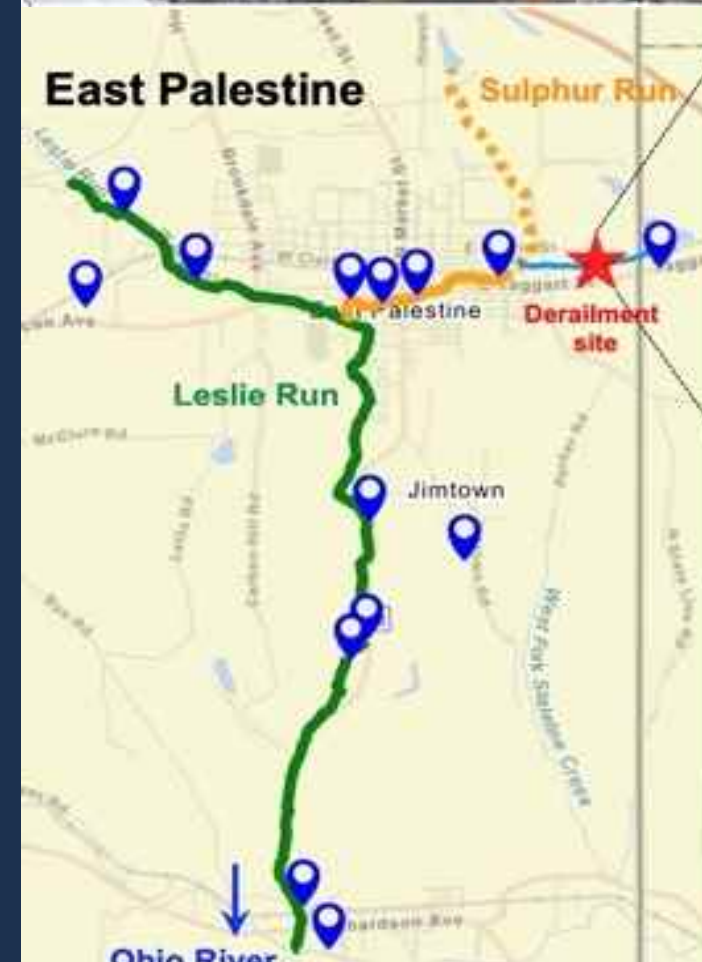


A World of Opportunity

*Assessing the Genetic Potential for Long Term
Bioremediation in East Palestine, OH*

