

Using Environmental Metabolomics to Improve Decision Making at Chlorinated Solvent Sites

Molecular Tools to Navigate Your Diagnostic Exploration

mi
microbialinsights





ONE DEGREE RULE



- A small change can help correct our course and get us to our goal.
- Understanding the black box of microbiology allows us to correct quickly and cost effectively.
 - What are the dominant organisms present?
 - What is the genetic potential for complete reductive dechlorination?
 - What organisms are competing for available donors?
 - Are compounds biodegrading?
 - What is likely to happen in the future?
 - What is the rate of degradation?



STATE OF REMEDIATION IN THE 1990'S

In situ bioremediation of chlorinated solvents

Microbiology (1998), 144, 599–608

Printed in Great Britain

**REVIEW
ARTICLE**

Bioremediation: towards a credible technology

Ian M. Head

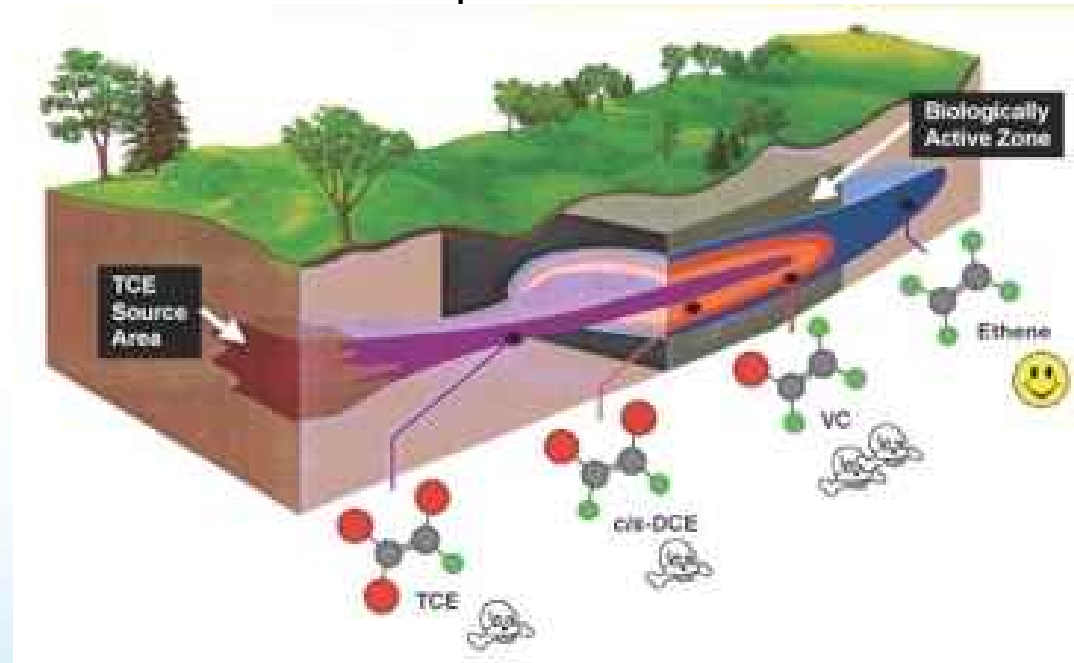
of chlorinated solvents and chlorinated aliphatic hydrocarbon contaminants in groundwater may soon become a reality. Both anaerobic reductive dehalogenation to non-chlorinated products and complete aerobic oxidation offer greatly increased promise for potential applications.

Current Opinion in Biotechnology 1993, 4:323–330



REMEDATION EFFORTS MUST LEAD TO COMPLETE DECHLORINATION

The toxicity of TCE transformation products increase as dechlorination proceeds.

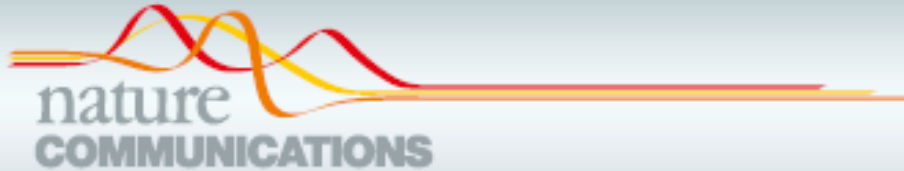


This is problematic if the process stalls once *cis*-DCE and/or VC is produced.



DATA GAPS

- qPCR and QuantArray provide quantitative data for microorganisms and gene targets
 - What about activity?
- Next Generation Sequencing provides a bigger picture of the overall microbiome
 - What about the health of the microbiome?
- Compound Specific Isotope Analysis and Stable Isotope Probing provide proof of contaminant degradation
 - What about predicting future degradation trends?




