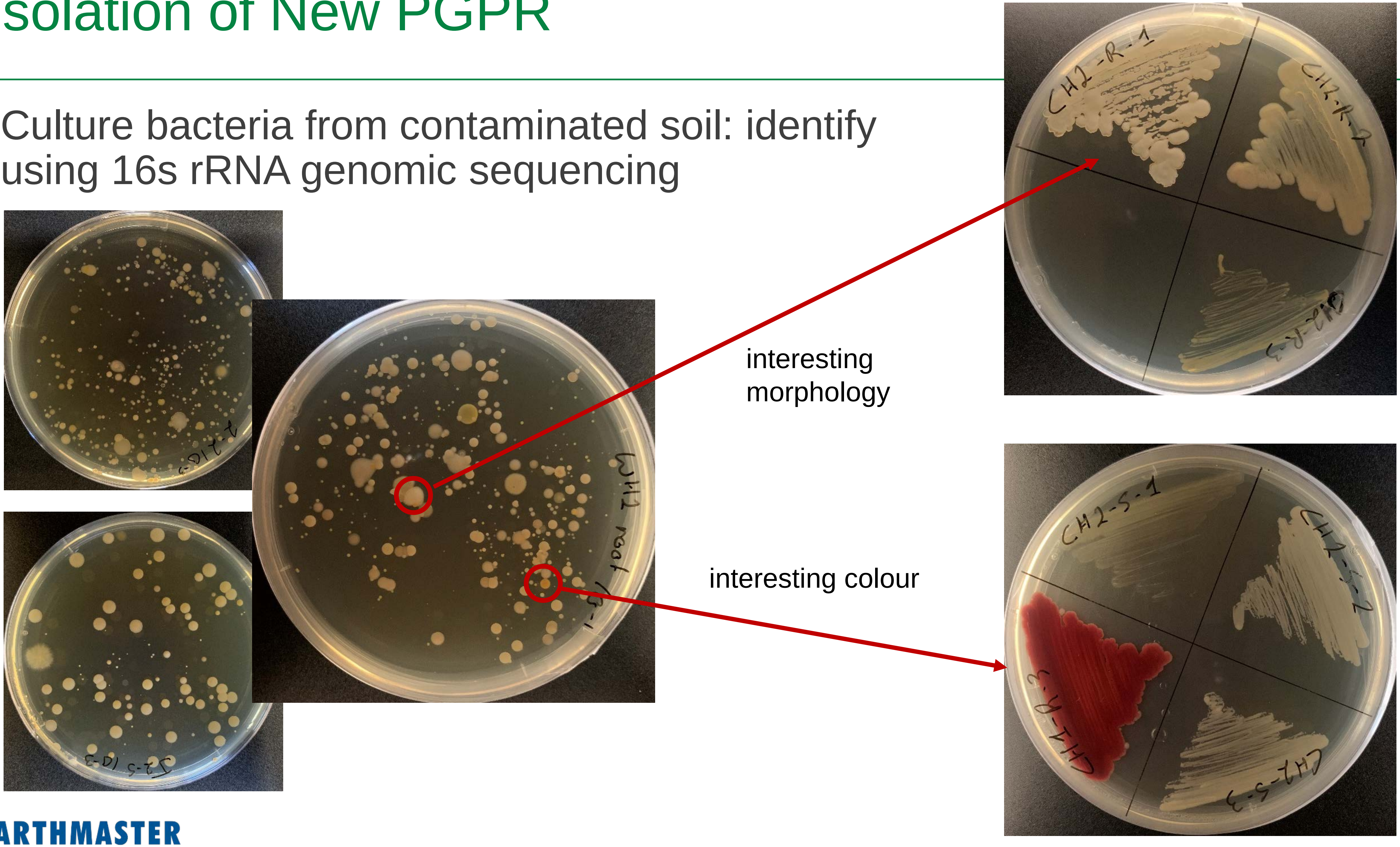
Isolation of New PGPR

Culture bacteria from contaminated soil: identify using 16s rRNA genomic sequencing