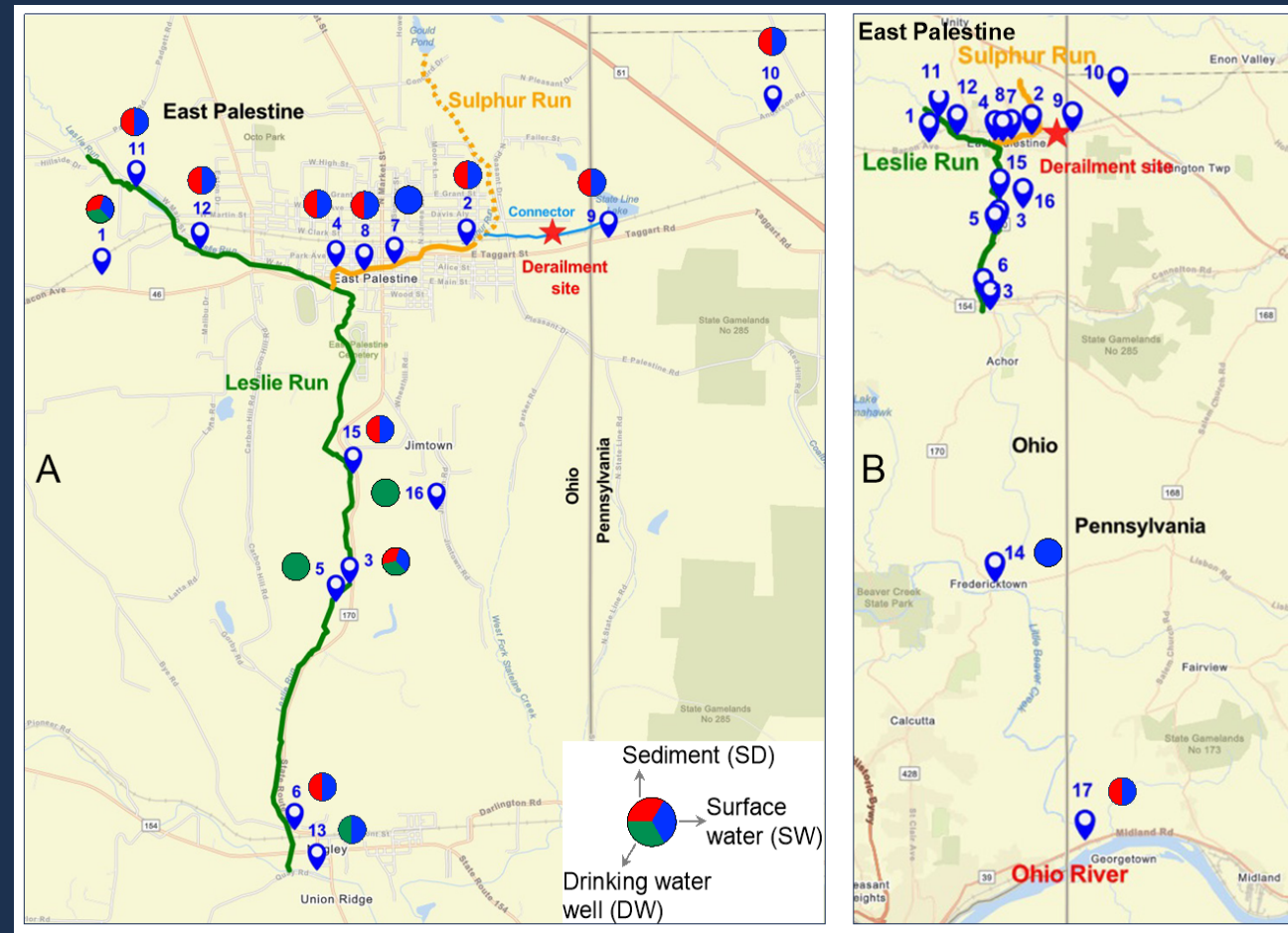
East Palestine Train Derailment

- February 3, 2023
- 150 car train derailed
- Released over 100,000 gallons of vinyl chloride (VC) and butyl acrylate (BA) which contaminated surface water and groundwater
- Leakage and elevated temperatures led to the decision for a controlled burn
- Primary surface water impacted was Sulphur Run
- Rapid Response funding from NSF to assess the potential for natural attenuation



Rapid Response Research Funding From NSF

- **June (128 days post)**
 - 16 surface waters
 - 18 sediments
 - 5 drinking wells
- **November (276 days post)**
 - 7 surface waters
 - 8 sediments
 - 4 drinking wells







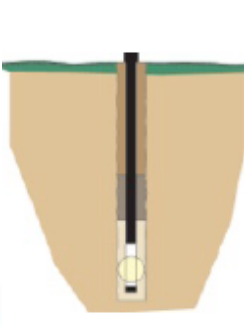
Analysis Performed

Microbial Insights

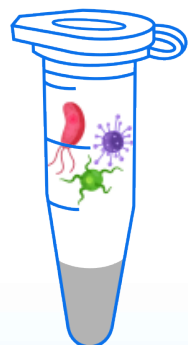
- Laboratory Extractions
 - QuantArray-Chlor
 - Next Generation Sequencing
- Field DNA Extractions
 - See talk by Sam Rosolina
June 6 in session F6
- QuantArray analysis of microcosm samples

University of TN

- Microcosm studies
 - See talk by Gao Chen
 - June 6 session H8
 - Next Generation Sequencing



Sample



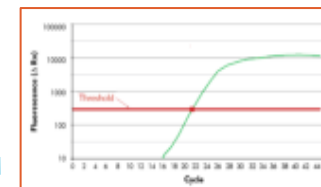
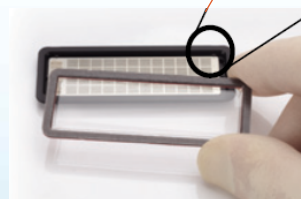
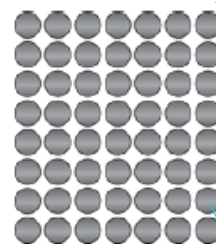
DNA
Extraction



