Phytoremediation and Microbial Degradation Pilot Studies for a Former Waste Water Pond in Northern California

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Aerial Photographs

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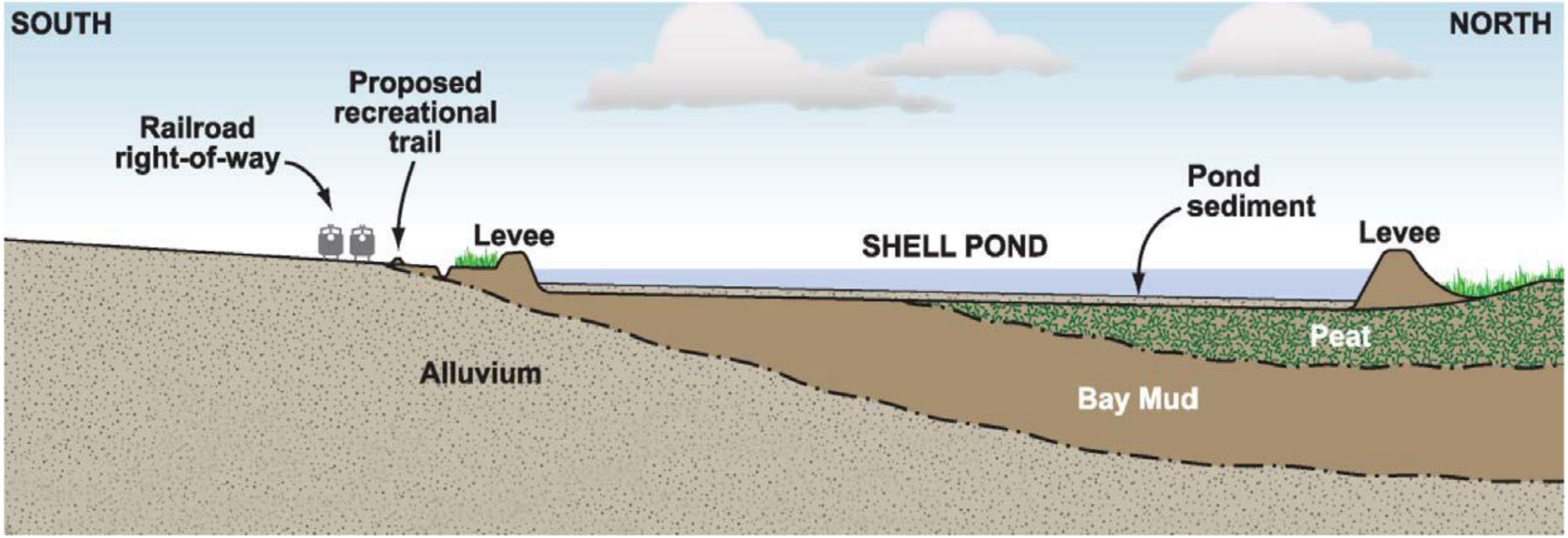
Conceptual Site Model and Remedy



Site Conditions

- Low mobility COCs (TPH, PAH, metals)
- Waste and underlying soil is low permeability
- No impacts beneath waste
- No groundwater impacts

Planned Remedy

- Dewater pond
- Establish plants
- Monitor progress, hotspot treatment as needed
- Long-term restoration of wetland

