



New Technology Briefings Superfund Research Program (SRP): Bioremediation

FRTTR Spring Meeting
June 5, 2020



*SRP Established 1986
SARA Legislation*

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- SRP Mandates -
Basic Research:
- Health Effects
 - Susceptibility/Risk
 - Detection Tools
 - Remediation Tools



Research Triangle Park, NC

National Institute of Environmental Health Sciences (NIEHS)

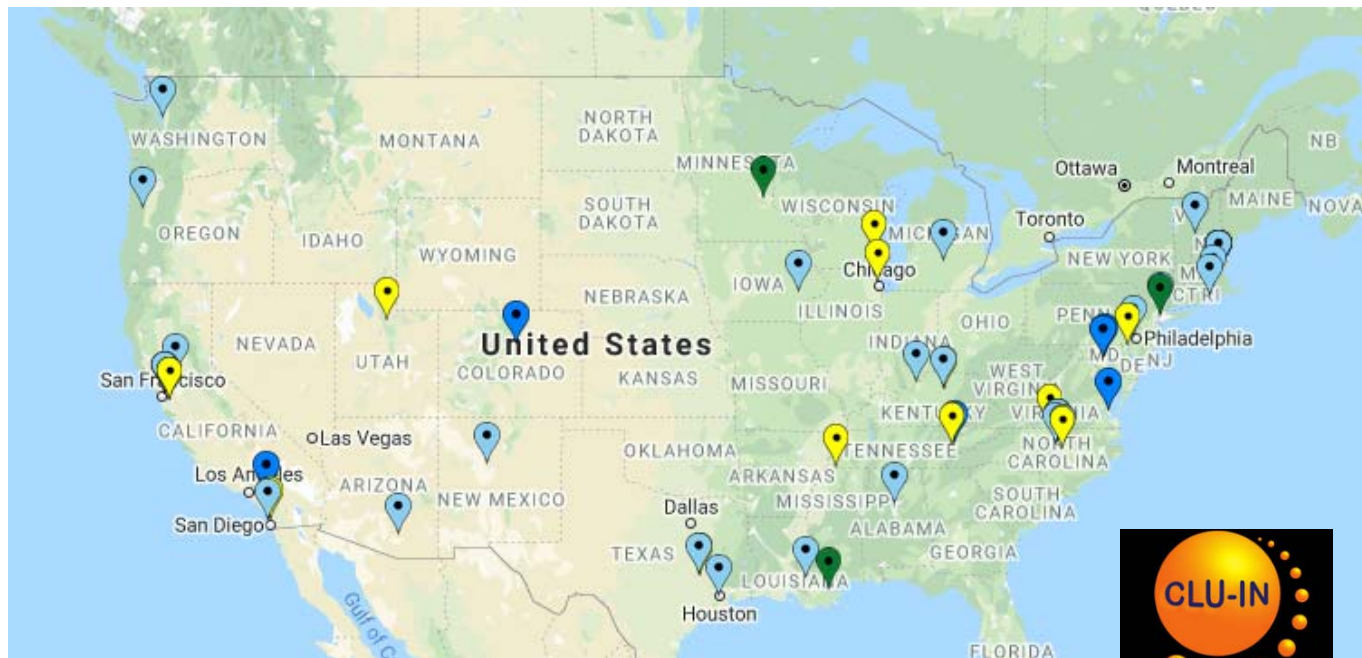
SRP's Early Bioremediation Research and Success Stories (1987 to today)

- **U Washington:** Pioneered use of genetically modified poplar and cypress trees to remediate **TCE**-contaminated groundwater. (Newman et al., Env Sci Technol, 1997; Gordon et al., EHP, 1998; Newman et al., Env Sci Technol, 1999; Featured in the NYT 4/7/2020)
- **UC Berkeley:** Showed that stable isotope can be used to track bioremediation success of **TCE**. (Alvarez-Cohen, ES&T, 2002)
- **U Iowa:** Investigating mechanisms involved in how plants and microbes degrade **PCBs**. Field-scale use of poplar trees in several remediation scenarios. (Mattes et al., Env Sci Pollut Res, 2018)
- **UC Davis:** Enhanced bioremediation of. Used naturally occurring bacteria and nutrient supplementation to enhance **MTBE** bioremediation in groundwater. (Hristova et al., App Environ Microbiol, 2001; Hristova et al., App Env Microbiol, 2003; Nakatsu et al., Int J Syst Evol Microbiol, 2006)
- **Microvi Biotech, Inc.:** Installed nitrate treatment systems in several California drinking water facilities. This bioreactor technology based off early SRP-funded project to sustainably remove **1,4-dioxane**.

