Sediment Bacteria Mining for Endophyte Inoculation and Phytoremediation Beneficial Reuse

Presented by Clara Austin - AECOM

Contaminated Sediment
Beneficial Use Workshop
Washington, DC

March 26, 2024

Technical Approach

- Phytoremediation and enhanced rhizodegradation (plant-assisted bioremediation) have been demonstrated to be suitable for dredged sediment reclamation and reuse
- Inoculation of bacteria (endophytes) is an innovative technology to ***enhance phytoremediation***
- **Proof-of-Concept:** Contaminant-specific degrading bacteria residing in dredged sediment may be adapted for use as plant inoculants

Benefits:

- Low-cost and sustainable techniques
- Increased aesthetic appeal of CDFs along shorelines
- Increase of carbon storage (using nature or plants)
- Transformation and degradation of contaminants
- High community acceptance

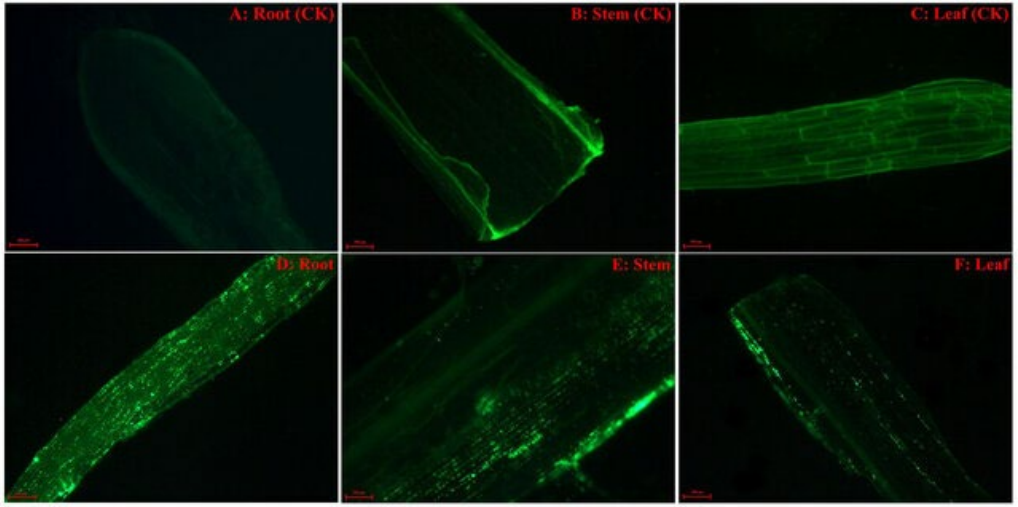
Army Corp's Calumet Harbor and River Confined Disposal Facility (CDF)

Endophytes

- Bacteria (*this study's focus*) or fungi that reside within the roots, stem and leaf tissue

Benefits:

- Reduce or remove contaminants
- Stress tolerance related to drought, temperature and salt
- Pathogen resistance
- Growth promotion



Visualization of inoculated endophyte, Pseudomonas sp. within plant tissues.

Sun, K., Liu, J., Gao, Y., Jin, L., Gu, Y., & Wang, W. (2014). Isolation, plant colonization potential and phenanthrene degradation performance of the endophytic bacterium Pseudomonas sp. Ph6-gfp1. Scientific Reports, 4(5462). https://doi.org/10.1038/srep054622