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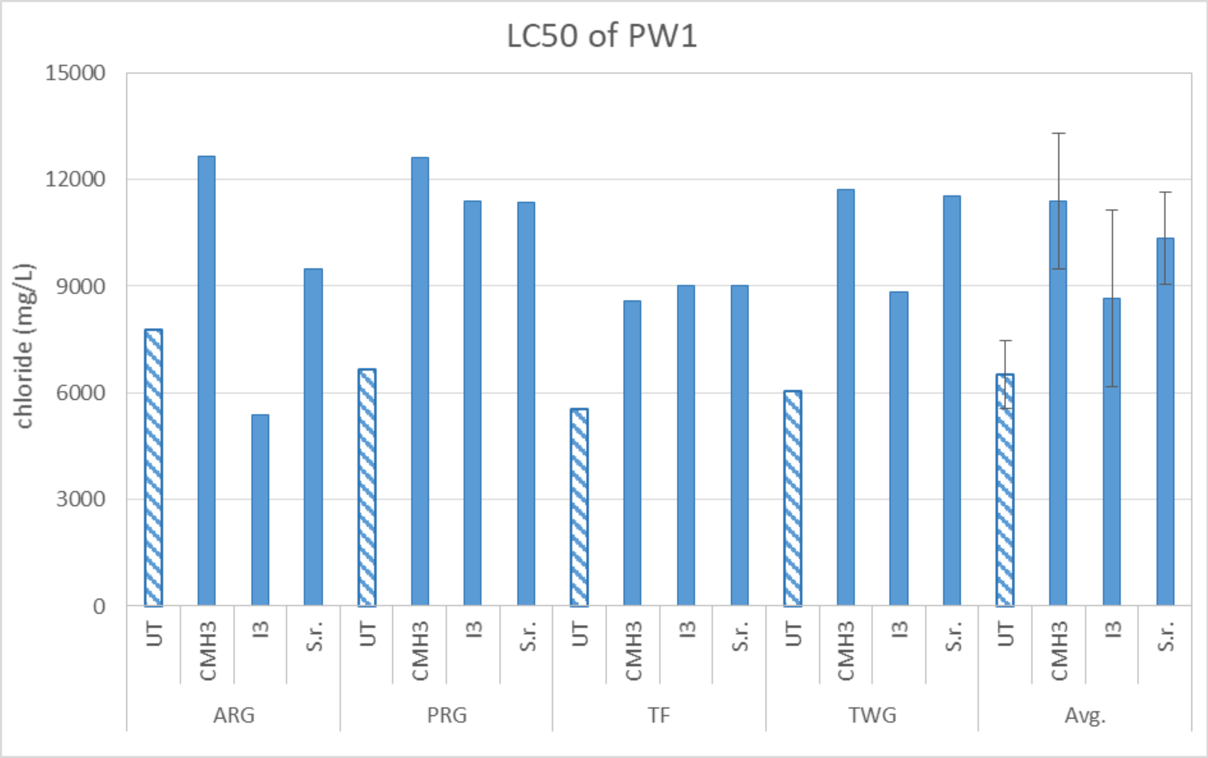
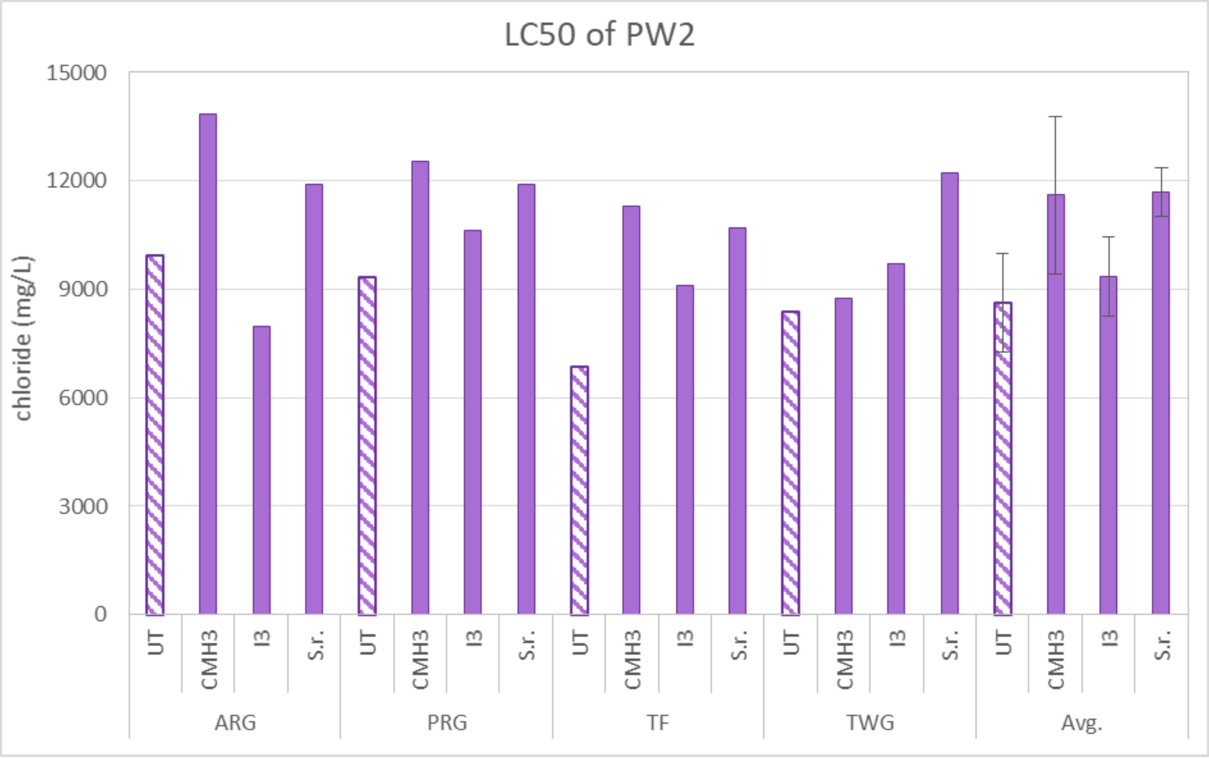
Quantifying the Effects of New PGPR – LC50





S.r. provides an equivalent protective effect as CMH3 for lethality of produced water.

