Assessing the Potential for Bioremediation through Formation and Fate of Metal Rich Granules in the Terrestrial Environment

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Research Team

ERDC-EL
• Dr. Sandra Brasfield, Research Biologist
• Dr. Robert Jones, Research Biologist
• Dr. Jennifer Seiter, Research Geochemist
• Dr. Fiona Crocker, Microbiologist

OSU
• Dr. Roman Lanno, Soil Ecotoxicologist
• Mr. Brandon Little, Graduate student
EL Current Technical Program – Core, Cross-Cutting, and Emerging Areas

Core

- Climate Change
- Environ’tl Sustainability of Materials
- Environmental Security
- Risk & Decision Science
- Environmental Sensing
- Environmental Restoration
- Environmental Resources
- Contaminant Mgmt & Munitions Response

Cross-Cutting

- Environmental Modeling & Forecasting

Emerging

- Systems Biology
- Emerging Capabilities
- Competencies, & Leadership Opportunities

Recognized

- Leadership Opportunities
Sustainable Materials Research Team
Environmental Risk Assessment Branch

**Aquatic Toxicology**
- Long-term exposure to explosives
- Biomimetics of contaminant bioavailability
- Impact of climate change to aquatic invertebrates
- Risk of contaminated sediment

**Terrestrial**
- Endocrine Disruption in reptiles
- Trophic transfer and bioaccumulation of metals
- Impact of munitions such as DNAN, TNT, RDX on earthworms

**Nanomaterials**
- Fate and ecotoxicology of nanomaterials

5,000 sq ft:
- culture facility
- toxicology laboratory
- biochemistry and analytical laboratories
Impacts of Lead (Pb)

• Most common metal contaminant on US Army small arms ranges is lead (Pb) – more than 3000 active small arms firing ranges

• Health concerns include inhalation and ingestion

• Potential for bioaccumulation in humans (soft tissue and bones), plants, and animals
Impact of Pb on Environmental Systems

- Pb transport pathways:
  - Airborne particulate
  - Storm water runoff, surface waters
  - Groundwater

- Potential for extensive impact to both aquatic and terrestrial organisms from Pb contaminated Army ranges
Current Remediation Methods

- **Off-range Disposal**
  - Exceeding 5 mg/L of Pb results in need to dispose of in hazardous waste land fill up to 100 x more cost

- **Physical Separation**
  - Mechanical sifting

- **Soil Washing**
  - Sifting combined with acid wash to dissolve soil particles, need to dispose of acid

- **Stabilization/Solidification**
  - Addition of ingredients to coat lead, rendering Pb immobile

Options are costly and often create a secondary source of waste
Bioremediation through MRGs

- Earthworms have ability to form Metal Rich Granules (MRGs)

- Earthworms store metals in subcellular compartments binding to phosphate, sulfur, and metallothioneins, ultimately rendering metals toxicologically inactive

- Excellent animal model due to abundance in environment and ease in creating stable laboratory cultures
Overview: MRG Process

- Metals are ingested
- Sequestered in subcellular compartments, rendered toxicologically inactive
- Excreted as biologically unavailable Pb into soil
- Need for further characterize MRG for long term assessment of bioremediation potential
- Determine if Pb can be re-released by bacterial degradation
MRG Extraction Process

1. Homogenize tissue
2. 5000 x g, 5 min. 4°C
   - (supernatant)
     - Cytosol, organelles, proteins

3. Pellet
4. 65°C, IN NaOH, 1 hr
   - Filter, glass wool
   - (debris)
     - Exoskeleton

5. 5000 x g, 10 min. 4°C
   - (supernatent)
     - Cellular Debris

6. P2 Metal-Rich Granules (MRGs)

Result: Fractionated MRG

- R.P. Jones et al 2009, Subcellular compartmentalization of lead in the earthworm, Eisenia fetida: Relationship to survival and reproduction
Purpose: Investigate Potential for Bioremediation through MRGs

**Previous Pb Trophic Transfer**

*Background:*
- Previous exposure have shown Pb in MRGs to be biologically unavailable when ingested by predators of terrestrial invertebrates

**Experimental Tasks:**
- Generate MRGs through Pb soil exposures and fractionation
- Characterize MRG mineral structures through synchrotron analysis
- Conduct bacterial microcosm exposures to determine potential for bacterial re-release
**OBJECTIVES:**

By inducing invertebrate MRG formation, we will assess:

1) mineral ultrastructure involved in making Pb biologically unavailable

2) potential for bacteria to release Pb back into the environment in a biologically available form

**PAYOFF:**

- Increased information about Pb will reduce uncertainty factors in risk assessment and unnecessary site cleanup, management costs, and range down time
- Potential for new metal bioremediation methods using soil invertebrates to stabilize metals contaminates
Experimental Procedure: Soil Exposure

- Exposure media: Field collected soil hydrated and amended with 4,000 mg/kg Pb(NO₃)₂ (n=3)
- Total of 285 worms exposed (95 per rep) to spiked soil
- Samples collected at 4 weeks from individual containers, depurated, and frozen for analysis
Experimental Procedure: Fractionation

Homogenize tissue

65°C, 1N NaOH, 1 hr

Filter, glass wool

5,000 x g, 10 min. 4°C

Pellet

5000 x g, 5 min. 4°C

(supernatant)
Cytosol, organelles, proteins

(debris)
Exoskeleton

(supernatant)
Cellular Debris

P2 Metal-Rich Granules (MRGs)
Synchrotron Use In Environmental Sciences

- utilizes focused light produced by electron acceleration near speed of light to observe matter at molecular scale

**Technique/Description**

- **X-ray Fluorescence (XRF):** chemical composition, elemental distribution

- **X-ray Absorption Spectroscopy (XAS):** chemical speciation (ex: oxidation state, sorbed VS. mineral form)

- **X-ray Diffraction (XRD):** Crystalline phase identification through fingerprinting

**Experimental Example**

- Tungsten and Calcium in Snail Shell

- Mercury speciation in soil

- Identification of Selenium and Arsenic mineralogy
• X-ray fluorescence (XRF) spectroscopy, X-ray absorption spectroscopy (XAS) data was collected at the X-ray microprobe GeoSoilEnviroCARS beamline the Advanced Photon Source at Argonne National Laboratory (ANL).
• Our proposal scored an “excellent” rating at this facility.
Synchrotron Characterization/Analysis for MRGs

- **X-ray fluorescence (XRF) mapping**
  - Elemental distribution and metal associations
    - Determination of Pb distribution of MRG’s and soil

- **X-ray Absorption Structure (XAS) Spectroscopy**
  - Chemical information including: speciation, binding surfaces and mechanisms.
    - Identification of Pb species

- **X-ray Diffraction (XRD)**
  - Identification of environmentally relevant crystalline compounds
    - Determine crystalline Pb species in MRG’s
The MRG in these XRF maps is higher in a number of different elements, including: As, Ca, Fe, Zn, Ti, and Pb
Cross Section Analysis

Pb/As

Zn

Fe

Relative Elemental Quantities in Worm Cross Section

Where the following describes the appropriate emission lines:

* is the Ka
** is the Kβ
+ is the La
++ is the Lβ
MRG Microbial Exposures

MRGs located within earthworm fractions

Speciation data collected

Construct microcosm exposures to determine potential for bacterial degradation
Role of Microorganisms in Pb MRG Fate

Soil plus Pb MRGs

+/- bacterial exopolysaccharide

Water extraction

MWCO filtration

HNO₃ extraction

Total Pb

Dissolved Pb,

Soil pH

Nutrients

Acid, Siderophore, or Exopolysaccharide

Pb⁺² mobilization

Sorption to cell surfaces

Uptake

Excretion

Effect of Soil Microorganisms on Pb MRG Bioavailability

Incubate Microcosms will mimic potential environmental re-release

Microcosms will mimic potential environmental re-release

Soil exposure to worms
Path Forward

- Continue to gain extensive Pb characterization information in soils and terrestrial organisms to fill speciation data gaps.

- If MRGs are environmentally stable/biologically unavailable, determine potential for bioremediation through stabilizing metals in soils.

- If bacterial re-release occurs, investigate methods which could halt the degradation.
Bio-remediation Possibilities
Chance to think outside-of-the box

Invertebrates generate MRGs, rendering Pb unavailable to predators, how can we leverage this?

- Potential for combining MRG generation with a current technique, i.e. sequester remaining Pb on range within MRGs to render toxicologically unavailable
- Utilize MRG formation to decrease harmful effects of Pb in soils; potential to reduce need to dispose of material in hazardous waste landfills
- If successful, potential to gain inexpensive, green method of Pb remediation
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