ARTICLE

<https://doi.org/10.1038/s41467-019-11311-9>

OPEN

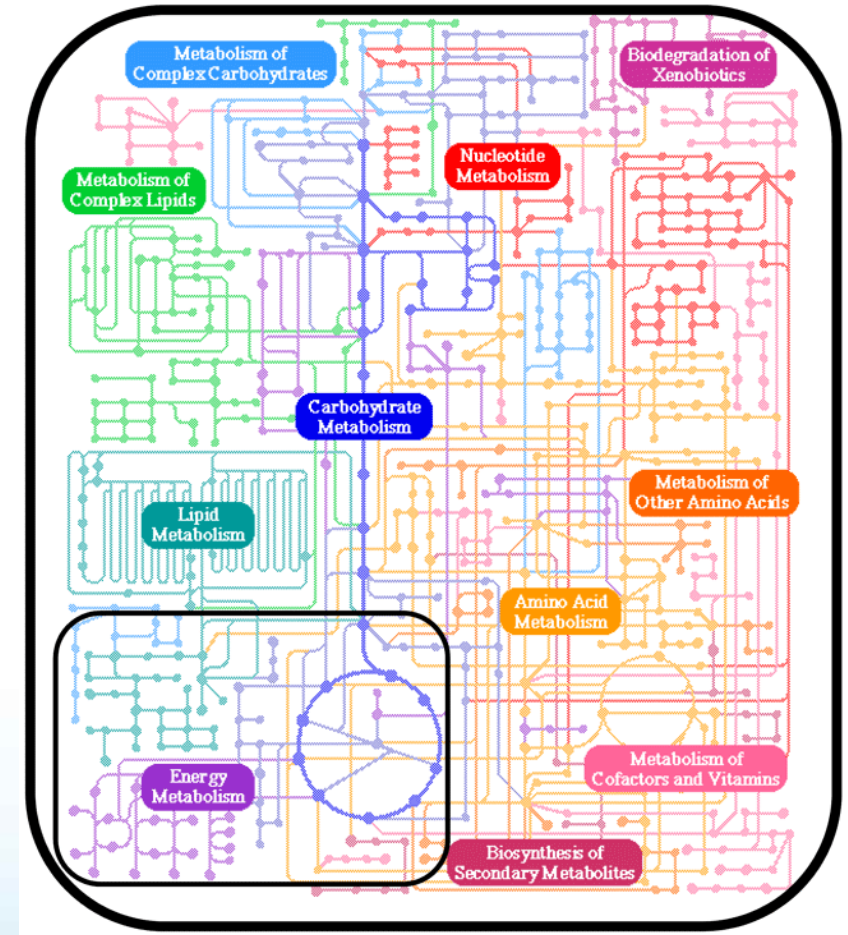
A metabolic profile of all-cause mortality risk identified in an observational study of 44,168 individuals

Joris Deelen  et al. [#]



METABOLOMICS

- Systematic study of the unique chemical fingerprints as the result specific cellular processes.
- Metabolome: the collection of all metabolites in a biological cell, tissue, organ or organism.
- Metabolic profiling: instantaneous snapshot of the cell physiology.



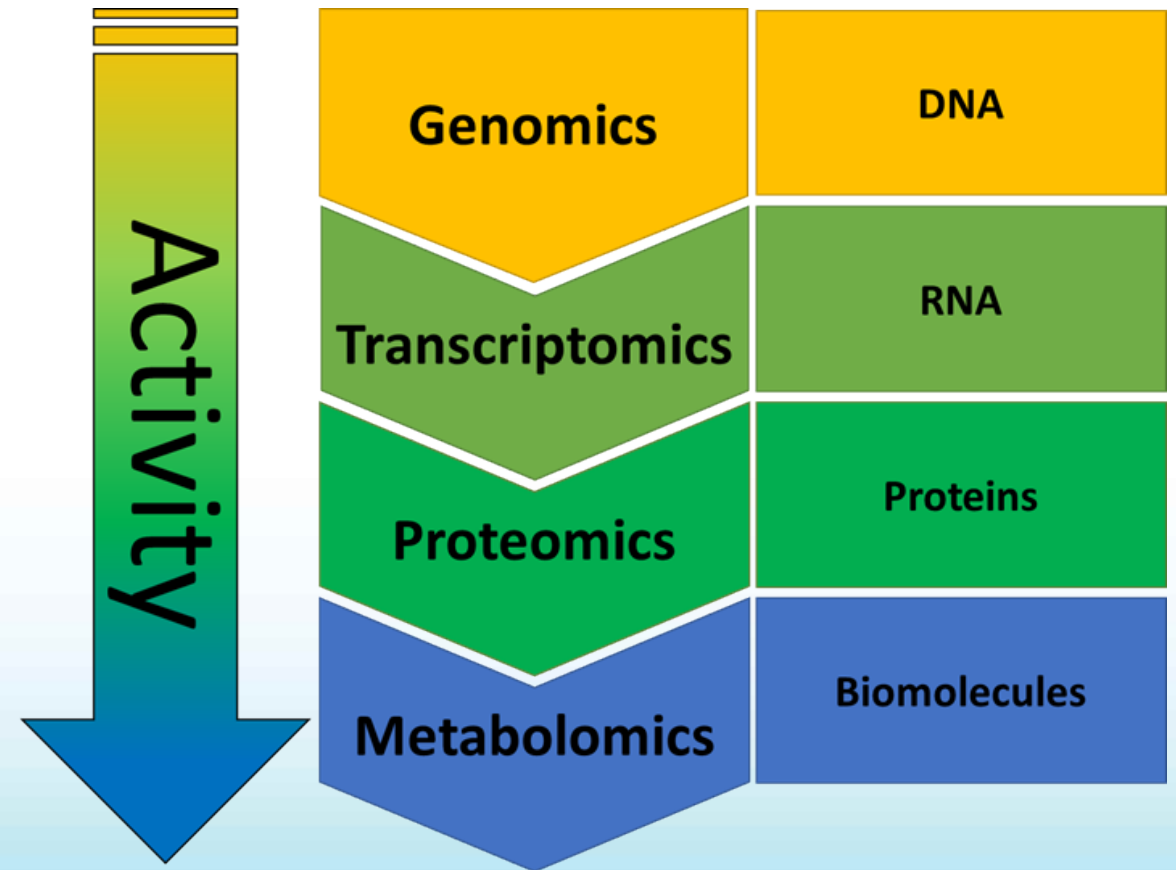
<http://www.urmc.rochester.edu/labs/Munger-Lab/>



