Phytoremediation of PHC and Salt Impacted Soil - Field Results, Soil Toxicity and New Research Using Soil Amendments to Accelerate Remediation

Bruce Greenberg, X-D Huang, P Mosley, K Gerhardt, X-M Yu, S Liddycoat, X Lu, M Hannaberg, B McCallum, G MacNeill, N Kroetsch, M DiNino, N Knezevich, P Gerwing, K Cryer, R Minks and B Poltorak

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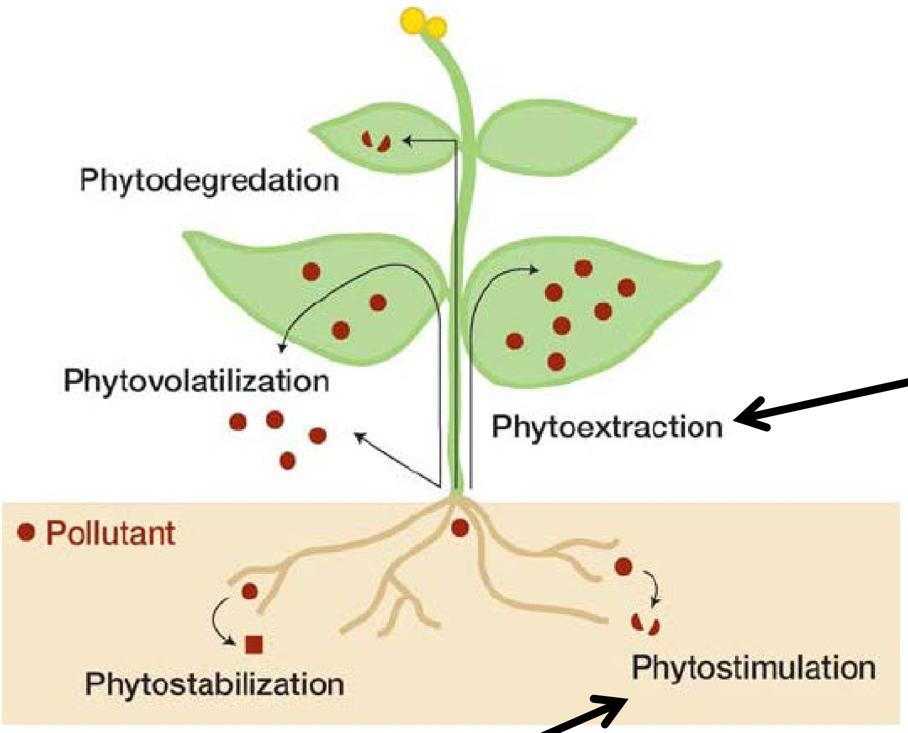
Overview of Today's Talk

- Overview of the Phytoremediation process
- CCME PHC analytical procedure
- New research on phytoremediation: Tier 2 risk assessment and soil ammendments

Phytoremediation:
A Scientifically Proven Solution

Arborist Neal Sturgis

Phytoremediation: Use of plants to remove impacts from soil, water or air



Salt

Rhizodegradation - PHC

- Volatilization
- Phytodegradation
- Chelation/compartment in leaves

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

Advantages of Phytoremediation

1. Soil quality improved.
2. Driven by solar energy - suitable to most regions and climates
3. **Cost effective** and technically feasible
4. **Plants** provide **sufficient biomass** for rapid remediation; **promote high rhizosphere activity**
5. Reasonable time frames - 2 to 4 years
6. Can be used effectively at **remote sites**
7. Greenhouse gas storage: **6 tonnes per ha per year**
8. Effective for remediation of PHC and salt – relevant to the energy industry

Development, Proof, and Full Scale Application of PGPR Enhanced Phytoremediation Systems (PEPS)

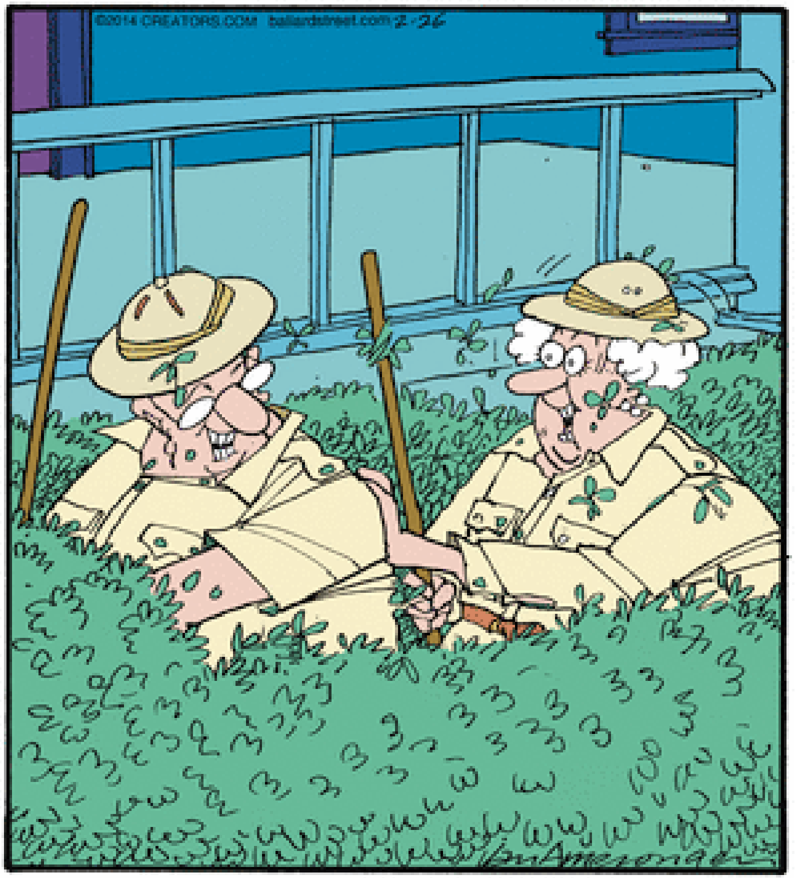
Over 14 years of research with full-scale field remediations at each stage of development and application > 30 sites, > 15 completed

1. **PHC:** sites in AB, BC, QC, MB, NWT, SK and ON (2004-13)
2. **Salt:** sites in SK, MB, AB and NWT (2007-13)

Performing full scale remediations for > 8 yrs

PEPS successful at > 30 sites

Key to Successful Phytoremediation: Aggressive Plant Growth



"You're right, Neal. Beating through the shrubs *is* like we're on safari!"

The key to phytoremediation success

- Transfer of the science from the lab to the field

1. Strategies for aggressive plant growth in impacted and poor quality soils at full scale sites
   – PEPS Deployment by highly trained scientists
2. Monitoring the progress of phytoremediation at each site – Following the chemistry
3. Continuous improvement of our phytoremediation systems through scientific research

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PGPR Enhanced Phytoremediation Systems (PEPS)

Aggressive plant growth strategies leads to remediation

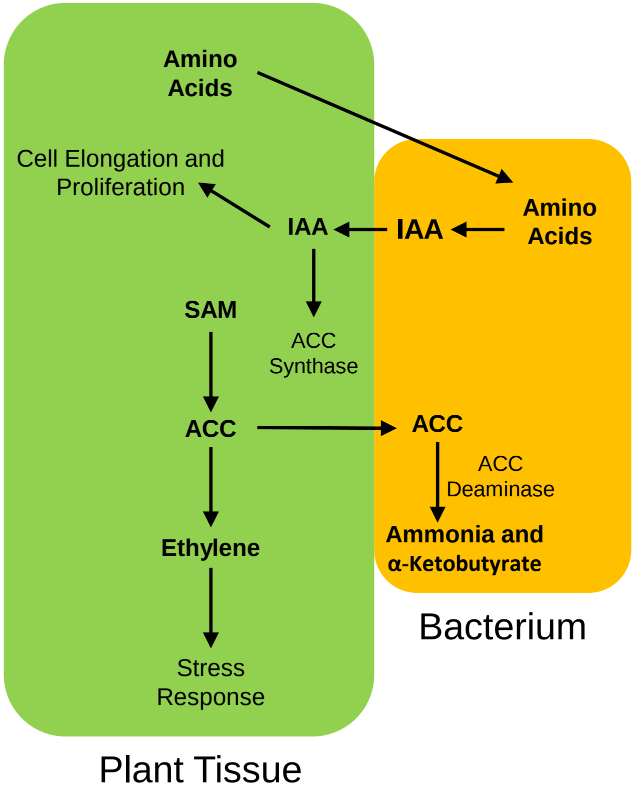
Physical soil treatment: Seed bed preparation

Phytoremediation: Growth of plants **with PGPR**

Monitoring and remediation assessment: Environmental chemistry to follow PEPS from start–to–finish

- PGPR: Plant growth promoting rhizobacteria.
- Prevent the synthesis of stress ethylene.
- PGPR are applied to the grass seeds prior to sowing
  $\rightarrow$ **NOT Bioaugmentation**
- Effect depth of remediation ~ 0.5 m

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



Adapted from Glick *et al*, 2004

Naturally occurring, non-pathogenic microbes (usually *Pseudomonads*)

Chosen initially based on ACC Deaminase activity and auxin (IAA) production

We have isolated PGPR from ON, AB, SK and the NWT (native to North America)

Can be isolated from soils anywhere

Phytoremediation of Petroleum

Georgina settles on a botanical response.

Track Record of PEPS Deployment at PHC Sites

Examples of Completed Sites

Site	Fraction	Date	Average (mg/kg)	% Remediation
Dawson 1	Light	Spring 2009	6500	91%
		Fall 2012	574	
	Medium	Spring 2009	2500	75%
		Fall 2012	624	
Dawson 2	Light	Spring 2009	6112	87%
		Fall 2012	771	
	Medium	Spring 2009	631	56%
		Fall 2012	275	
Dawson 3	Light	Spring 2009	6648	96%
		Fall 2012	246	
	Medium	Spring 2009	2483	97%
		Fall 2012	321	
Peace River	Medium	Spring 2007	492	62%
		Fall 2008	190	
Quebec City	Medium	Spring 2009	517	48%
		Fall 2009	270	
Swan Hills	Light	Spring 2009	1700	83%
		Fall 2011	283	
	Medium	Spring 2009	2950	57%
		Fall 2011	1258	
Steinbach	Light	Spring 2008	761	44%
		Fall 2008	335	
Hinton 2	Medium	Spring 2007	900	44%
		Fall 2008	500	
Edson	Medium	Spring 2007	1500	33%
		Fall 2008	1000	
Nota Creek	Light	Spring 2011	549	85%
		Spring 2013	84	
	Medium	Spring 2011	515	64%
		Spring 2013	186	

Ave rate of remediation: 35 % per year

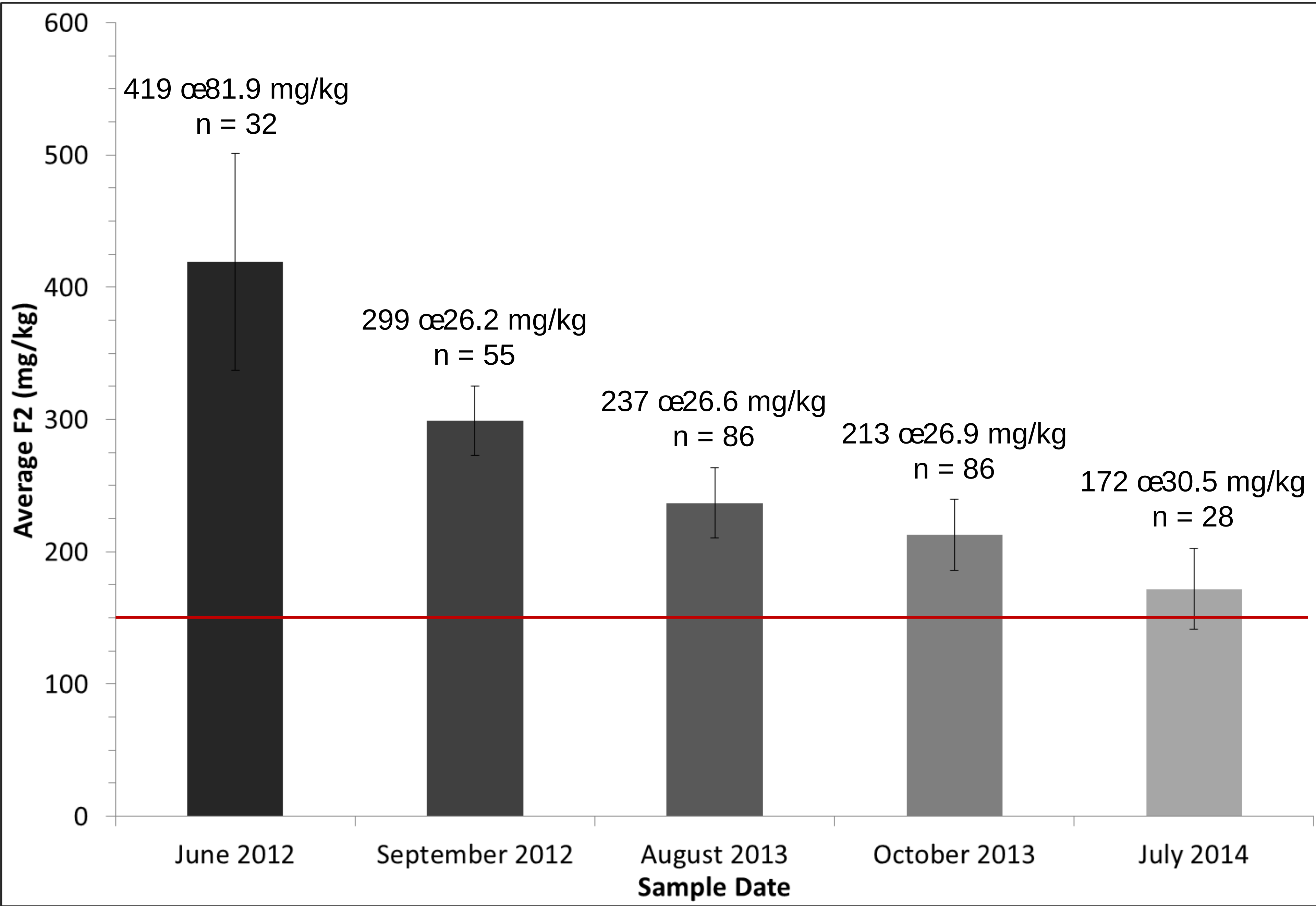
Track Record of PEPS Deployment at PHC Sites