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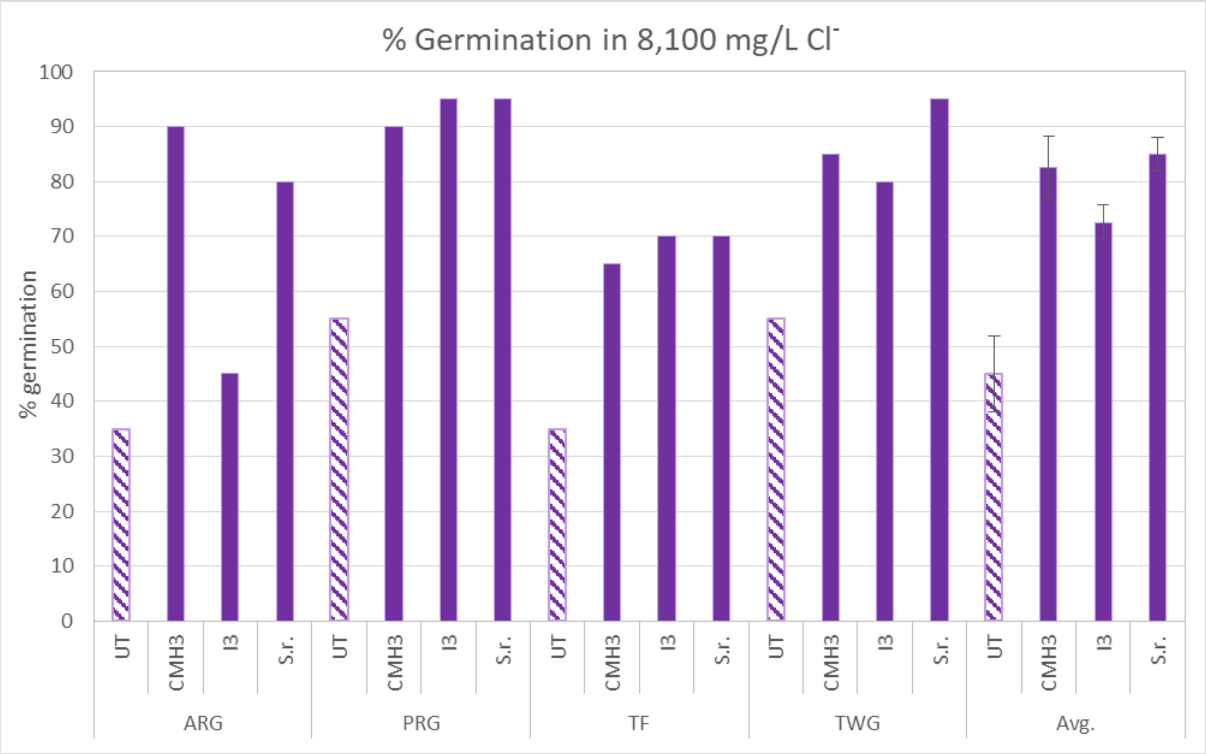
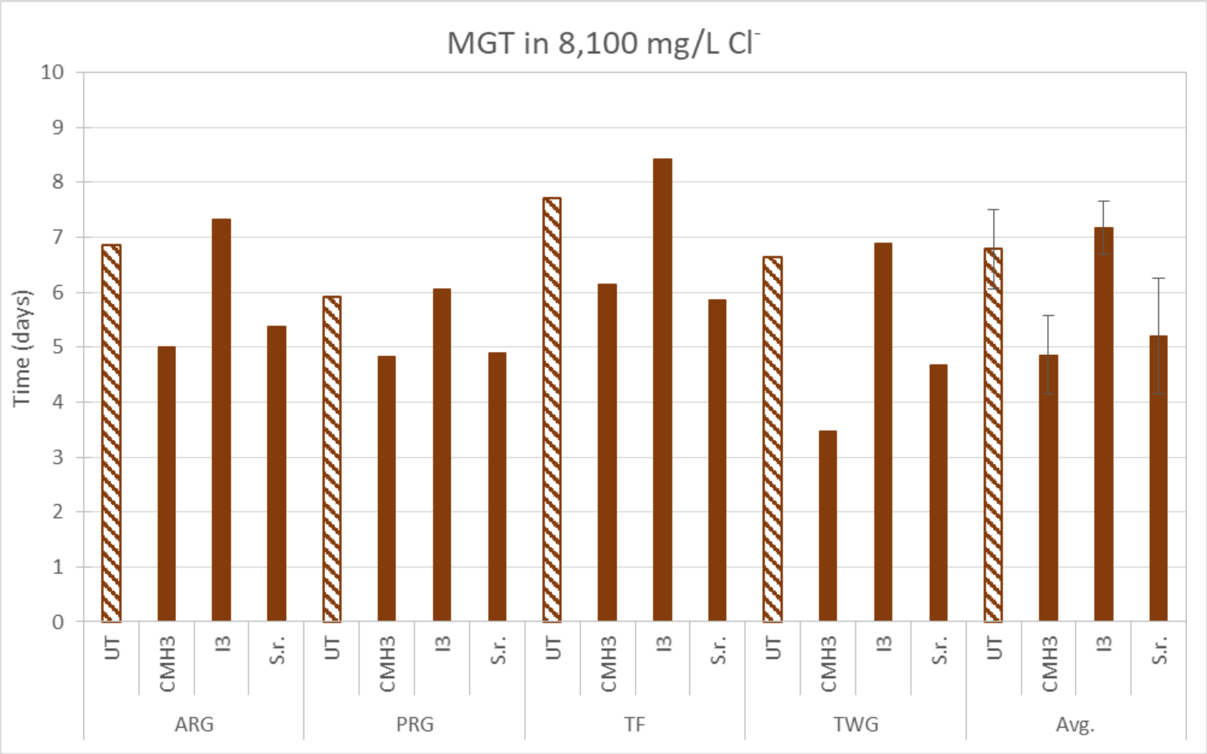
Quantifying the Effects of New PGPR - Germination





