SiREM
Leading Science · Lasting Solutions

Monitoring Metals/Metalloid Reduction and Their Impacts in Aquatic Environments Through Molecular Genetic Tools

Jeff Roberts and Phil Dennis (SiREM)
Silvia Mancini, Andrew Holmes, Rachel James (Geosyntec Consultants)

Presented by Jeff Roberts
Remtech Banff AB
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siremlab.com

Introduction to SiREM

Founded in 2002 in Guelph, ON

Provide products and testing services to support and improve site remediation

Further information: siremlab.com

SiREM Service Areas

Treatability Testing

treatability studies

SiREMNA™

Characterization/Monitoring

- *Molecular Testing*

gene trac®

- *Passive Samplers for Vapor and Pore Water*

WATERLOO MEMBRANE SAMPLER

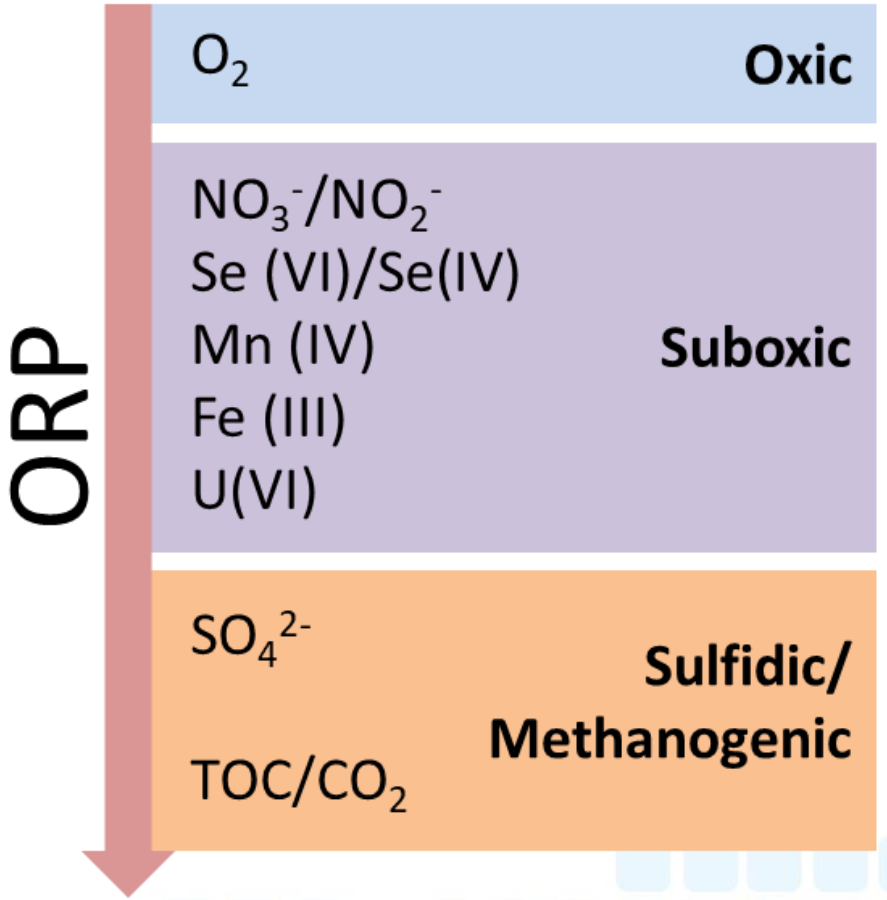
SP3

Bioaugmentation

KB-1®

Potential Contaminants of Concern

- Metal cations (e.g. cadmium, lead, zinc)
- Transition metals (iron, manganese, copper, chromium, mercury)
- Non-metals (sulfur, nitrogen, selenium)
- Metalloids (arsenic, antimony, selenium)
- Actinides (uranium)
- Nitrate, nitrite
- Phosphate
- Sulfate
- Perchlorate, chlorate
- Explosives nitrogen residues
- Acidity



Microbial Community Characterization is Evolving

- Culture based methods (e.g., plating) - used since 1890's
- Clone libraries/Sanger Sequencing - widespread use by late 1990's
- Denaturing gradient gel electrophoresis (DGGE) -1990's
- Quantitative PCR early 2000s
- Next generation sequencing (NGS) of 16S rRNA amplicons - 2010's

Plus eDNA profiles of non-microbial species in aquatic systems ~2015

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Determining the Biotic Composition of Water Using Genetic Methods