Several of these stories featured in Suk et al., EHP, 2018



Highlights: SRP Research in Bioremediation



Bioremediation Grantees (25 Projects):

- Multi-Project Centers
- Biogeochemical Interactions
- Small Business

Recent Webinars:

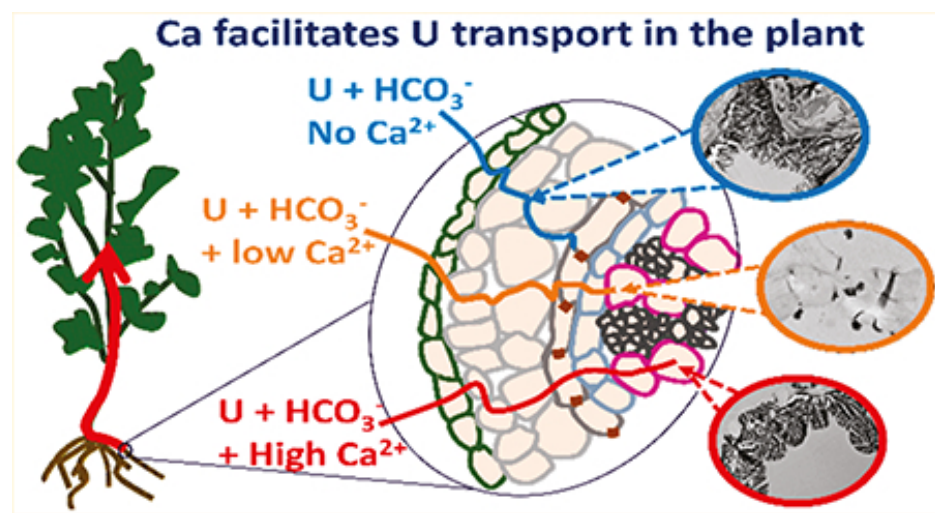
- Biogeochemical Interactions Grantees (Spring 2019)
- Bioremediation Webinar Series (Fall 2019)
- FRTR Presents: Bioremediation Part 1 (May 29, 2020)

Center Grantees: University of New Mexico P42ES025589

Immobilization of U, As, and Co-occurring Metals in Mine Wastes

Jose Manuel Cerrato, University of New Mexico

- Developing strategies to immobilize **arsenic, uranium, and metal mixtures** in mining waste
- Investigating reactions and mechanisms at molecular level to understand macro-scale processes influencing water quality
- Manipulating rhizosphere environment to alter microbiome-plant interactions controlling metal uptake
- Approach: in-vitro and greenhouse experiments; working at Jackpile-Paguate Uranium Mine - Laguna Pueblo, New Mexico
- Progress: Calcium in carbonate water inhibits the transport and precipitation of U in the root and facilitates transport and translocation toward shoots
(El Hayek et al., ACS Earth Space Chem, 2019)