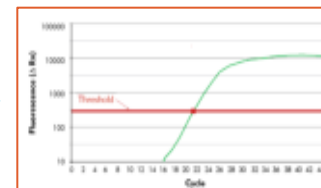
DNA



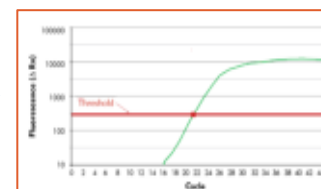
Sub Array
Amplification



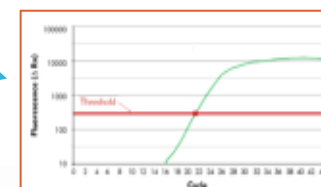
DHG



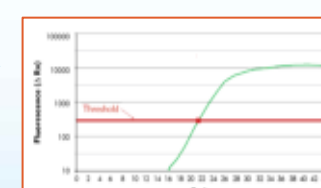
DHC



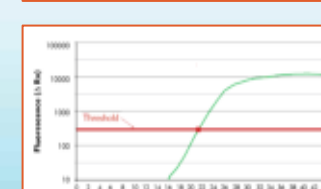
BVC



VCR



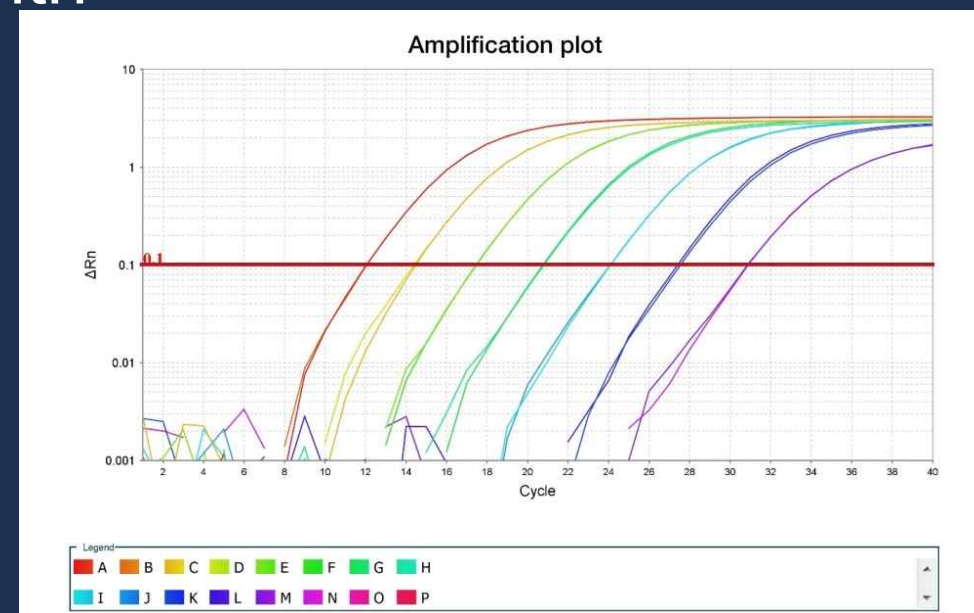
TCE



ETN

QuantArray[®]-Chlorinated

- qPCR assays for 29 targets associated with chlorinated solvent degradation
- Degradation of vinyl chloride
 - Aerobic
 - etnC and etnE
 - Anaerobic
 - *Dehalococcoides*
 - tceA, vcrA and bvcA
 - *Dehalogenimonas*



Bio-Extract™ Field Kit

- Start with a Bio-Flo® filter
- Field compatible with no need for electrical equipment
- Extract multiple samples together
- Ship extracts to the lab for analysis or use FieldQuant for further evaluation on site