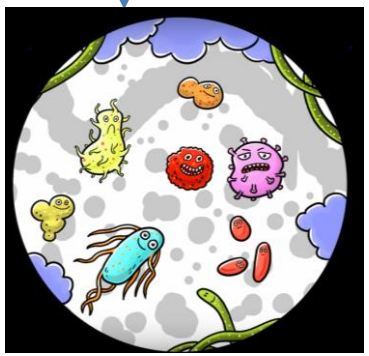
Quantitative
PCR Tests

eDNA Methods

Quantify pre-selected targets:

- Organisms e.g., Anammox bacteria
- Functional genes
  e.g., denitrification

NGS
Amplicon
Sequencing



Characterization of non-microbial biota by Sequencing Cytochrome C Oxidase gene

Characterize entire microbial community by sequencing 16S rRNA gene

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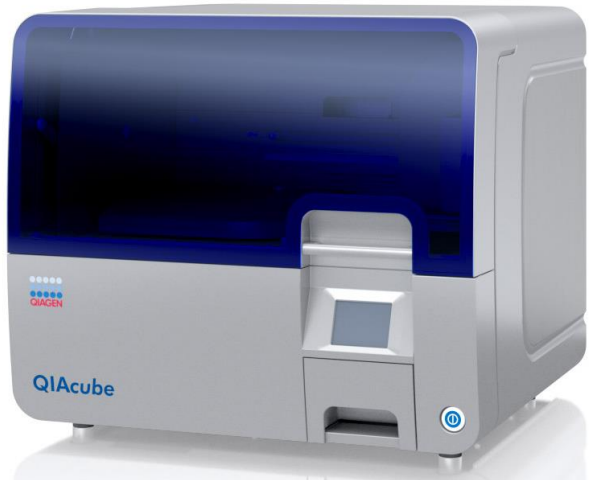
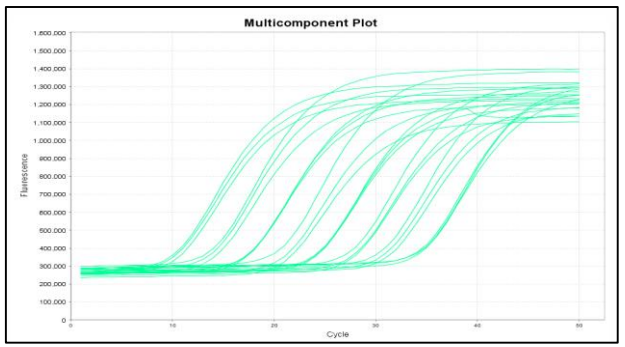
Gene-Trac® qPCR and NGS Overview