METABOLOMICS

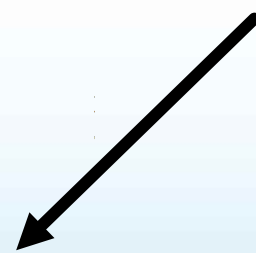
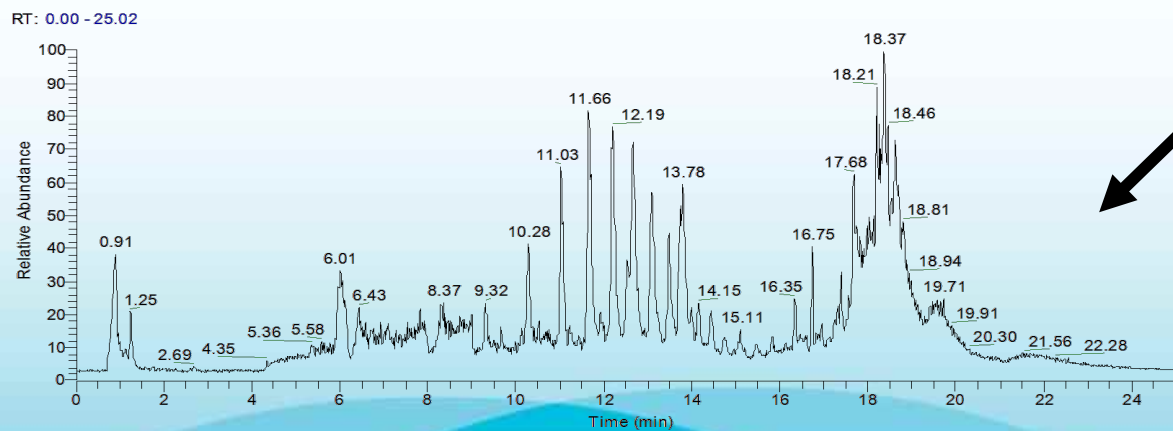
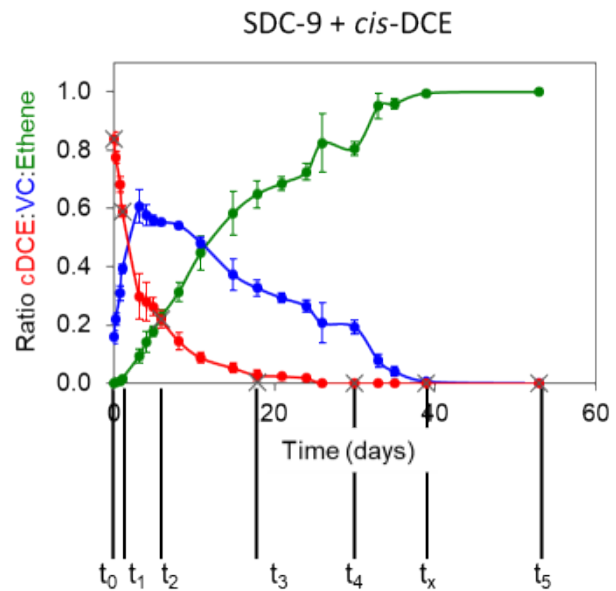
- Analysis of all small molecules (MW<1100) within an environmental sample
- Identification of 80-100 known compounds
- Comparison of the overall metabolic profile
- Statistical Analysis and pattern recognition
 - Predictive capabilities
 - Activity of key degraders

- Metabolomics - analysis of thousands of small molecules from a biological sample
- Trends and patterns can be used to predict the health and activity of the entire microbiome
- Allows for a broad screening of reductive dechlorination potential



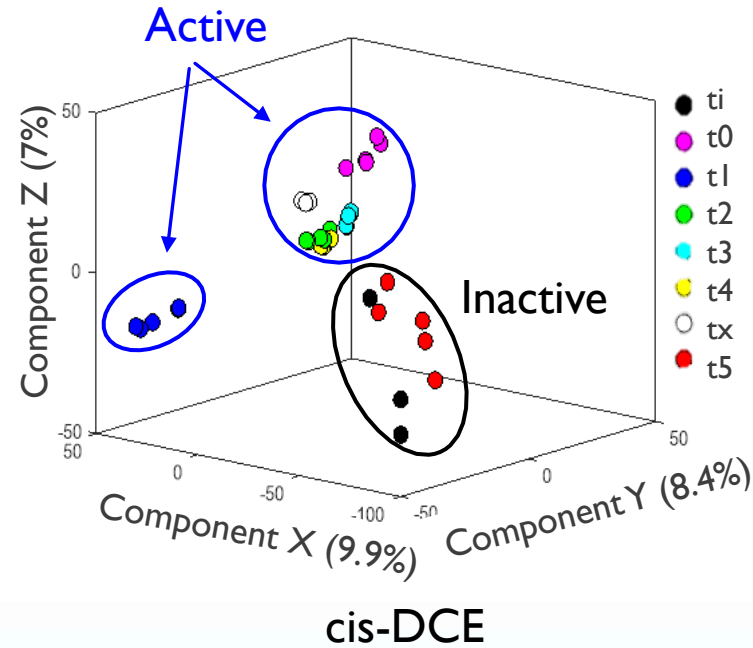
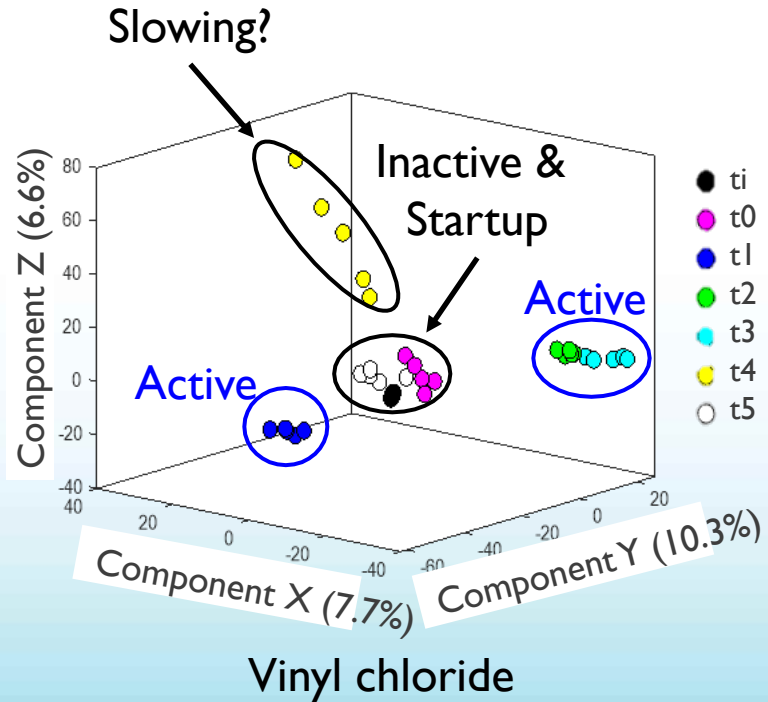