S.r. equivalent to CMH3 for decrease in MGT and increase in % germination

- Why use a combination? Different modes of action may give a compounding advantage

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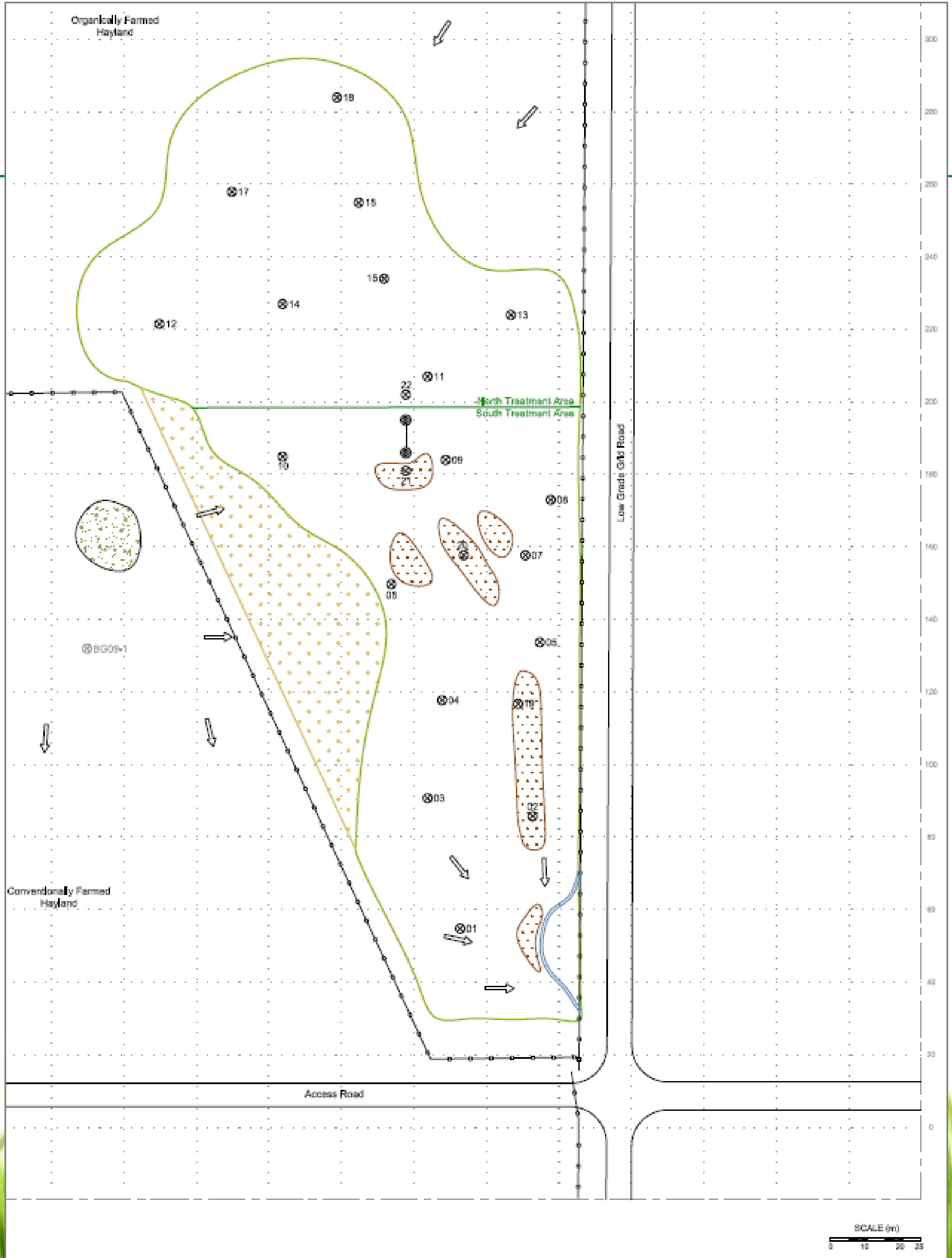
Weyburn 08-31

