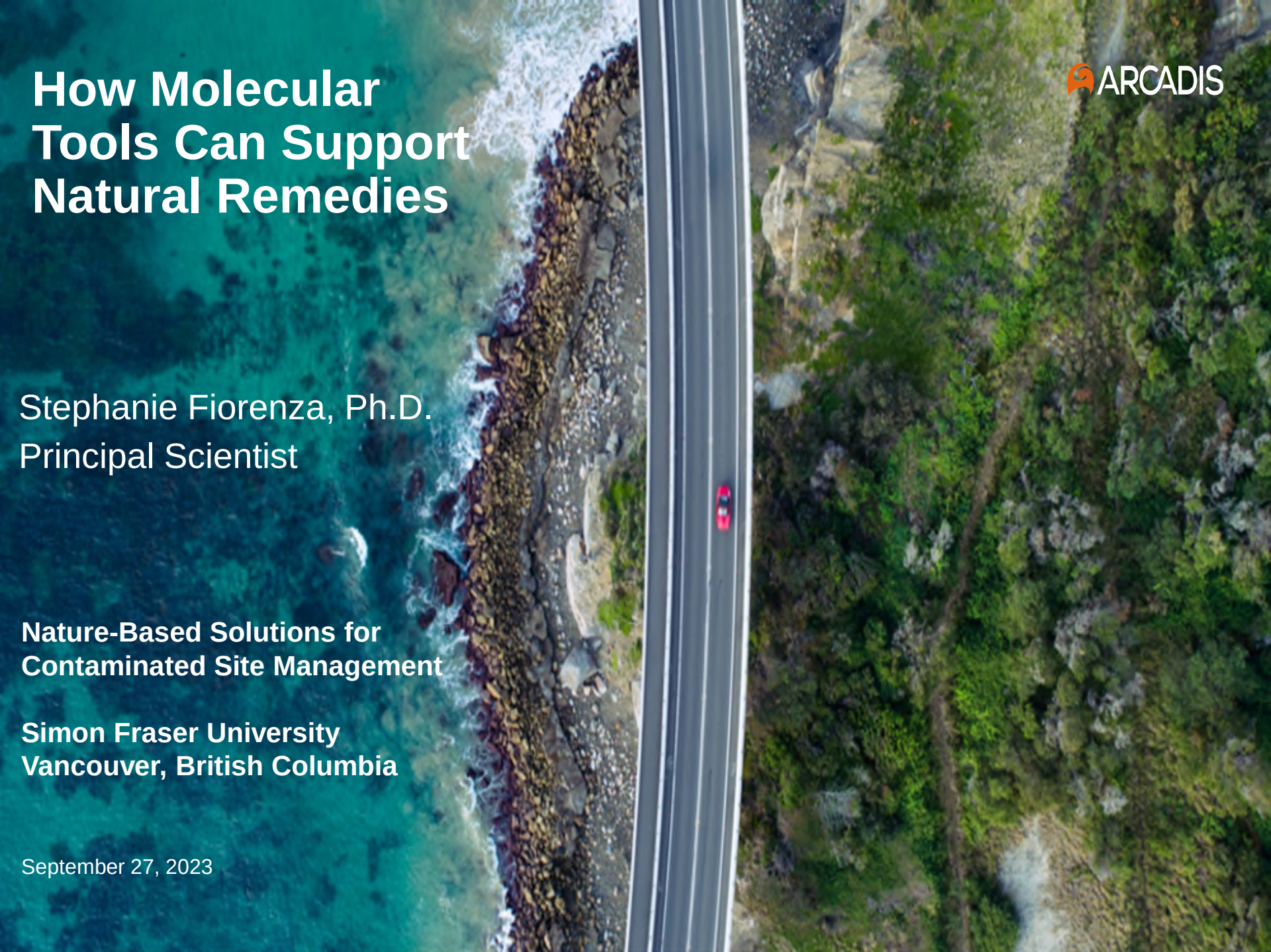
How Molecular Tools Can Support Natural Remedies



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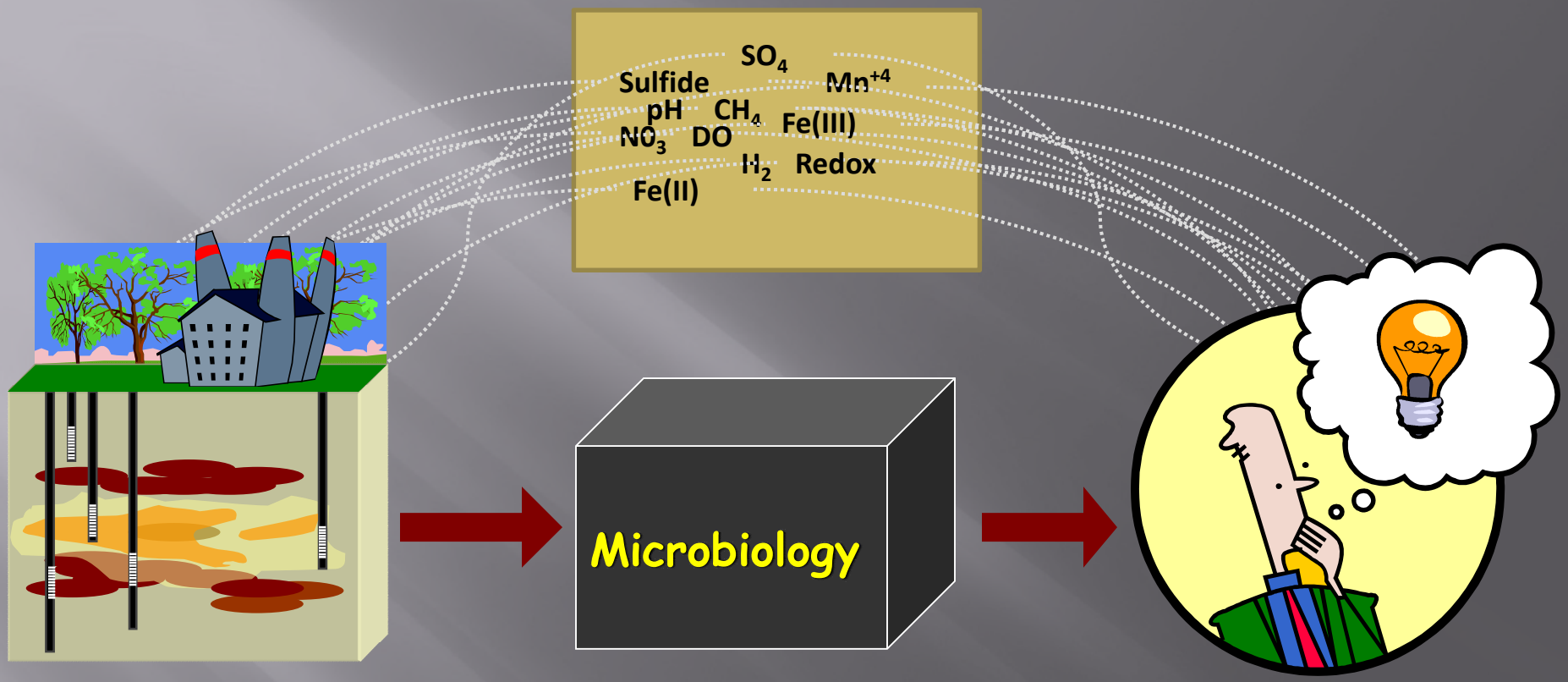
Principal Scientist

Nature-Based Solutions for Contaminated Site Management

Simon Fraser University
Vancouver, British Columbia

September 27, 2023

Conceptual Site Model



Nature-based remediation relies on biodegradation

Biodegradation relies on microorganisms that have specific requirements

- **Oxygen:** Aerobic, anaerobic and facultative
- **Temperature:** psychrophiles, mesophiles, thermophiles
- **pH:** Neutrophile, acidophiles
- **Metabolic requirements:** Specific electron donors /acceptors, trace nutrients
- **Contaminants:** some support growth, some inhibit or are toxic, concentration dependent

Advantages of Interrogating Microbiome

Parallel Data - complement traditional parameters such VOCs, organic carbon, field parameters etc.

Selective - specific PCR primers find the “microbial needle in the haystack”

Sensitive – PCR - based methods are capable of detecting a few microbes (gene copies) when contaminant impacts may not be apparent

Predictive of potential functions even if not yet expressed or still “growing up”

Hi Resolution Data show where biodegradation activities are (and are not) occurring

Microbes are **Bio-monitors** - MBTs that can tell you about geochemical conditions in the subsurface- NGS provides detailed microbial profiles ideal for this purpose.