SBIR METABOLOMICS STUDY





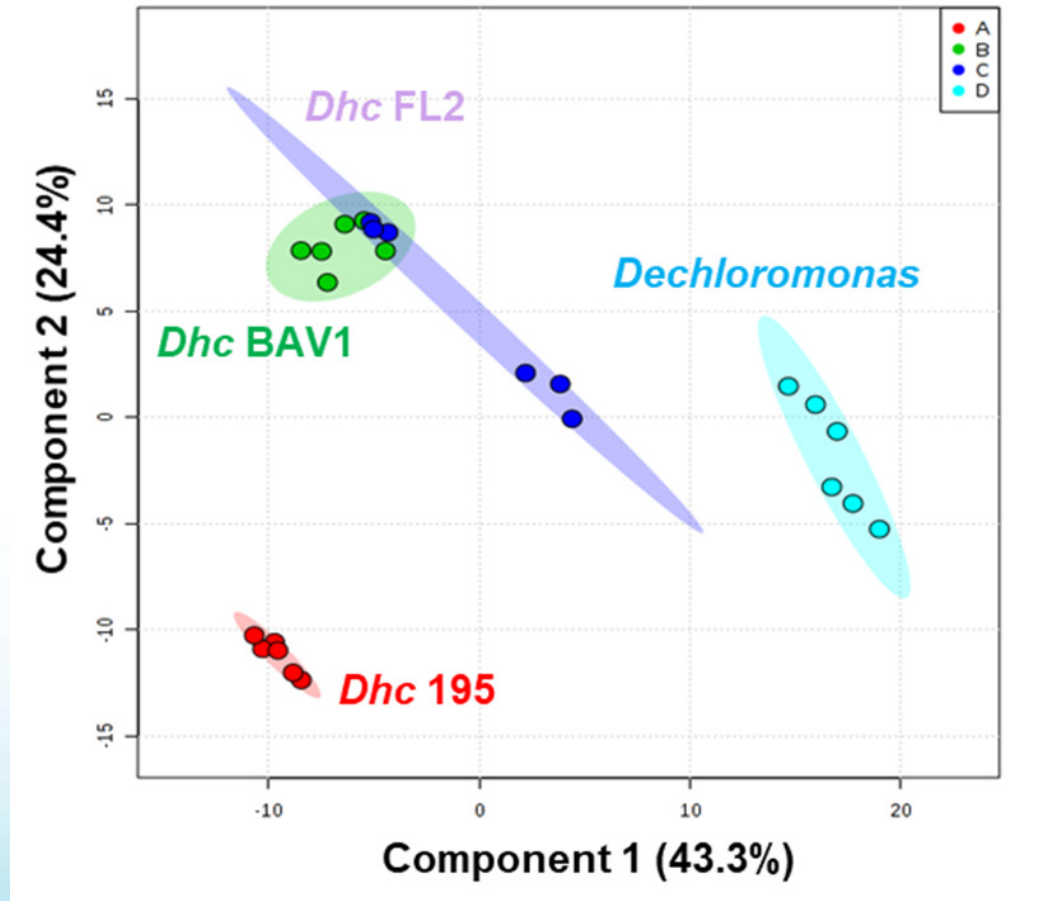
SBIR METABOLOMICS STUDY



- **Commercial Bioaugmentation consortium (SDC-9)**
- **Sampling performed in intervals to capture stage of dechlorination**
- **Over 10,000 metabolites identified**
- **Both the known and unknown metabolites were analyzed using PLS-DA plots**
- **Differences in stages of dechlorination**