Field qPCR

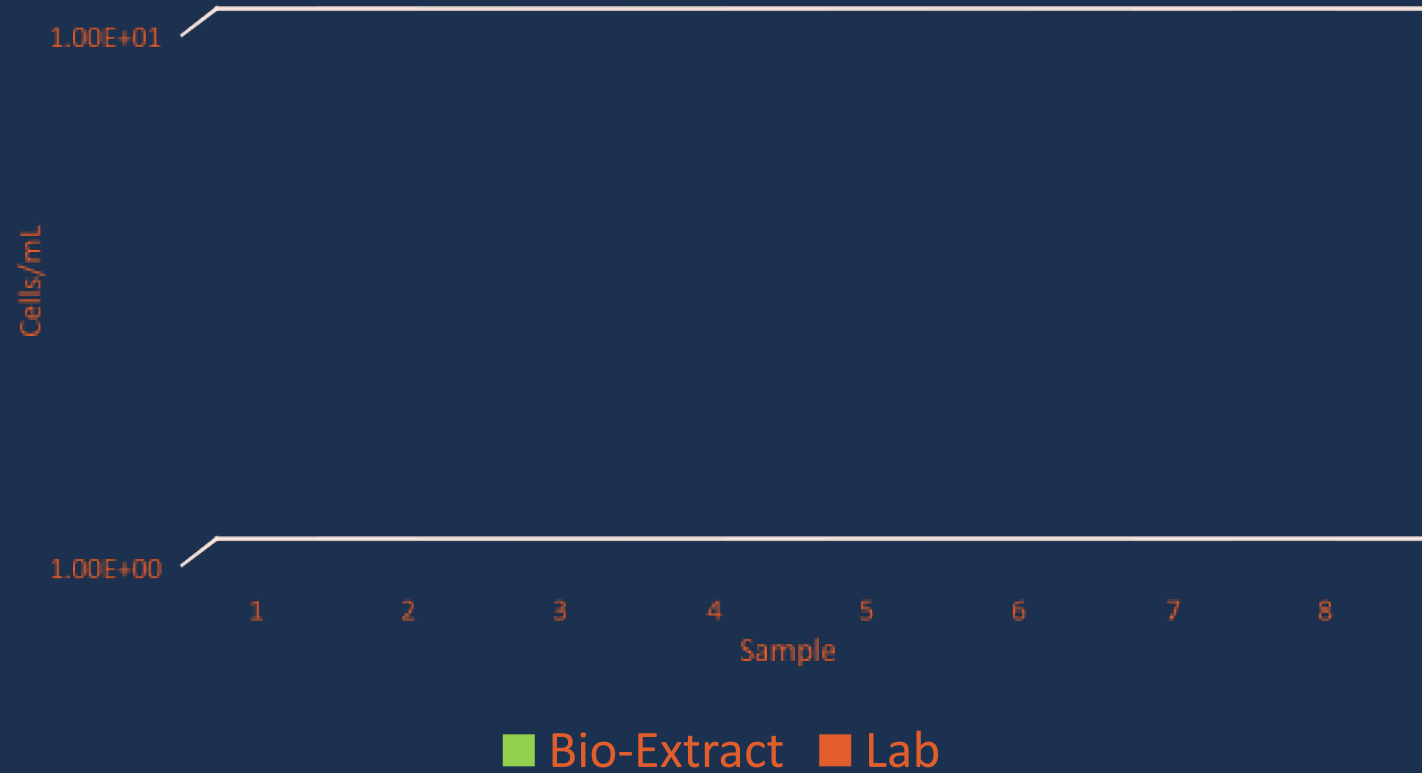


- Same qPCR assays as run in the lab
- Able to be used in extreme weather environments
- Simple to use
- Ideal for Remote/Emergency Response
- Rapid Reporting
- Flexibility