Adapted from P. Dennis

MBTs aid in site characterization, remedy selection and implementation in NBS

- Among the lowest-cost remediation approaches
- Compatible with low impact passive systems
- Sustainable remediation e.g., low carbon footprint

Microorganisms respond in real time to changing subsurface conditions

ation

nature based
passive system
better sustainability metrics

A nature-based
passive system has
better sustainability metrics

Reasons to Use Molecular Microbial Tools

Answer questions such as:

What microorganisms are present? (DNA)

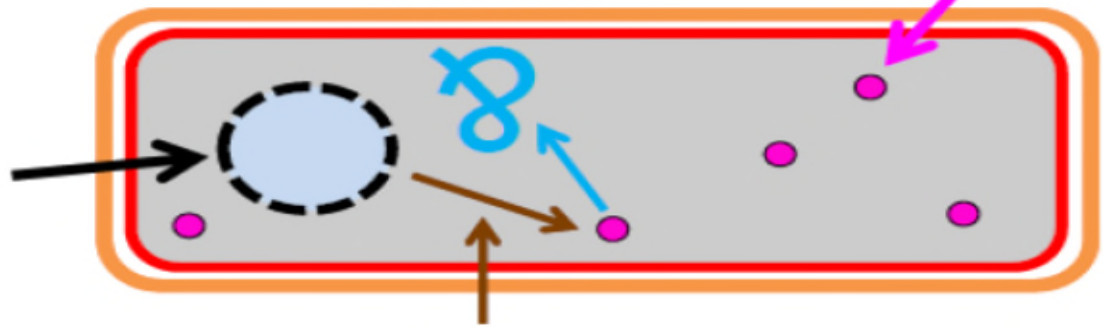
- Are there known degraders of the contaminant of interest?
- What kind of metabolism do they have?

Can they biodegrade the contaminant(s) of interest? (DNA)

How much degradation activity is occurring? (mRNA – qPCR)

Is there conclusive evidence of degradation (proteins)?

Bacterial Cell



(META)PROTEOMICS

Tells you:
Activity

'Enzymes'
Dehalogenases
Oxygenases

Protein

Ribosome

Protein production centers

DNA

Information storehouse

mRNA

Takes information from DNA to Ribosomes

Tells you:
Presence of microbes & metabolic genes

Tells you:
Active microbes and genes being made

qPCR
MICROARRAYS
(META)GENOMICS

(META)TRANSCRIPTOMICS

From K. Kucharyk

DNA Replication

- Strands separate
- DNA replication occurs on the template strand
- DNA polymerase (enzyme) adds matching base at the 3’ end of the template

DNA Transcription to RNA

- Strands separate
- RNA polymerase transcribes template DNA to an RNA copy, the ‘message’ or messenger RNA (mRNA)

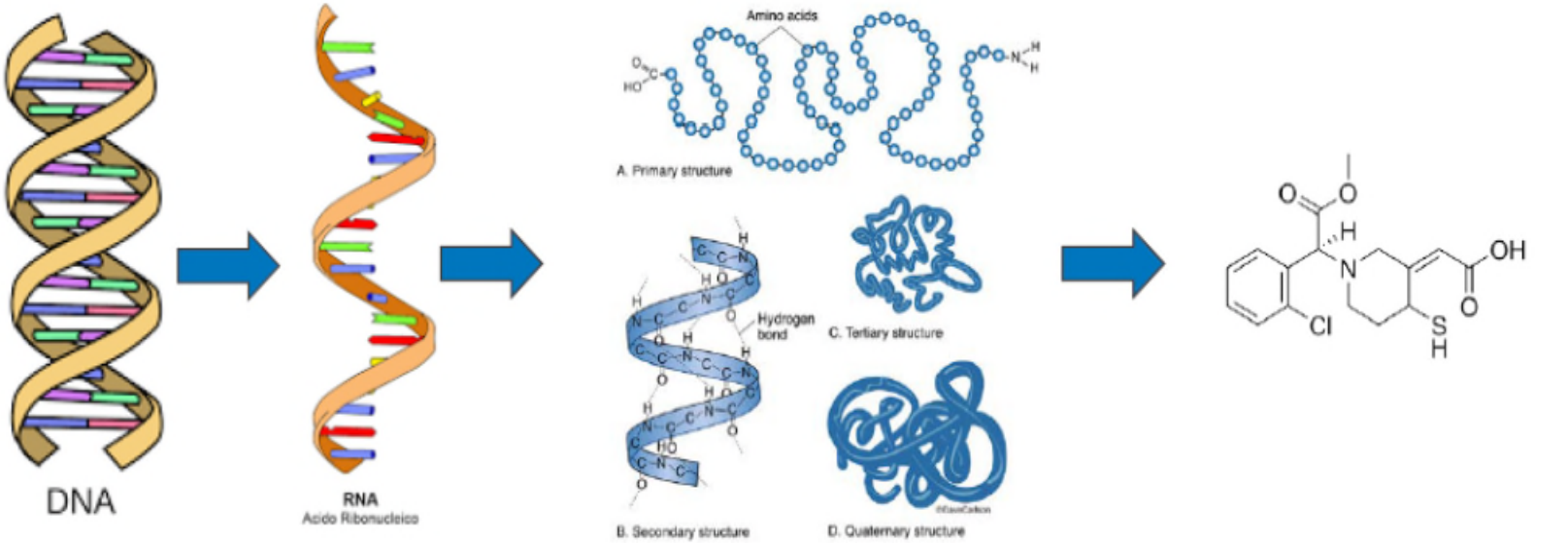
RNA Translation to Protein

- Translation of mRNA to protein occurs in the ribosome
- transfer RNA brings amino acids to the growing protein

Applicable MBTs

PCR
qPCR
Arrays
NGS

RT-qPCR



GENOMICS

TRANSCRIPTOMICS

FUNCTIONAL GENOMICS

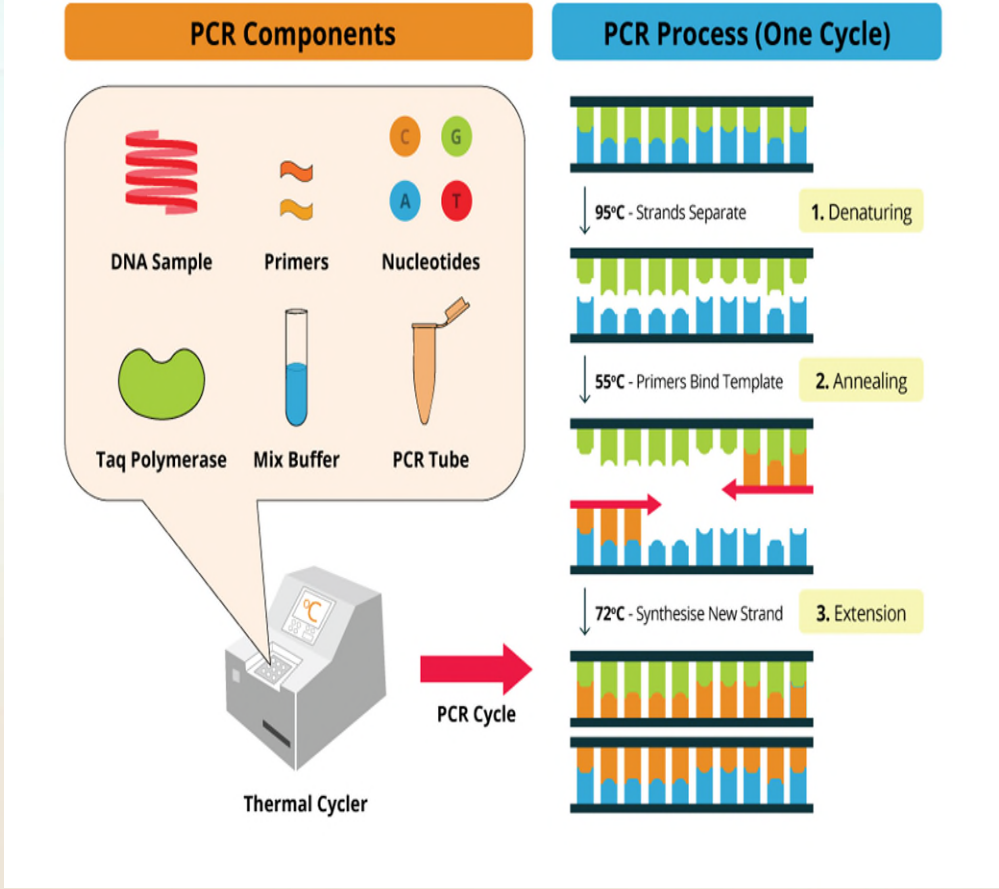
PROTEOMICS

METABOLOMICS

Modified from K. Kucharyk, Battelle 2019

Polymerase Chain Reaction (PCR)