SBIR METABOLOMICS STUDY





SBIR METABOLOMICS STUDY

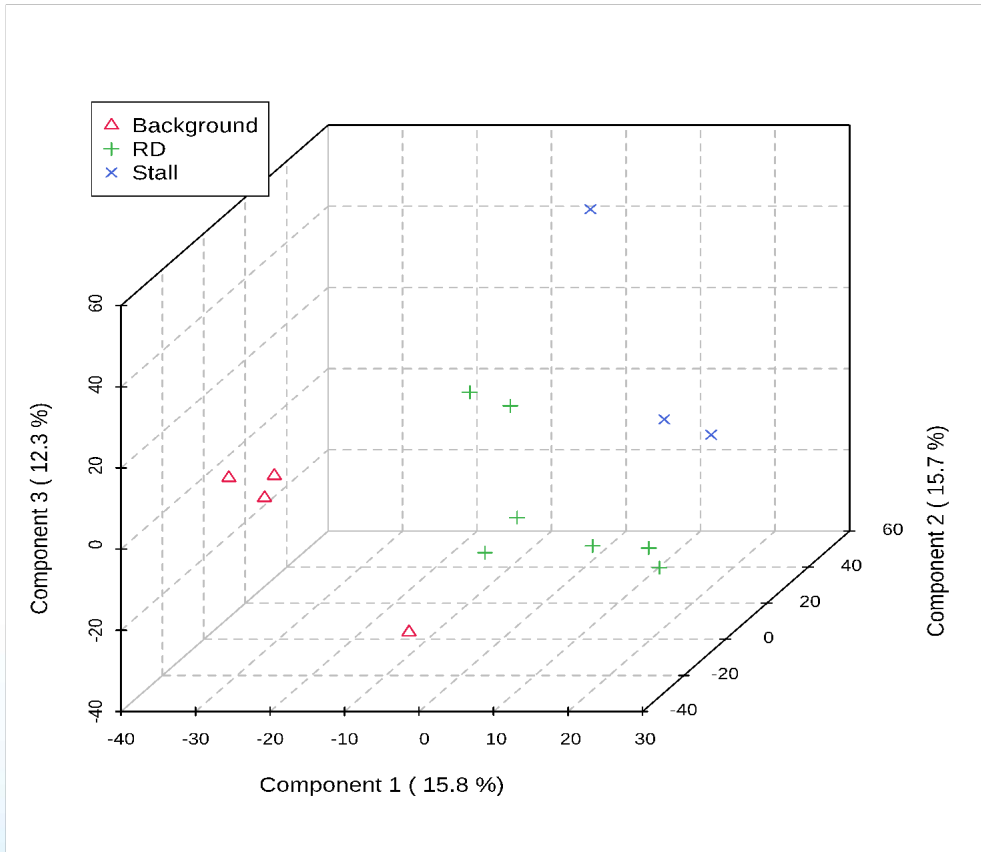


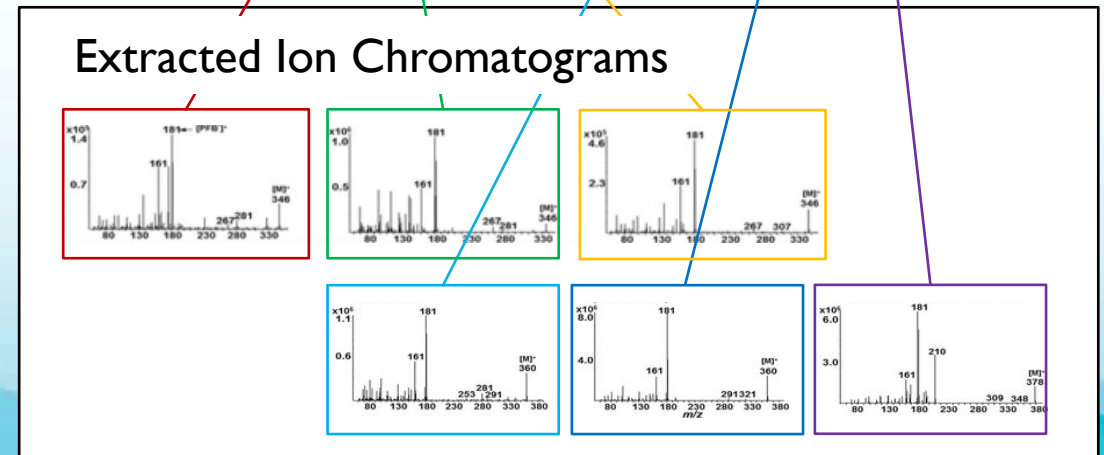
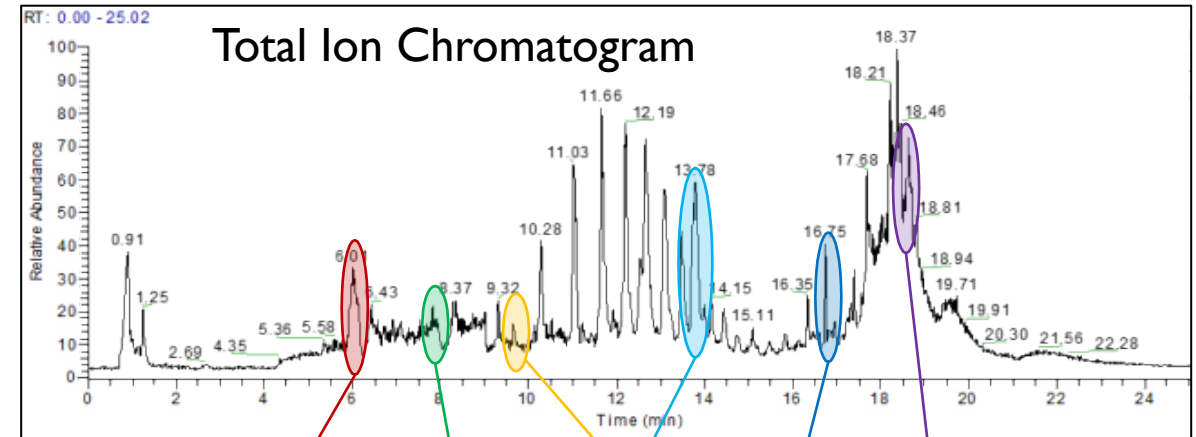
Figure 10: 3D scores plot between the selected PCs. The explained variances are shown in brackets.