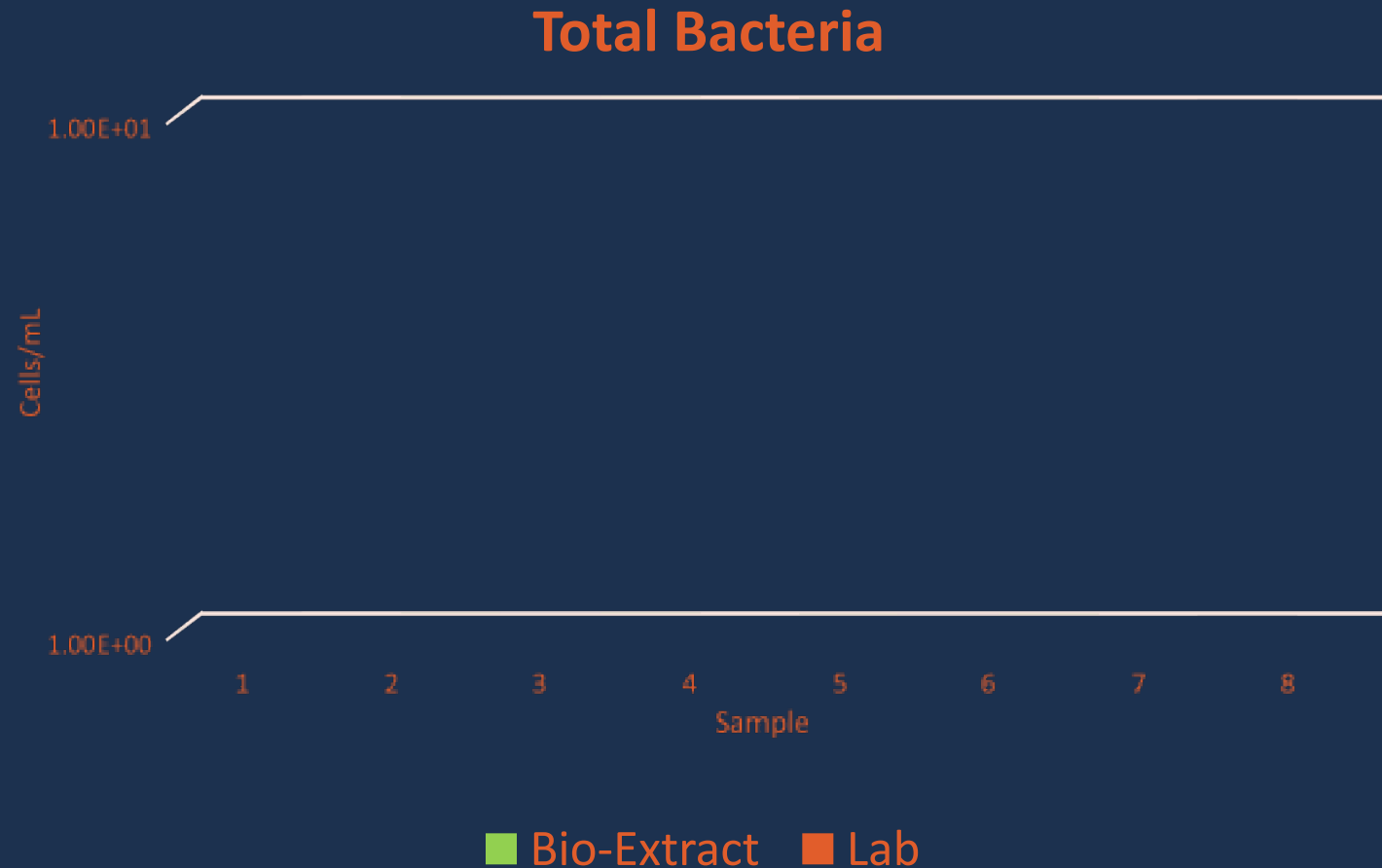


Comparison data—Lab to Field

Sulfate Reducing Bacteria



Comparison data—Lab to Field



Aerobic Vinyl Chloride Oxidation

- entC and entE biomarkers for aerobic degradation of vinyl chloride (VC) were detected only in sediment samples
 - 35% and 18% frequency respectively
- Microcosms
 - all 11 sediment sampling locations consumed VC after 20-40 day lag phase
 - VC was completely degraded in these microcosms after 40-80 days
 - Sequencing of the microcosms showed a high relative abundance of *Mycobacterium*
 - qPCR analysis of etnC and etnE biomarkers ranged from E+04 to E+08 cells/mL
- Demonstrated potential for attenuation of VC at locations throughout Sulphur Run and Leslie Run



Anaerobic Degradation --DHC

June

- 22/39 (56%) positive for DHC
- 56% of surface waters
- 44% of sediments
- 100% of drinking wells

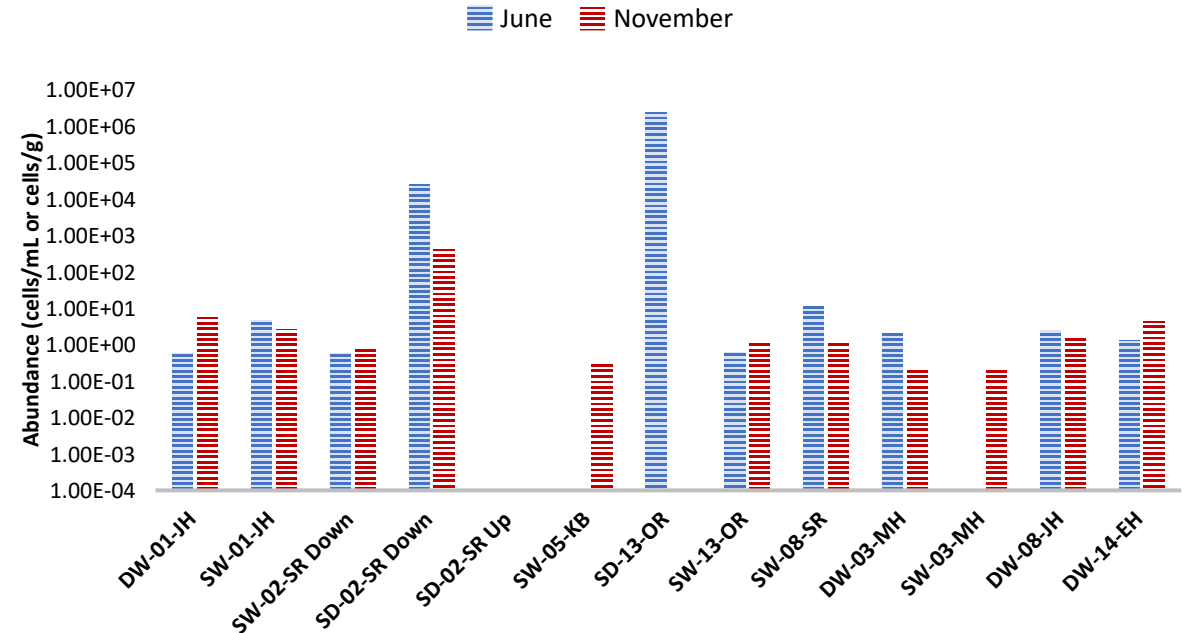
November

- 16/22 (73%) positive for DHC
- 100% of surface waters
- 63% of sediments
- 100% of drinking wells

DHC abundance remained consistent with 3 exceptions

- 2 surface waters no detection in June but low in November
- 1 sediment high in June but not detected in November

DEHALOCOCCOIDES (DHC)



June

- 22/39 (56%) positive for DHC
- 56% of surface waters
- 44% of sediments
- **100% of drinking wells**