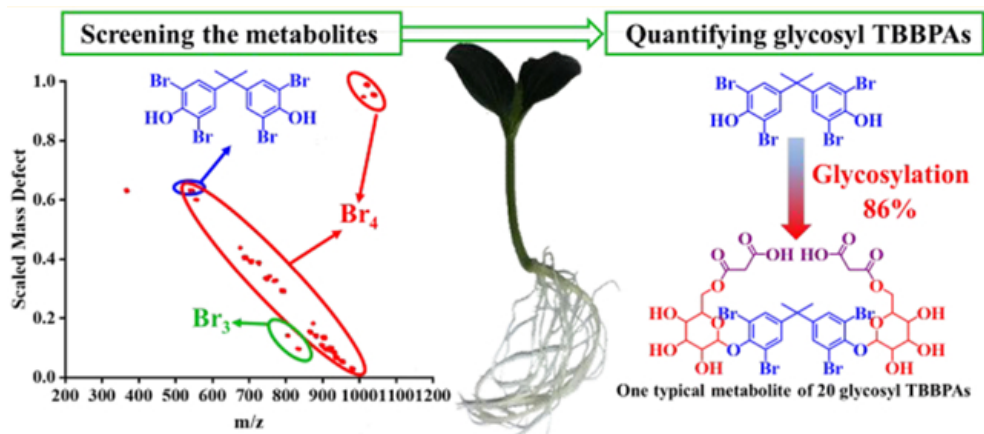


Center Grantees: University of Iowa P42ES013661

Mitigating Airborne PCB Emissions from Sediments with Black Carbon Materials and PCB-Degrading Biofilms

Tim Mattes and Jerry Schnoor, University of Iowa

- Mechanisms of dechlorination for several legacy and emerging contaminants (Schnoor received 2019 ACS Award for Innovation).
- PCB dechlorination hotspots and reductive dehalogenase genes in sediments from a contaminated wastewater lagoon (Mattes et al. 2018 Environ Sci Pollut Res Int)
- Exploring black carbon and biofilms to mitigate **PCBs**. Demonstrated dechlorination potential and identified candidate genes to serve as biomarkers of PCB dichlorination (Ewald et al., Environ Sci Pollut Res Int, 2019)
- Discovered pumpkin seedlings can break down tetrabromobisphenol A **TBBPA** (Hou et al., Environ Sci Technol, 2019)



Center Grantees: University of Arizona P42ES004940

Exposures, Health Impacts, and Risk for Mine Waste Contamination

Phytostabilization Technology for Mining Wastes in Arid and Semiarid Environments: Plant-Microbe-Metal Indicators to Predict Sustainability

Raina Maier, University of Arizona

- **Compost-assisted phytostabilization for mine tailings** containing **arsenic and lead** in arid environments.
- Combining microbiome and plant transcriptome analyses to identify key microbes important for plant establishment and survival. (Young et al., *Microbiome*, 2018; Yu et al., *New Phytologist*, 2018; Dayama et al., *BioRxiv*, 2019)
- Other Bioremediation Projects:
 - **High-throughput cultivation**/screening for cultures of interest; synthetic microbial communities (Paul Carini)
 - **Arsenic** sequestered in the root exterior and interior vacuoles in the root zone of *Prosopis juliflora* (mesquite) (Jon Chorover) (Hammond et al., *Environ Sci Technol*, 2018)
 - Investigating physical and biogeochemical processes controlling migration of **mine-drainage contaminants** in groundwater using innovative methods (Mark Brusseau) (Araujo and Brusseau, *Environ Sci Process Impacts*, 2019; Guo et al., *Hydrogeology Journal*, 2019; Jiang et al., *Water Resour Res*, 2019)

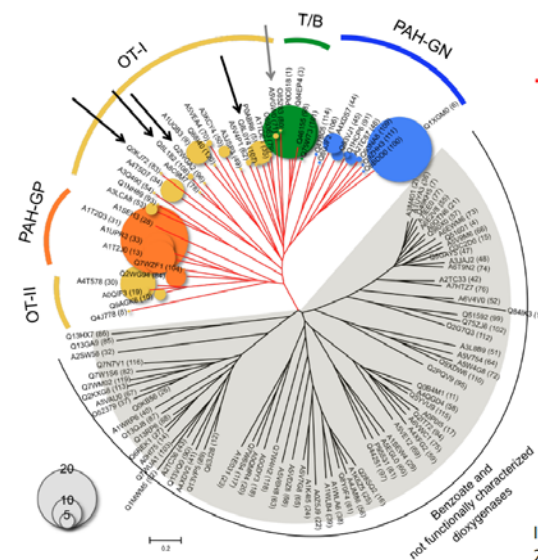


Center Grantees: Michigan State University P42ES004911

Molecular Insight into Dioxin Degradation by Microbes and Microbial Communities

Gerben J. Zylstra (Rutgers University) James Tiedje (MSU)

- Characterizing the microbial response to dioxin to understand the limitations on environmental detoxification
- Developing a comprehensive profile of microbial community metabolic capabilities for degradation
- Bioavailability of clay-adsorbed dioxin to *Sphingomonas wittichii* RW1 and its associated genome-wide shifts in gene expression
- Developed Microbial Genomes Atlas – www.enve-omics.gatech.edu (Rodriguez et al Nucl Acids Res, 2018)



Protein tree of dioxinases

OT-I contains small clusters that attack dioxin (gray) and dibenzofuran (black) compounds (clusters 71, 107, 96, 108, 83).