Total Petroleum Hydrocarbons

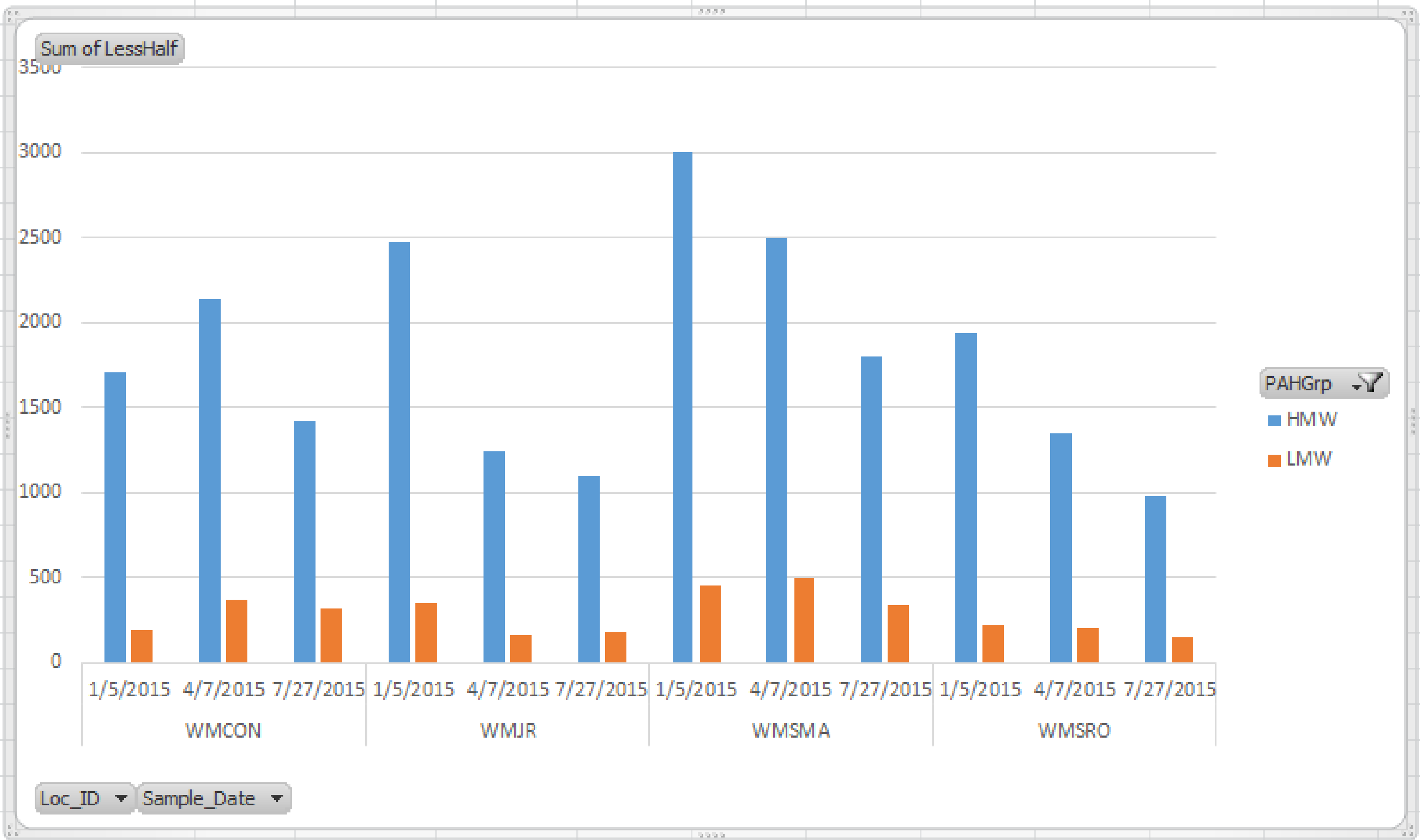
	Entrix Sampling, April 2009 (mg/kg)		PG&E Sampling, February 2013 (mg/kg)	% Reduction	Entrix Sampling, April 2009 (mg/kg)	PG&E Sampling, February 2013 (mg/kg)	% Reduction
Total Petroleum Hydrocarbons (mg/kg)	SP-1-SED	SP-1-SED-DUP	#5 Pond Soil SSE RL = 5.0		SP-3-SED	#6 Pond Soil SE RL = 5.0	
C6	18	ND	ND	100.0	370	ND	100.0
C7	21	ND	ND	100.0	370	ND	100.0
C8	9.9	ND	ND	100.0	370	ND	100.0
C9-C10	36	1.9	ND	100.0	34	ND	100.0
Total TPH as Gasoline	84.9	1.9	0	100.0	1144	0	100.0
C11-C12	100	5.2	ND	100.0	130	ND	100.0
C13-C14	190	4.5	ND	100.0	270	ND	100.0
C15-C16	200	17	ND	100.0	660	ND	100.0
C17-C18	250	25	ND	100.0	1,100	ND	100.0
C19-C20	130	23	ND	100.0	1,200	ND	100.0
C21-C22	100	64	ND	100.0	1,100	5.5	99.5
C23-C24	69	65	ND	100.0	1,200	ND	100.0
C25-C28	72	240	ND	100.0	2,300	5.1	99.8
Total TPH as Diesel	1111	443.7	0	100.0	7960	10.6	99.9
C29-C32	100	260	ND	100.0	2,500	ND	100.0
C33-C36	99	180	7.1	96.2	2,300	7.9	99.7
C37-C40	36	73	ND	100.0	2,000	ND	100.0
C41-C44	40	120	6.1	93.9	1,600	ND	100.0
Total TPH as Motor Oil	275	633	13	97.8	8,400	8	99.9
C6-C44 Total	1,471	1,079	13	99	17,504	19	100