Samples



DNA Extraction

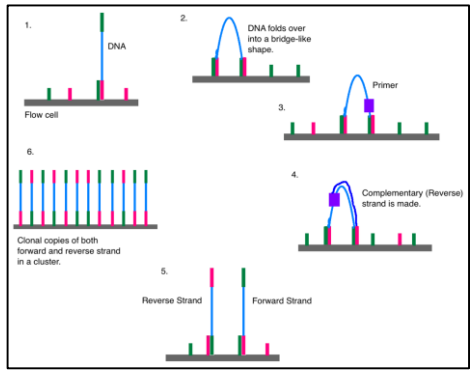
qPCR



Quantification

qPCR
Enumeration of specific targets

NGS



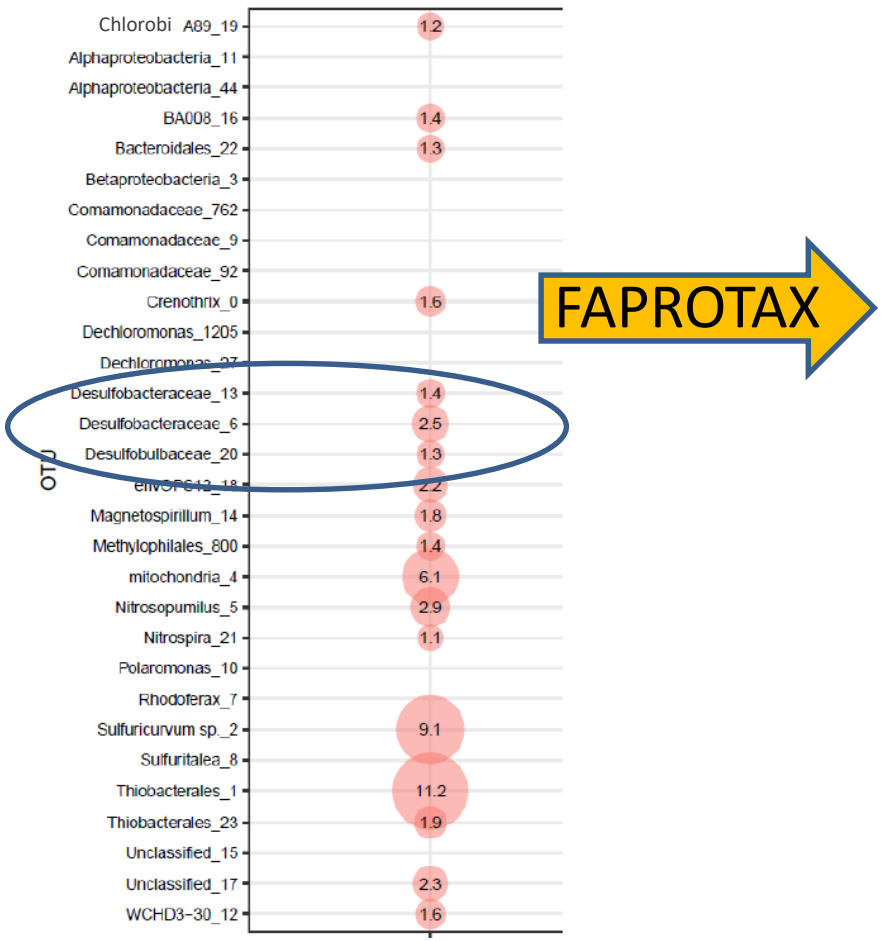
Illumina Sequencing

Bioinformatics

NGS
Comprehensive list of microbes and proportion

NGS Microbial Community Analysis Data

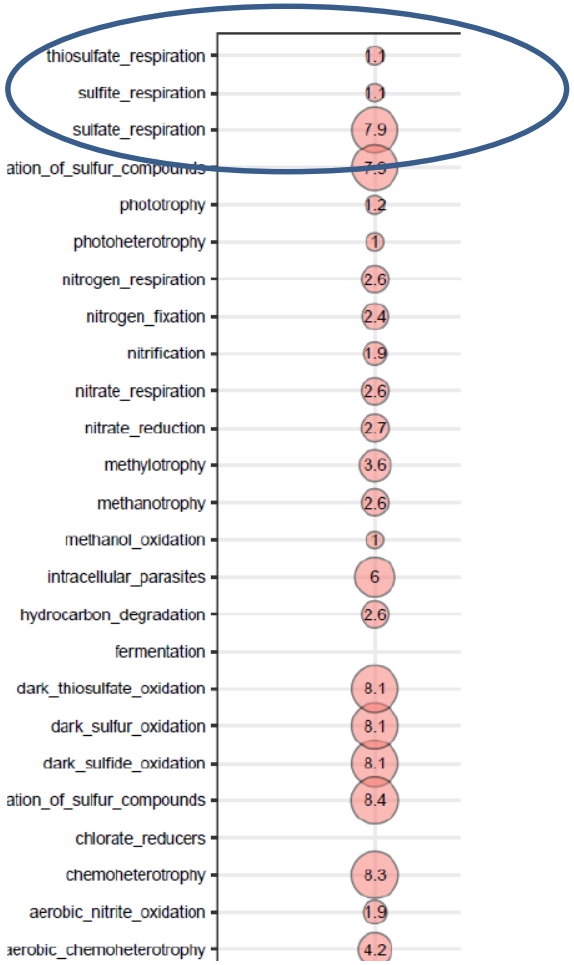
Microbial Taxa %



Sample A

FAPROTAX

Functional Groupings



Sample A

Enumeration Table

Taxonomic Designation	OTU ID	per/L
p__Chlorobi; c__BSV26; o__A89	19	6.E+06
c__Alphaproteobacteria	11	3.E+06
c__Alphaproteobacteria	44	3.E+05
o__Bacteroidales; f__BA008	16	7.E+06
o__Bacteroidales	22	7.E+06
c__Betaproteobacteria	3	1.E+05
f__Comamonadaceae	762	5.E+05
f__Comamonadaceae	9	6.E+04
f__Comamonadaceae	92	2.E+06
g__Crenothrix	0	8.E+06
g__Dechloromonas	1205	0.E+00
g__Dechloromonas	27	1.E+05
f__Desulfobacteraceae	13	7.E+06
f__Desulfobacteraceae	6	1.E+07
f__Desulfobulbaceae	20	7.E+06
c__Anaerolineae; o__envOPS12	18	1.E+07
g__Magnetospirillum	14	9.E+06
o__Methylophilales	800	7.E+06
o__Rickettsiales; f__mitochondria	4	3.E+07
g__Nitrosopumilus	5	1.E+07
g__Nitrospira	21	6.E+06
g__Polaromonas	10	2.E+05
g__Rhodoferax	7	1.E+06
g__Sulfuricurvum; s__kujiense	2	5.E+07
g__Sulfuritalea	8	2.E+05
o__Thiobacterales	1	6.E+07
o__Thiobacterales	23	1.E+07
Unclassified	15	0.E+00
Unclassified	17	1.E+07
c__Parvarchaea; o__WCHD3-30	12	8.E+06