Project Goals:

- Re-vegetate the grassland site to at least 70% of background levels.
- Reduce soil salt levels over time to allow for sustainable plant growth.
- 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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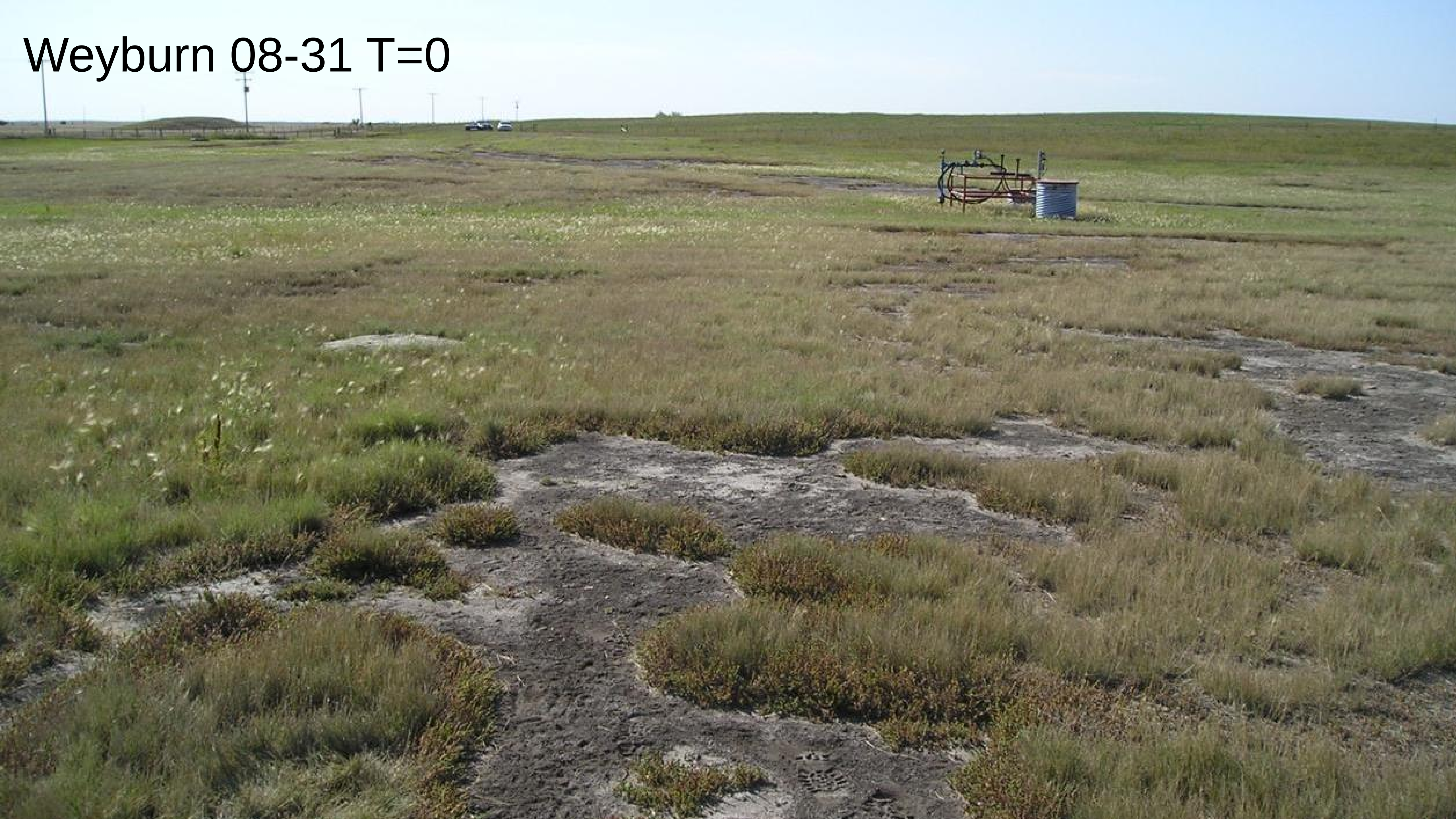
Weyburn 08-31 T=0



Weyburn 08-31 - Three Months After Seeding

Weyburn 08-31 - Year 2

Predicting Salt Remediation Based on Field Deployment

Parameter	Value
Annual Decrease in EC_e	1 to 2 dS/m
Na^+ and Cl^- Uptake into Foliage	65 g/kg dry mass
Na^+ : Cl^- Ratio in Plant Foliage (mass basis)	25:75
Na^+ and Cl^- Removed from Project Sites in Foliage	150 kg/ha
Change in EC_e Accounted for by Foliar Uptake of Salt	Up to 95%

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Reclamation Applications – Laboratory Studies

Environmental growth chamber studies using salt contaminated soil.