PAHs and Metals

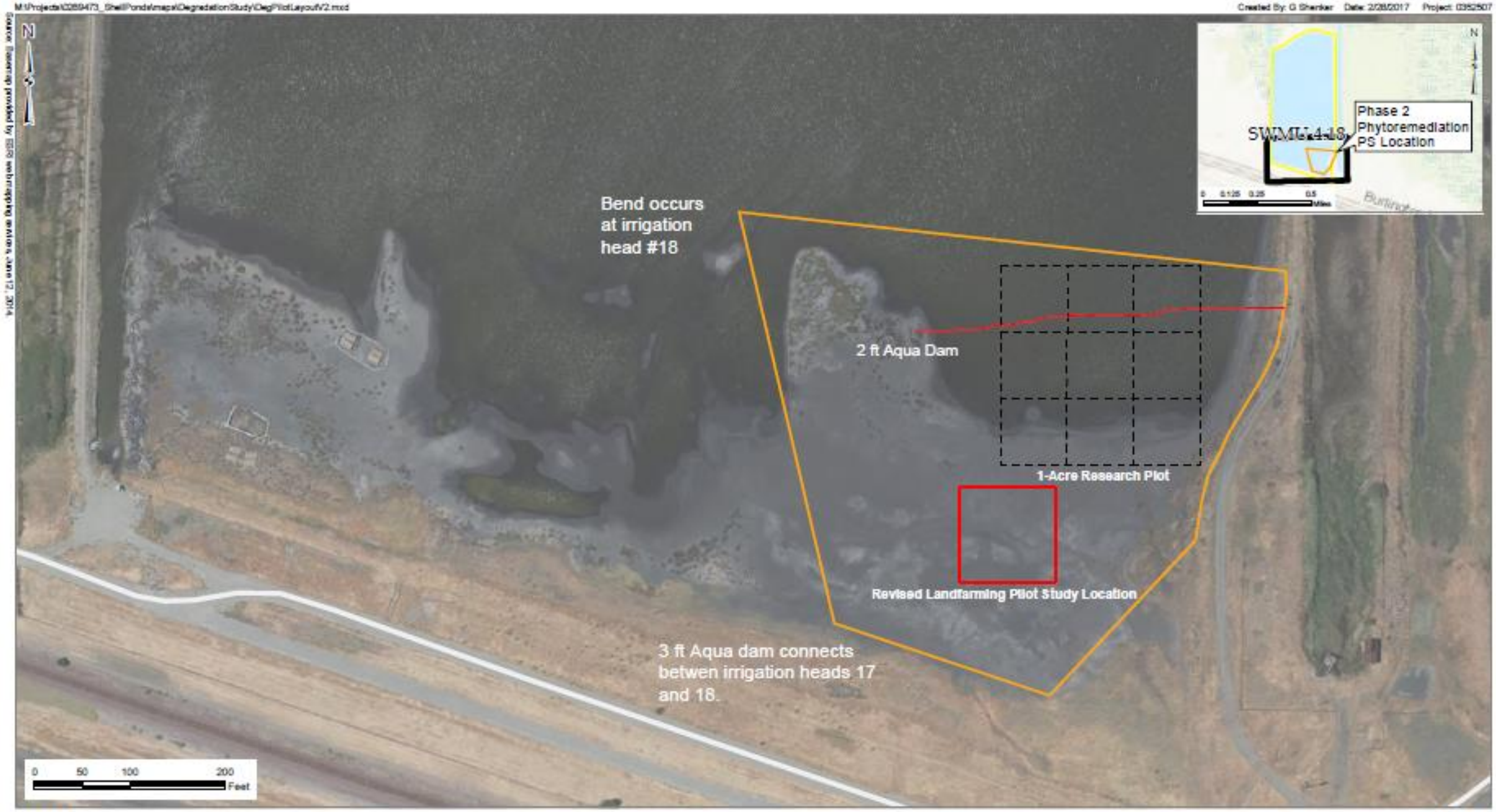
	Entrix Sampling, April 2009 (mg/kg)		PG&E Sampling, February 2013 (mg/kg)	% Reduction	Entrix Sampling, April 2009 (mg/kg)	PG&E Sampling, February 2013 (mg/kg)	% Reduction
Acenaphthylene	2.5	1.8	ND	100	1.3	1.5	-15.4
Benzo (a) Pyrene	3.2	2.6	2	55.6	5.2	0.59	88.7
Benzo (g,h,i) Perylene	26	22	ND	100.0	94	6.4	93.2
Fluoranthene	6.3	5	0.77	91.3	5.2	3.2	38.5
Indeno (1,2,3-c,d) Pyrene	4.2	3.3	ND	100.0	14	1.2	91.4
Phenanthrene	5.3	3.2	ND	100.0	1.6	2.9	-81.3
Pyrene	23	17	3.8	87.9	19	16	15.8