Examples of Sites in Progress

Site	Fraction	Date	Average (mg/kg)	% Remediation
Hinton 1	Light	Spring 2010	1254	75%
		Fall 2012	318	
	Medium	Spring 2008	2850	26%
		Fall 2012	2120	
Beaver River	Light	Spring 2010	3658	55%
		Fall 2012	1664	
	Medium	Spring 2010	1335	32%
		Fall 2012	912	
Red Earth 1	Light	Fall 2011	916	82%
		Fall 2013	163	
	Medium	Fall 2011	2394	58%
		Fall 2013	1015	
Red Earth 2	Light	Fall 2011	752	71%
		Fall 2013	215	
	Medium	Fall 2011	1740	36%
		Fall 2013	111	
Red Earth 3	Light	Fall 2011	620	50%
		Fall 2013	309	
	Medium	Fall 2011	1537	10%
		Fall 2013	1391	
Hinton 3	Light	Fall 2011	159	57%
		Fall 2013	68	
	Medium	Fall 2011	2233	35%
		Fall 2013	1461	
St. Leon	Light	Spring 2012	41	-1%
		Fall 2013	42	
	Medium	Spring 2012	2633	32%
		Fall 2013	1786	
	Light	Spring 2012	132	28%
		Fall 2013	95	
	Medium	Spring 2012	1887	26%
		Fall 2013	1399	

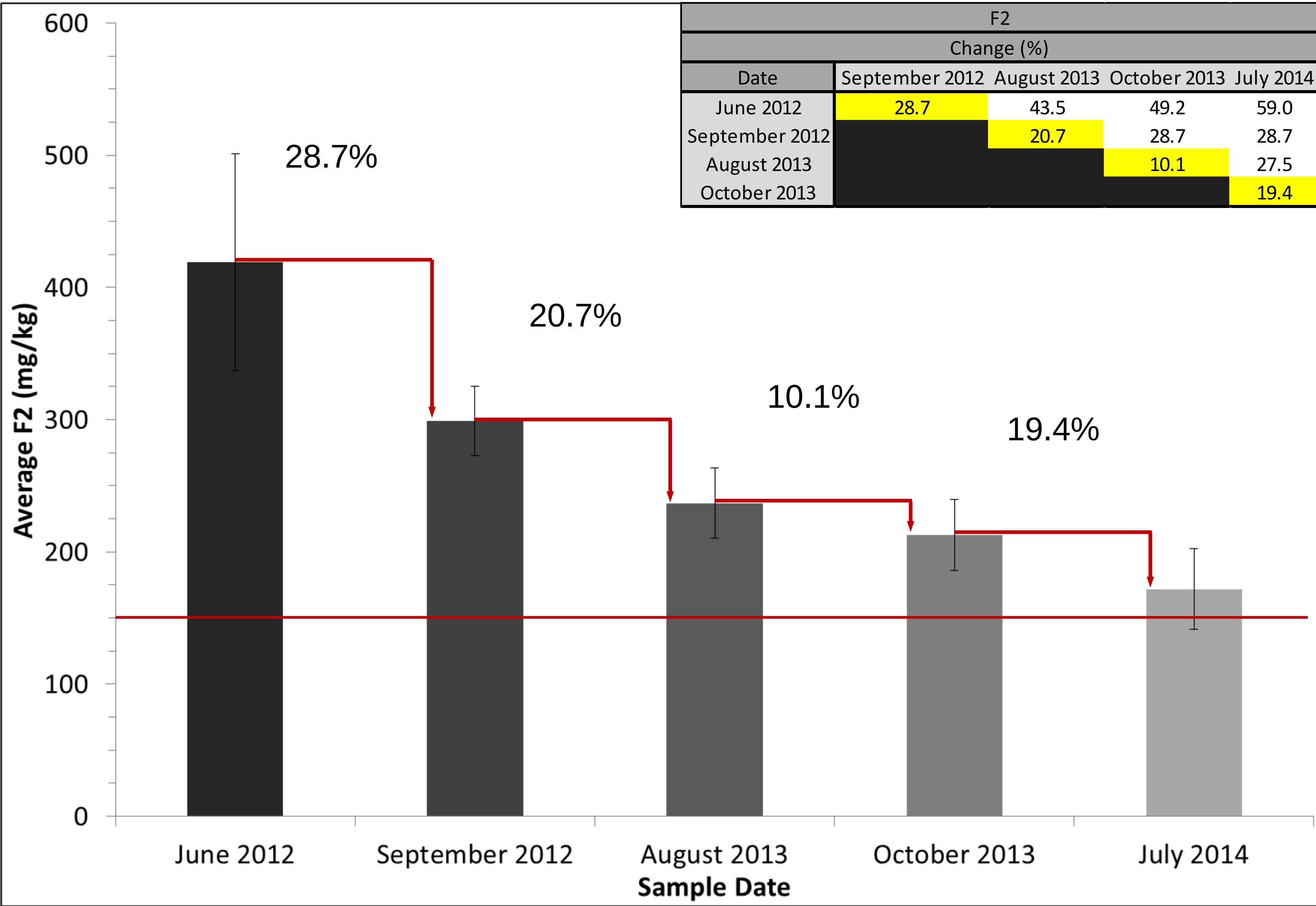
Site	Fraction	Date	Average (mg/kg)	% Remediation
Oakville	Light	Spring 2012	197	70%
		Fall 2013	60	
	Medium	Spring 2012	4676	64%
		Fall 2013	1688	
	Light	Spring 2012	1064	64%
		Fall 2013	388	
	Medium	Spring 2012	24046	51%
		Fall 2013	11719	
	Light	Spring 2012	302	44%
		Fall 2013	168	
	Medium	Spring 2012	16098	45%
		Fall 2013	8843	
	Light	Spring 2012	161	63%
		Fall 2013	60	
	Medium	Spring 2012	2980	44%
		Fall 2013	1657	
Edson 3	Light	Fall 2013	310	N/A
		-	-	
	Medium	Fall 2013	646	N/A
		-	-	
Edson 4	Light	Fall 2013	311	N/A
		-	-	
	Medium	Fall 2013	298	N/A
		-	-	
Norman Wells	Light	Spring 2013	208	75%
		Fall 2013	53	
	Medium	Spring 2013	366	45%
		Fall 2013	200	