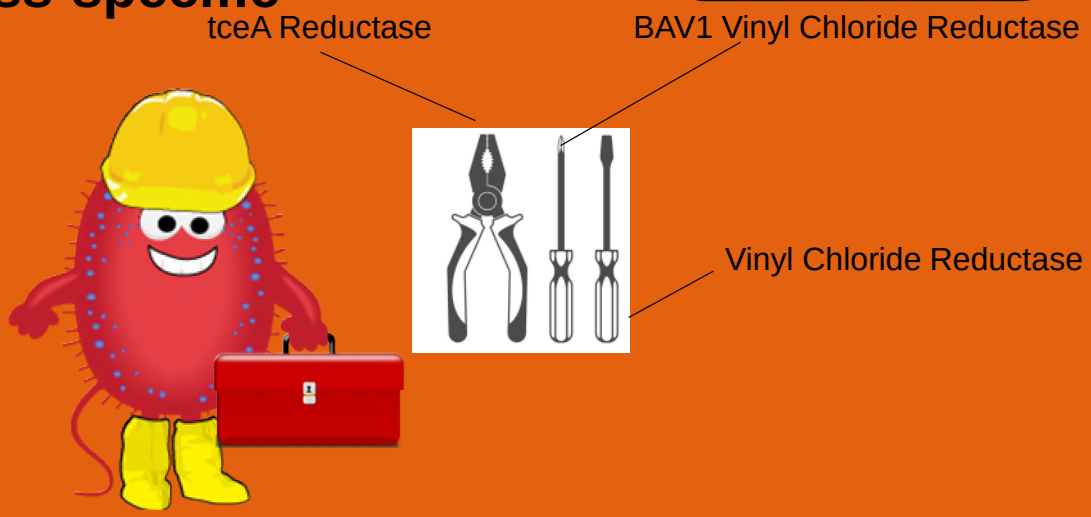
- **Denature** DNA at 95˚C
- Add primers to bind to separated DNA
- **Anneal primers** at 45 – 65 ˚C. lower temp may be less specific binding
- **Extension of DNA** segments at 72 ˚C
- Use electrophoresis to compare amplicons



Quantitative Polymerase Chain Reaction (qPCR)

Advantages

- **Targeted** – looking for specific genes
  - Taxonomic genes (name tags)
  - Functional genes (tools in the toolbox)
- **Biodegradation Process-specific**
  - Contaminants
  - Geochemistry
    - Competing electron acceptors
    - Redox conditionsd



Source information: Microbial Insights, Inc. unless otherwise stated

Reverse transcriptase qPCR (RT-qPCR)

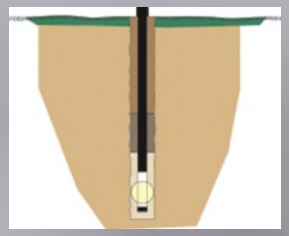
- RNA converted to cDNA using enzymes called reverse transcriptase
- The cDNA can now be used in PCR or qPCR.

Comparison with traditional microbiology

	qPCR	RT-qPCR	Culture
Reduced detection time	Yes	Yes	No
Reduced costs	Yes	Yes	No
Viable pathogens detected	Yes	Yes	Yes
Detects viable but nonculturable	Yes	Yes	No
Detects only viable microbes	No	Yes	Yes
Quantitative results	Yes	Yes	Yes

How does QuantArray® work?

Sample Collection

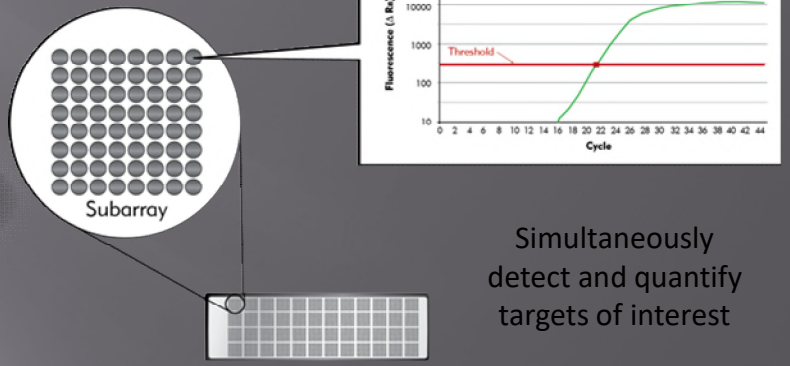


Groundwater, soil, or Bio-Trap samplers collected and shipped overnight on ice (4c)

DNA Extraction

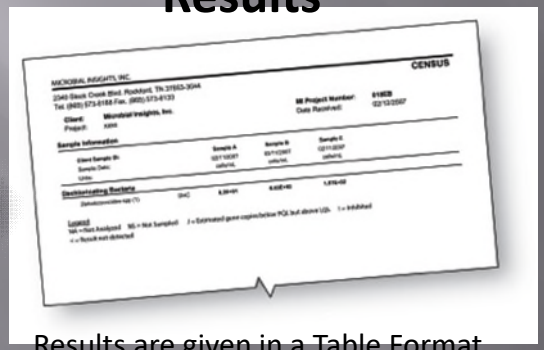
DNA extracted from sample upon arrival

Amplification

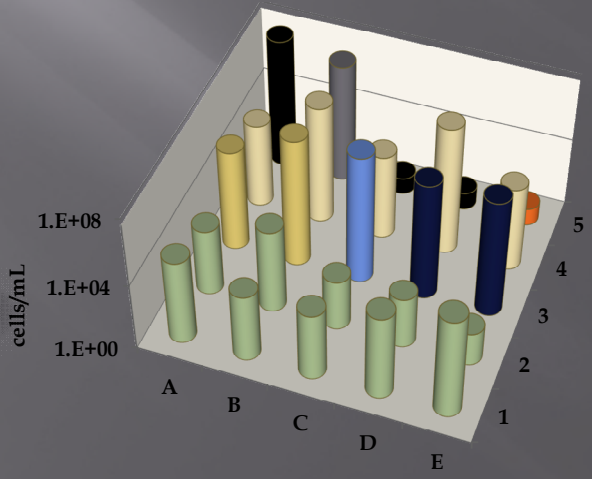


Simultaneously detect and quantify targets of interest

Results



Results are given in a Table Format



Results are integrated and compared to other parameters to evaluate site management decisions

Amplifies functional genes
DNA – what is possible
mRNA – what is active

Modified from Microbial Insights

QuantArray®-Petro

Sample Information
Aerobic BTEX and MTBE (cells/mL)
Toluene 3- and 4-Monooxygenases (RMO)
Toluene 2 Monooxygenase (RDEG)
Phenol Hydroxylase (PHE)
Toluene/Benzene Dioxygenase (TOD)
Xylene/Toluene Monooxygenase (TOL)
Ethylbenzene/Isopropylbenzene Dioxygenase (EDO)
Biphenyl/Isopropylbenzene Dioxygenase (BPH4)
Methylibium petroliphilum PM1 (PM1)
TBA Monooxygenase (TBA)
Aerobic PAHs and Alkanes (cells/mL)
Naphthalene Dioxygenase (NAH)
Phenanthrene Dioxygenase (PHN)
Alkane Monooxygenase (ALK)
Alkane Monooxygenase (ALMA)
Anaerobic BTEX (cells/mL)
Benzoyl Coenzyme A Reductase (BCR)
Benzylsuccinate synthase (BSS)
Benzene Carboxylase (ABC)
Anaerobic PAHs and Alkanes (cells/mL)
Benzoyl Coenzyme A Reductase (BCR)
Naphthylmethylsuccinate Synthase (NMS)
Naphthalene Carboxylase (ANC)
Alklysuccinate Synthase (ASSA)
Other (cells/bead)
Total Eubacteria (EBAC)
Sulfate Reducing Bacteria (APS)

microbialinsights

qPCR vs Next Generation Sequencing

qPCR

- Requires knowledge – have to know what you're looking for and have correct sequence
- Many qPCR assays available
- Functional genes
- Detects low abundance organisms