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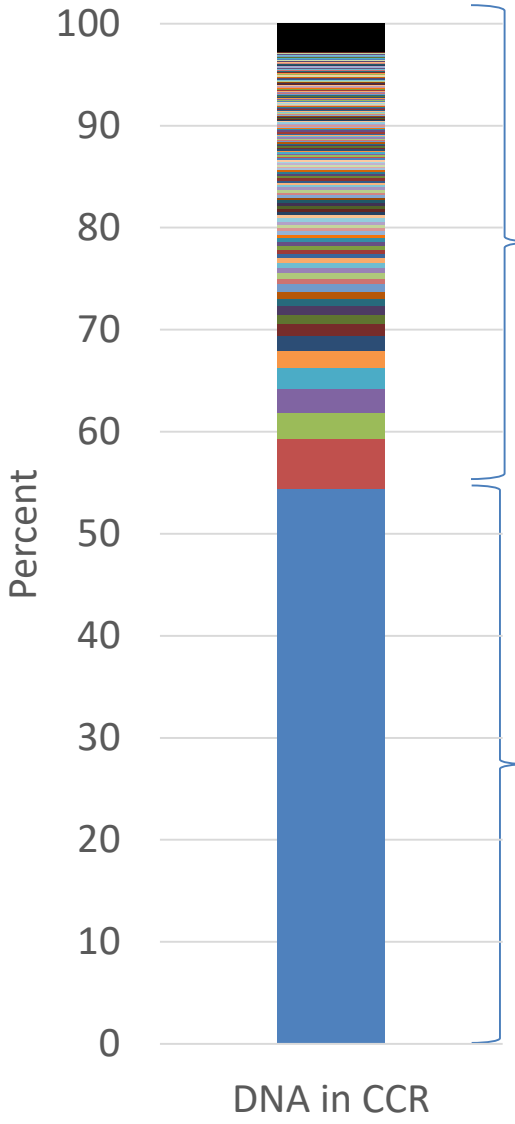
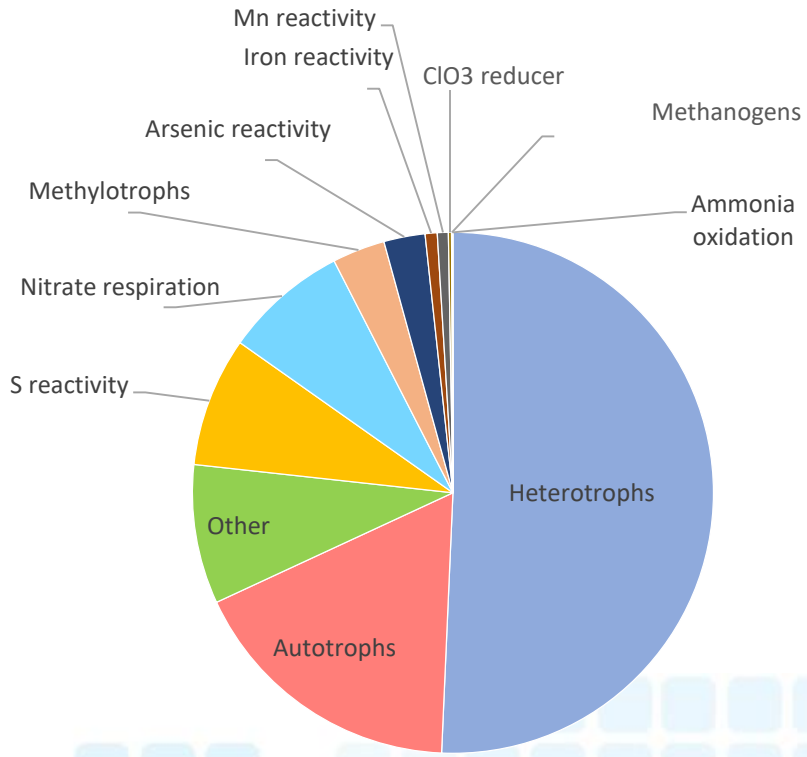
Example: Complex microbial communities offer valuable biogeochemical reactivity to support bio-stabilization