Average F2 Concentrations for Six Sites in Alberta

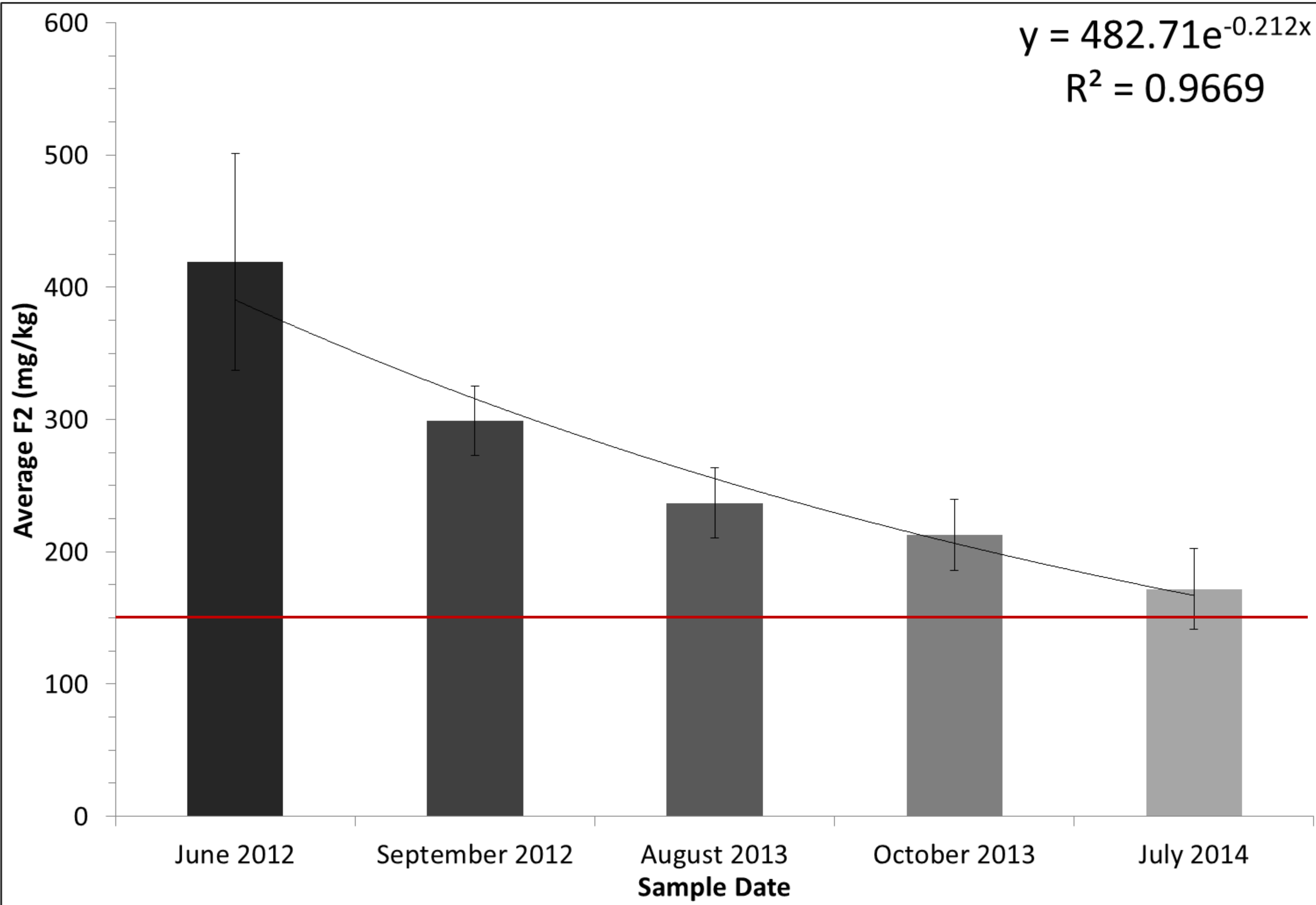


Average F2 Remediation for Six Sites in Alberta

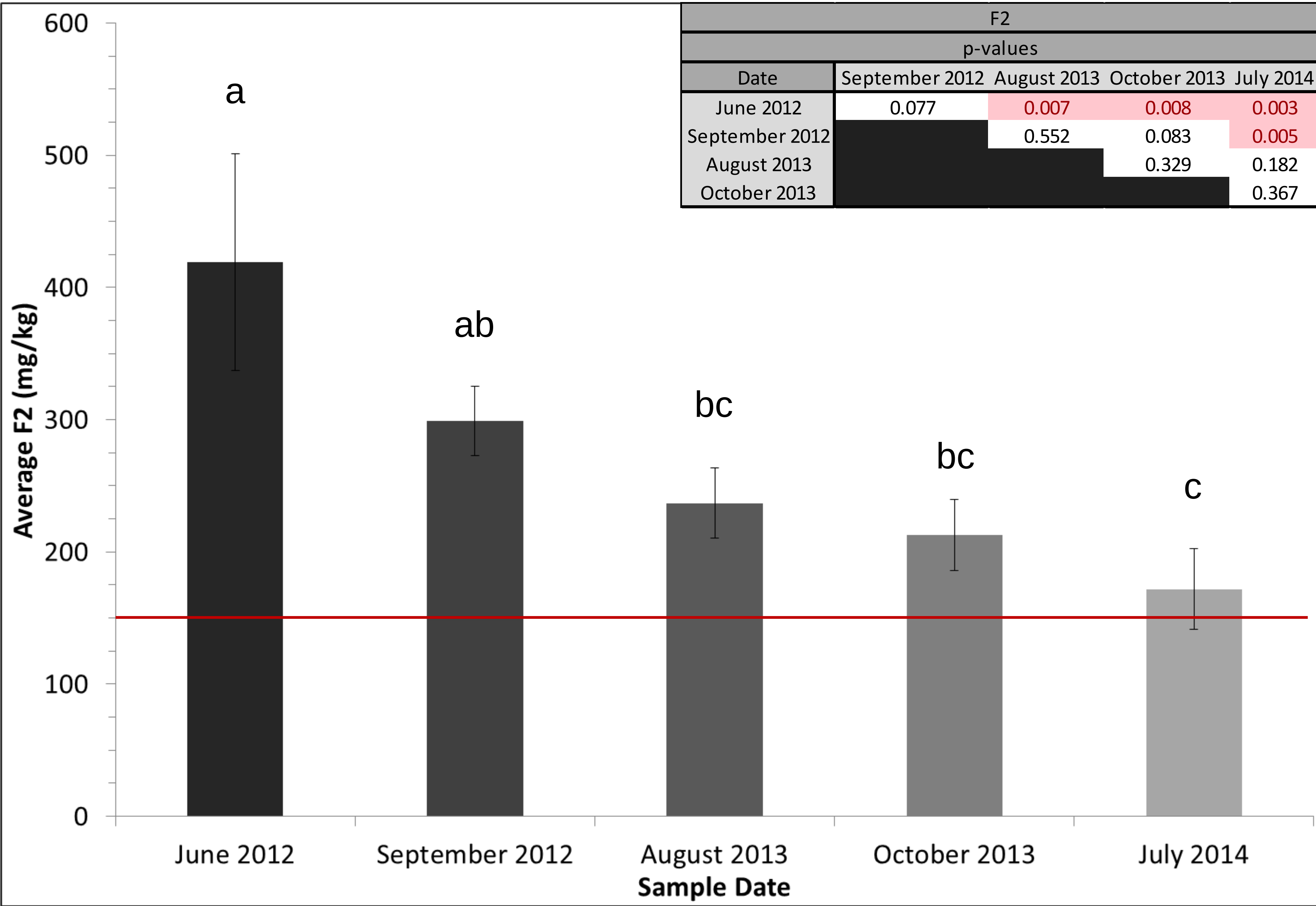
F2				
Change (%)				
Date	September 2012	August 2013	October 2013	July 2014
June 2012	28.7	43.5	49.2	59.0
September 2012		20.7	28.7	28.7
August 2013			10.1	27.5
October 2013				19.4



F2 Remediation Trend for Six Sites in Alberta

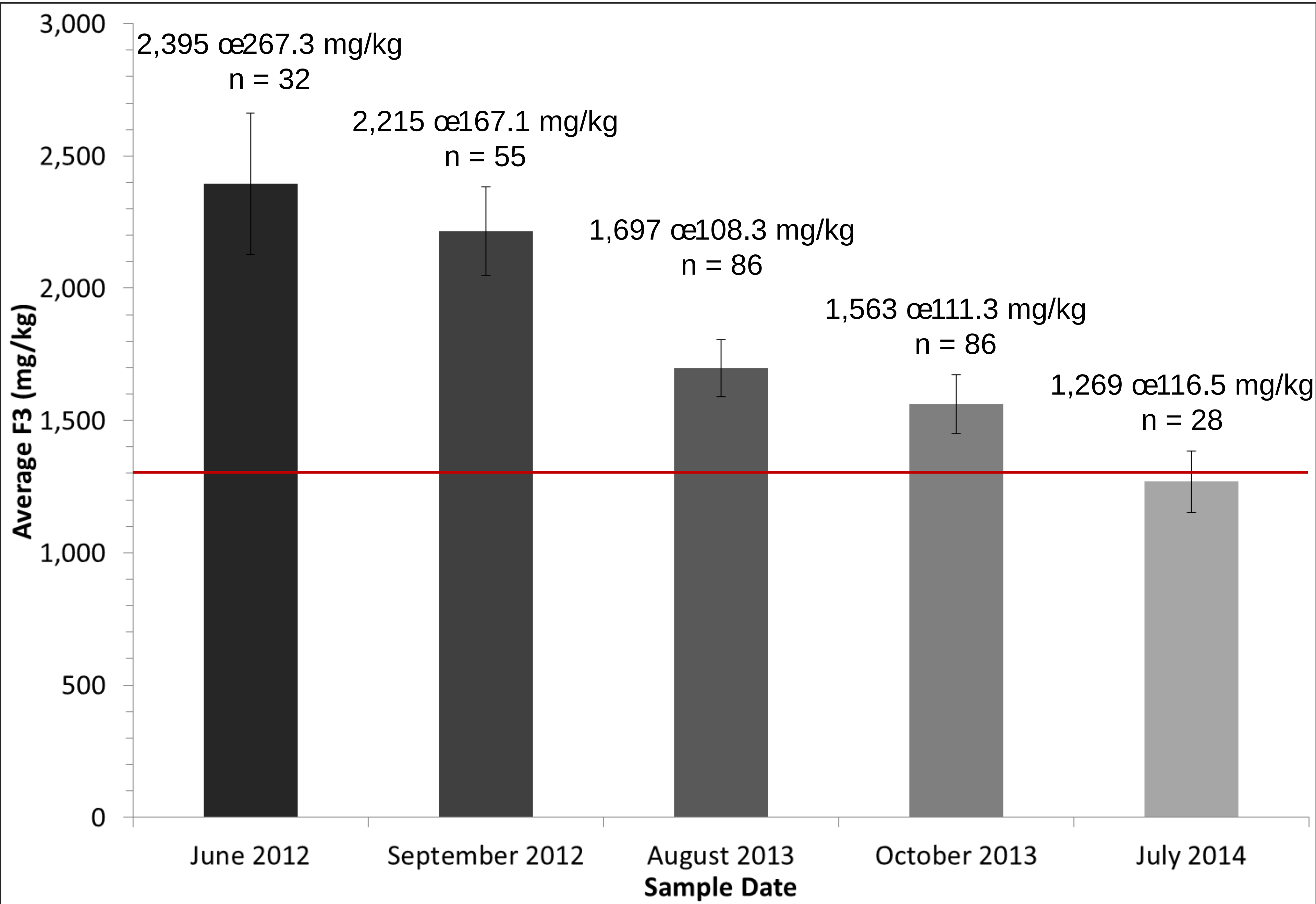


F2 Remediation Statistics for Six Sites in Alberta

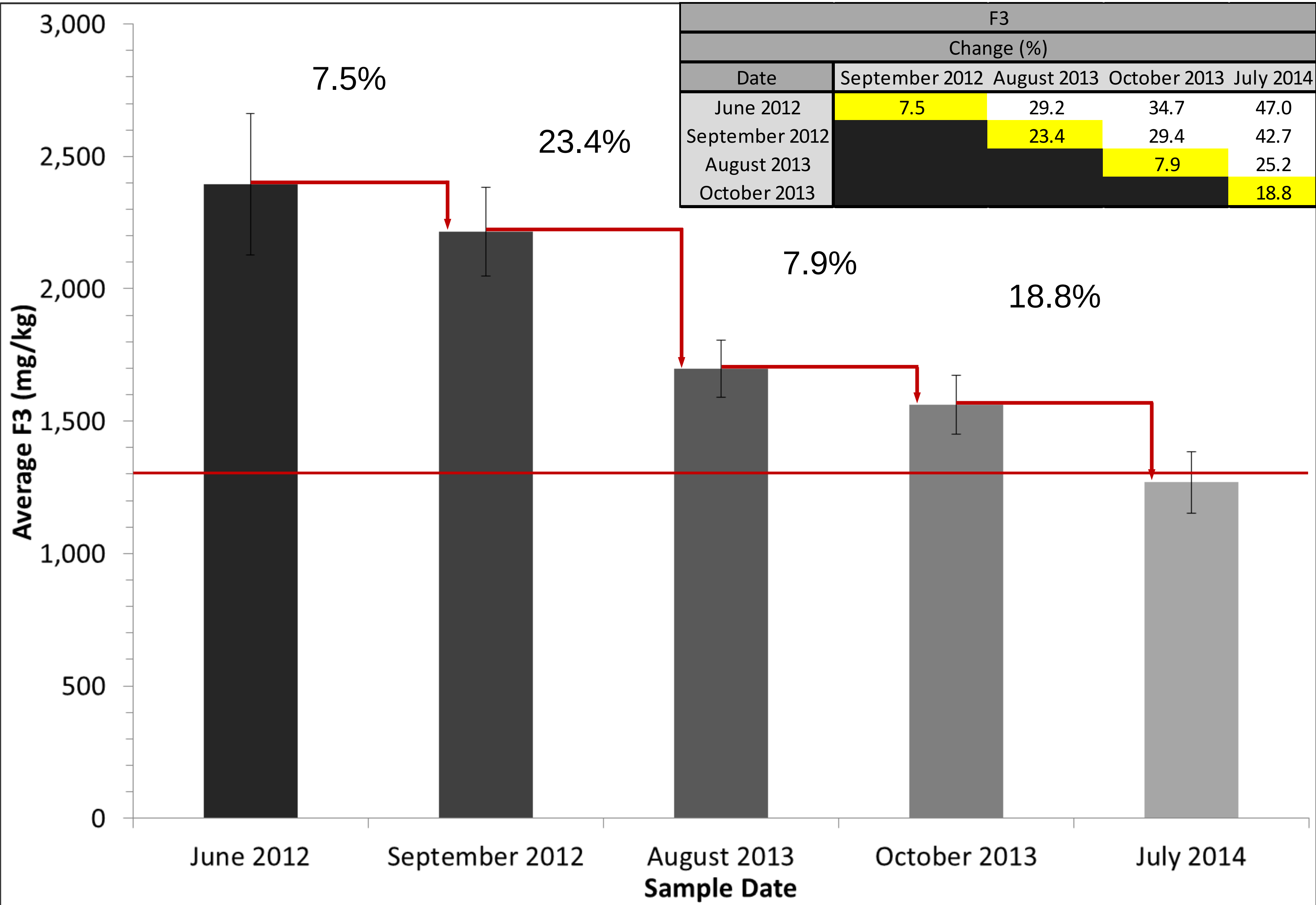


F2				
p-values				
Date	September 2012	August 2013	October 2013	July 2014
June 2012	0.077	0.007	0.008	0.003
September 2012		0.552	0.083	0.005
August 2013			0.329	0.182
October 2013				0.367