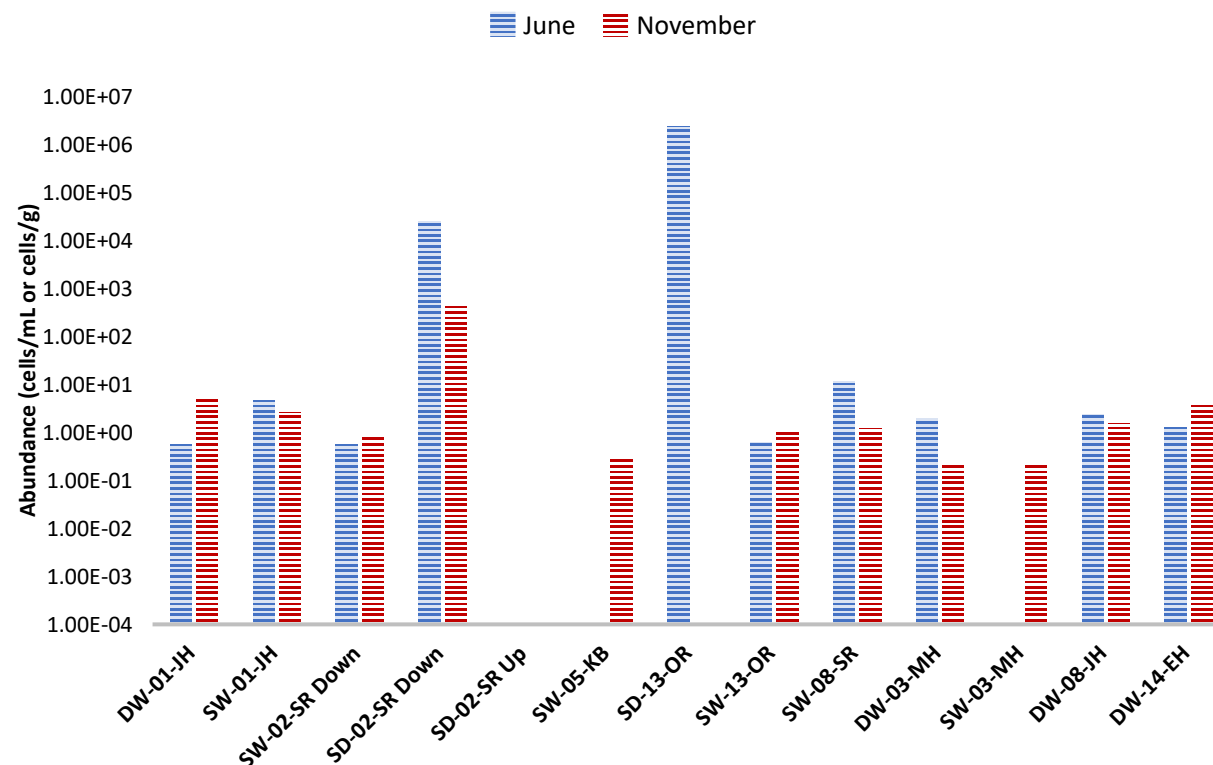
November

- 16/22 (73%) positive for DHC
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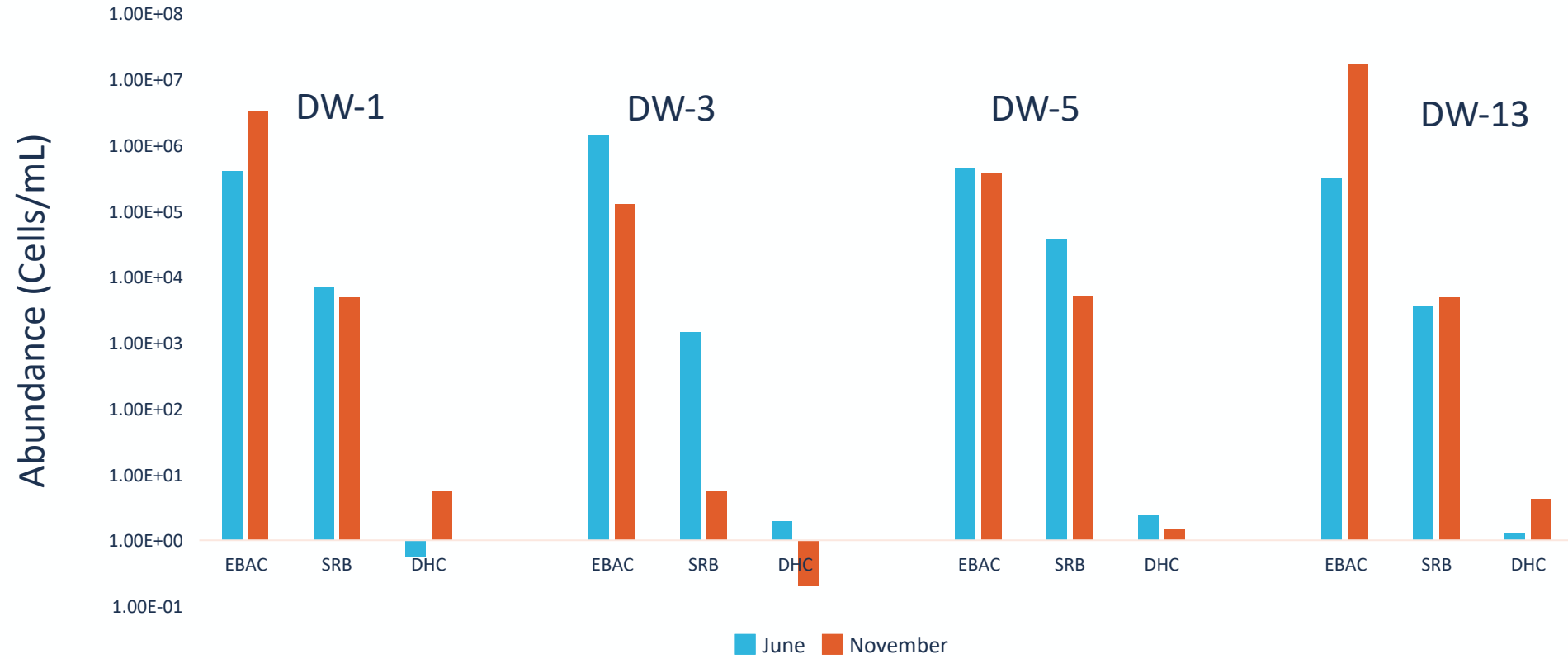
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DEHALOCOCCOIDES (DHC)



Drinking Water Wells



Sulfide in Drinking Wells

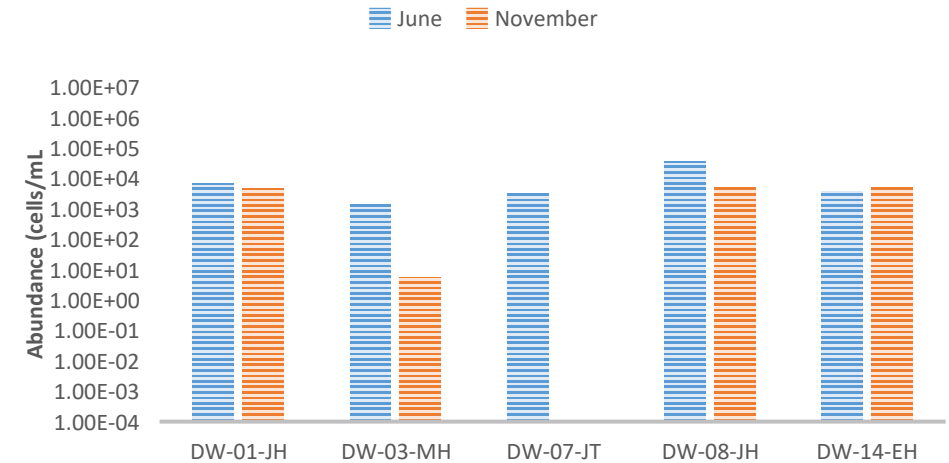
- Sulfide detected via Pepto-Bismol test
- SRB detected in all drinking wells
- Electron donors likely stimulating sulfate reducing bacteria (SRB)
 - BA and glycols from the accident
 - Ethene from reductive dechlorination
- Wells likely impacted by disturbances from the accident
- Unclear how due to lack of hydrogeological assessments