46% of DNA with species identified

54% of DNA with 500 unidentifiable species

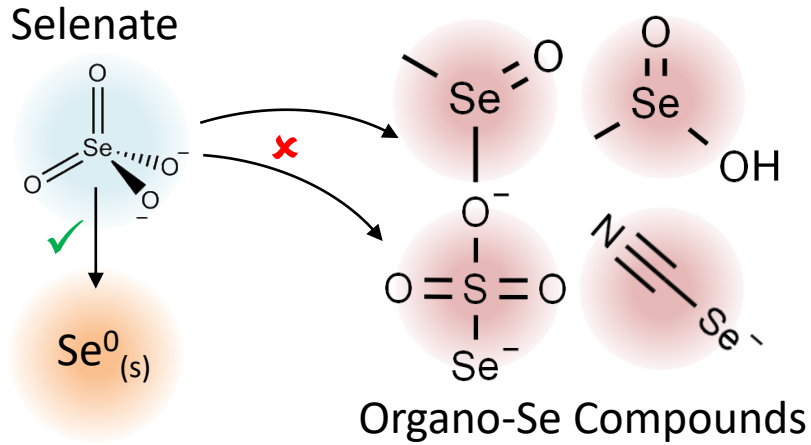
847 species
At least 60 species known to interact with metals, likely many more



The Selenium (Se) Conundrum

The 'Essential Toxin'

1. **Treatment Challenge** – Selenate's [Se(VI)] low affinity for adsorption necessitates reduction for removal; risks organo-Se generation
2. **Ecotoxicity Risks** – Se bioaccumulates in aquatic life; organo-Se generated can be 100,000x more bioavailable, increasing toxic effects
3. **Regulatory Driver** - Evolving strict regulations of [Se] < 1 µg/L remain near detection limits of state-of-the-art analytical methods



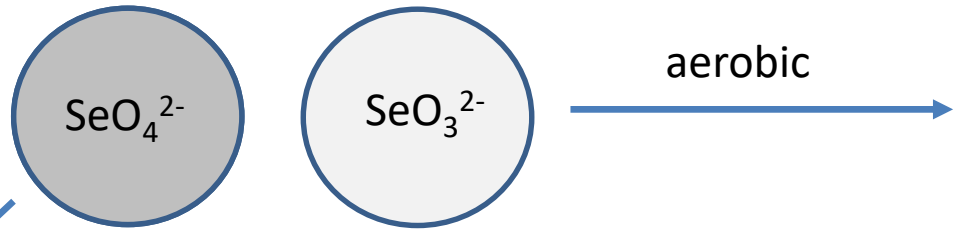
Steam Electric Power
Flue gas desulfurization wastewaters (FGDW) contains Se.

Agriculture Operations
Recycling of manure for fertilizer leads to high Se in farm land.

Mining Operations
Mountain-top removal (MTR) mining releases Se from subsurface.

Biological Selenium Reduction

A wide variety of microorganisms reduce selenium compounds under anaerobic and aerobic conditions