Average F3 Concentrations for Six Sites in Alberta

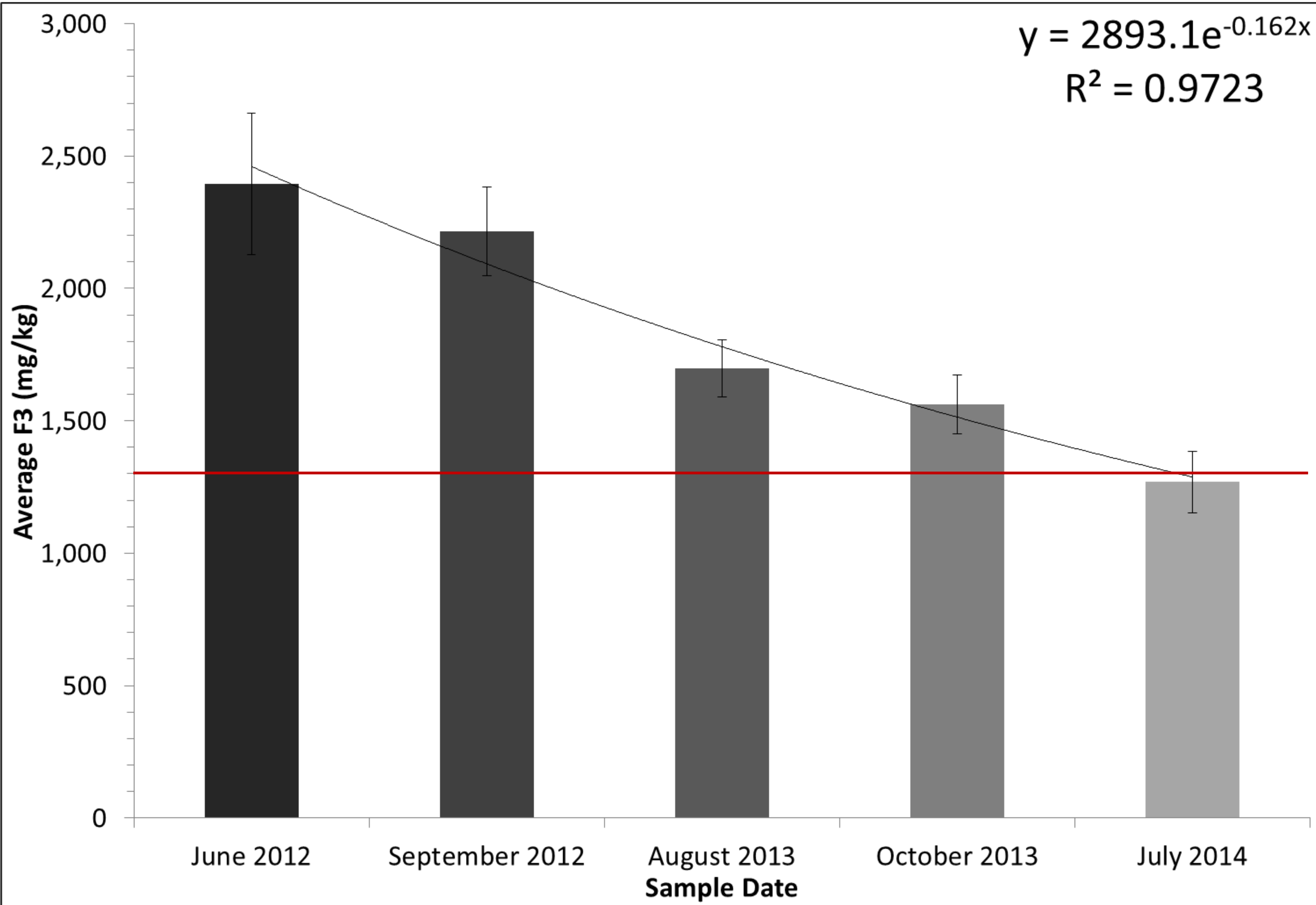


Average F3 Remediation for Six Sites in Alberta



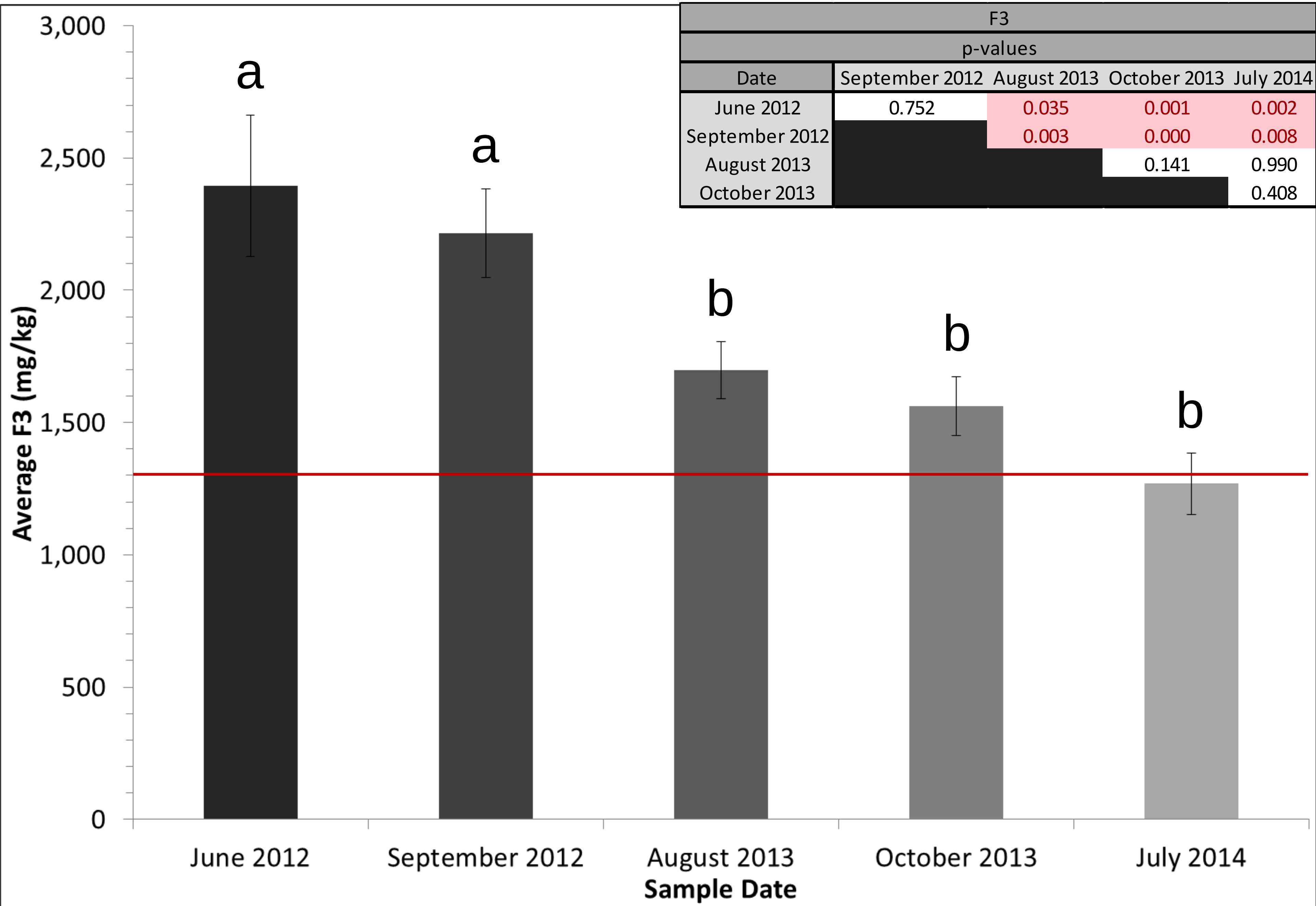
F3				
Change (%)				
Date	September 2012	August 2013	October 2013	July 2014
June 2012	7.5	29.2	34.7	47.0
September 2012		23.4	29.4	42.7
August 2013			7.9	25.2
October 2013				18.8

F3 Remediation Trend for Six Sites in Alberta



F3 Remediation Statistics for Six Sites in Alberta

F3				
p-values				
Date	September 2012	August 2013	October 2013	July 2014
June 2012	0.752	0.035	0.001	0.002
September 2012		0.003	0.000	0.008
August 2013			0.141	0.990
October 2013				0.408



Representative PHC-impacted Site in Alberta

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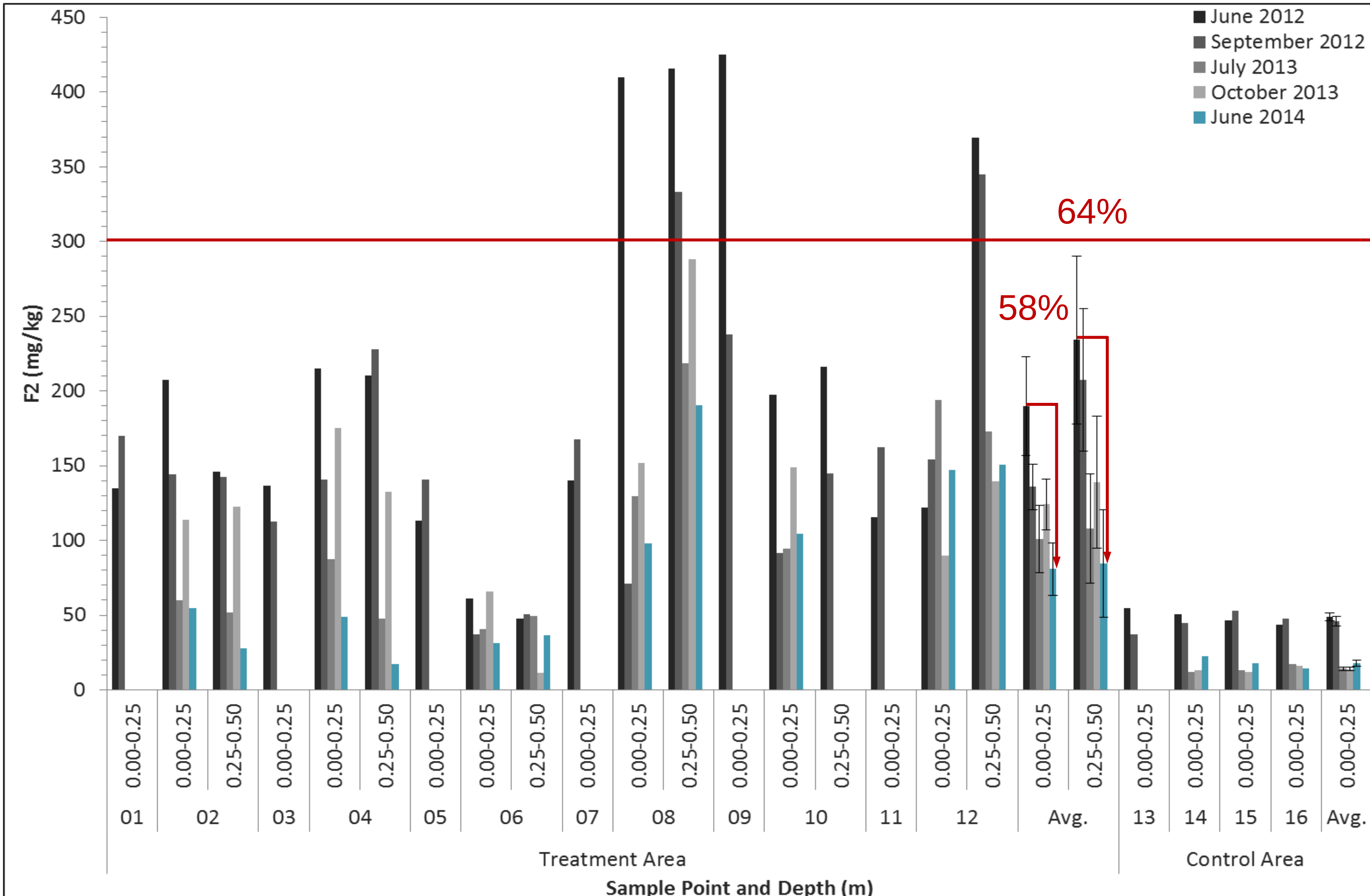
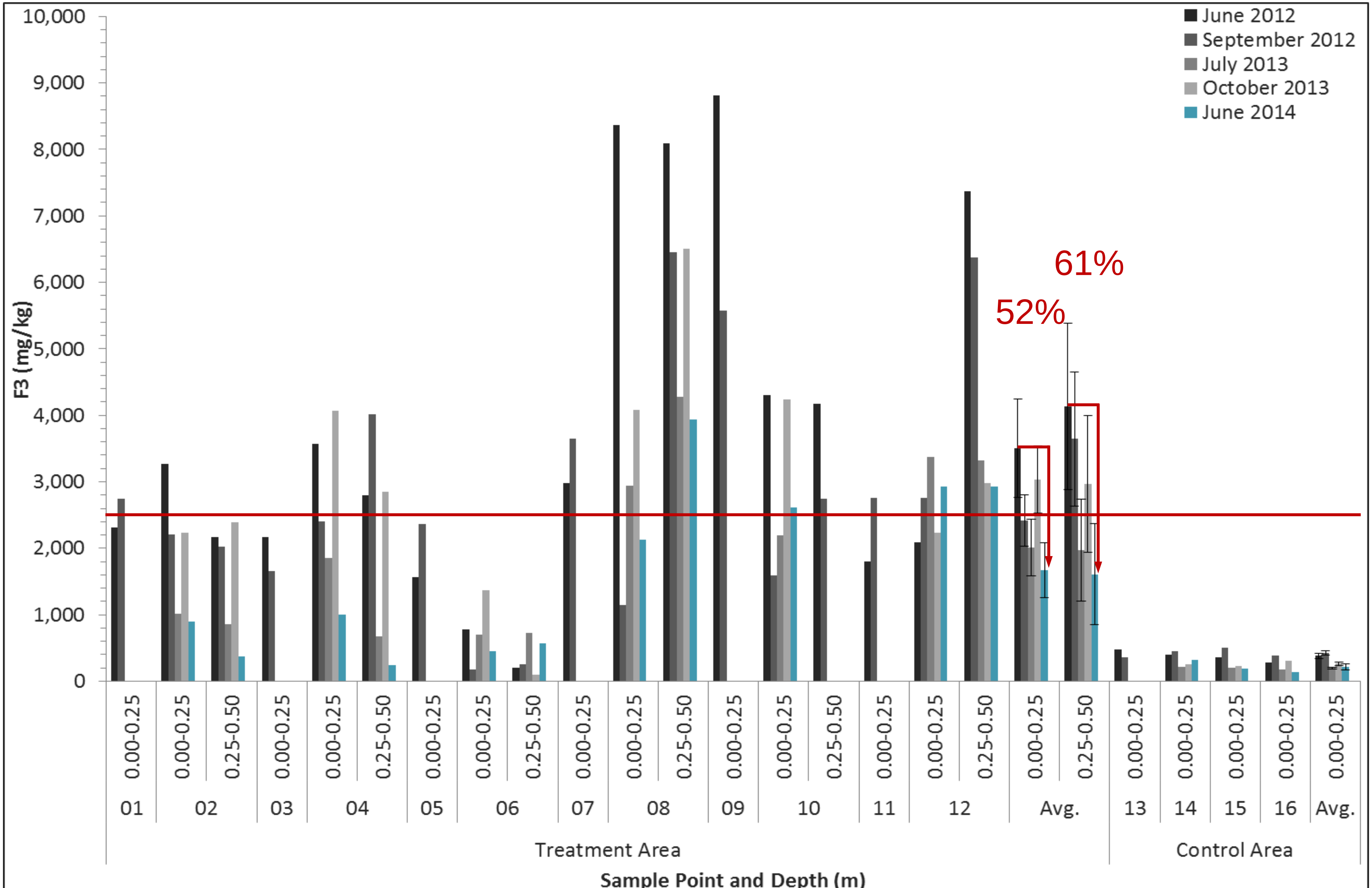
Representative PHC-impacted Site in Alberta