- **Three chlorinated sites**
 - **Belleville Industrial (NJ)**
 - **Former Vickers Facility (MO)**
 - **Earnhart Site (TN)**
- **Different stages of Dechlorination**
 - **Active RD**
 - **Stall**
 - **Background**
- **Over 12,000 metabolites identified**
- **PLS-DA plot clearly distinguishes each stage**
- **VIP scoring identified 5 metabolites that could be sentinel for robust RD!**



- Each sample's pattern is compared against a database
- MI's in-house database
- Made up of well-characterized samples with known reductive dechlorination classifications
- Incorporation of machine learning to predict unknown samples based on the database

Thousands of features per sample

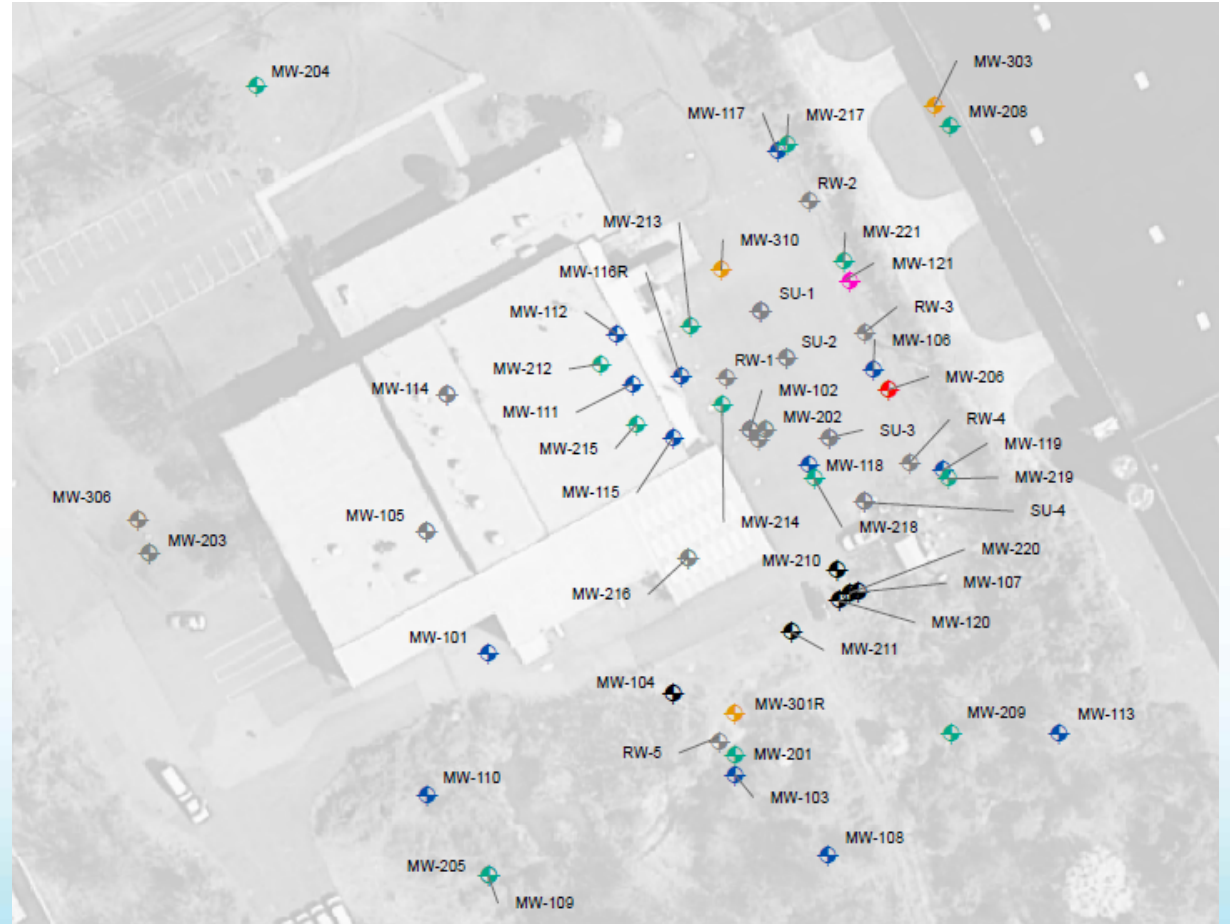




MetaArray™ Case Study



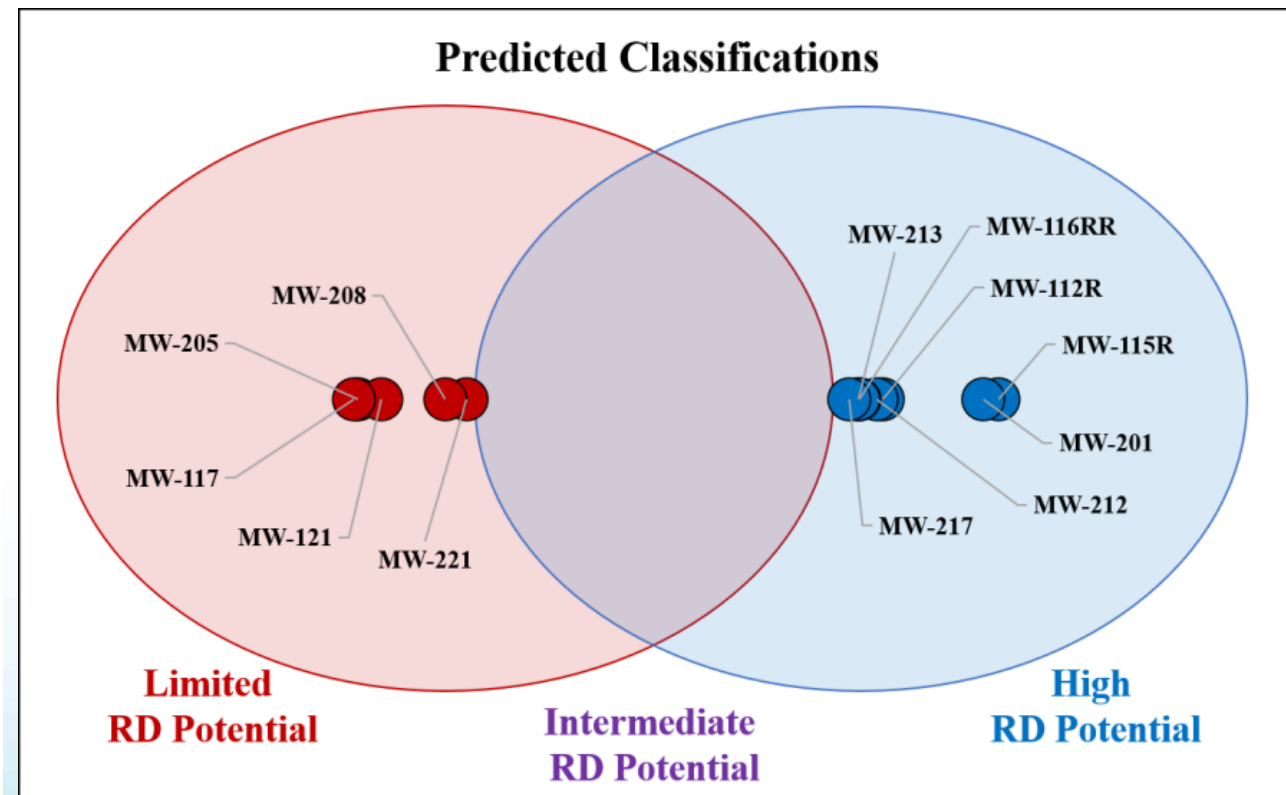
- Chlorinated solvent site
- QuantArray-Chlor and MetaArray
- Site chemistry, geochem, and history were not shared before analysis