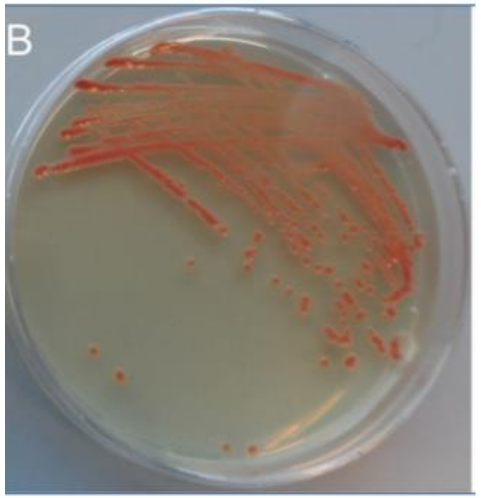


Specialized-Anaerobic

e.g., Bacillus selenitireducens

Non-Specialized Anaerobic
Denitrifiers/Sulfate Reducing bacteria

e.g., Thauera selenatis
Desulfovibrio desulfuricans



Comamonas testosterone
e.g., Pseudomonas stutzeri

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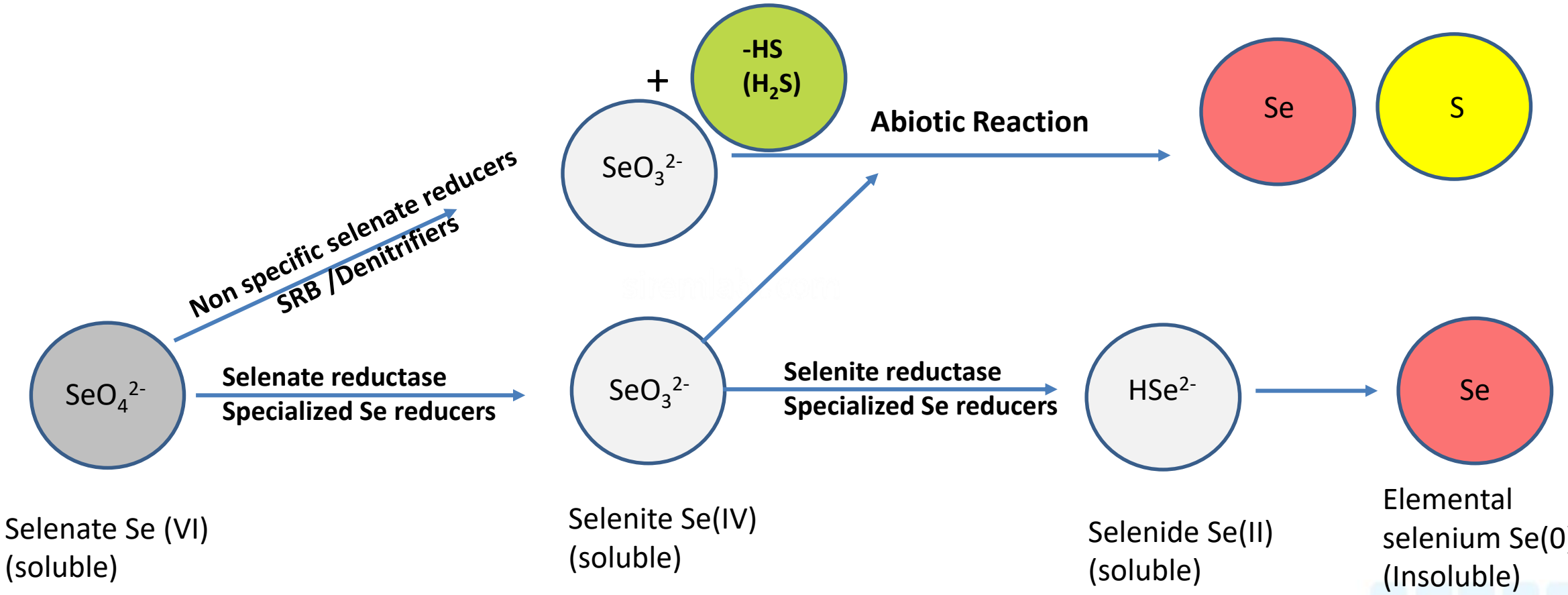
Selenium Immobilization Pathways



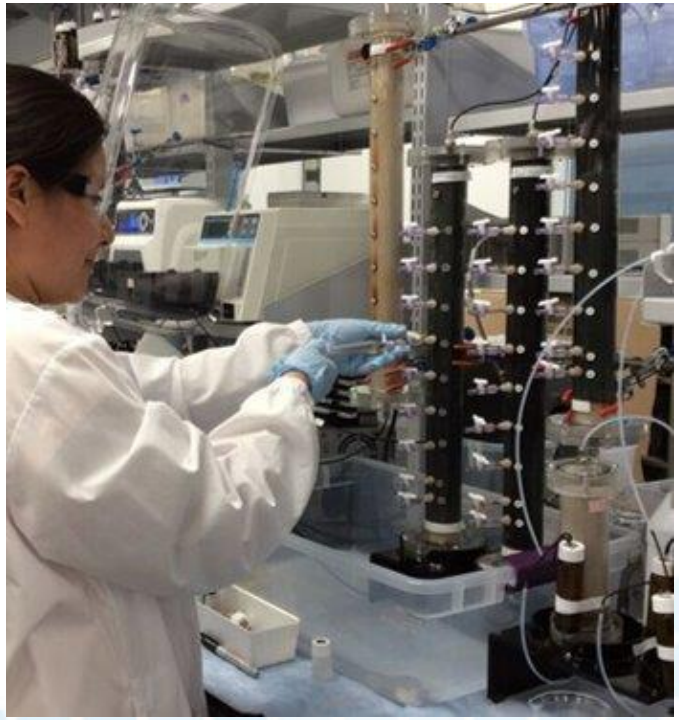
Molecular Tests for Selenium Targets

- **Gene-Trac Selenate Reductase:** qPCR Test for genes that convert selenate to selenite
- **Gene-Trac SRB:** qPCR test for sulphate reducing bacteria that non-specifically reduce selenium compounds and biogeochemical precipitation (via H_2S)
- **Gene-Trac Denitrification:** qPCR **t**ests for nitrate reducing microbes that non-specifically reduce selenium compounds
- **Gene-Trac NGS-** comprehensive microbial characterization that can detect all of the above groups