	Entrix Sampling, April 2009 (mg/kg)		PG&E Sampling, February 2013 (mg/kg)	Entrix Sampling, April 2009 (mg/kg)	PG&E Sampling, February 2013 (mg/kg)
EPA 6010B Title 22 Metals (mg/kg)	SP-1-SED	SP-1-SED-DUP	#5 Pond Soil SSE	SP-3-SED	#6 Pond Soil SE
Chromium	80.8	98.5	53.7	29.2	35.3
Cobalt	43.4	52.3	25.5	3.46	29.4
Copper	64.9	71	46.7	42.3	45.8
Lead	61.5	66.1	50.5	98.7	24.8
Molybendum	23.5	0.086	18.4	1.32	49.4
Nickel	101	109	60.3	21.7	57.2
Mercury	0.867	1.13	0.611	1.58	0.362

UCB Phytoremediation Experiments



Phase 2 Pilot Study



Legend

- Revised Phase 2 Phytoremediation Pilot Study Area (~4.5 Acres)
- Phase 2 Phytoremediation Research Plot Area - 1 Acre
- Pilot Study Areas - 100 ft X 100 ft

Notes:
One-Acre Research Plot is 210' x 210'

Figure 1
Research Plot Configuration
T=0 Summary
Shell Pond
Bay Point, California