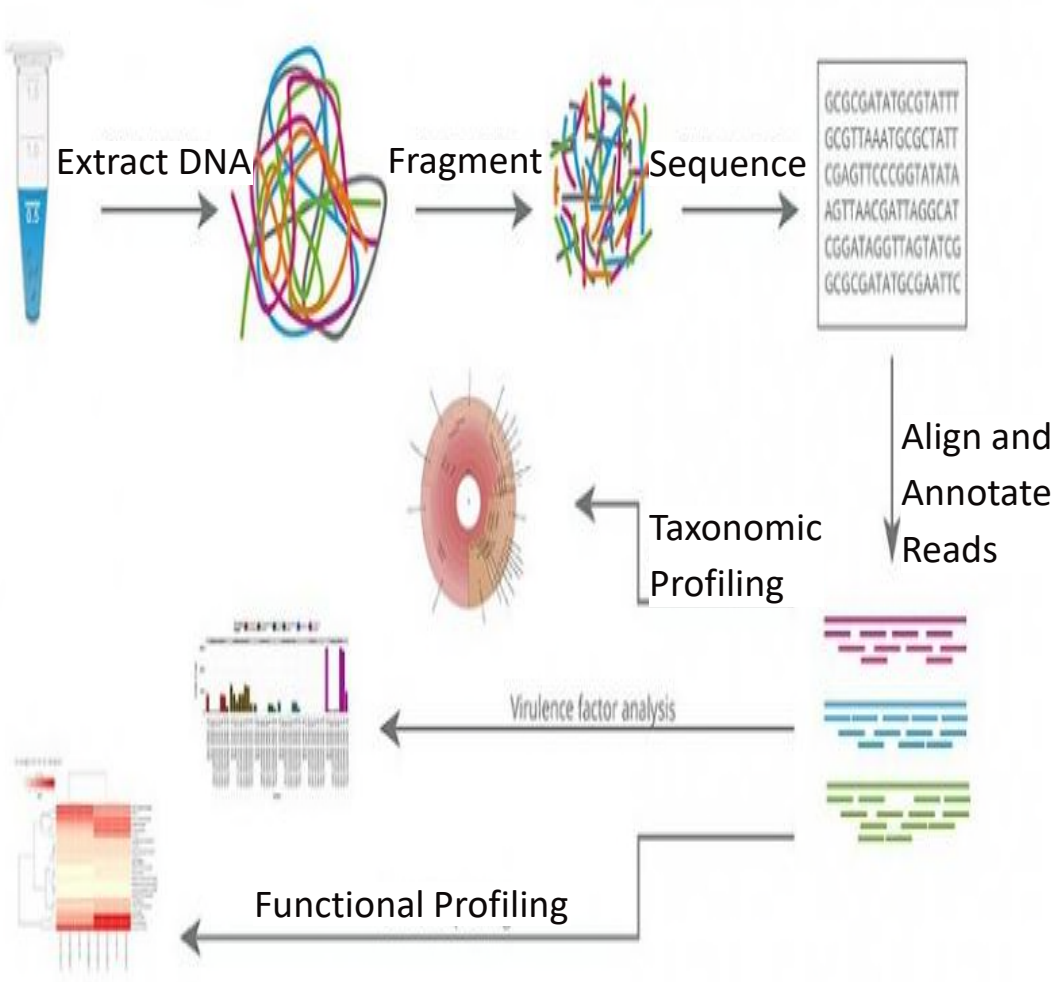
NGS

- Exploratory
- Who is there?
- Resolution to genus level
- Data rich

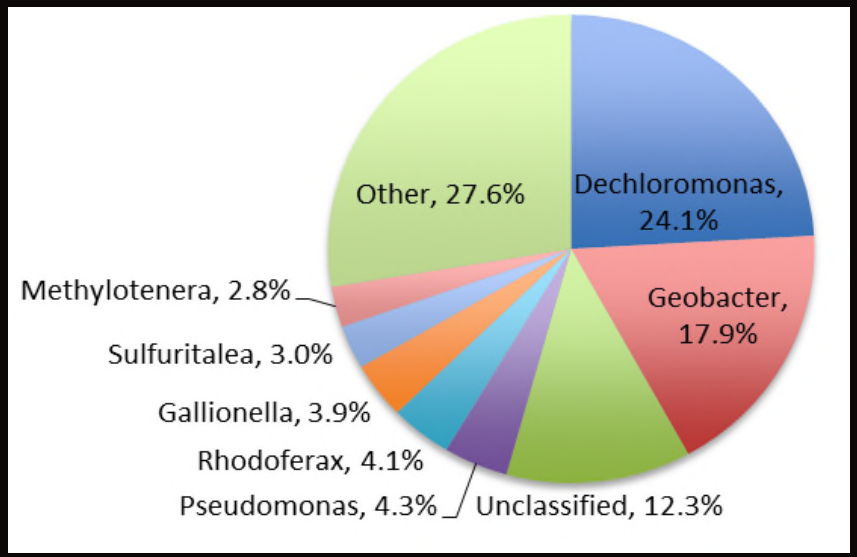
Source information: Microbial Insights, Inc. unless otherwise stated

Metagenomics

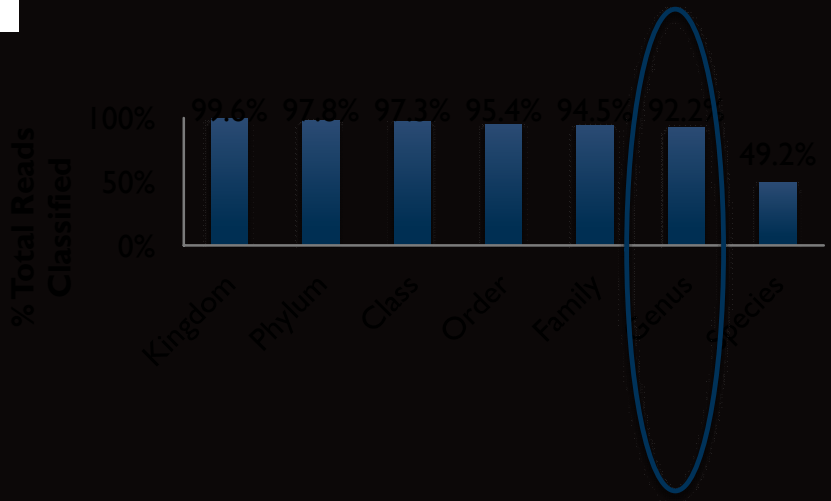
- Identifies nonculturable microorganisms
- Allows greater understanding of microbial community diversity
- Next generation sequencing (NGS)



Example NGS Results



Sample ID	Reads Passing Quality Filtering	% Reads Classified to Genus	Shannon Genus Diversity
MW6	607,795	92.2%	3.0
MW7	577,170	93.6%	2.9
MW8	719,650	93.7%	2.3
MW9	736,200	94.1%	2.3
MW10	734,080	93.6%	2.7



- Snapshot of community
- Genus level
- Relative quantitation)
- May require statistical analyses

Source information: Microbial Insights, Inc. unless otherwise stated

Nature Based Remediation

Applications of Molecular Biological Tools

Phytoremediation

Site:

Pipeline rupture leading to release of diesel in remote area

Prescribed burn during emergency response to pipeline breach of diesel

Courtesy of Barry Harding, AECOM, 2023

MBTs Used for Site Characterization and Remedy Selection

Physical Setting:

Sagebrush Steppe

Saline Alkali Soils

High Na+, Cl-

MBTs Used:

Next Generation Sequencing with 16s RNA

qPCR array (QuantArray Petro)

Spill Area, Salt Crusts, Background (control)

Results:

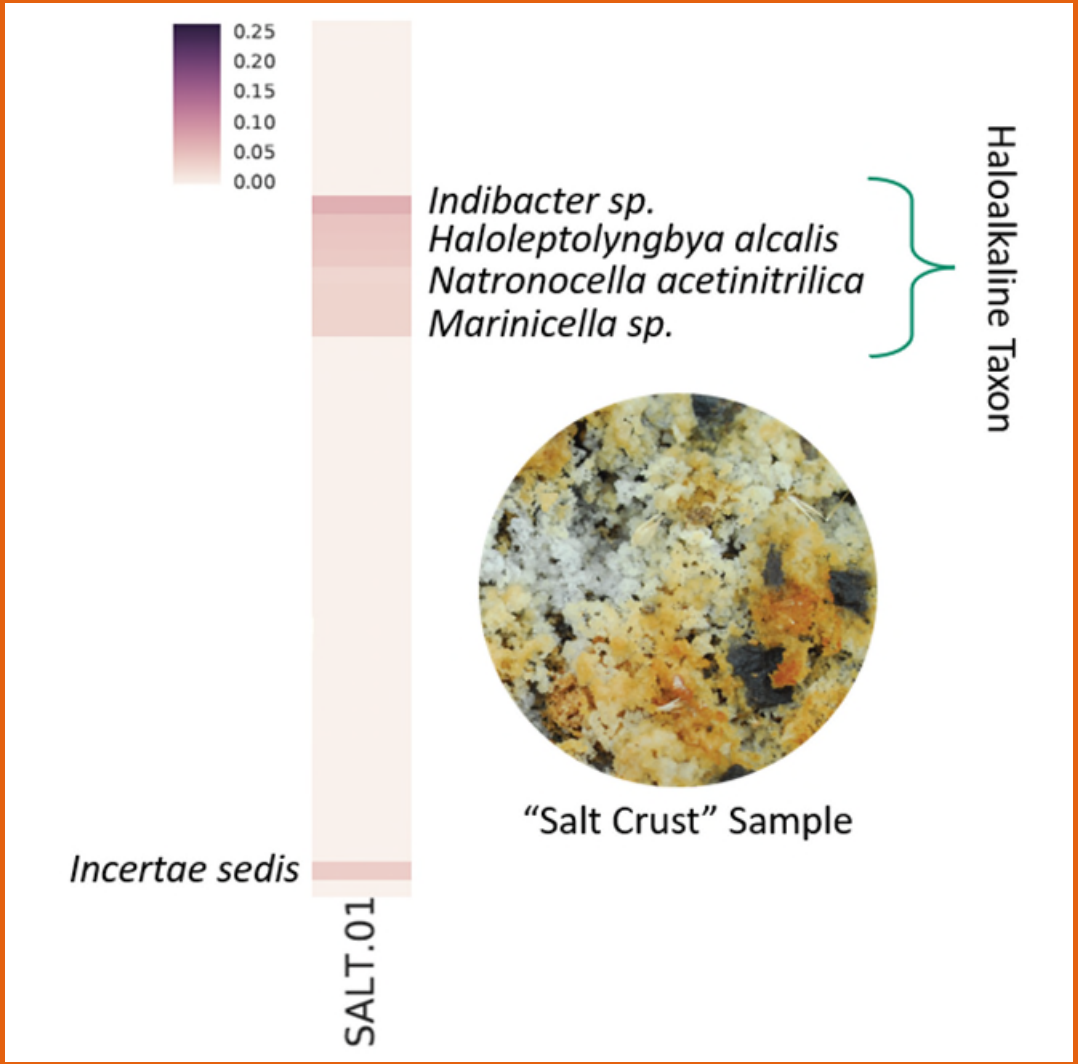
Halophilic and Halotolerant bacteria in soils (*Indibacter sp., Haloleptolyngbya alcalis, Natronocella acetinitrilica, Marinicella sp.*