Sample Description			Salinity									
Sample Location	Sampling Date	Sample Depth (m bgl)	Saturation (%)	Calcium (mg/kg)	Chloride (mg/kg)	ECe (dS/m)	Magnesium (mg/kg)	Potassium (mg/kg)	SAR	Sodium (mg/kg)	Sulfate (mg/kg)	pH
Soil Analysis												
Sample 1	May-19-20	grab	48.0	440	2300	18.0	250	92.0	19.0	1400	1500	7.5
Sample 2	May-19-20	grab	53.0	460	2100	16.0	240	96.0	18.0	1400	1600	7.5
Sample 3	May-19-20	grab	49.0	410	2300	18.0	240	88.0	18.0	1300	1400	7.6
Average	May-19-20	grab	50.0	437	2233	17.3	243	92.0	18.3	1367	1500	7.5

Grass Plugs – Day 0

Blue grama grass (*Bouteloua gracilis*)

control

2 ml PGPR

10 ml PGPR

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

Pots contained salt contaminated soil.

Pots were not fertilized.

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

Growth was monitored for 7 weeks.

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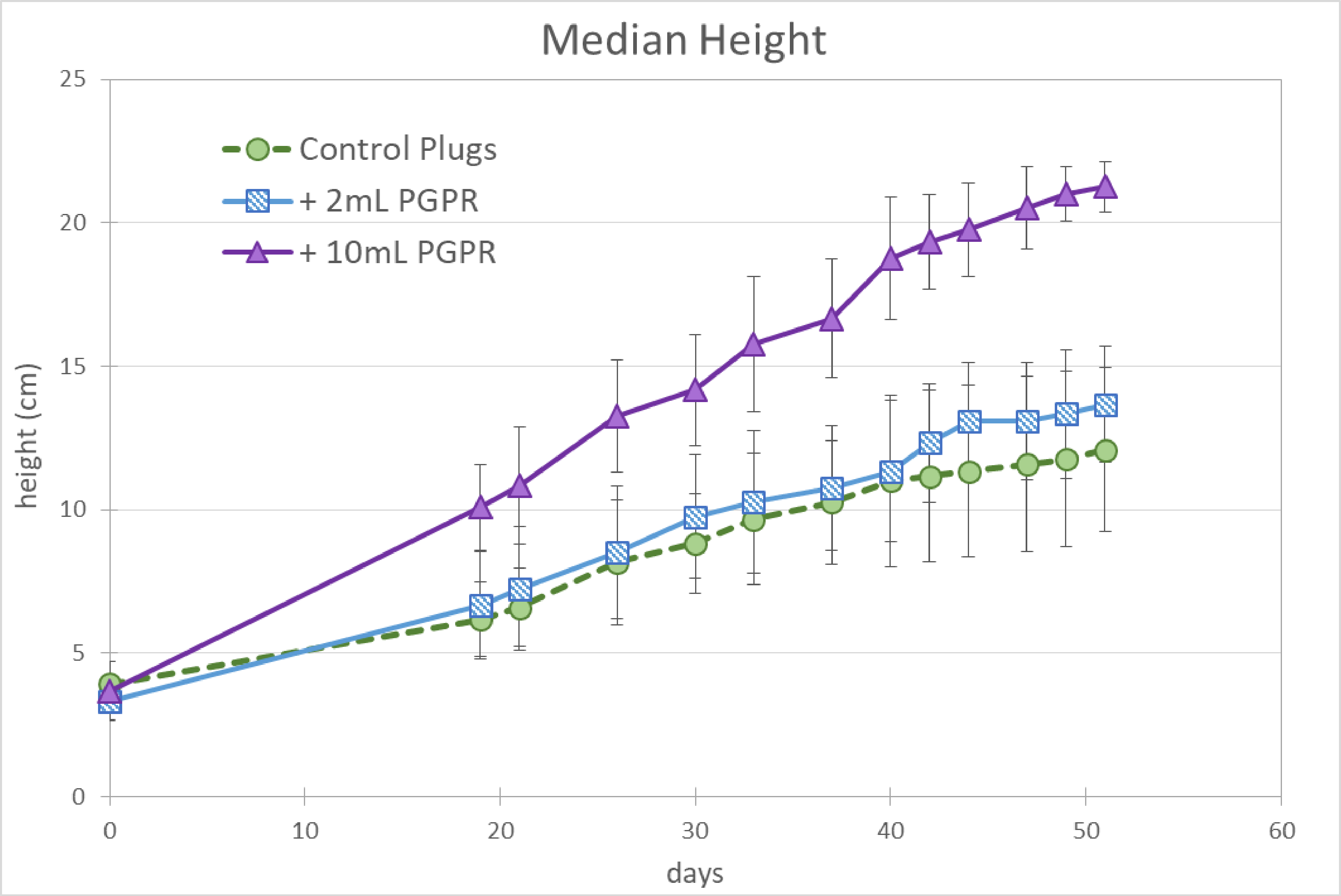
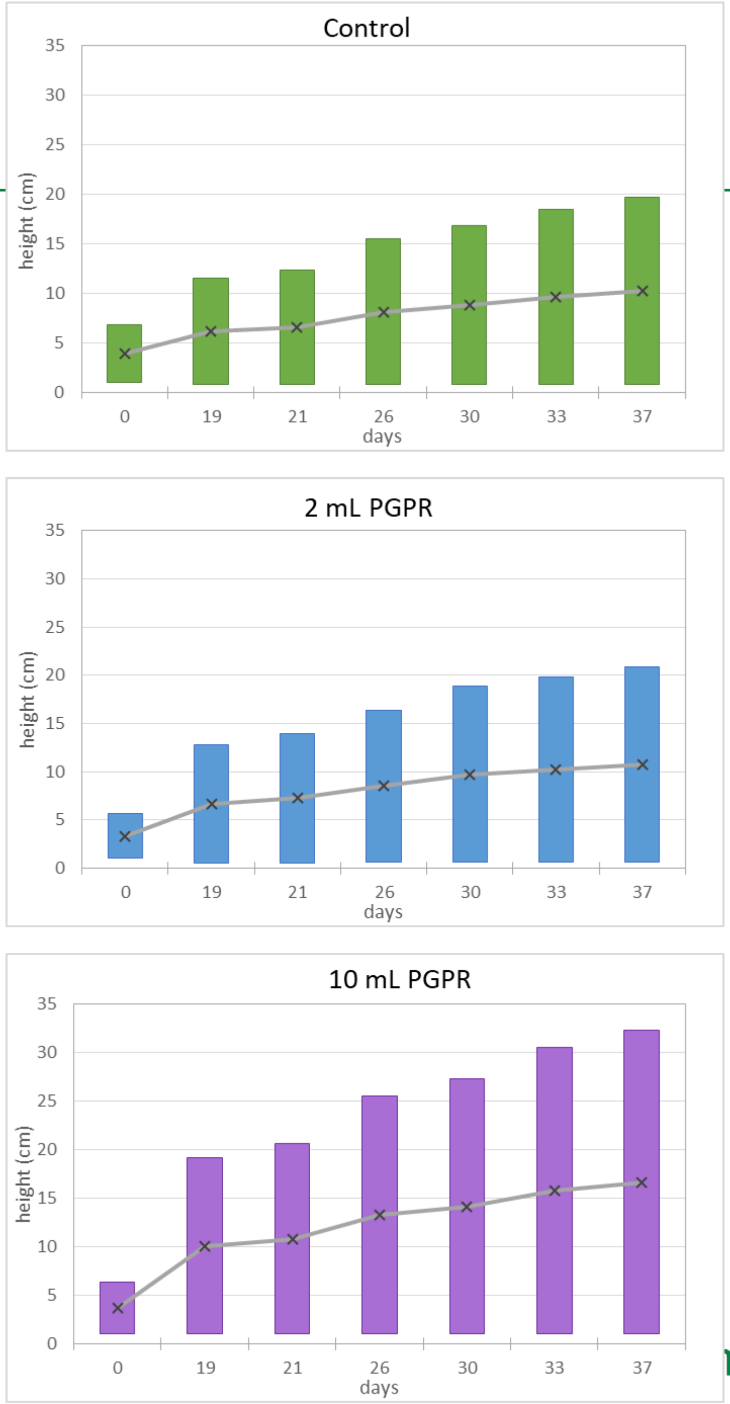
Plant Height