Treatability Testing

Evaluate technologies including:

- Anaerobic and aerobic bioremediation
- *In situ* chemical reduction (e.g., ZVI)
- *In situ* chemical oxidation (e.g., permanganate, persulfate)
- Physical/chemical/biological method for metals



Batch and Column Studies

- Evaluation of Commercial Amendments for metals treatment (Cr(VI), Cd, Co, Pb, Mn, Ni, Zn)
  - Some examples include EHC-M© (ZVI), SRS-M© (emulsified veg oil metal formula), Apatite II™ (from fish bones), GeoBind™ (ferric FeO, and Na, Al and Si oxides), and many more
- ZVI PRB for As Treatment
- In situ Attenuation for Zn and Metals Treatment
  - Reactive materials of 1) carbon substrates for SRB, 2) Zeolite as adsorptive sequestration

Flow Through Reactors

- Used to;
  - Evaluate operational and system upset parameters
  - Monitor Redox conditions
  - Test amendments
  - Permitting support

Mercury Background

- Mercury is a toxic metal –causes neurological, kidney, skin, digestive and immune system effects
- Mercury in sediment comes from both natural and anthropogenic sources.
- Mercury in the environment does not break down over time, it changes form
- Mercury used in production of caustic soda and chlorine in pulp and paper bleaching
- Mercury is typically emitted in inorganic form, but can be converted to methylmercury the most toxic form with health risks for fish, wildlife, and humans.