Environmental Resources Management
www.erm.com
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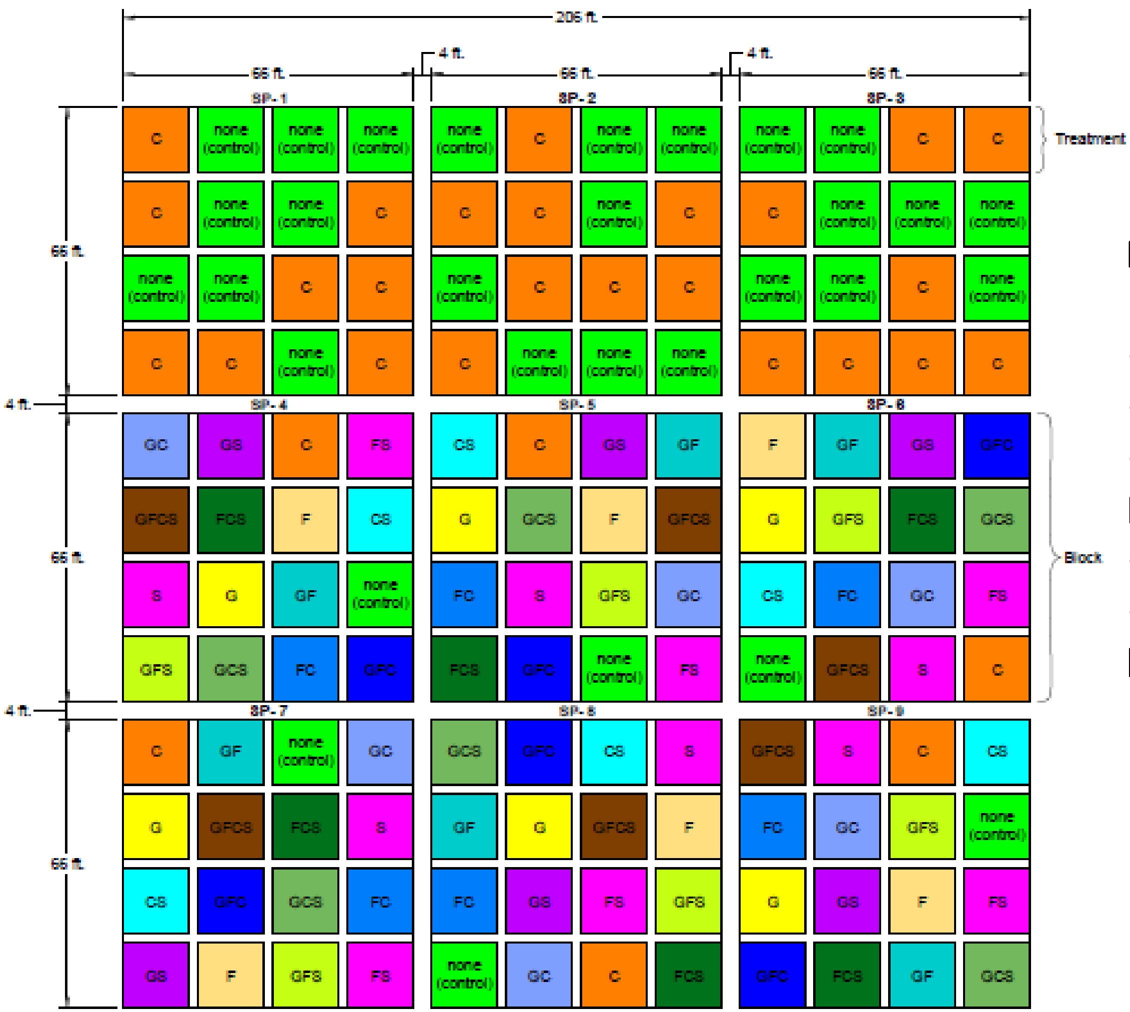
Block Design and Plant Selection

9 Block Randomized Complete Block (RCB) Design



Plant Seeds used in the Seed Mixes

- *Distichlis spicata* (saltgrass), Facultative
- *Jaumea carnosa* (jaumea), Obligate
- *Sporobolus airoides* (alkali sacaton), Facultative
- *Atriplex patula* (fat hen), Facultative Wet
- *Hordeum brachyantherum* (meadow barley), Facultative Wet

T=0 TPH (C6-C44) and HMW PAHs

Table A – Total TPH and HMW PAHs in Sediment

Block Number	Analyte	Minimum Concentration	Maximum Concentration	Average Concentration	Standard Deviation
		milligrams per kilograms (mg/kg)			
Block SP-1	TPH	2,700	33,000	17,731	7,371
	HMW PAH	8	879	266	227.72
Block SP-2	TPH	13,000	33,000	21,000	5,668
	HMW PAH	90	507	388	466.92
Block SP-3	TPH	22,000	180,000	72,688	49,594
	HMW PAH	140	834	421	166.35
Block SP-4	TPH	640	35,000	17,584	9,145
	HMW PAH	32	833	338	186.5
Block SP-5	TPH	6,300	41,000	21,269	8,336
	HMW PAH	241	949	442	202.96
Block SP-6	TPH	11,000	23,000	16,813	3,779
	HMW PAH	133	852	480	166.09
Block SP-7	TPH	4,400	23,000	11,088	4,650
	HMW PAH	17	669	334	155.62
Block SP-8	TPH	5,900	19,000	13,263	4,020
	HMW PAH	275	1,010	460	189.78
Block SP-9	TPH	6,200	25,000	13,325	3,746
	HMW PAH	300	6,407	966	1,430.72