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Grass Plugs - Day 33

control

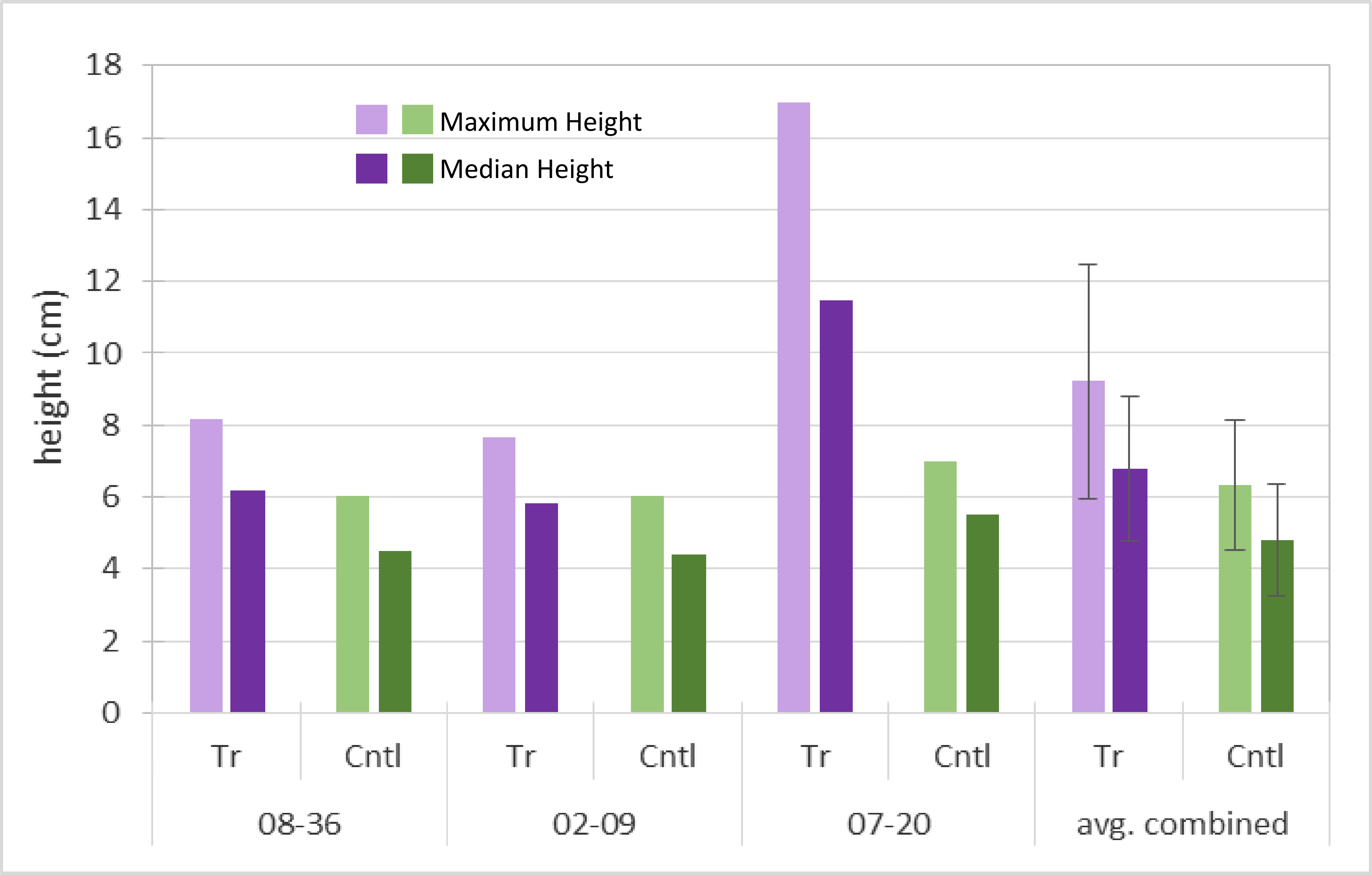
2 ml PGPR

10 ml PGPR

The 10 mL treatment volume was chosen for a small preliminary field trial at reclaimed well sites.

Not shown - The 2 mL and 10 mL treatments were repeated with L-tryptophan. No advantage was observed by including L-tryptophan.

Reclamation Applications – Preliminary Field Trial



Reclamation Applications – Preliminary Field Trial

Site 08-36

treated control

Site 02-09

treated control

Site 07-20

treated control

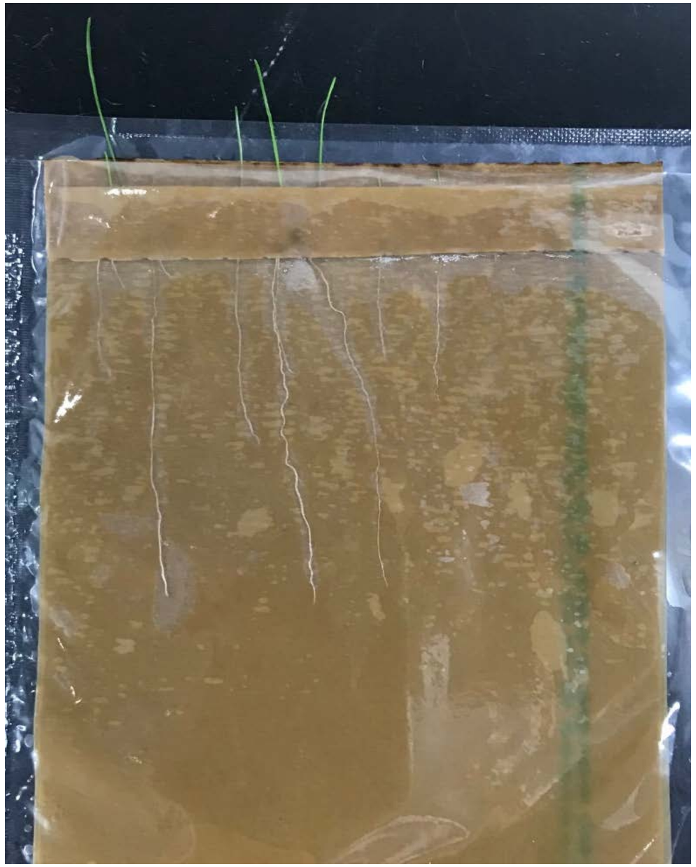
Plot Description		Plug Health	
		# Poor	# Good
08-36	Treated	1	3
	Control	3	0
02-09	Treated	0	3
	Control	2	1
07-20	Treated	0	5
	Control	1	1
Combined	**Treated**	**1**	**11**
	Control	**6**	**2**

Next Steps

Additional lab studies with PGPR combinations for seed

- Rooting pouches
- Growth chamber studies
    - Soil up to 50 dS/m
- Field deployment

Additional lab studies for grass plugs



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Next Steps

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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?