Research Study Objectives

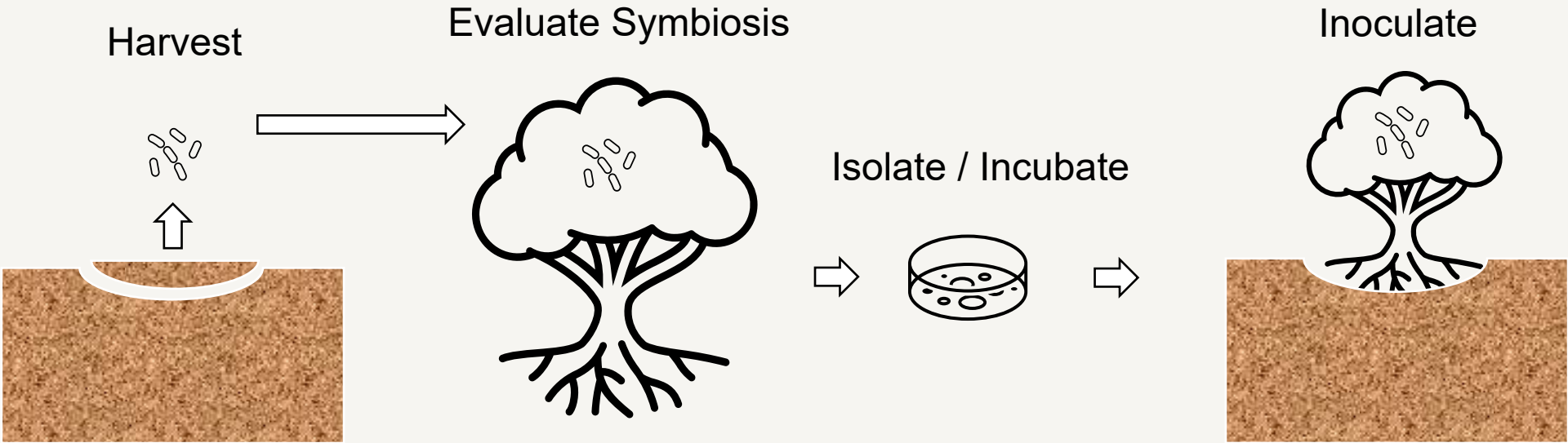
- Better understand the microbial population, biodiversity and microbiology of the dredged sediment,
- Evaluate if any beneficial bacteria are taken up from the sediment into the plant tissue, and
- Identify if any bacteria would be amendable for endophyte inoculation to enhance phytoremediation processes,
- Eventual reapplication of depleted sediments – *not within this scope.*

Notes:

- Broad Agency Announcement
- W912HZ-22-BAA-01
- Techniques for Contaminated Dredged Material Disposal and Treatment (EL-10)
- Chief Agency POC: David Moore

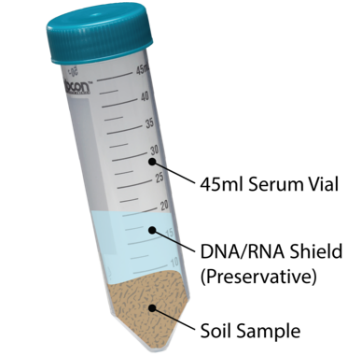
Long-range Sediment Re-Use Premise

1. Contaminant-specific degrading bacteria residing in dredged sediment may be adapted for use as plant inoculants
2. Remediation of sediments via bacteria-mediated phyto-transformation might be enhanced through bioaugmentation of plants using contaminant-degrading endophytes.



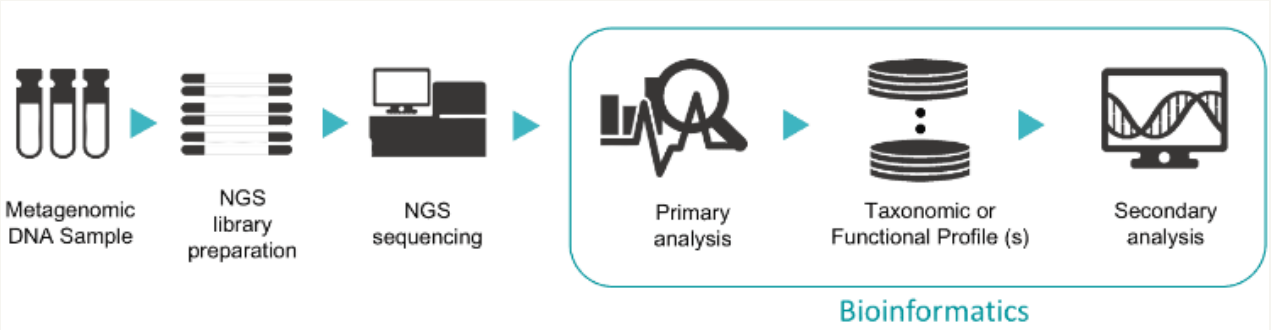
Next Generation Sequencing
Targeting 16s Ribosomal RNA Gene (Bacteria)

- NGS performed by Zymo Research, Irvine California
- Quick-16S™ Proprietary Primer Set V3-V4
- Use of chloroplast filter to remove plasmid noise
- Baseline data file 1.10 GB