Distribution of TPH and PAHs

TPH

Block 1

16,000	9,800	24,000	8,200
10,000	26,000	20,000	25,000
33,000	21,000	19,000	20,000
2,700	16,000	18,000	15,000

Block 2

16,000	21,000	17,000	28,000
19,000	25,000	19,000	23,000
33,000	26,000	13,000	16,000
16,000	21,000	14,000	29,000

Block 3

37,000	22,000	36,000	27,000
30,000	45,000	54,000	29,000
23,000	120,000	110,000	150,000
180,000	70,000	120,000	110,000

Block 4

16,000	26,000	640	32,000
18,000	21,000	4,000	3,700
19,000	15,000	23,000	35,000
15,000	17,000	22,000	14,000

Block 5

22,000	41,000	25,000	16,000
6,300	19,000	28,000	31,000
29,000	14,000	19,000	25,000
22,000	13,000	19,000	11,000

Block 6

13,000	13,000	14,000	19,000
16,000	13,000	19,000	16,000
22,000	15,000	21,000	22,000
13,000	23,000	19,000	11,000

Block 7

12,000	23,000	8,200	20,000
11,000	11,000	10,000	9,800
4,400	6,800	5,200	12,000
9,000	14,000	10,000	11,000

Block 8

9,500	16,000	18,000	18,000
7,800	17,000	15,000	11,000
12,000	12,000	17,000	11,000
15,000	5,900	8,000	19,000

Block 9

16,000	12,000	14,000	15,000
14,000	25,000	13,000	12,000
13,000	15,000	11,000	12,000
13,000	12,000	6,200	10,000

PAH

Block 1

144	76	176	134
130	256	300	121
258	133	358	760
8	254	265	879

Block 2

214	295	192	184
427	239	90	208
390	225	196	421
507	261	2,148	215

Block 3

380	140	342	384
372	632	321	622
407	537	417	441
834	294	182	427

Block 4

192	306	32	193
452	833	245	177
332	146	354	347
385	337	483	595

Block 5

387	358	408	323
241	937	532	382
263	266	341	398
476	433	381	949

Block 6

233	600	597	376
495	510	587	567
443	370	293	133
516	576	537	852

Block 7

337	379	307	566
17	301	329	487
210	191	336	669
134	259	390	431

Block 8

516	451	748	298
520	528	311	1,010
326	355	360	275
374	584	426	276

Block 9

594	528	696	805
303	389	1,243	643
300	475	502	806
319	1,111	331	6,407

Agronomic Data

Key Agronomic Elements	Data Summary	Data Interpretation
pH	Average across Research Plot = 7.3, with some blocks yielding pH levels up to 8	Levels are higher than the optimal range (6.3 to 6.8)
Soluble salt	Average across Research Plot = 14.85 mmho/cm	Levels are higher than the optimal value (<2 mmhos/cm)
Nitrate-Nitrogen	Average across Research Plot = 6 ppm, with some blocks yielding levels <1 ppm	Levels are lower than optimal value (>10 ppm)
Phosphorus (P)	Average across Research Plot = 111 ppm	Levels are acceptable and conducive for plant growth
Potassium (K)	Average across Research Plot = 551 ppm	Levels are acceptable and conducive for plant growth
Sulfate	Average across Research Plot = 1,092 ppm	Levels are higher than the optimal range (25 to 35 ppm)
Sodium base saturation (% of cation exchange capacity for K, Mg, Ca, Na)	Average across Research Plot = 10,795	Very high levels result in low calcium and magnesium base saturation