Several petroleum degraders and rhizospheric bacteria identified, including *B. Mojavensis*

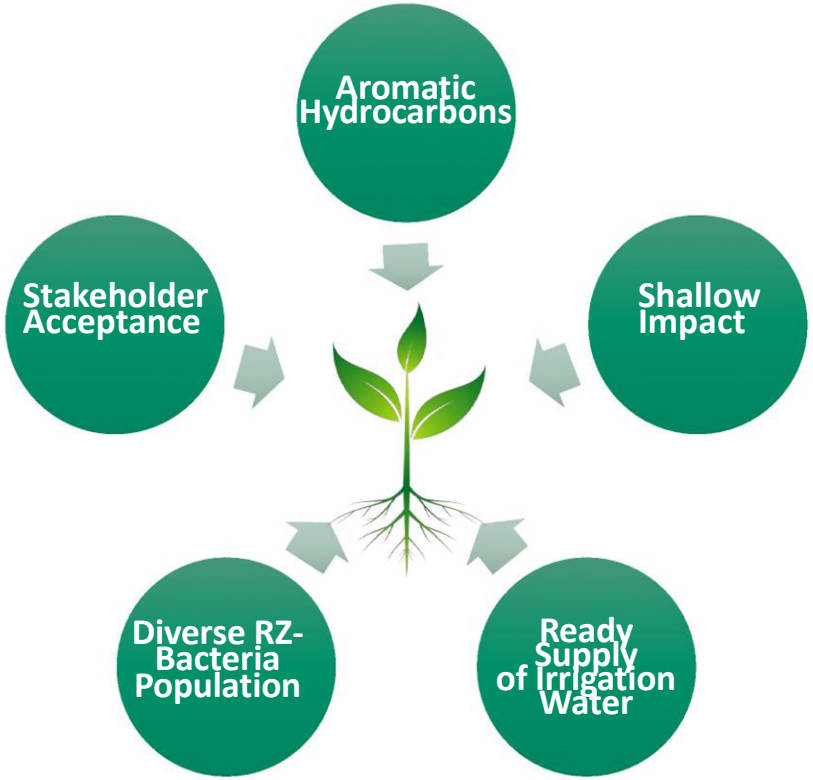
Aerobic and Anaerobic BTEX degraders (toluene/benzene monooxygenases (RMO/RDEG)

Sulfate Reducing Bacteria

Illustration of NGS Results



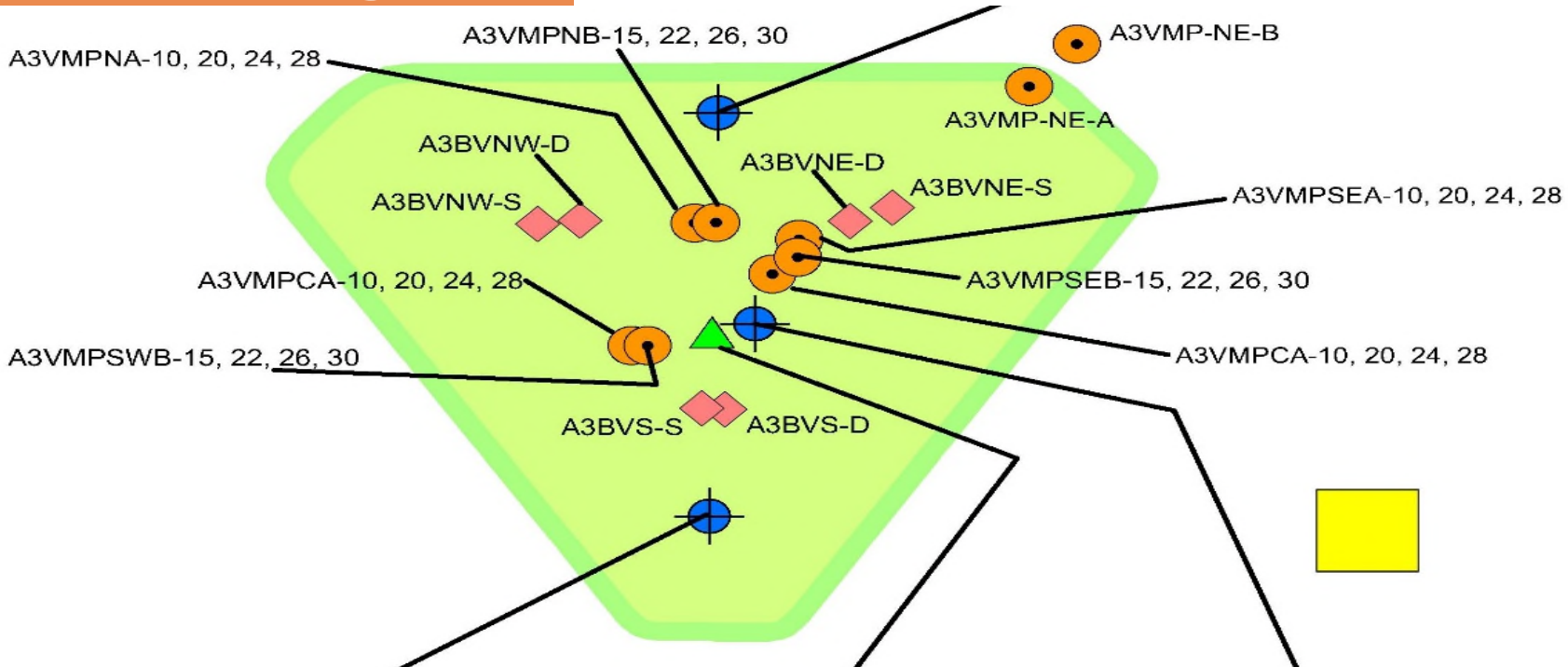
Conditions Conducive for Rhizodegradation



Post-Implementation Spring 2023

MBTs Used during Remedy Implementaion

Bioventing



A3PZ-S-GW (6/14/2018)	
Parameter	Result
Benzene	12.9
Ethylbenzene	1.54
Toluene	0.644
Xylene (Total)	6.55
TPH-GRO	54
TPH-DRO	18.6

A3AS-C-GW (6/15/2018)	
Parameter	Result
Benzene	0.0068 J
Ethylbenzene	ND
Toluene	0.0056 J
Xylene (Total)	ND
TPH-GRO	1.6 J
TPH-DRO	8.6

A3PZ-C-GW (6/15/2018)	
Parameter	Result
Benzene	11.9
Ethylbenzene	1.73
Toluene	0.636
Xylene (Total)	5.38
TPH-GRO	47.6 J
TPH-DRO	22.3

MBTs Used:

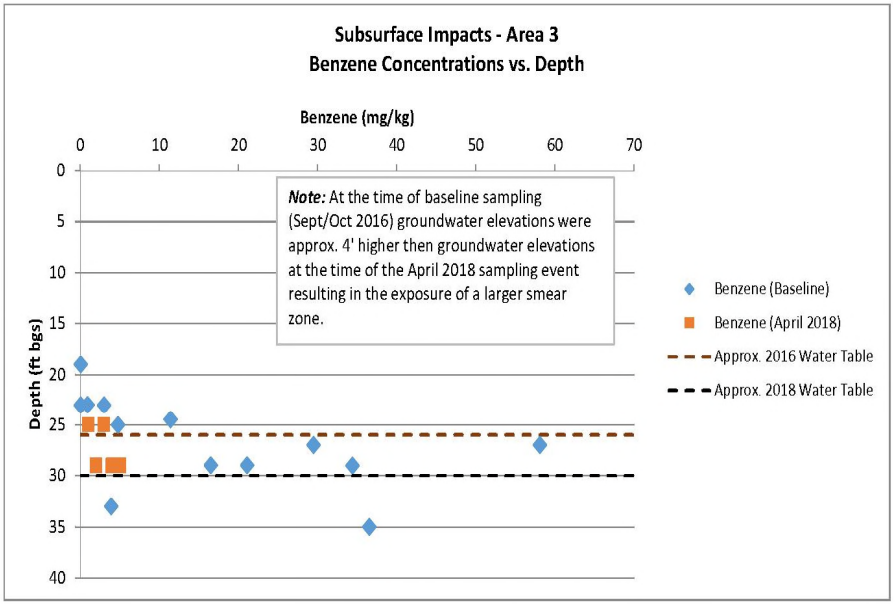
- **qPCR – QuantArray Petro**
- **RTqPCR – QuantArray Petro**