No Ice Needed

NGS workflow

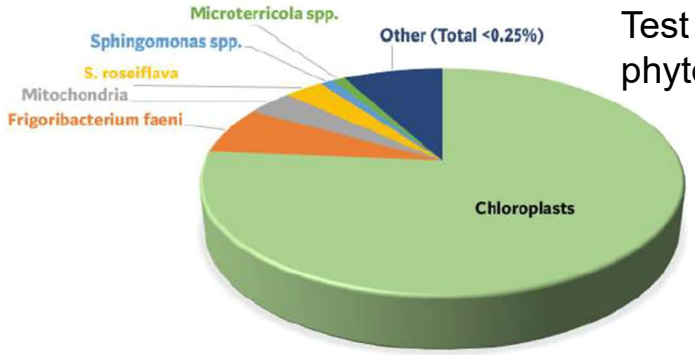


Lessons Learned – NGS of Plant Tissues

- Analysis of plant tissues bacteria using NGS targeting 16S rRNA
- Chloroplasts and mitochondria genetically similar to bacteria and cause interference and/or misinterpretations

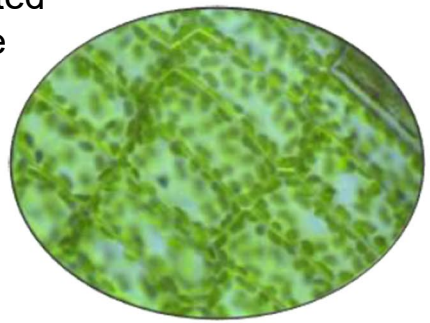
Remedy:

- Apply a "chloroplast and mitochondria filter" and primer modifications



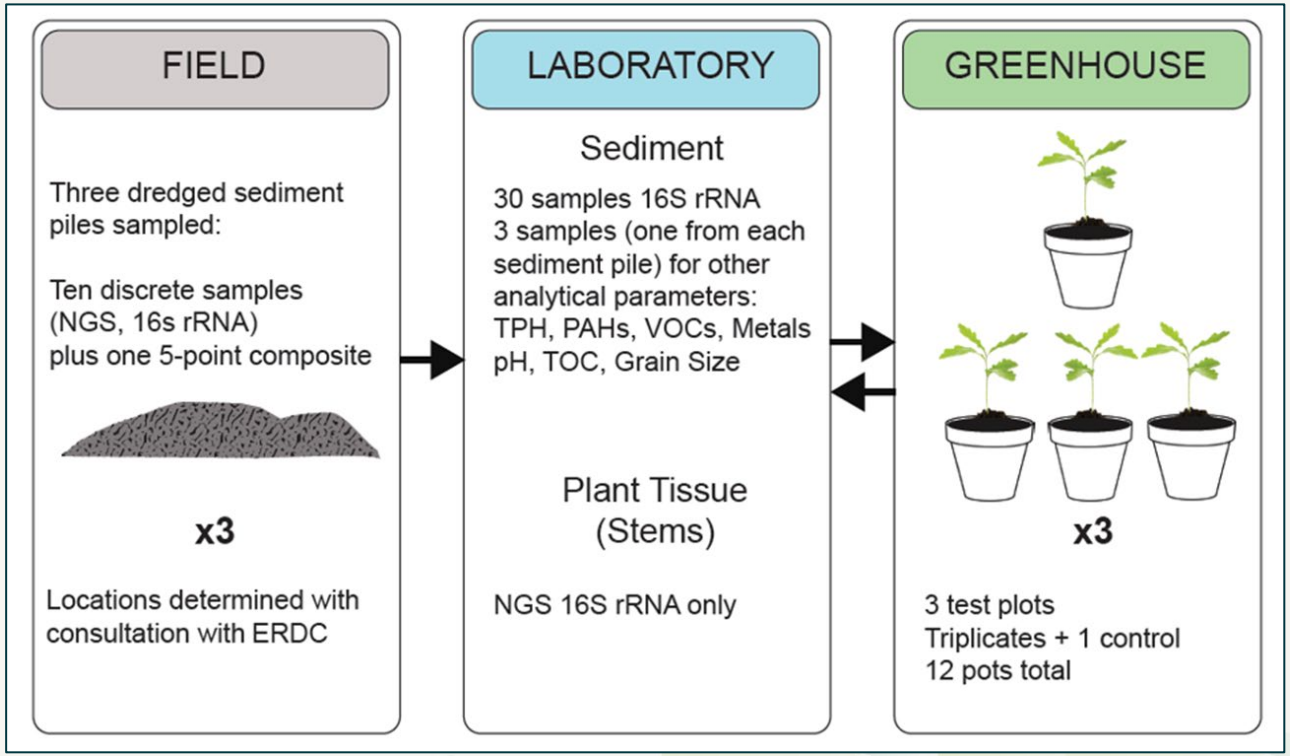
16S rRNA amplicon sequencing of leaf tissue derived from hybrid poplar trees, Bristol, PA Site. Showing predominance of chloroplast DNA.