OT-II is mainly phthalate dioxinases (30)

Iwai, Johnson...Tiedje 2011, AEM, Appl. 77: 3551

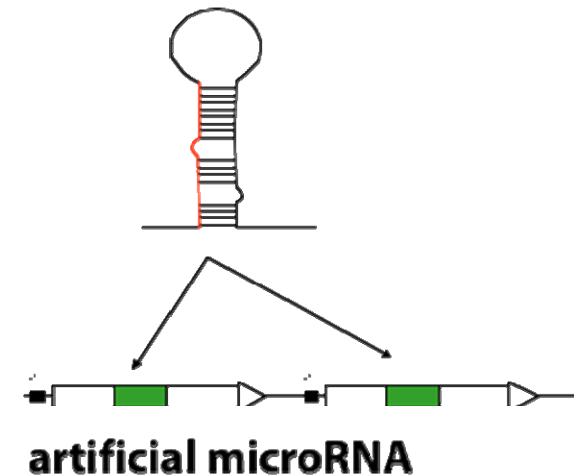
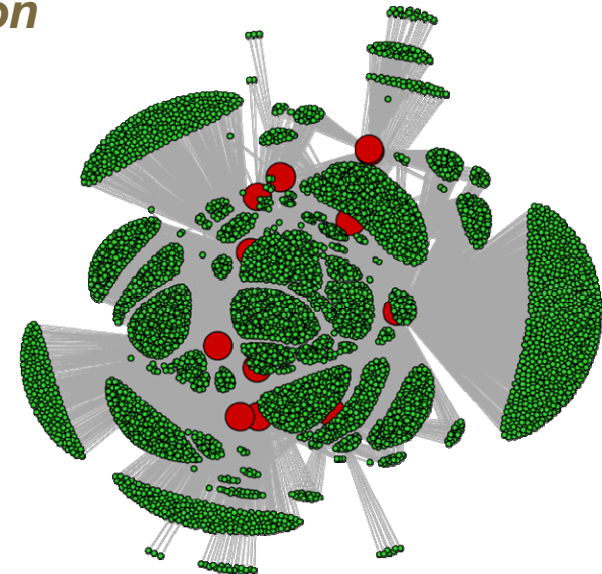
(Chai et al., Sci Total Environ, 2020; Sallach et al., Sci Total Environ, 2019; Fu et al., Environ Pollut, 2018; Ahn et al., Ann Microbiol, 2017; Stedtfeld et al., J Environ Manage, 2017; Chai et al., PLoS One, 2016)

Center Grantees: UC San Diego P42ES010337

Molecular Mechanisms of Heavy Metal Detoxification and Engineering Accumulation in Plants

Julian Schroeder, UC San Diego

- Metal transport in plant cells – e.g. phytochelatins
- Machine Learning Approaches
 - New powerful screen to identify new genes, gene families, and network principles that function in **heavy metal** and **arsenic** resistance
 - Developed genome-wide artificial microRNA libraries that can identify the genes, signal transduction pathways, and mechanisms underlying heavy metal(loid) accumulation in plants (Hauser et al., Plant Cell, 2013)
 - The UCSD artificial microRNA database is available online at: <http://phantomdb.ucsd.edu/>



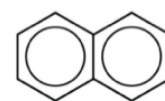
Center Grantees: Duke University P42ES010356

Engineering Physico-Chemical Environment to Enhance Bioremediation of Developmental Toxicants in Sediment Fungal-Bacterial Biofilms Claudia Gunsch, Heileen Hsu-Kim, Rytas Vilgalys, Duke University

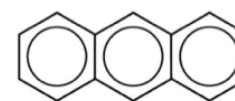
- Identified types of fungi abundant in the presence of **PAHs** (Czaplicki et al., Remediation, 2016)
- Biochar and activated carbon promote biodegradation of **TBBPA** and may be helpful for removing other harmful contaminants (Lefevre et al., Water Res, 2018)
- Developing a strategy for "precision bioremediation" to identify specific targets for genetic bioaugmentation – inserting the relevant genes into native organisms (Redfern et al., J. Haz Mat, 2019)