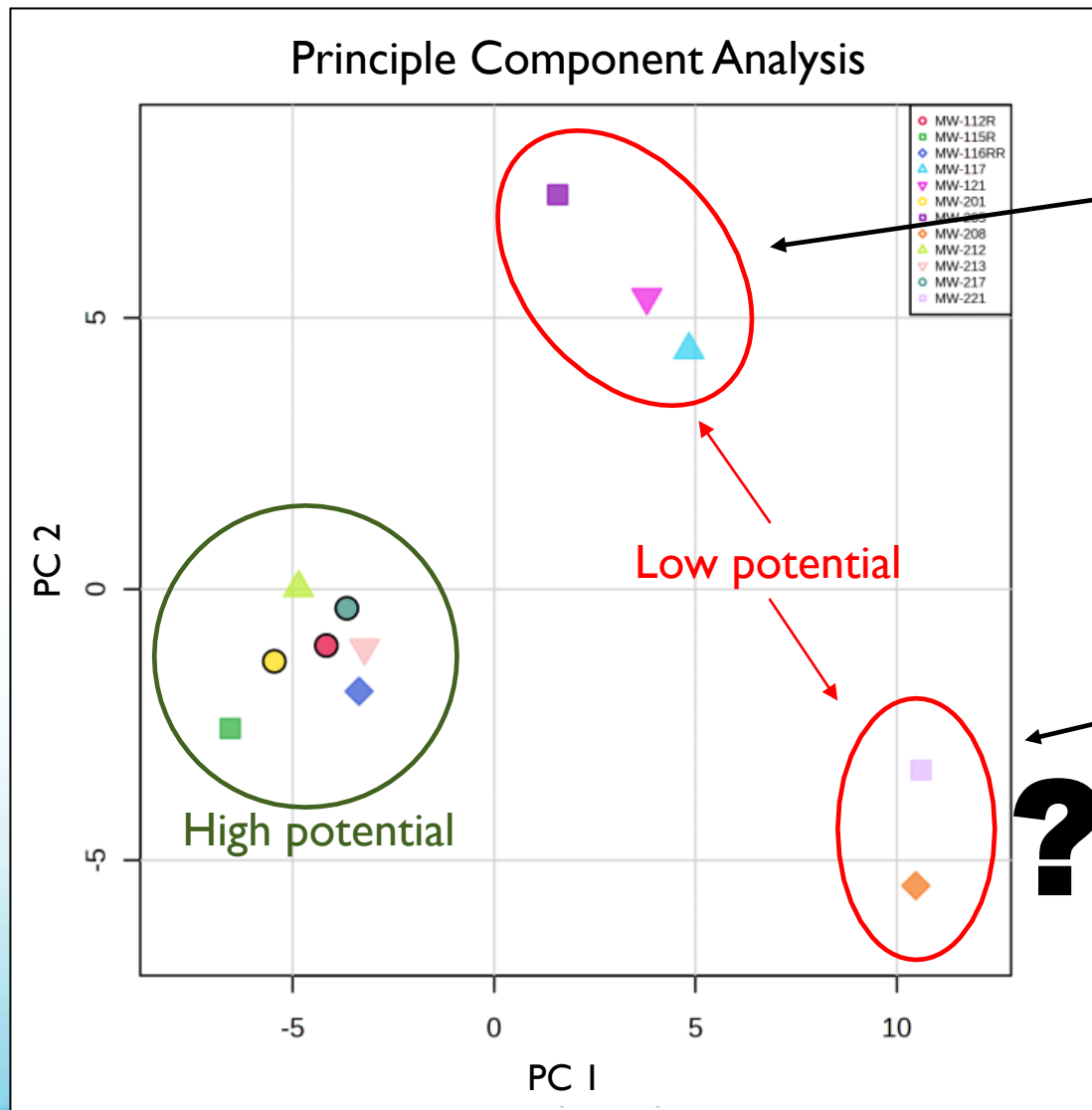
MetaArray™ Case Study

Sample Name	Predicted Class	SVM Model Accuracy ¹
MW-115R	High Reductive Dechlorination Potential	80%
MW-212	High Reductive Dechlorination Potential	80%
MW-121	Limited Reductive Dechlorination Potential	80%
MW-221	Limited Reductive Dechlorination Potential	80%
MW-116RR	High Reductive Dechlorination Potential	80%
MW-213	High Reductive Dechlorination Potential	80%
MW-117	Limited Reductive Dechlorination Potential	80%





MetaArray™ Case Study



Low Potential & Low DHC

Low Potential & 10³ cell/mL DHC

?



MetaArray™ Case Study

High Potential

Heavy treatment Area
Excavation
EVO Soil Mixing
ZVI/Hydraulic Fracking

Low Potential & 10^3 cell/mL DHC

Bedrock wells
Share the same fracture line