Test results from non-related phytoremediation field site



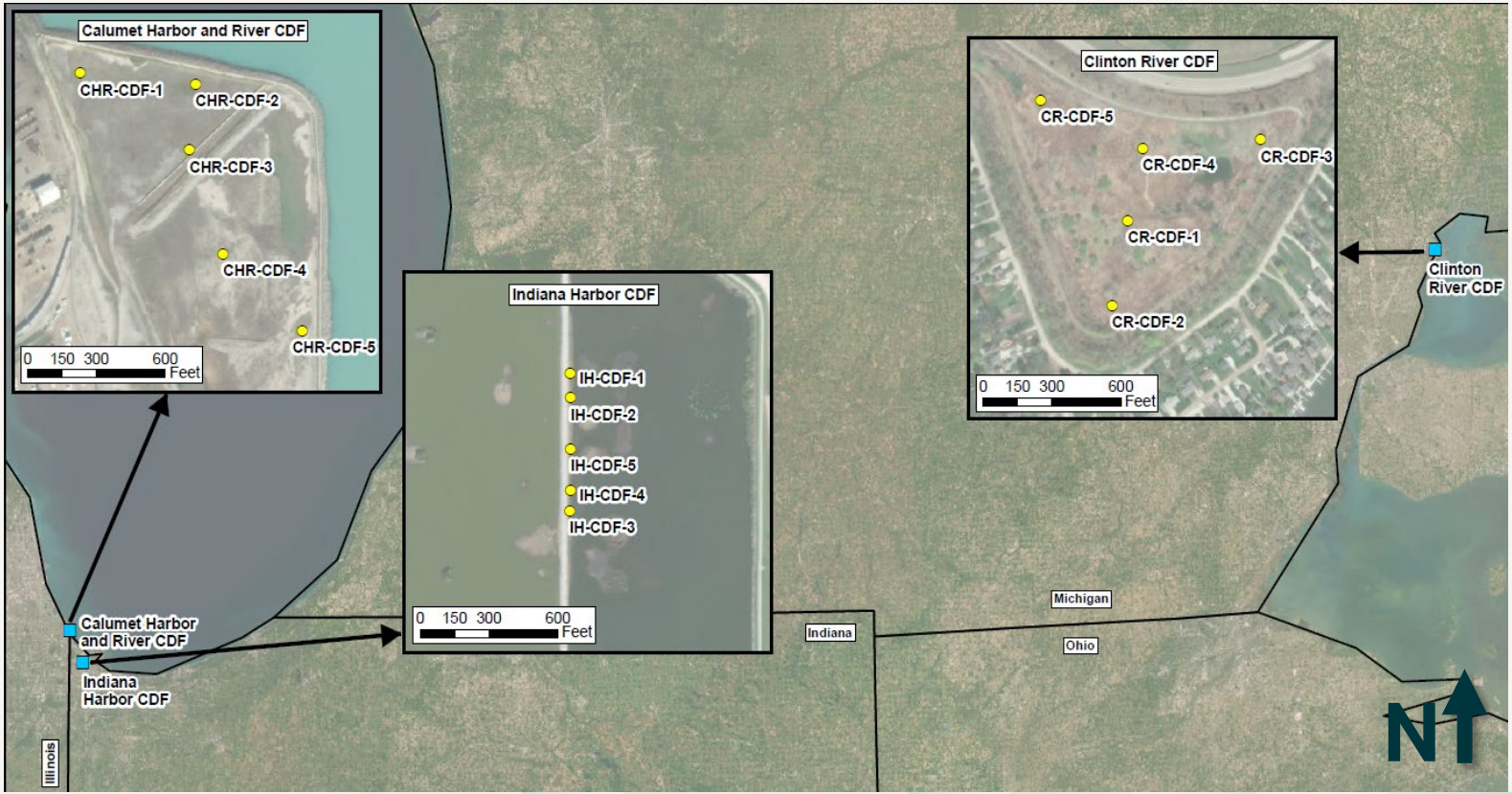
Chloroplasts in tree leaf. Chloroplast size approximately 5 µm. Source: https://en.wikipedia.org/wiki/Cytoplasmic_streaming