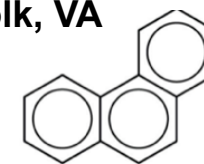
**Republic Creosoting,
Elizabeth River in Norfolk, VA**



Naphthalene



Anthracene



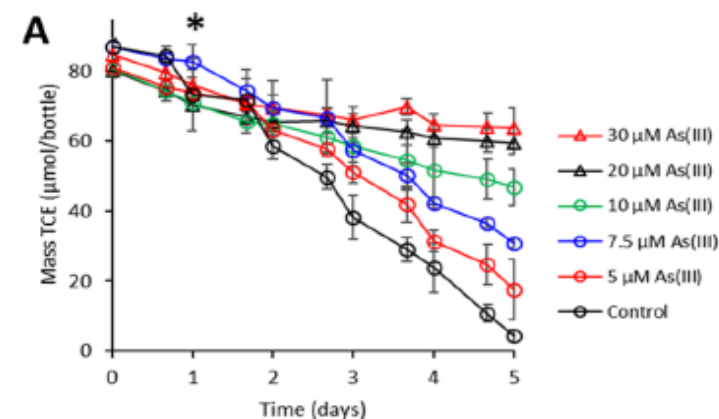
Phenanthrene

Center Grantees: UC Berkeley R01ES024255, P42ES004705

Microbial Communities that Bioremediate Chemical Mixtures

Lisa Alvarez-Cohen, UC Berkeley)

- Systems biology approach to **TCE** bioremediation
- TCE-degrading microbes interact with co-existing organisms (Mao et al., *Env Sci Technol*, 2017; Men et al., *Env Sci Technol*, 2017)
- TCE biodegradation inhibited by **arsenic** but overcome by supplemental nutrients (Gushgari and Alvarez-Cohen, *Env Sci Technol*, 2020)
- Explored **PFAS** effects on TCE degradation (Weathers et al., *Env Sci Technol*, 2016)
- **Biogeochemical Interactions Grant**: Effects of sulfate reduction on TCE bioremediation; (Mao and Alvarez-Cohen, *Appl Environ Microbiol*, 2017; Men et al., *Appl Environ Microbiol*, 2017)



Amount of TCE degraded by bacteria decreased over time with higher As(III) concentrations (Image from Gushgari & Alvarez-Cohen, 2020)

Biogeochemical Interaction Grantees

Biogeochemical Controls over Corrinoid Bioavailability to Organohalide-Respiring Chloroflexi

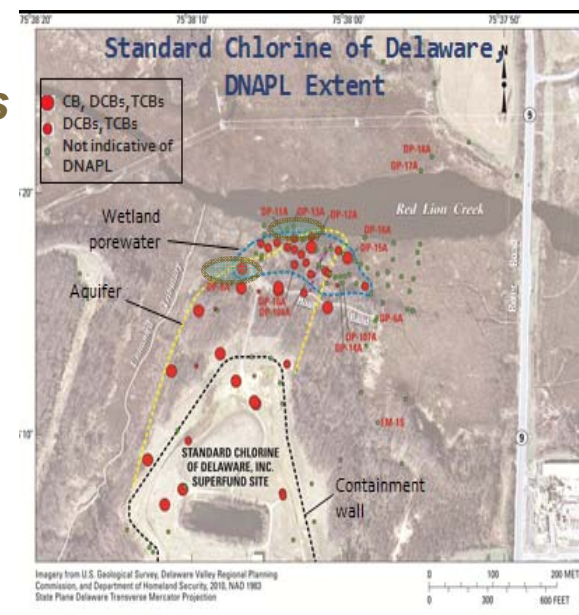
Frank E. Loeffler, University of Tennessee, R01ES024294

- Discovered new cobamide structure used in bacterial **TCE** dechlorination (Yan et al., Nat Chem Biol, 2018)
- Discovered that nitrous oxide, commonly found in groundwater, inhibits bacterial reductive chlorination of **PCE**, **cis-1,2-dichloroethane**, and **vinyl chloride** (Yin et al., Environ Sci Technol, 2019)

