## Sediment microbial community profiles along a metal-laden leachate gradient

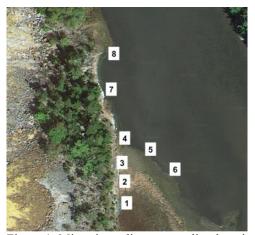
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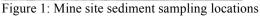
The Callahan Mine US EPA Superfund site in Brookfield Maine is a metal contaminated tidal estuary. We analyzed sediment microbial community structures along a heavy metal gradient formed by a slagheap leachate outflow. A steep cline in sediment community structure along the intertidal zone between the leachate outflow and pool was apparent.

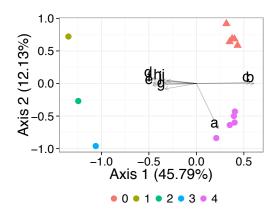
Heavy metal contamination of waterways and estuaries is persistent within the United States. Microbes are useful as a biochemical tool to aid in remediation of contaminated sediments<sup>1</sup> and as indicators of ecosystem health<sup>2</sup>. In this study we evaluate the differences in microbial community composition between the Callahan Mine site and a reference site (Horseshoe Cove) as remediation processes at the mine site are initiated.

Samples were collected in sterile 50-ml centrifuge tubes and thoroughly homogenized before sub-sampling for DNA isolation. Sampling locations included the mine site (n=8, Fig 1) and a near by reference site (n = 5). Microbial DNA from sediment samples was extracted and purified with the PowerSoil® (MO-BIO) isolation kit. Libraries of 16S amplicons were re-sequenced in a massively parallel manner to yield distinct operational taxonomic units from pyrosequencing reads. Statistical analyses were completed in R (version 3.0.3).

Microbial communities closest to the leachate outflow were significantly different from the reference site and mixing zone communities (Fig 2, permutation ANOVA, variance=0.85, F=9.11, p=0.01). As expected, all samples were dominated by species from Bacteroidetes (Mine 35.56±11.62%, Reference 23.52±6.33%) and Proteobacteria (Mine 43.71±9.75%, Reference 50.34±3.46%) phyla. Interestingly, all samples with the exception of sites 2 and 3 had relatively high abundance of sulfur- and sulfate-reducing bacteria (Deltaproteobacteria). The reference and mixing zone samples contained 15.95±4.85% and 13.98±2.10% respectively, and site 1 (closest to leachate) had 11.17% abundance of Deltaproteobacteria. Sites 2 and 3 yielded 0.96% and 1.24% each. Sulfur- and sulfate-reducing bacteria are important in bioremediation of heavy metal contaminated soils and were expected to be fairly abundant in sites with higher aqueous heavy metal burdens.







Mine Sediment ▲ Reference Sediment

Figure 2 (right): Principal coordinate analysis (PCOA) of mine and reference sediment microbial communities based on Bray-Curtis dissimilarity. Sites colored 1-3 represent near-leachate locations #1-3 in Fig 1; sites colored 4 represent mixing/further distance, sites #4-8 in Fig 1. Vector key: a, *Flavobacterium*; b, *Pseudomonas*; c, *Cytophaga*; d, *Geopsychrobacter*; e, *Sphingobacterium*; f, *Aquiflexum*; g, *Flexibacter*; h, *Flavisolibacter*; i, *Altererythrobacter*; j, *Polaromonas*.

- 1. **Mulligan CN, Yong RN, Gibbs BF**. Remediation technologies for metal-contaminated soils and groundwater: an evaluation. *Engineering Geology*. 60:193-207, 2001.
- 2. **van Bruggen AHC, Semenov AM**. In search of biological indicators for soil health and disease suppression. *Applied Soil Ecology*. 15:13-24, 2000.