Typical mid-season PEPS plant growth relative to indigenous plant growth

Representative PHC-impacted Site in Alberta - F2



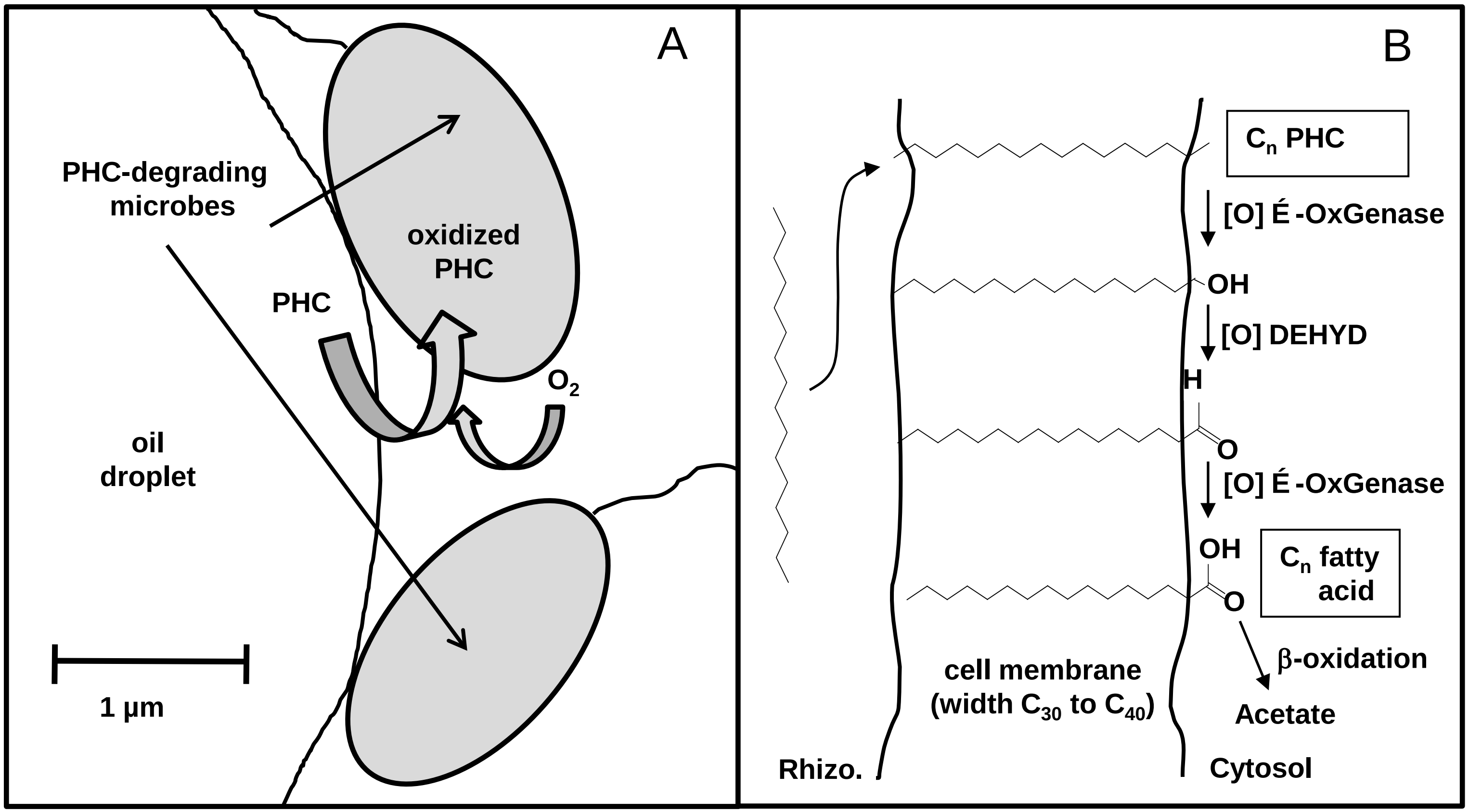
Representative PHC-impacted Site in Alberta - F3