Dual-Biofilm Reactive Barrier for Treatment of Chlorinated Benzenes at Anaerobic-Aerobic Interfaces in Contaminated Groundwater and Sediments

Edward Bouwer, Johns Hopkins University
R01ES024279

- Evaluating flow-through barrier with granular activated carbon coated with anaerobic and aerobic microorganisms for breaking down **chlorobenzenes** and **benzene** contaminants
- Featured in Michelle Lorah (USGS) FRTR presentation (5/29/2020)



Biogeochemical Interactions Grantees

Development of in-situ Mercury Remediation Approaches Based on Methylmercury Bioavailability

Upal Ghosh, UM Baltimore County (R01ES024284)

- Identifying biogeochemical characteristics that make **mercury**-contaminated sites suitable for remediation with activated carbon
- Designing sorbent amendment/capping strategies that reduce methylmercury bioavailability

(Schwartz et al., Environ Sci Process Impacts, 2019)



Biogeochemical Framework to Evaluate Mercury Methylation Potential During in-situ Remediation of Contaminated Sediments

Heileen Hsu-Kim, Duke University (R01ES024344)

- Studying sediment microorganisms that methylate **mercury** and factors that can control and reduce toxic methylmercury production
- **Passive sampling strategies** to measure mercury bioavailability and biomethylation

(Hsu-Kim et al., Ambio, 2018; Ndu et al., Environ Sci Technol, 2018; Wyatt et al., Environ Sci Technol, 2016)

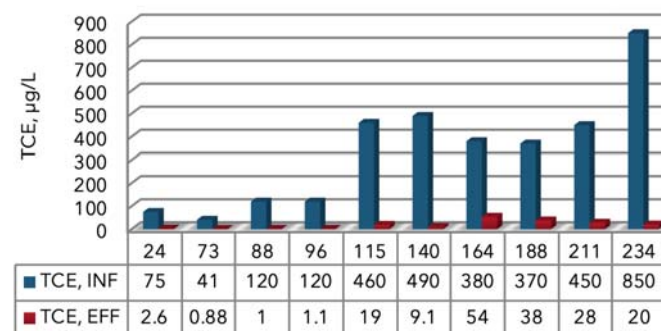
Small Business Grantees

Biocatalyst Platform Technology for Enhancing Cometabolic Biodegradation

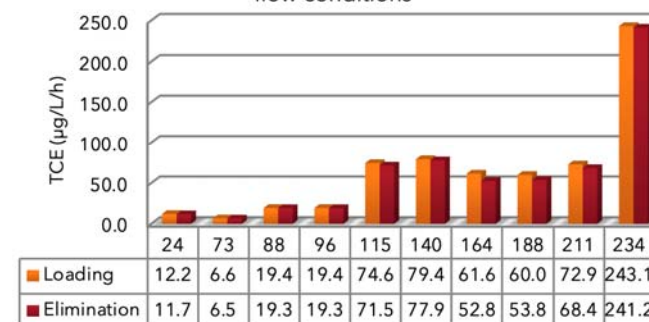
Fatemeh Shirazi, Microvi Biotechnologies
(R44ES024670)

- **Bioreactor:** Development of a continuous flow prototype for the degradation **TCE**
- Application of computational methodologies to detect key interactions
 - Better capture individual heterogeneity
 - Customizable platforms for wide range of bioremediation situations and data inputs
 - Integration with other modeling tools
 - Leverage data from next-generation tools and techniques for translation into practical applications
 - “An Agent-Based Modeling Platform for Environmental Biotechnology” (R41ES026541)