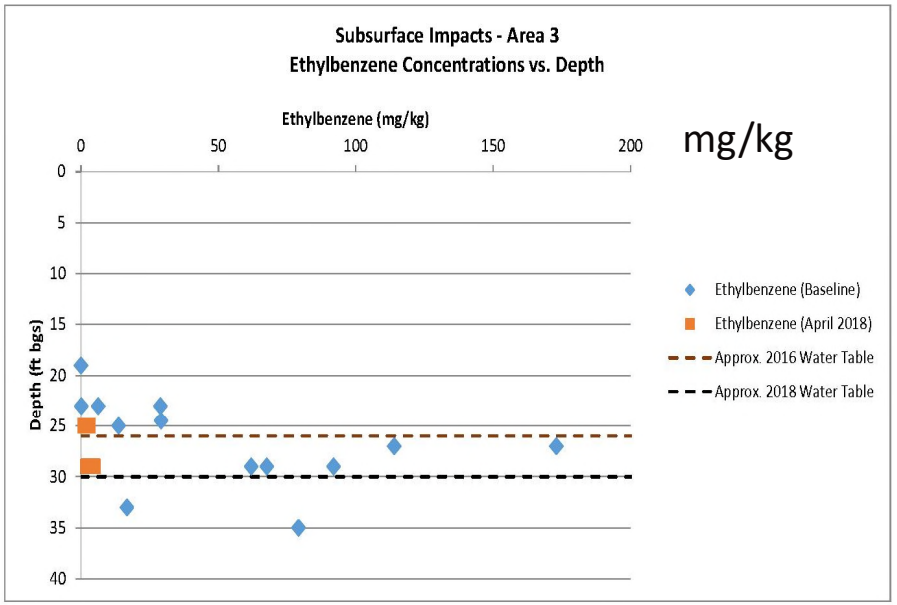
Former Refinery Site

AREA 3 (BIOVENT) PILOT TEST
SOIL ANALYTICAL RESULTS

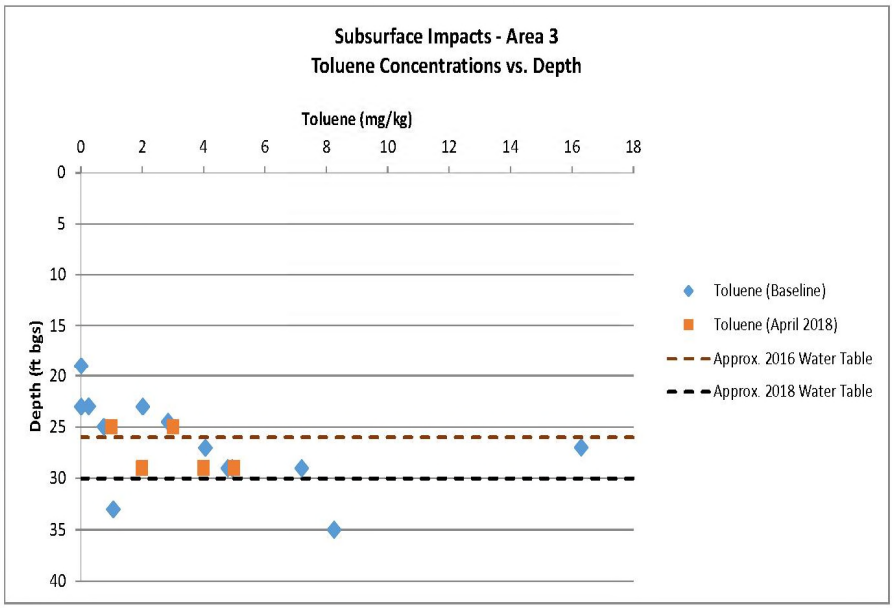
Blue – baseline

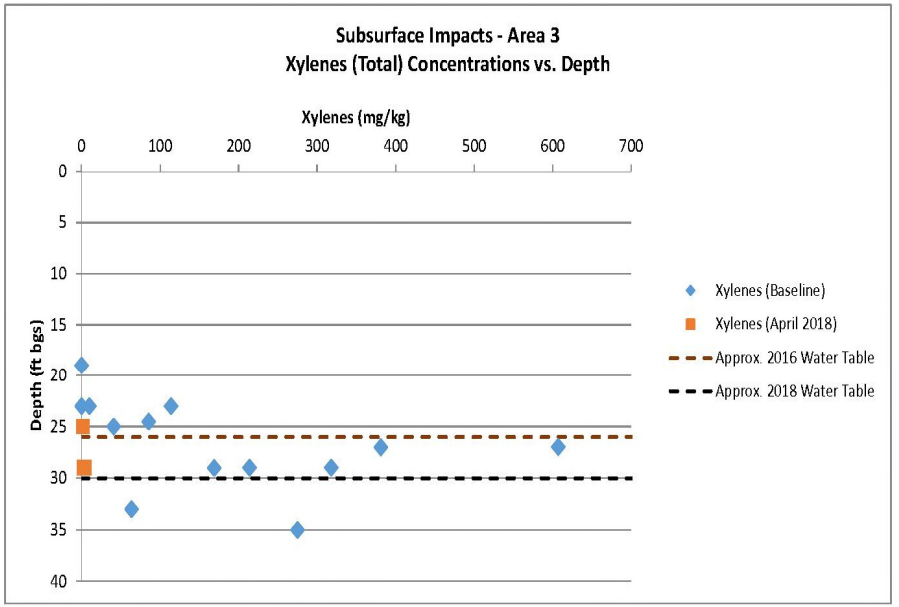
Orange – Two years of treatment





mg/kg



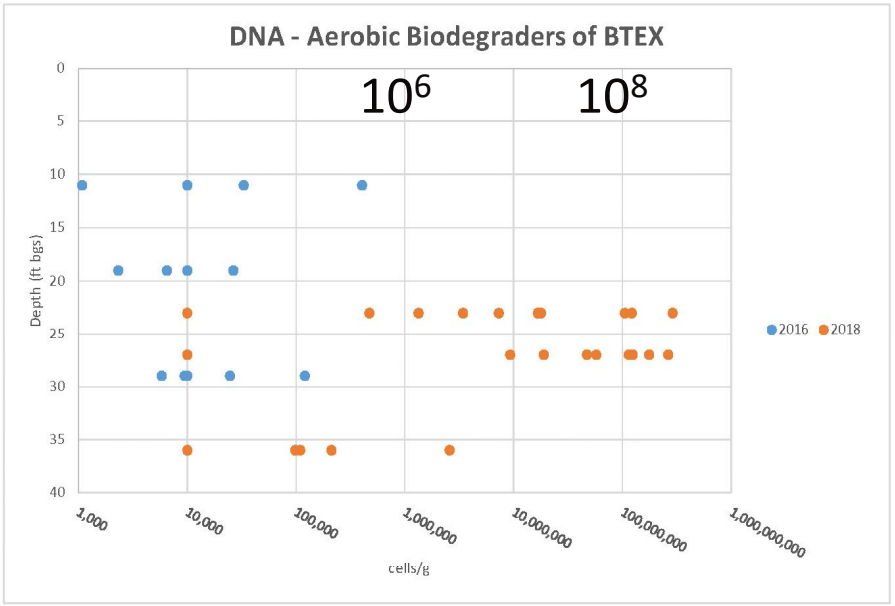


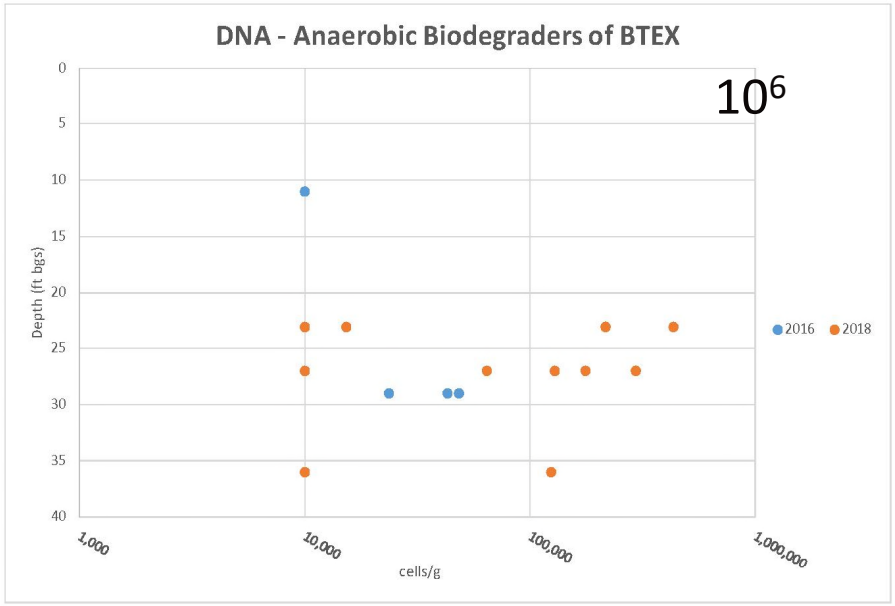
DNA

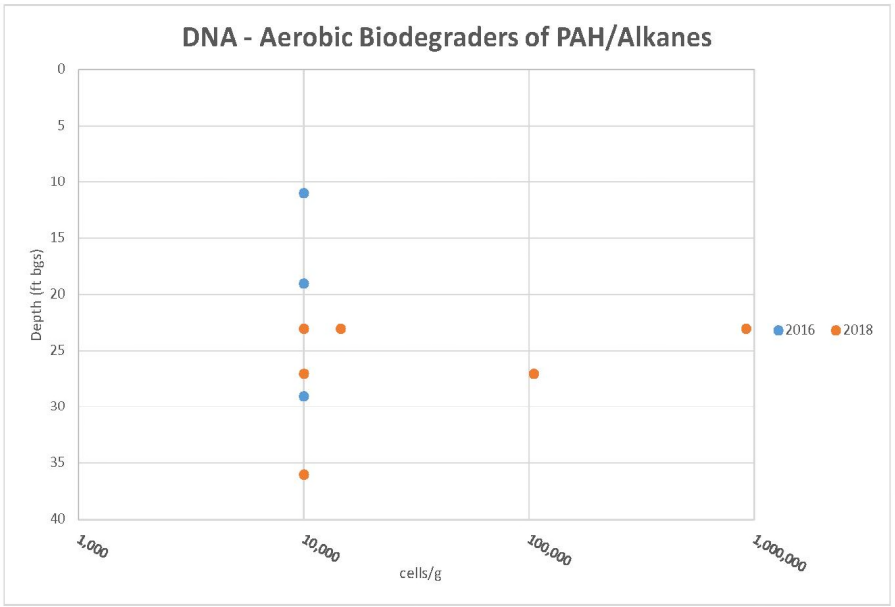
AREA 3 (BIOVENT) PILOT TEST
QUANTARRAY RESULTS - DNA

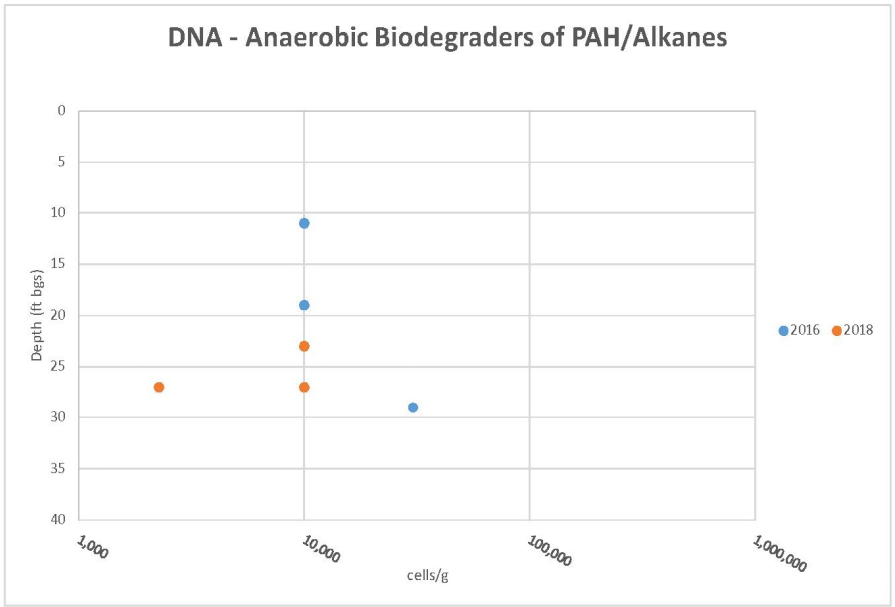
Blue – baseline

Orange – Two years of treatment

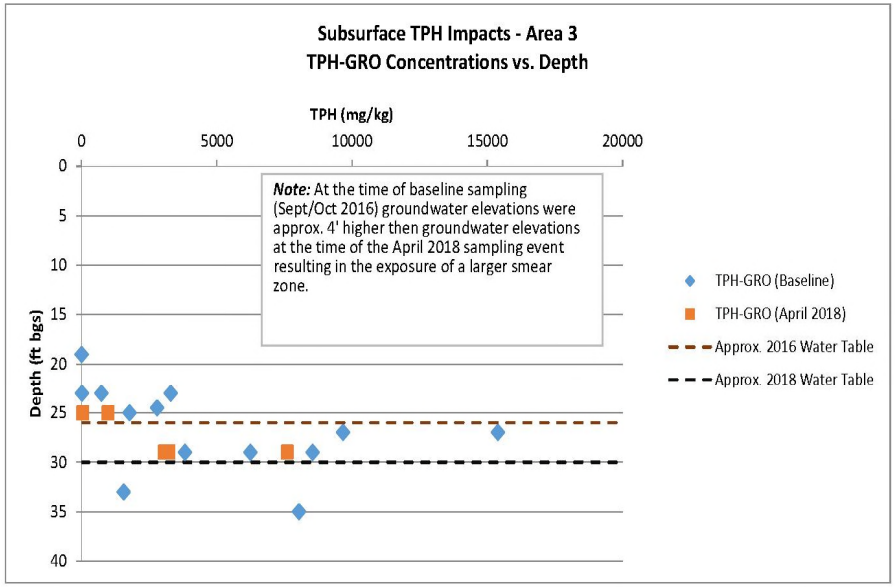


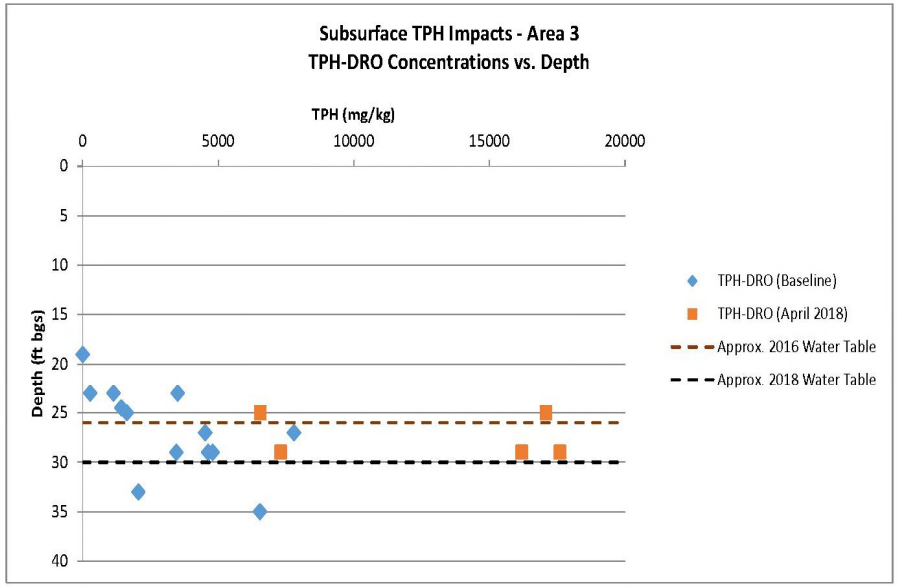






AREA 3 (BIOVENT) PILOT TEST
SOIL ANALYTICAL RESULTS





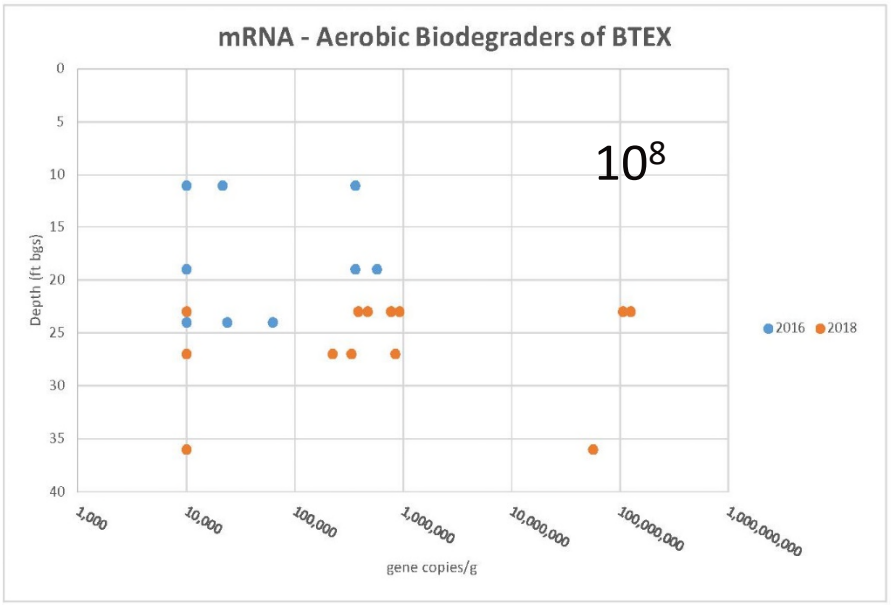