Background

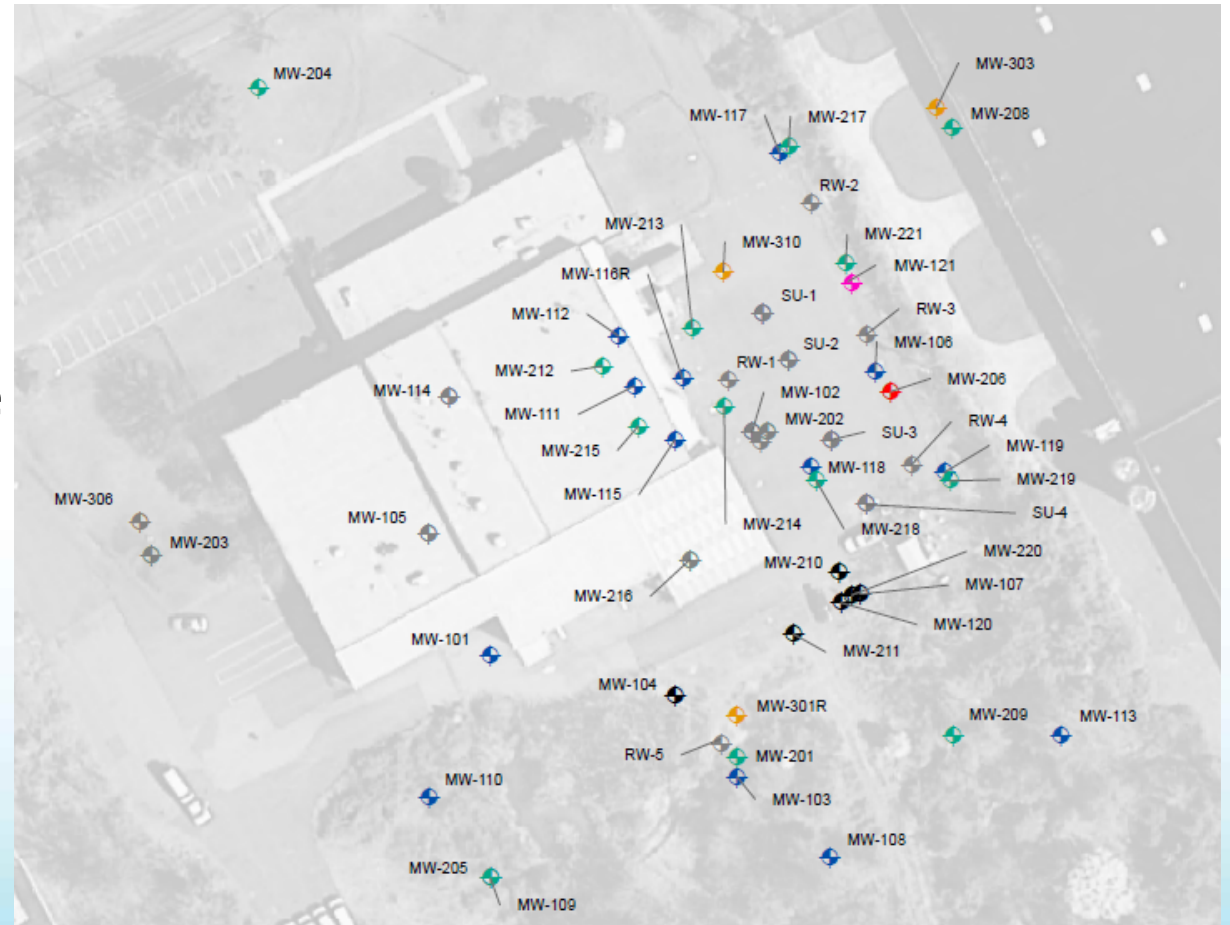




MetaArray™ Case Study



- Next steps
 - Second sampling event
 - Compare results
 - Has the DHC and functional gene abundance changed
 - Does MetaArray indicate in the lower potential wells
 - Use the MBT results to identify the locations with issues
 - Reassess strategy in these locations





CONCLUSIONS

- Machine learning will continue to grow our databases and understanding of the biological metabolome.
- Metabolomics has the ability to help us get closer to rates of degradation.
- Key sentinel metabolites likely exist to help us define degradation potential and predict processes or community health.



THANK YOU!

dtaggart@microbe.com
(865) 573-8188
www.microbe.com