Tiered Study Approach



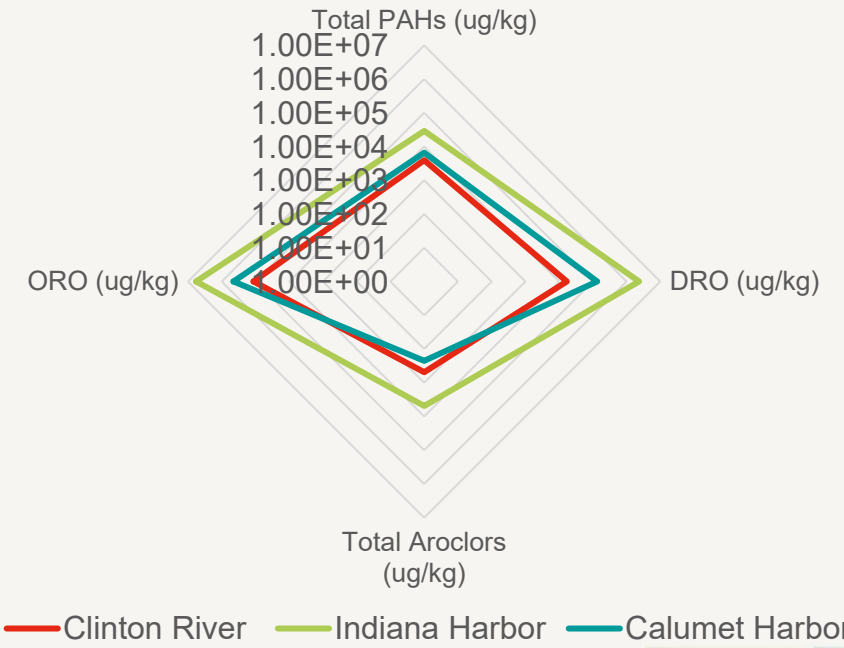
AECOM collecting dredged sediment from the Indiana Harbor Confined Disposal Facility