Environmental Molecular Diagnostics

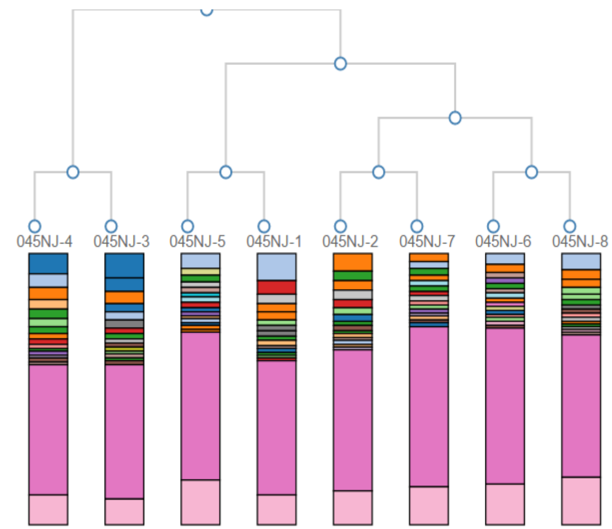
- Next Generation Sequencing (NGS) - High-throughput DNA-based analysis to discern composition of microbial community collected T=0 and T=end
- Quantarray Petro – Identify microbial enzyme activity specific to TPH/PAH degradation collected T=0 and T=end
- Stable Isotope Probing (SIP) – Provide a direct line of evidence that identifies the fate of 13C labeled COCs collected T=end
- Biotraps to be collected and analyzed by SIP at T=end
- Results will be combined with time-series PAH and TPH data for first-order degradation rates

Example of high throughput gene sequencing NGS

PG&E

Environmental Molecular Diagnostics

- NGS analysis indicates varied microbial population including halophilic (*Halothiobacillus*), sulfate reducing (*Desulfosarcina*), TPH and PAH degraders
- Also identified microbes typically only found on sea floor indicating diversity of microbial community
- E. coli and Salmonella observed also indicating diversity of microbial community
- Quantarray data showed elevated aerobic and anaerobic microbial enzymatic activity including toluene, naphthalene and phenol degrading enzymes providing evidence of degradation of Site COCs by indigenous microbial community



Hierarchical Clustering Dendrogram from Microbial Insights NGS laboratory

Winter 2017 – The Drought has Ended

Before 12/05/2016

After 01/31/2017

Spring 2017 - Recovery

Sustainability and Safety Benefits

Avoided environmental impacts & safety risks by applying phytoremediation instead of off-site disposal

Item	Data
Volume of soil to remediate	200,000 cubic yards
Weight of soil to remediate	320,000 tons
Soil Disposal Trucking Trips	13,913 truck trips
Total Trucking Miles	194,783 miles
Trucking Days	174 days
Off-Site Disposal Cost	$30,000,000+
CO_2 Emissions	767 tons
Trucking Accidents Avoided	0.3 accidents damage, injury, fatality

Stakeholder Engagement

- Identify stakeholder values, perceptions, and concerns – listen to them
- Develop key messages and work with community members that address their concerns and ours (before they are rolled out)
- Communicate in their language (no jargon, short, clear, and concise, keep it simple)
- Understand the importance of trust and credibility
- People's perception is their reality, which is largely based on where they get their information and from whom they trust

Summary

- TPH (640 to 180,000 mg/kg), PAHs (8 to 6,407 mg/kg), and metals (variable) were detected in all 9 blocks. T=0 results will be compared T=end
- T=0 fingerprinting data established baseline concentrations for a detailed suite of VOCs and SVOCs
- T=0 microbial data show elevated levels of anaerobic PAH enzymes, which indicate a presence of anaerobic microbes capable of degrading PAHs
- NGS analysis show that the microbial population is dominated by a robust population of proteobacteria (gram negative)
- Metals were detected in the plant tissue from all 6 reference plots and will be compared to T=end Research Plots

Summary

- This approach embodies the green remediation elements.
- The approach is innovative and probably the first of its kind for a project of this size and scope.
- The science indicates that the remedial goals can be achieved, but the time will need to be measured in years.
- Impacts to the community will be minimal and they will benefit by having a former industrial wastewater facility returned to a wetland.
- Stakeholder engagement ***must be a strategic level need*** rather than a support level role.