Blue – baseline

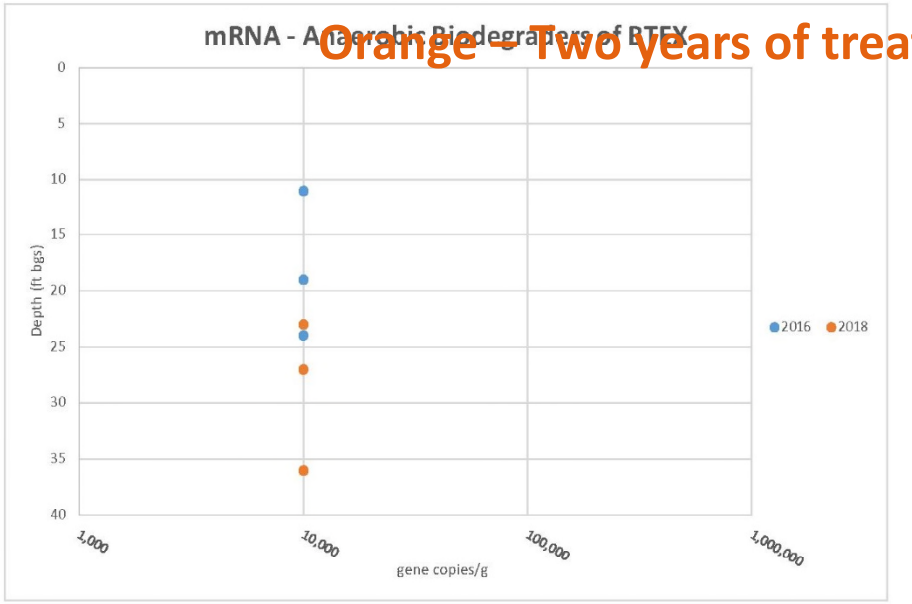
Orange – Two years of treatment

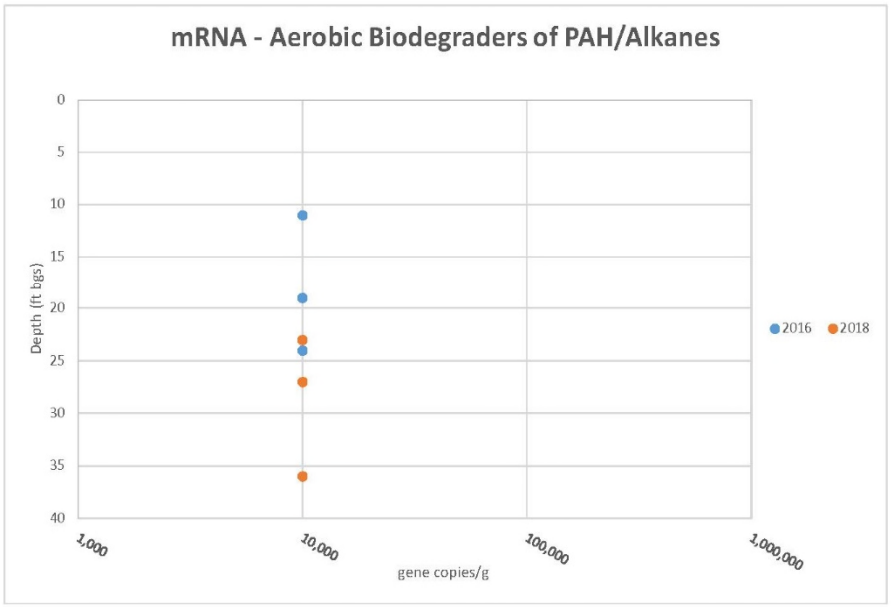
AREA 3 (BIOVENT) PILOT TEST
QUANTARRAY RESULTS - mRNA

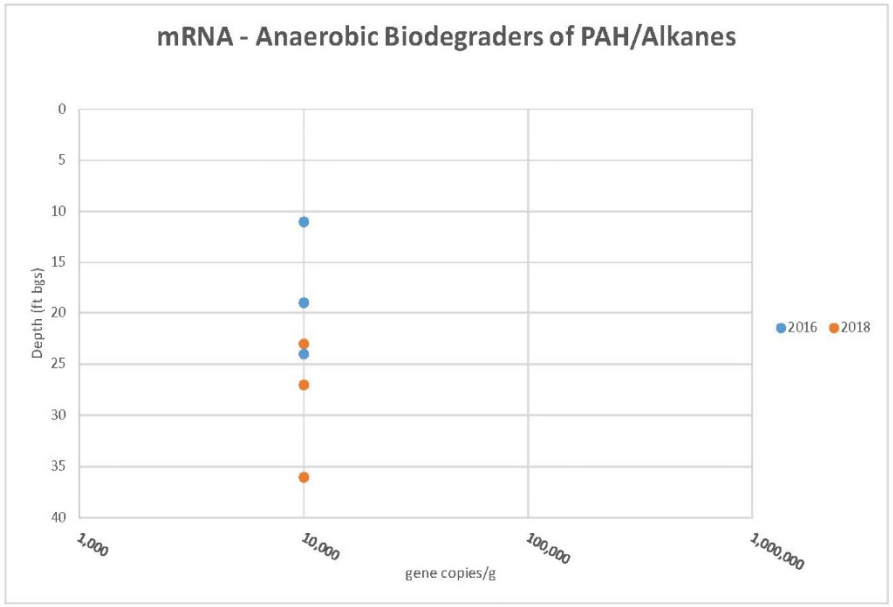
Blue – baseline

Orange – Two years of treat

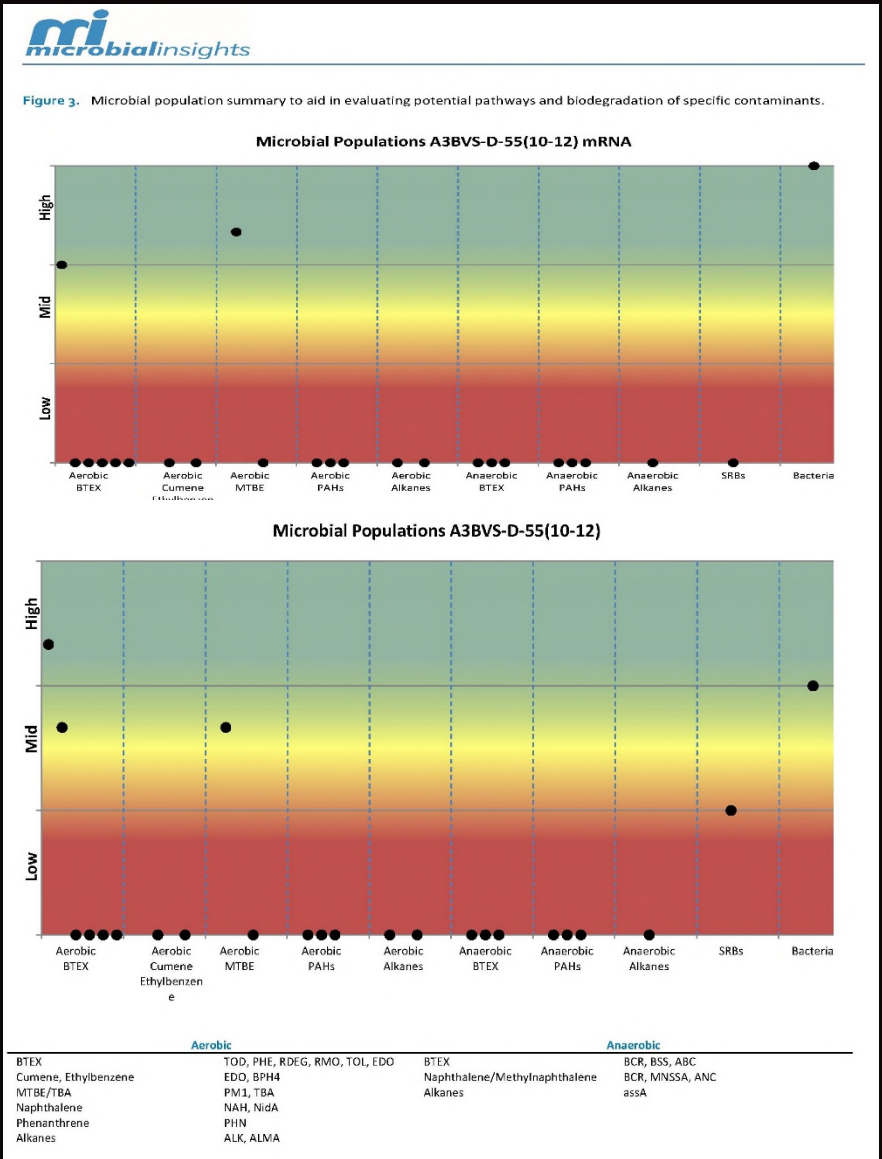








Bioventing Test Results



BTEX and TPH – GRO soil concentrations decreased following the two-year pilot test

TPH – DRO soil concentrations increased following the two-year pilot test

QuantArray – Petro (qPCR – DNA) showed increased levels of aerobic BTEX degrading gene targets, anaerobic BTEX degrading gene targets, and aerobic PAH degrading gene targets.

QuantArray – Petro (RT-qPCR – mRNA) showed increased levels of aerobic BTEX degradation activity (only).

Limitations of MBTs

Knowledge

- Used less frequently, more education may be needed up front
- DNA-based MBTs do not differentiate between living and dead cells

Sample collection and handling

- Samples must remain cold during shipment
- DNA should be extracted within 48 hrs of sampling, and the same day a sample is received at the lab

Interpretation

- Data requires context
- Significant change is an order of magnitude or more
- Understanding when a result is high, medium, or low and insignificant

Thank you for your attention

Questions?

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