H₂S-NEGATIVE:
no black
precipitate
formed



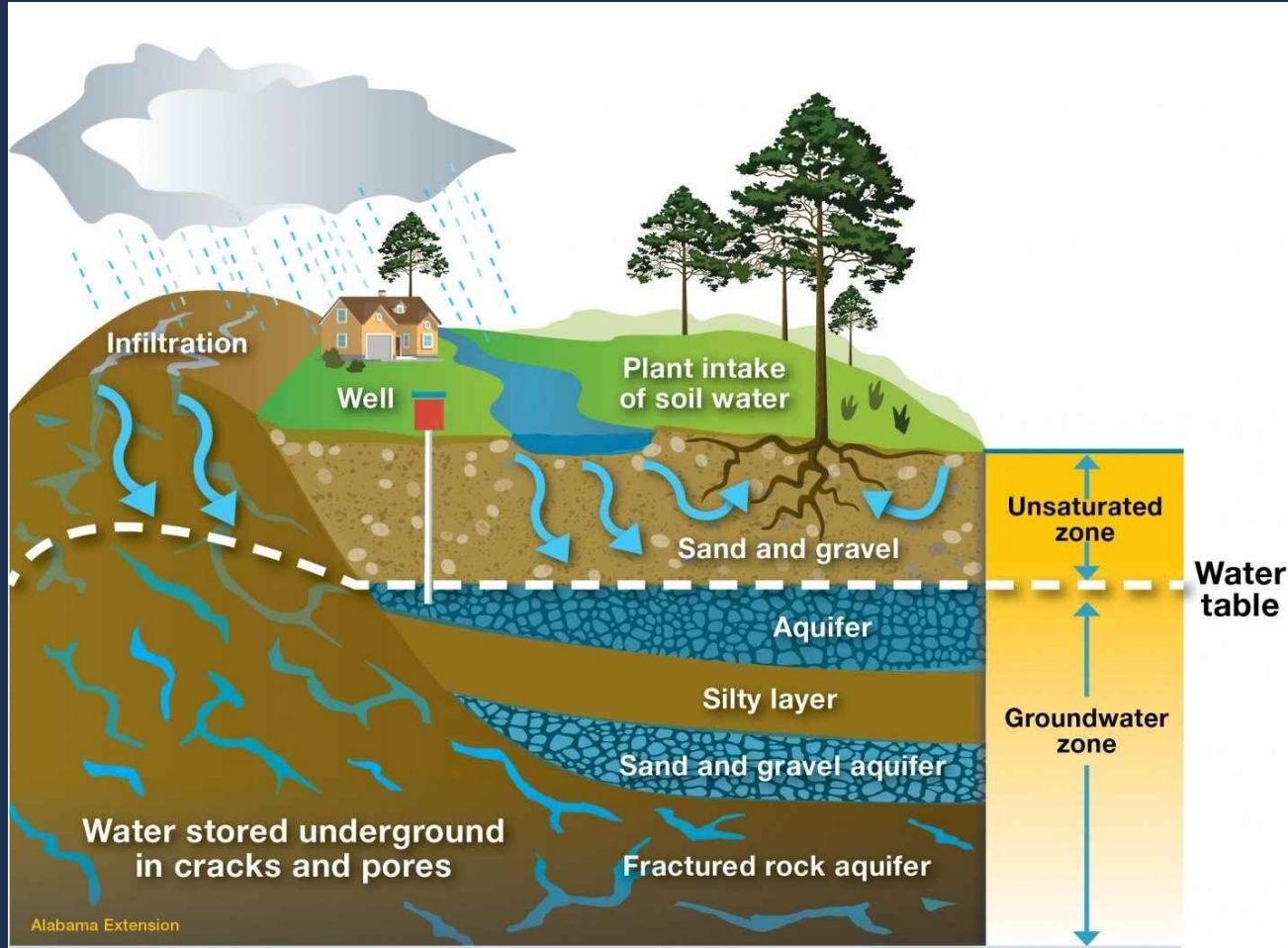
H₂S-POSITIVE:
black precipitate
formed

SULFATE REDUCING BACTERIA (APS GENE)



Comparison of qPCR results for APS between June 2023 and November 2023

Hydrogeological Assessments



- Provide an understanding of
 - Regional hydrology
 - Geology
 - Land use
 - Water quality
 - Principal aquifers and aquitards
- Serve as a foundation for
 - Understanding potential uncertainties
 - Identifying data gaps to further refine the understanding of the hydrogeological setting

Recommendations and Lessons Learned

- Full hydrogeological survey of impacted and surrounding watershed
 - Include assessment of risks of contaminant migration from spill site to drinking water wells
- Include natural attenuation in decision making process
 - More immediate sampling and analyses
 - Include MBTs
 - May provide a better understanding of the resident microbiome response
- Make emergency assessment and guidance documents more readily available



- If possible, avoid combustion of released contaminants.
- Clearer communication with members of the impacted or potentially impacted communities
 - East Palestine community members were provided conflicting information, even months after the derailment.
 - Those who provide drinking water testing need to clearly explain what the results mean within each testing report.
- Increased response time for funding agencies
 - The NSF RAPID funding was critical for achieving the objectives and gathering the information within this study. Even within a funding source specifically designed for quick implementation, the delay resulted in our first sampling event occurring four months after the contaminant release. A quicker response will allow research to take place during pivotal, early windows.
- Develop an ITRC guidance document for the emergency response to large-scale contaminant release
 - In the case of East Palestine, a broad and simple guidance document on what steps to take and what steps to avoid during emergency response could have contributed to a safer outcome.



Special thanks

Rapid Response Research Grant National Science Foundation

The staff of Microbial Insights

The Loeffler Lab at the University of TN

Science and Technology Department at Inter Amercian University of Puerto Rico

Whelton Lab at Purdue University





THANK YOU!

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