TCE degradation under continuous flow conditions



TCE loading vs elimination capacity under continuous flow conditions

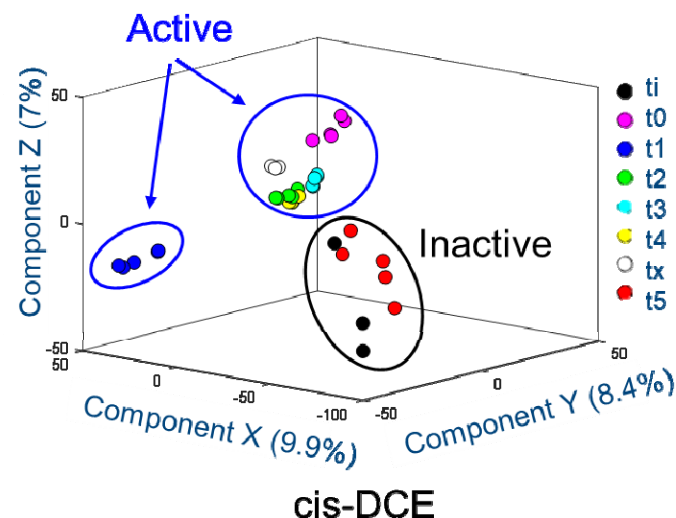


Small Business Grantees

Expanding the Tool Box: Environmental Metabolomics Improves Decision Making and Management of Contaminated (Superfund) Sites

Dora Taggart, Microbial Insights, (R43ES030669)

- Sampling metabolomes from the environment and **comparing profiles from different contaminated sites**
- Identification of 80 – 100 known compounds in samples; thousands of unknown compounds
- Use statistical analysis and pattern recognition to predict and understand activity of key degraders





Small Business Grantees

Novel Rhamnolipid Surfactants for Recovery of Critical Elements and Remediation of Metal Contaminated Waste Streams

Chett Boxley (Glycosurf) and Raina Maier (U Arizona), R44ES029423

- Developing green rhamnolipid surfactants for efficient and cost-effective removal of **heavy metals and rare earth elements** from wastewater
- Produces high-purity and high-performance glycolipids, for example rhamnolipids, using renewable sugar molecules



Other Grantees: Monitoring Remediation Success

Beyond Parent Compound Disappearance in the Bioremediation of PAH-Contaminated Soil

Mike Aitken, UNC Chapel Hill (P42ES005948)

- Metabolites of **PAH** bioremediation and soil toxicity
(Vila et al., Sci Total Environ, 2020; Chibwe et al., Sci Total Environ, 2017)

Identification of Remediation Technologies and Conditions that Minimize Formation of Hazardous PAH Breakdown Products at Superfund Sites

Staci Simonich, Oregon State (P42ES016465)

- High throughput toxicity testing for **PAH** bioremediation metabolites
(Kramer et al., Environ Sci Technol, 2019; Titaley et al., Environ Sci Technol, 2019; Trine et al., Environ Sci Technol, 2019; Schrlau et al., Environ Sci Technol, 2017)

Optimizing Bioremediation for Risk Reduction Using Integrated Bioassay, Non-Target Analysis and Genomic Mining Techniques

Tom Young, UC Davis (P42ES004699)

- Bioassay-based approaches to test toxicity reductions from bioremediation
(Black et al., Environ Sci Process Impacts, 2019; Parry et al., Water Res, 2016)

Other Grantees: Past Research Projects

Using Microbial Induced Calcite Precipitation by Indigenous Soil Bacteria to Reduce Mobility of Lead in Soil

Malcolm Burbank, BioCement Technologies (R43ES025132)



- BioCement stabilizes metals in soil (e.g., **lead, barium, cadmium, cobalt, manganese, strontium, zinc**)
- Alters engineering characteristics while reducing mobility; process is carbon neutral to carbon negative
- BioCement is commercially available; currently testing the use of BioCement to treat munitions-impacted soil



Novel Mechanism of Uranium Reduction Via Microbial Nanowires

Gemma Reguera, Michigan State University (R01ES017052)

- Developed patented device based on metal reduction by Geobacter species
- Removes **uranium** from water – and tested at Oak Ridge National Laboratory

(Cologgi et al., PNAS, 2011; Cologgi et al., Appl Environ Microbiol, 2014; Reguera, Biocem Soc Trans, 2012; Cosert et al., MBio, 2019)



Summary: SRP Research in Bioremediation

Who

- Bacterial/fungal/plant systems
- Microbiome, biofilms

What

- Contaminants: inorganics, organics
- Emerging, mixtures

How – Innovative Mechanisms

- New approaches to find key players
- Innovation in understanding function
- Omics/Computational approaches
- Assessing effectiveness, metabolite toxicity

When

- Conditions where bioremediation are optimal
- Identifying new ways to optimize conditions

Thank you!
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