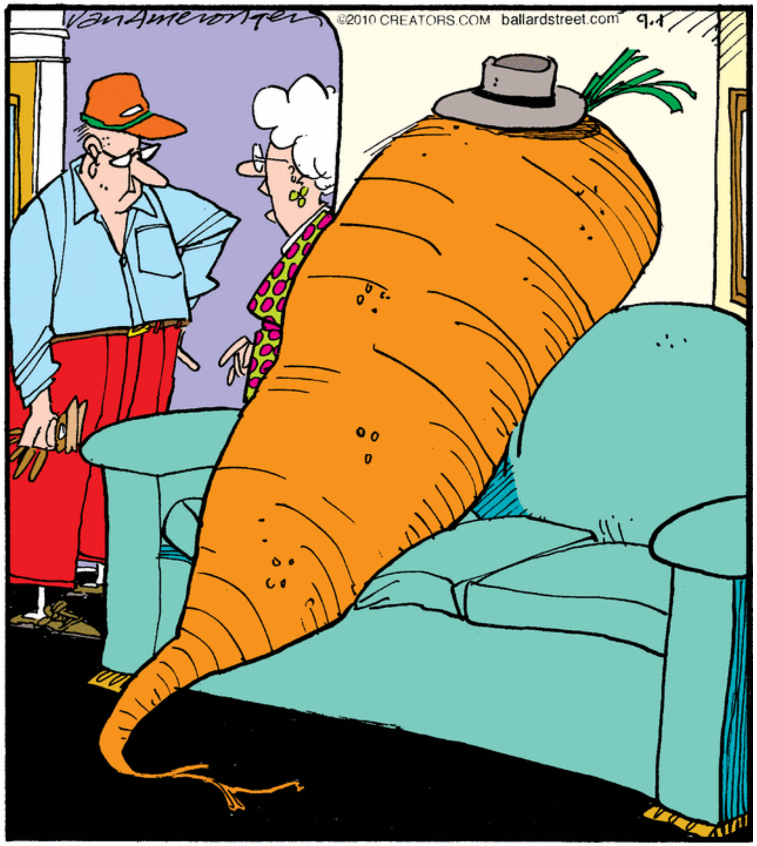
Phytoremediation of PHC

(A) Microbial aerobic PHC degradation – rhizosphere supported by plants

(B) Microbial oxygenation pathway of PHC to form a fatty acid

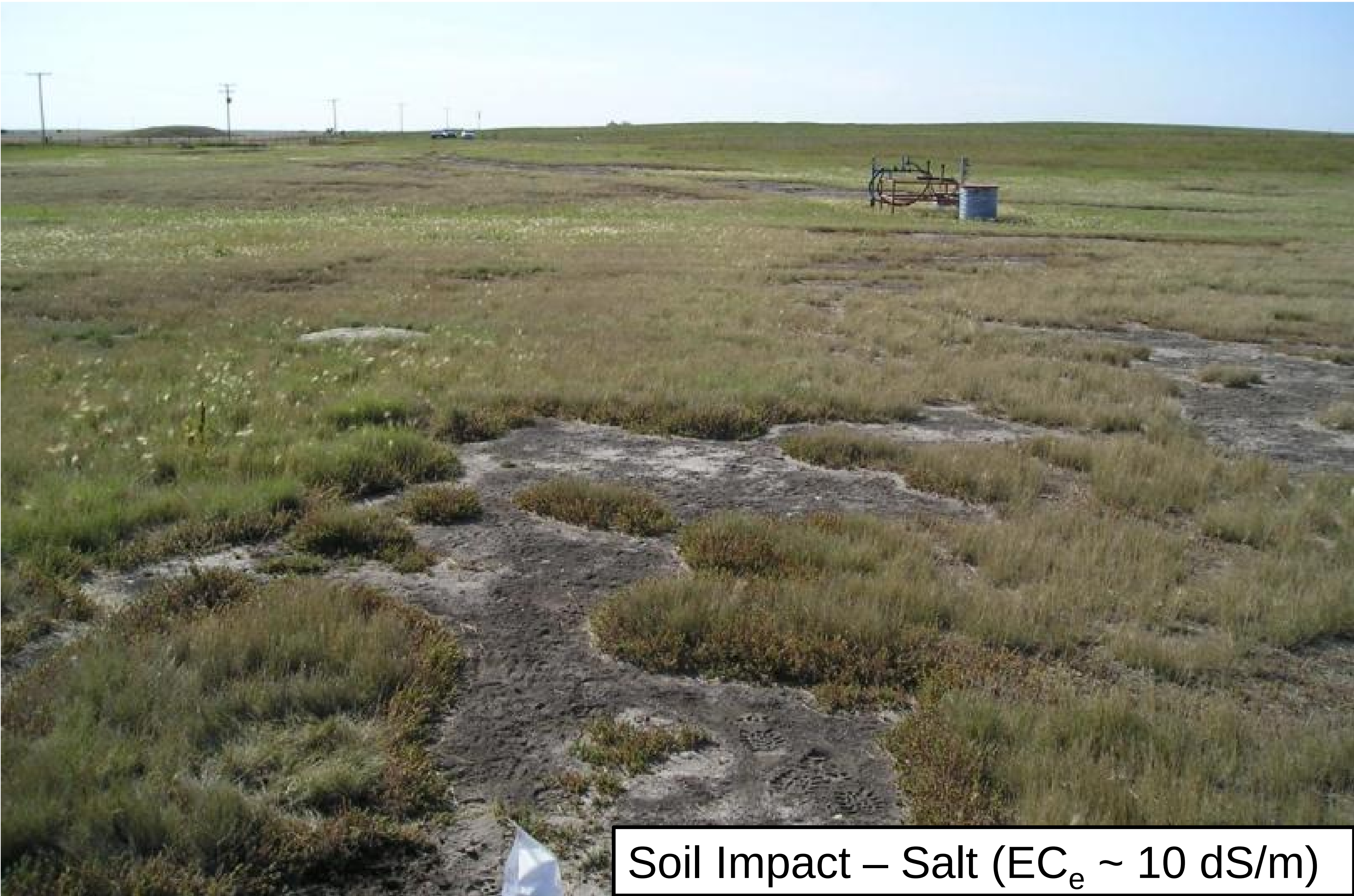


Phytoremediation of Salt

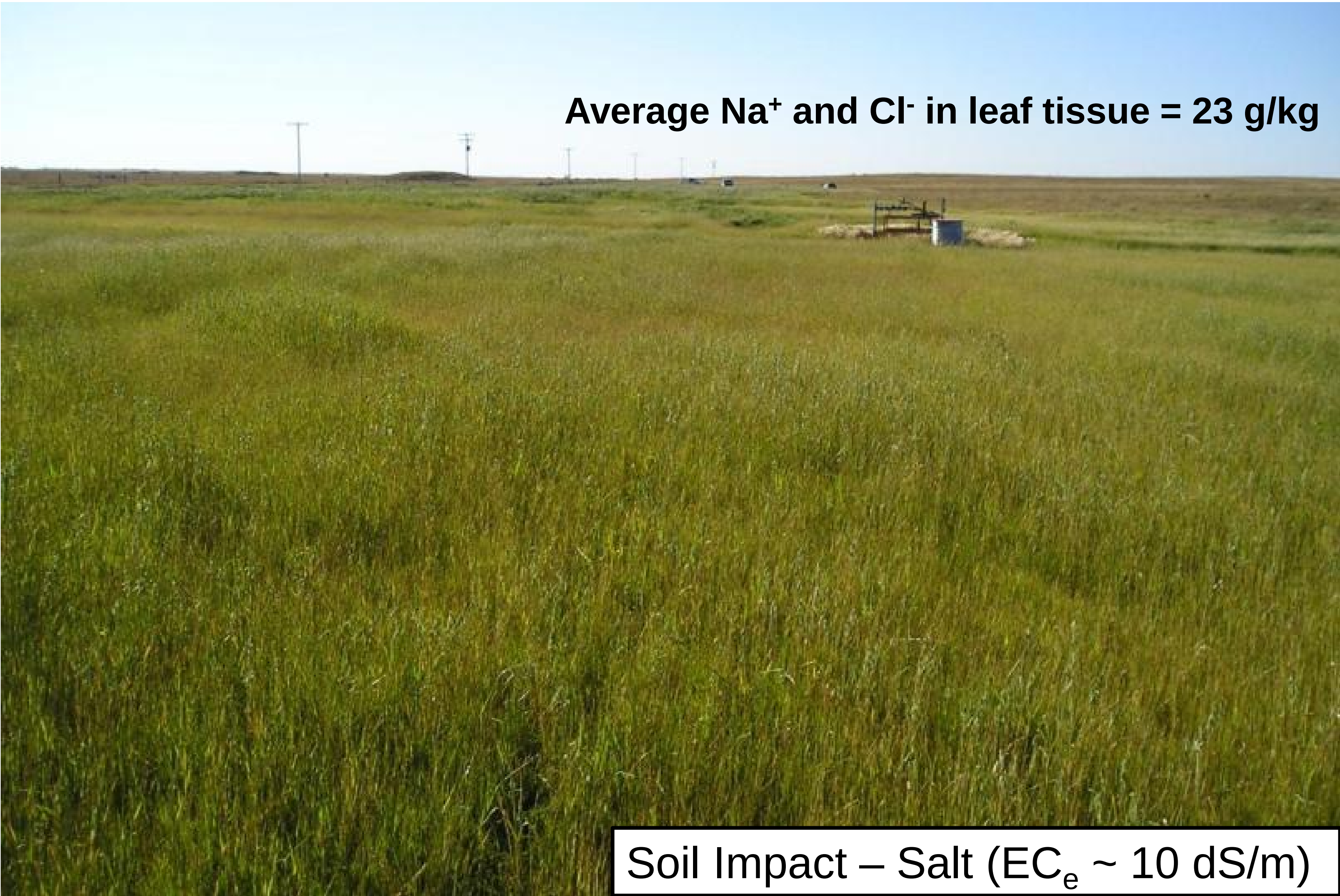


"Gardening pride aside, it's still a carrot and belongs outside."

Weyburn, SK - 2: Before PEPS deployment



Weyburn, SK - 2: PEPS deployment – 3 Months



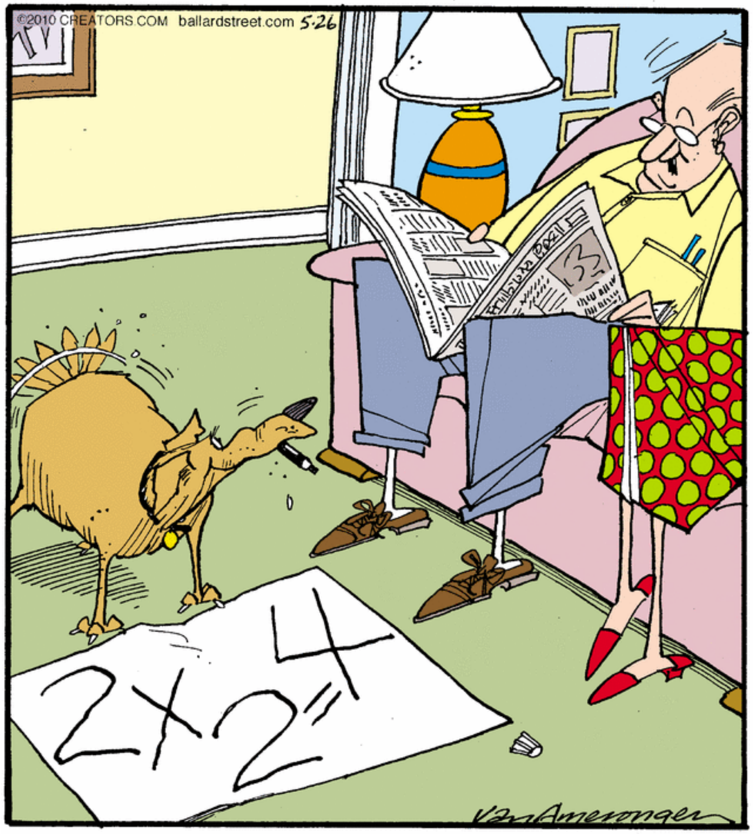
Salt Remediation by PEPS

Parameter	Value
Annual Decrease in EC_e	1 to 2 dS/m
Na^+ and Cl^- Uptake into Foliage	29 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%

With Abundant Plant Growth: PEPS results in PHC and Salt Remediation

- At least twice as much plant biomass due to PGPR
  - Root growth to 50 cm below ground level
- Remediation monitored using CCME PHC and salt analytical methods
- 30 to 40% remediation per year with PEPS; Twice as fast as plants without PGPR
- For PHC remediation, rhizosphere microbes consume PHC
  - elevated 10 to 100 fold with the PEPS
- Salt sequestered to foliage
  - Foliage harvested and removed from site
- No shortcuts for successful phytoremediation
  - Plant growth enhanced by meticulous site preparation and use of PGPR
  - Data validation to properly assess remediation at every stage
  - Only proven scientific methodologies used in PEPS
  - Patience – Allow time for nature to run its course

Research on the CCME PHC Technique for Assessment of Phytoremediation



"That's the kind of quality thinking we've come to expect from you, Reggie!"

Site Specific Risk Assessment
Soil Toxicity Assessment

- CCME Tier 1 generic criteria are the gold standard
- Tier 1 criteria is based mostly on spiked and/or unweathered soils
- CCME allows for Tier 2 risk assessments
- Does soil toxicity change following phytoremediation and do the soils become non-toxic before Tier 1 criteria are met?
- To assess this:
  - How toxic are authentic weathered soils before PEPS?
  - How toxic are authentic weathered soils after PEPS?

Assessment of Soil Toxicity Before Phytoremediation

- F2: 50 to 3,000 mg/kg F3: 200 to 10,000 mg/kg
- All toxicity assays follow Environment Canada (EC) guidelines
- Plant toxicity assays
  - Barley, Red Clover, Alfalfa and Cucumber
  - End Points
    - Seedling emergence
    - Root growth
    - Shoot growth
- Soil invertebrate assays
  - Springtails (*Folsomia candida - Colembola*)
  - End Points
    - Adult Survival
    - Juvenile production

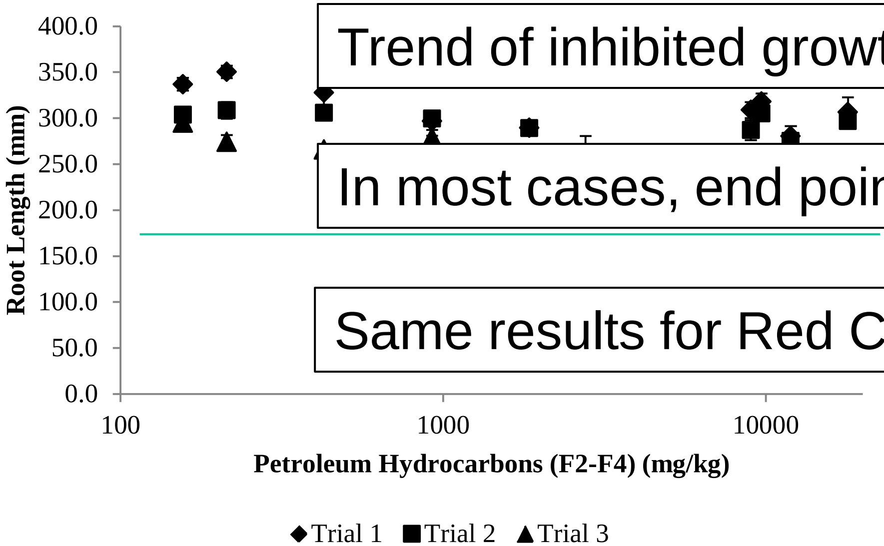
In consultation with Gladys Stephenson and George Dixon

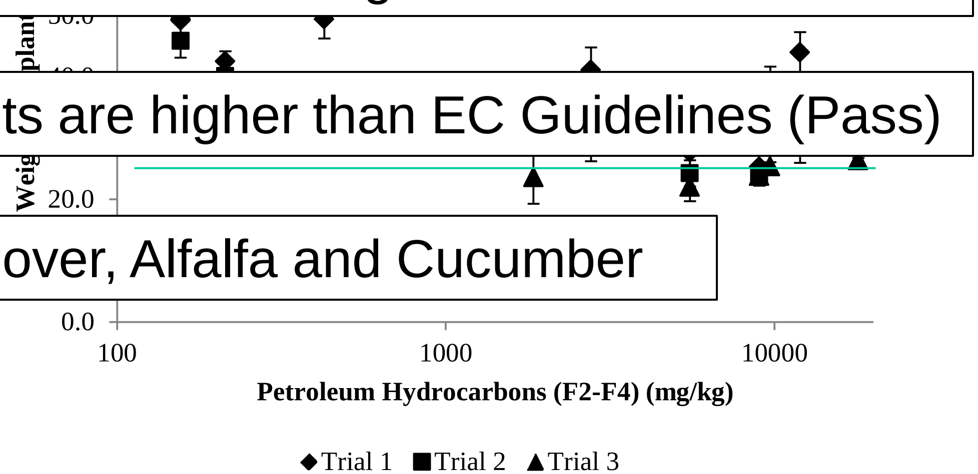
Barley Root and Shoot Growth – Before PEPS

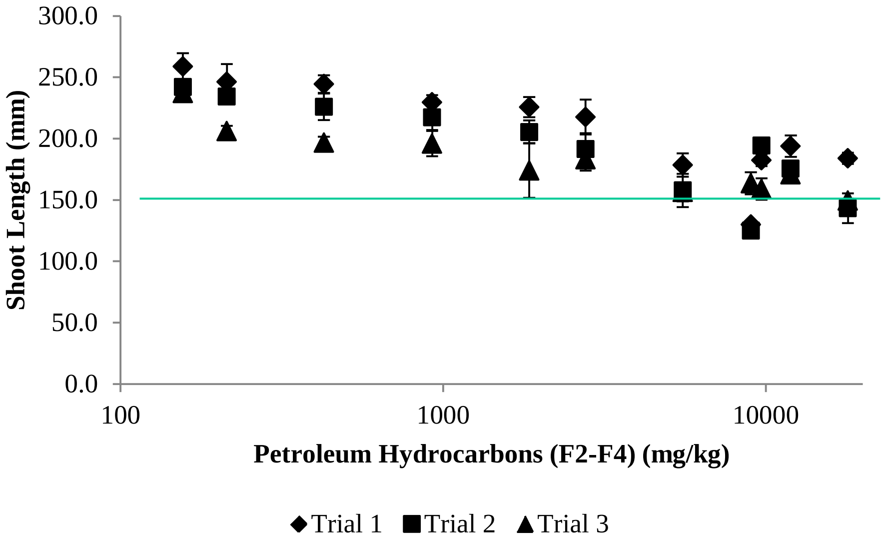
Trend of inhibited growth with increasing concentrations of PHC

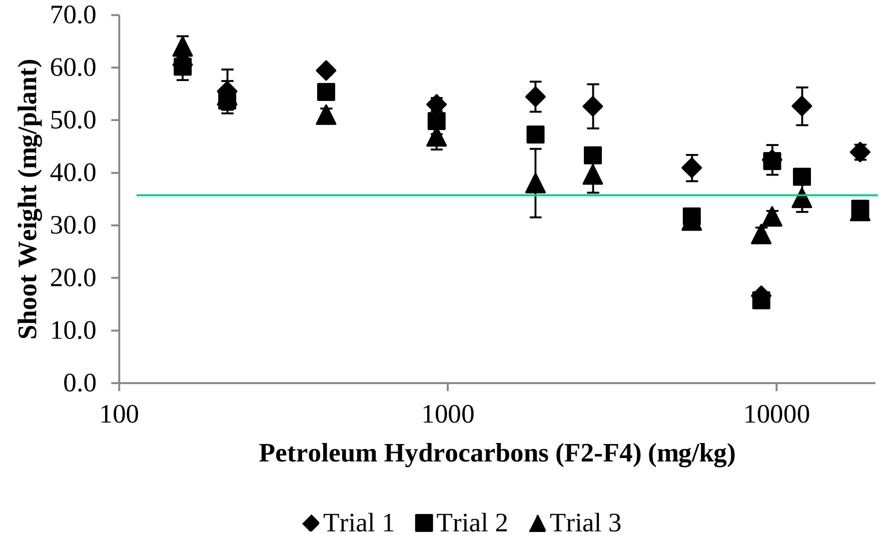
In most cases, end points are higher than EC Guidelines (Pass)