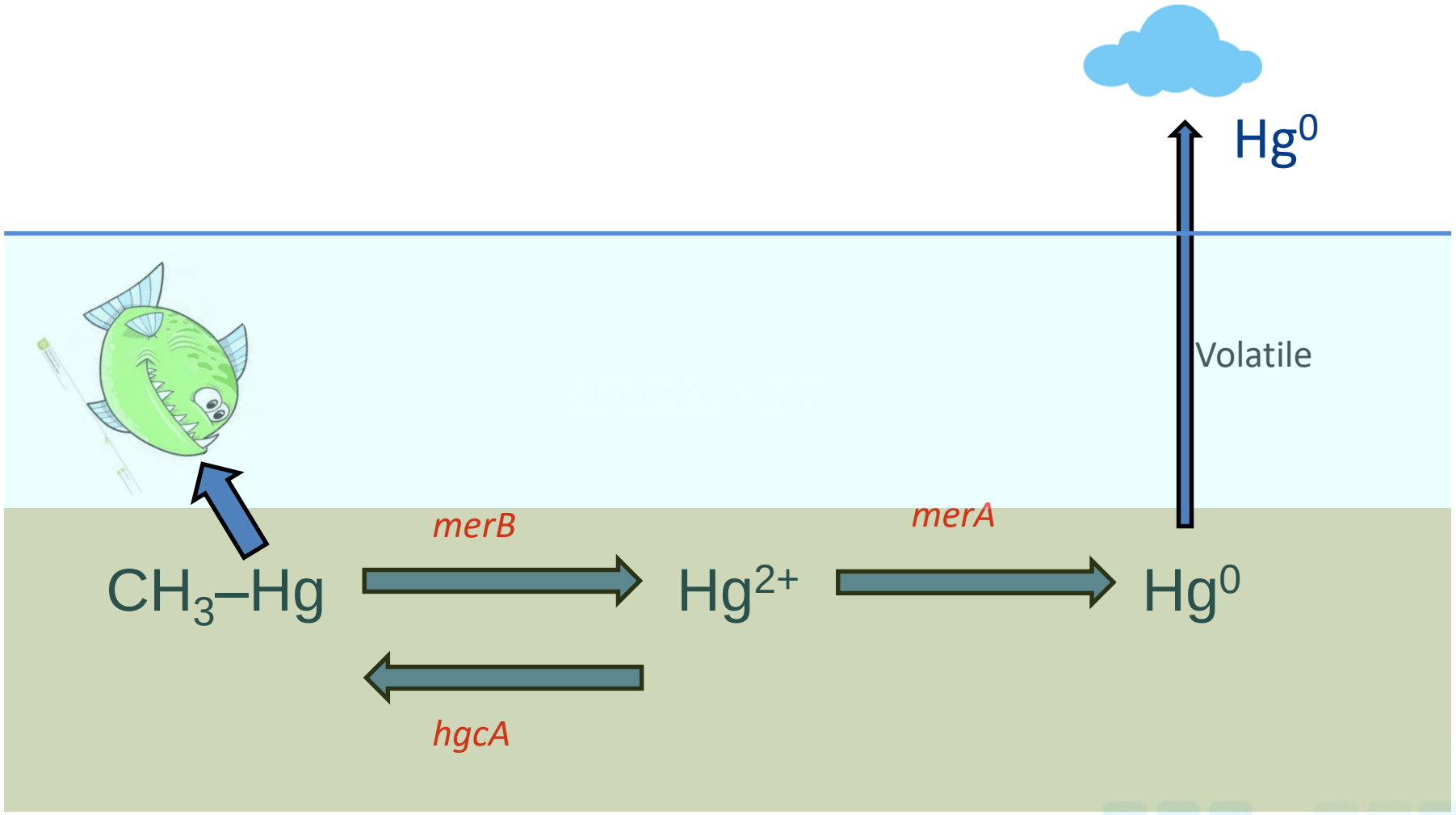
$$H_3C—Hg^+ \ X^-$$

Methylmercury

Molecular targets Relevant to Mercury Metabolism

Target	Molecular Target	Comment	Reference
Sulfate reducers	*dsrA*	Gene-Trac® SRB Test	Benoit et al. , 2003
Geobacter	16S rRNA gene	Gene-Trac® Geo	Lu et al., 2016
Mercury Methylation Corrinoid Protein	*hgcA*	High correlation with mercury methylation in diverse taxa including Firmicutes, Chloroflexi, and Methanomicrobia	Liu et al., 2014
Mercury(II) reductase	*merA*	Catalyzes the reduction of Hg^{2+} to Hg^{0}. converts toxic mercury ions into relatively inert (but volatile) elemental form.	Poulain et al., 2015
Organomercurial Lyase	*merB*	Cleaves the C-Hg bond produces Hg^{2+}	Lu et al., 2016 Liebert et al., 1997

Mercury Methylation/Demethylation



New Jersey Site Study Area

- Tidal estuary with multiple adjacent industries in NJ
- 22 River Miles, 1,500 acres of marshland
- PCBs, mercury from multiple industrial sources
- Conducted treatability and pilot studies for capping and amendment addition for risk reduction

qPCR testing in NJ Site Sediment

Sample	*merA* copies/g	*merb* copies/g
NJ Sediment-1	1E+08	5E+05
NJ Sediment-2	9E+07	9E+06
NJ Sediment-3	9E+07	2E+06

qPCR tests indicated that the Site sediment microbial community had the genes required for mercury demethylation (*merB*) and for reduction of Mercury II (*merA*)- suggest detoxification is possible

eDNA The Basics!

Traditional Bioassessment Surveys vs eDNA

Traditional Bioassessment Surveys :

- Include collection and identification of whole organisms (e.g., fish, benthic macroinvertebrates, benthic algae) are time consuming, expensive, potentially disruptive to the environment.

- eDNA requires just a water or sediment sample, required less specialized knowledge for identification and can supplement traditional bioassessment at lower cost

CCDB
Canadian Centre for DNA Barcoding

SiREM | siremlab.com

eDNA Process Overview

5) Species identification by database searches of cytochrome oxidase sequences

BARCODE OF LIFE DATA SYSTEM v4

4) Next generation sequencing of PCR amplicons

3) PCR amplify only the genes for taxonomic ID (e.g., cytochrome c oxidase for animals)

2 Extract Total DNA

1) Sample water or sediment etc.

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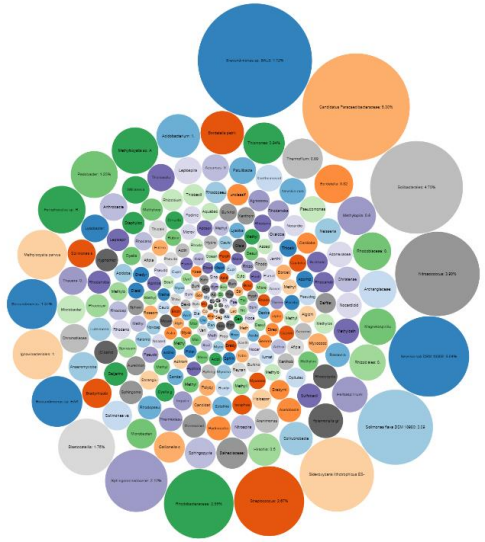
Conclusions

- Growing number of molecular tools for characterizing impacts and treatment of metals and metalloids
- These tools are increasingly being applied to characterize:
  - Individual microbes/functional genes (qPCR)
  - Whole microbial communities (NGS); and
  - Non microbial species (eDNA)
- These leading-edge tools will continue to evolve to be even more powerful



Questions?

jroberts@siremlab.com
519-515-0840

www.siremlab.com