Confined Disposal Facilities in the Michigan Great Lakes

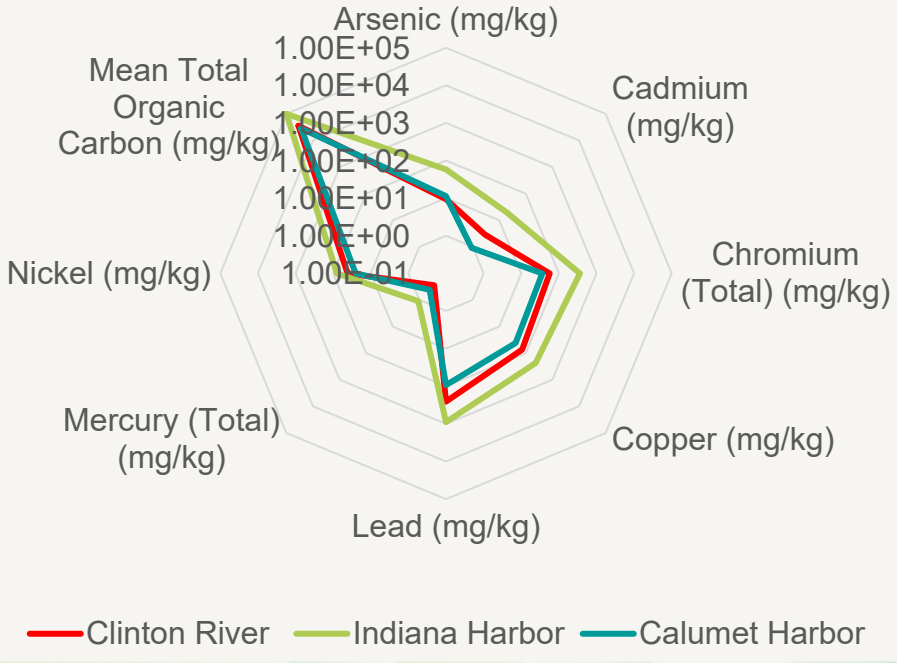


Baseline Sediment Results from the CDFs

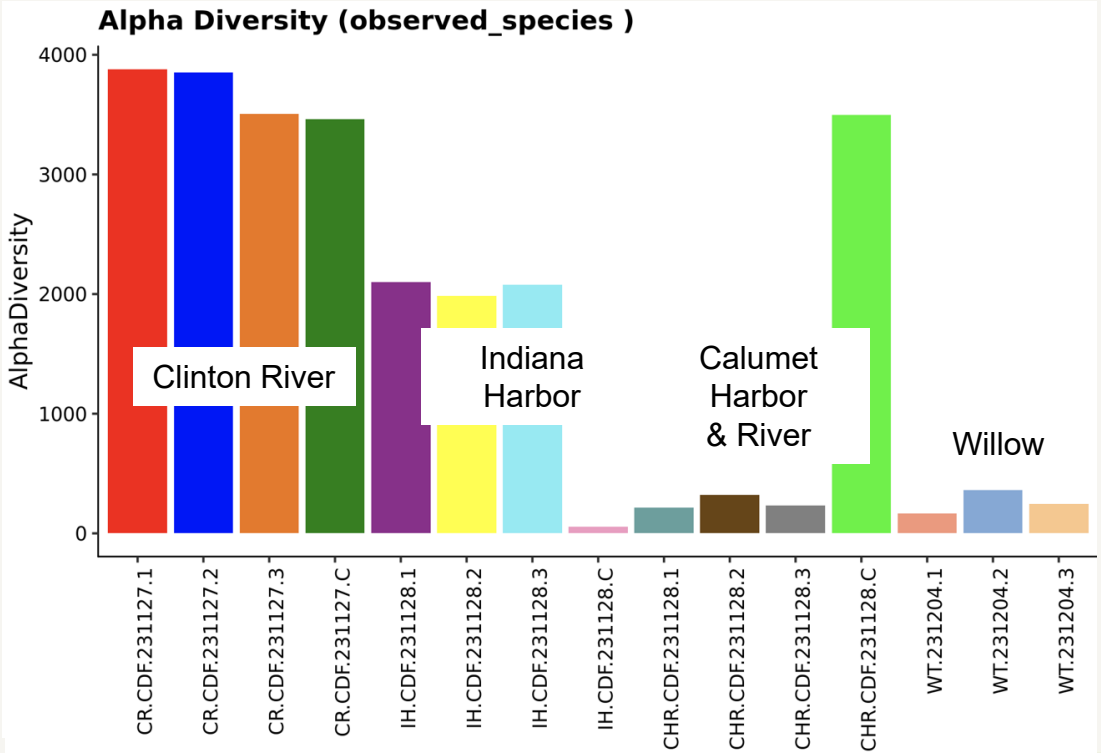
Comparison of Organic Contaminants