Same results for Red Clover, Alfalfa and Cucumber

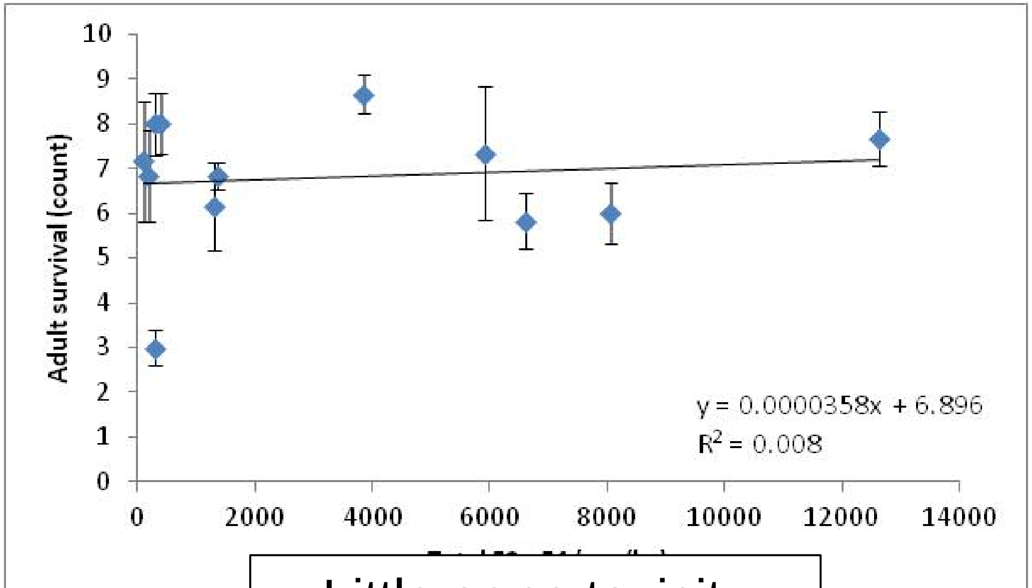




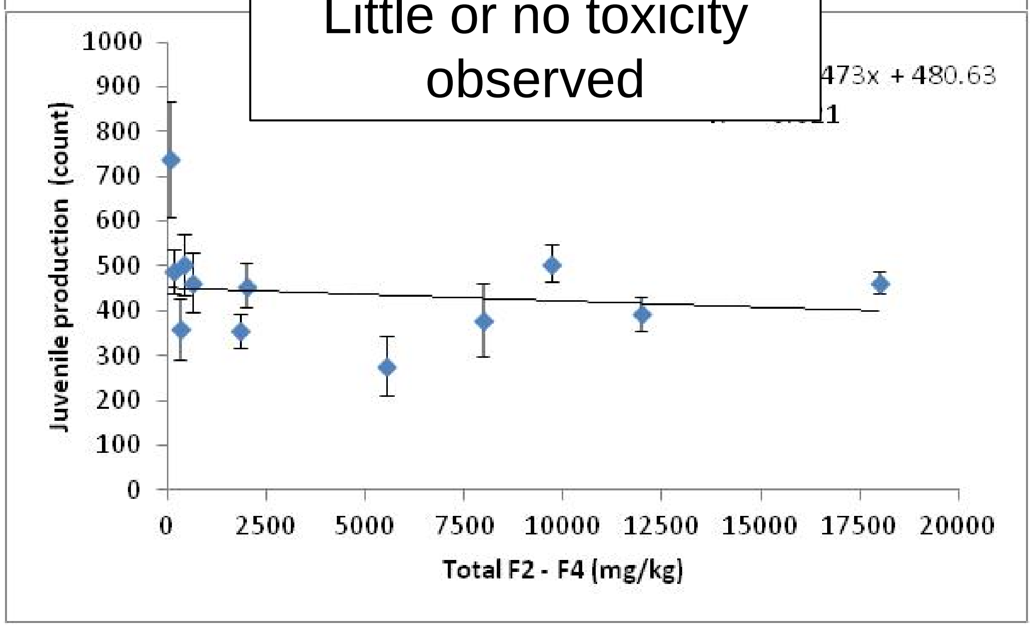




Colembola Adult Survival Before PEPS



Little or no toxicity observed



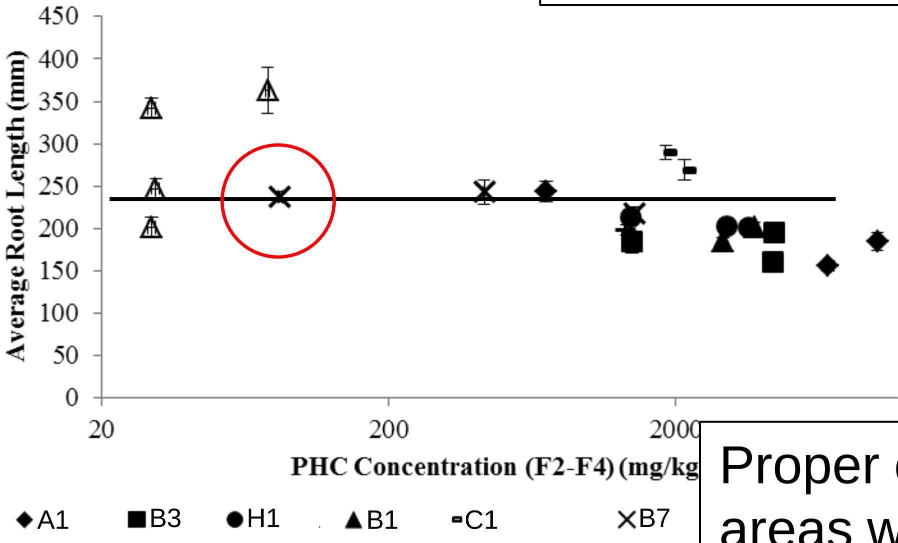
Colembola Juvenile Production Before PEPS

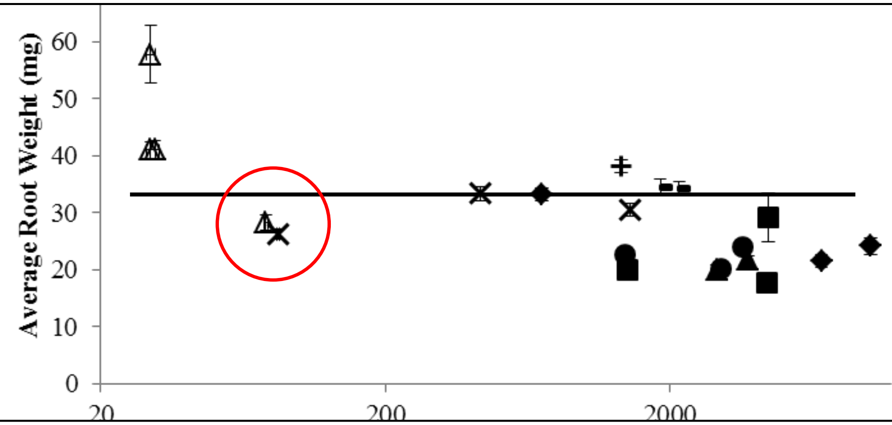
Plant and *Colembola* Toxicity Following PEPS

- Field Soils were obtained from seven sites
- F2: 10 to 5,200 mg/kg F3: 64 to 4,800 mg/kg
  - BC, ON and AB
  - PGPR enhanced phytoremediation had been preformed on these sites for a varying number of years
  - Selected soils with low, medium, and high PHC impacts
  - Control soils (unimpacted) were collected where possible

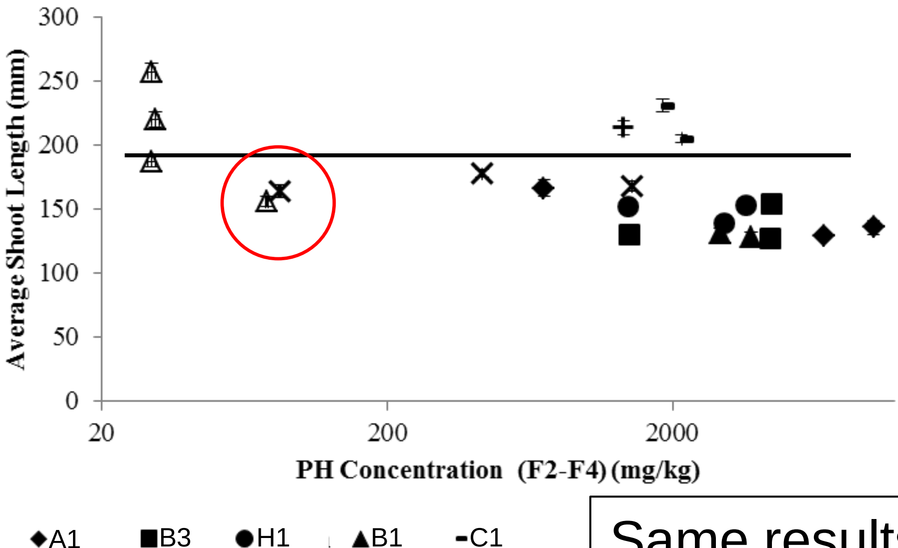
Barley Root and Shoot Growth – After PEPS

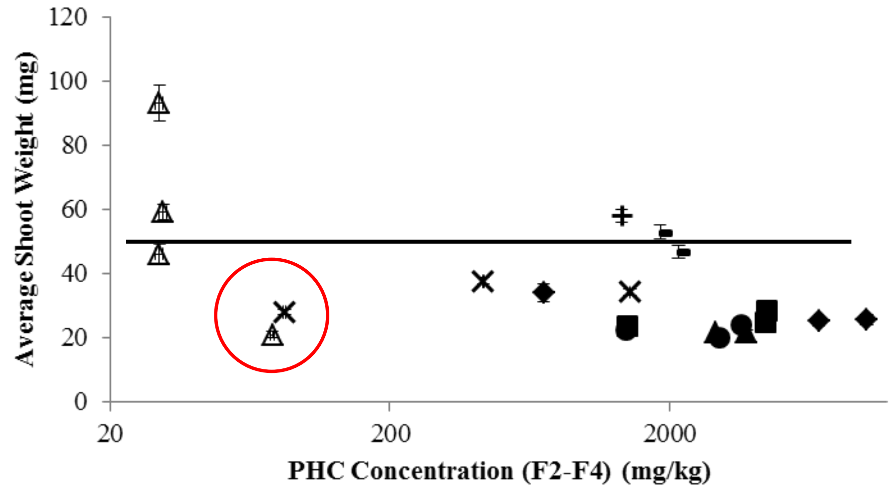
Toxicity does not correlate well with PHC concentration





Proper controls with same soil properties – areas with very low PHC (less than 150 mg/kg)





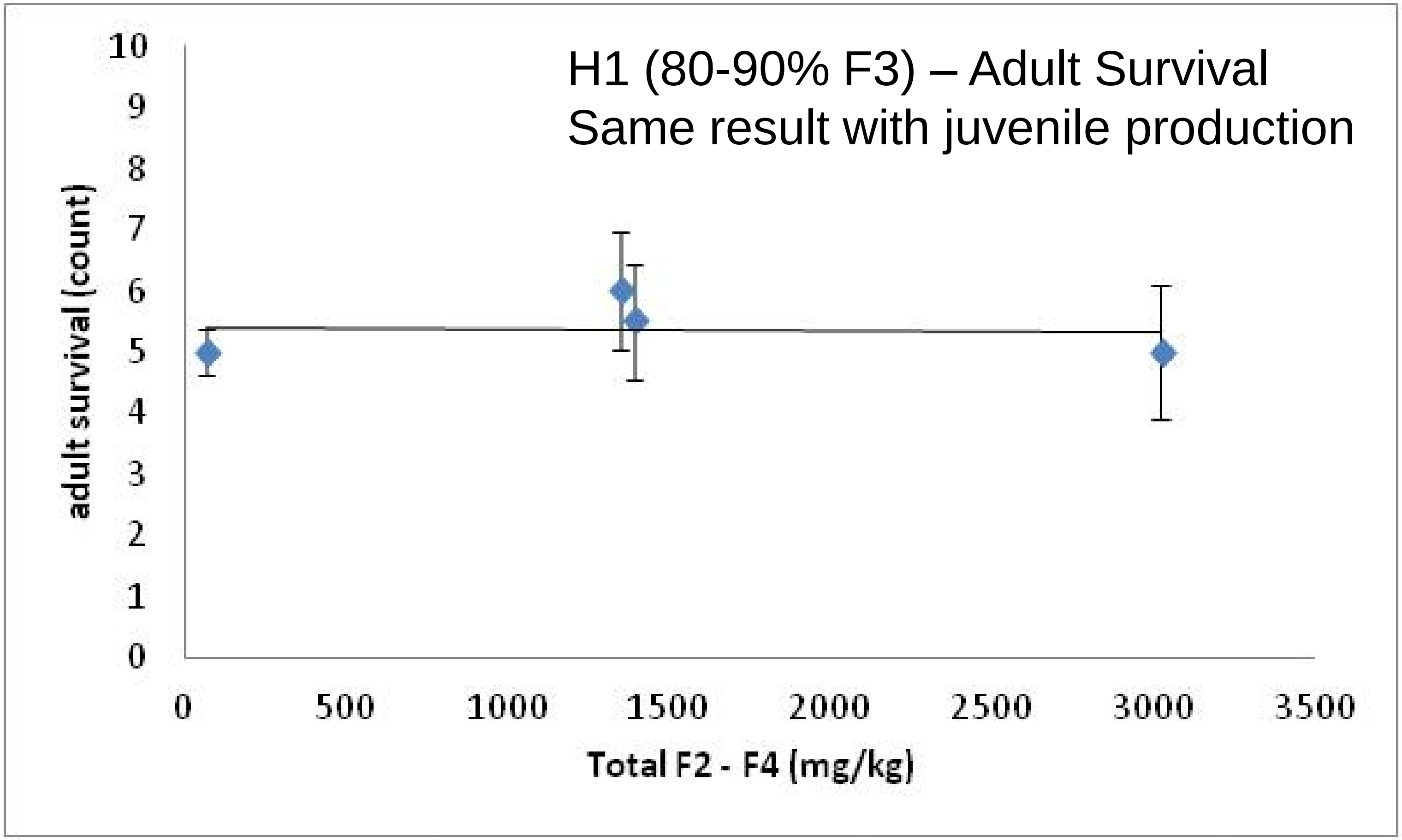
Same results for Red Clover, Alfalfa and Cucumber

Site	Sample	Petroleum Hydrocarbon Concentration (mg/kg)			25th Percentile
		F2	F3	Total	Field Control[1]
B3	3	**170**	1,200	1,412	83
	9/11	**1,000**	**3,300**	4,385	76
	6	**240**	**4,050**	4,430	89
A1	3	80	580	709	85
	4/5	**3,500**	**3,200**	6,810	48
	8	**5,200**	**4,800**	10,120	63
H1	25	150	1,200	1,392	78
	33	**340**	**2,600**	3,024	83
	15	**610**	**2,900**	3,598	83
P1		47	1,000	1,347	88
B1	19	**730**	**2,100**	2,930	66
	12	**1,300**	**2,400**	3,770	69
C1	CPH	19	1,300	1,849	103
	CPL	32	**1,600**	2,152	102
B7	11	10	64	84	83
	3	37	370	431	97
	7	**170**	1,200	1,442	92

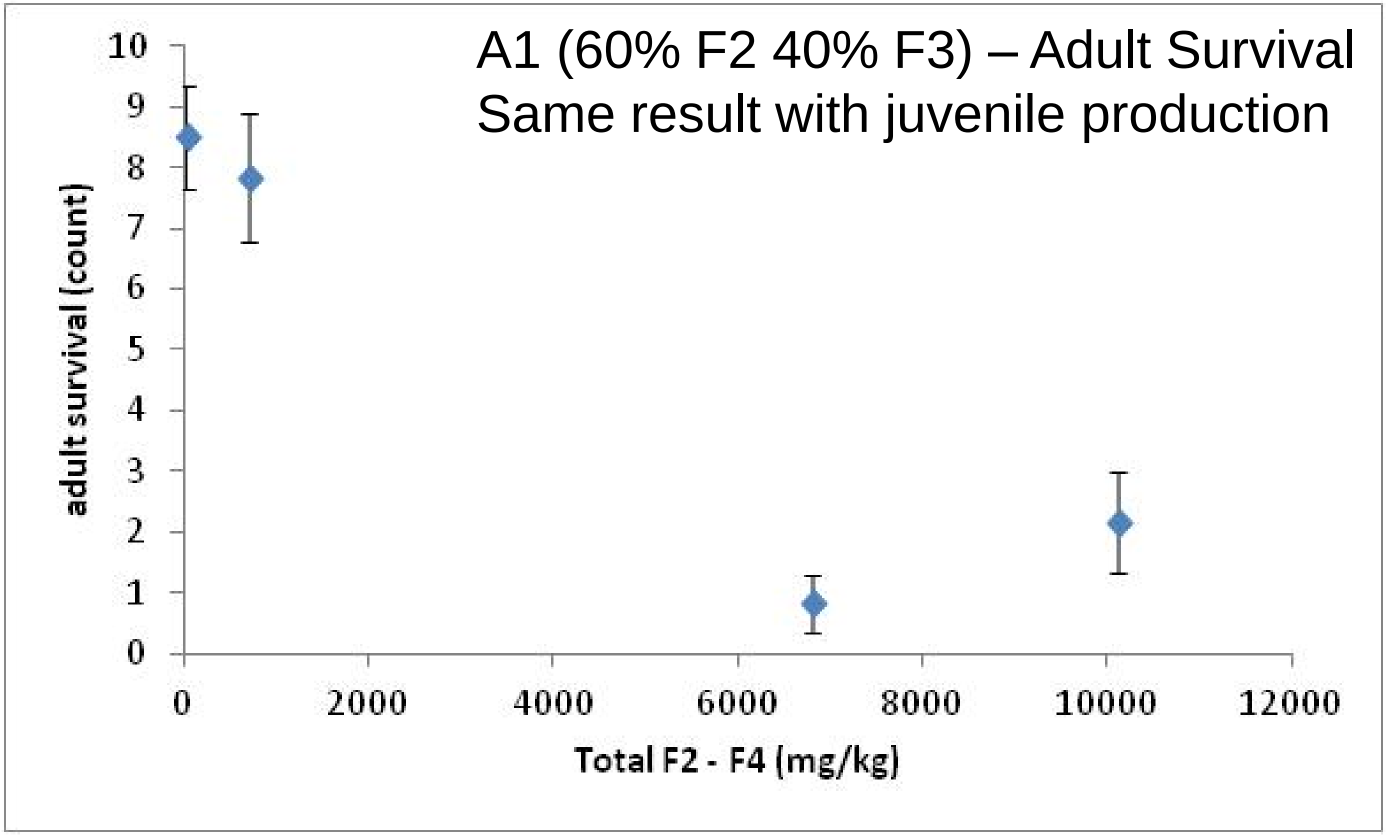
[1] Field Control was the average of A1-3, H1-25, P1, C1, B7-11 and B7-3 – Areas with PHC less than 150 mg/kg

Only samples with high F2 show toxicity after PEPS treatment

Toxicity to *Colembola* After PEPS – Low F2



Toxicity to *Colembola* After PEPS – High F2



Summary of Tier 2 Risk Assessment

- Weathered soil before PEPS treatment had low toxicity that correlated with PHC levels
- Toxicity of PEPS treated soils did not correlate with PHC levels
- After PEPS treatment, toxicity can be observed if F2 is present at high levels
- In most cases, F2 remediates after 1 or 2 years of PEPS treatment
- Tier 2 approach after PEPS – Realistic option

Enhanced Phytoremediation

- Increase soil O_2 levels
- Surfactant to enhance PHC bioavailability
- Increase rate of remediation

Enhanced Oxygen Levels

- Provide a source of O_2 to low O_2 soils
- MgO_2 and CaO_2 not very soluble in water
- Allows slow release of O_2
- Increase rate of PHC metabolism

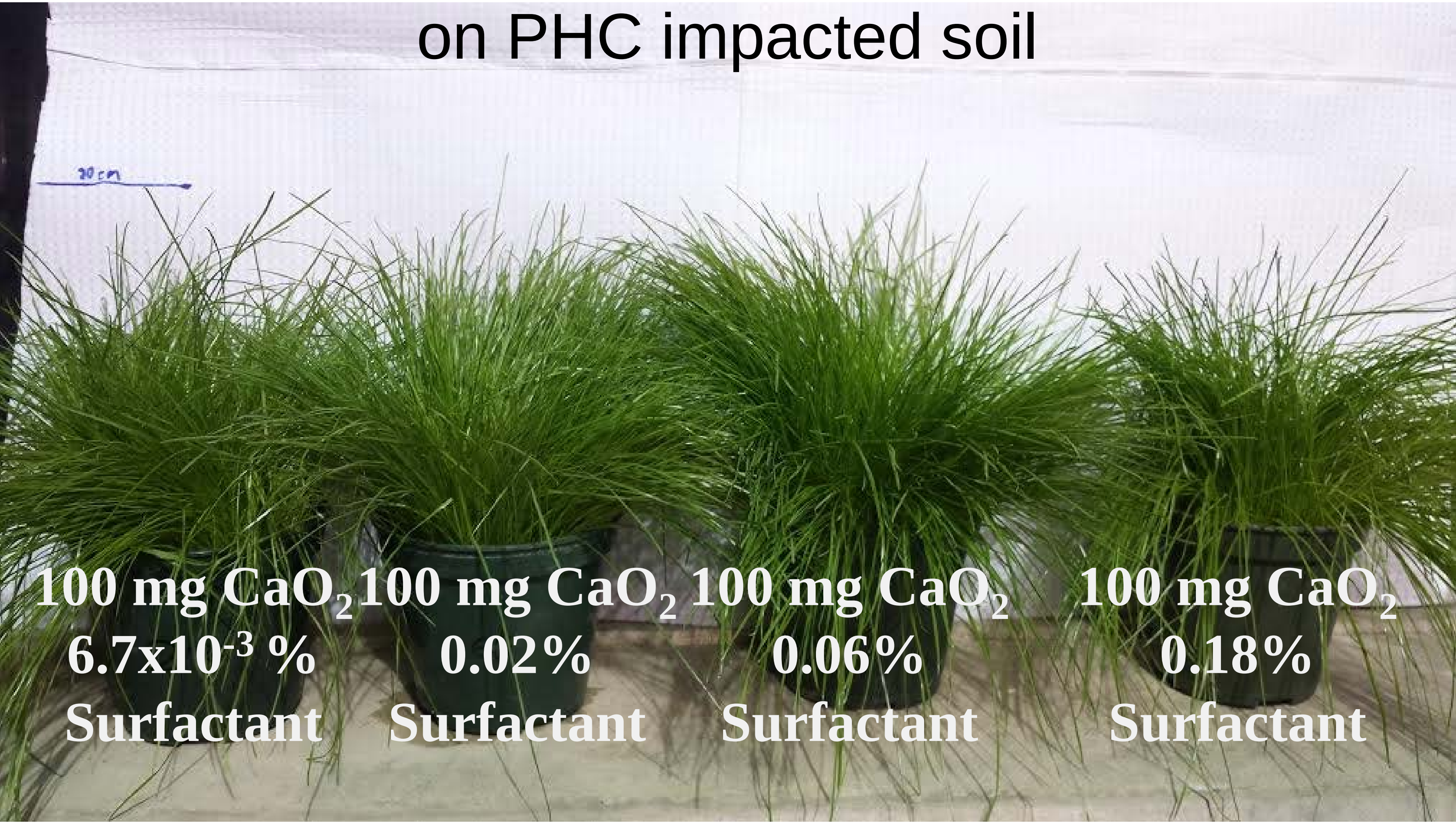
Considerations

- Could raise pH of soil out of the optimal range of 5-8 for bacteria
- Might need to increase the pH buffering capacity by adding phosphate buffers (fertilizer)
- Half life could be too short for long term phytoremediation project

Surfactants

- Lower surface tension between 2 liquids or liquid and a solid
- Allows PHC to become more bioavailable for metabolism
  - SDS
  - Rhamanolipid (biosurfactant)
  - Petroleum sulfonates (commercial products) (do not interfere with CCME analyses)

Plant growth with CaO_2 and Surfactant on PHC impacted soil



Conclusions

- PEPS proven to work at PHC, PAH and/or salt impacted sites.
- Remediation at all sites have been successful; > 30 sites.
- Phytoremediation costs < half the cost of landfilling.
- Liability is reduced, not transferred to a landfill.
- Cost effective at remote sites.
- Enhanced CCME BOC method – Only BOC is removed.
- Phytoremediation to Tier 1 standards in most cases.
- Tier 2 approach – will work – After PEPS brings F3 levels d 2500 mg/kg and F2 < 500 mg/kg no toxicity.
- CaO_2/surfactants do not impact on plant growth.

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FIDO REMEDIATION?

<u>You're</u> doing it wrong.

Thank you

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