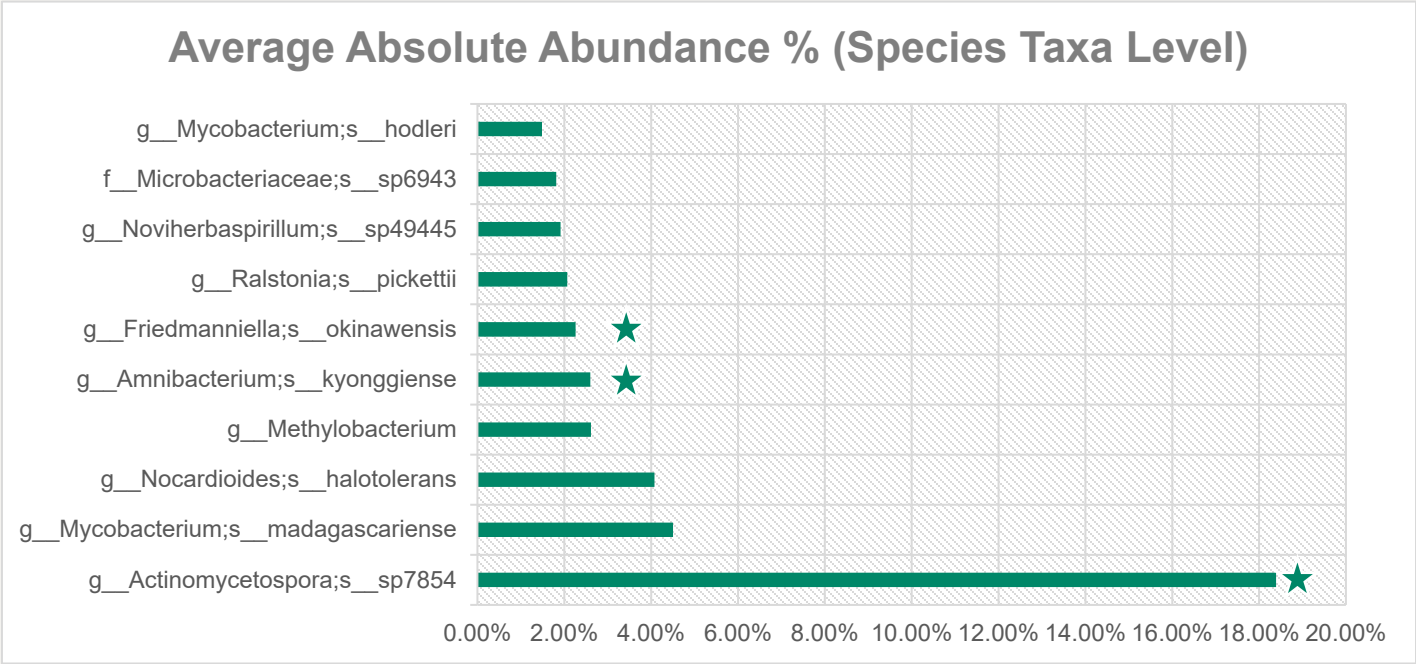
Comparison of Inorganic Contaminants



Observed Bacteria Species Counts

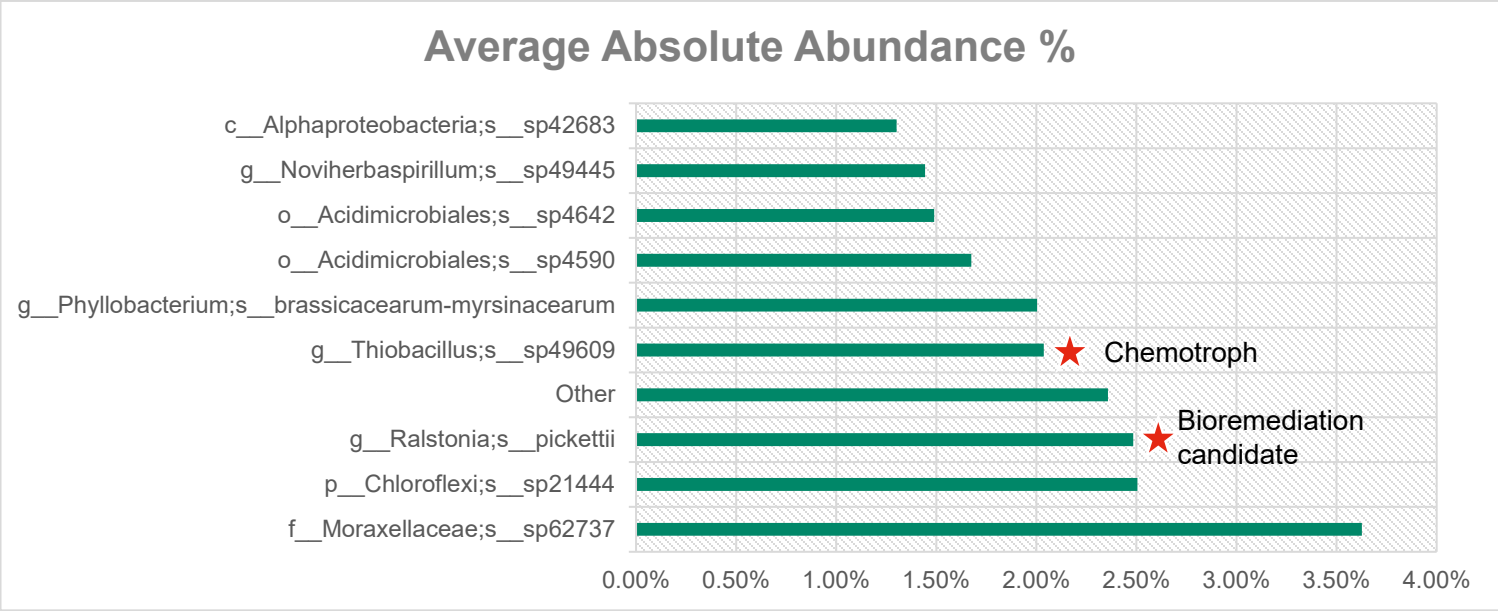


Baseline Sequencing of Bacteria in Willow Stems
(Showing top 10 identified taxa)



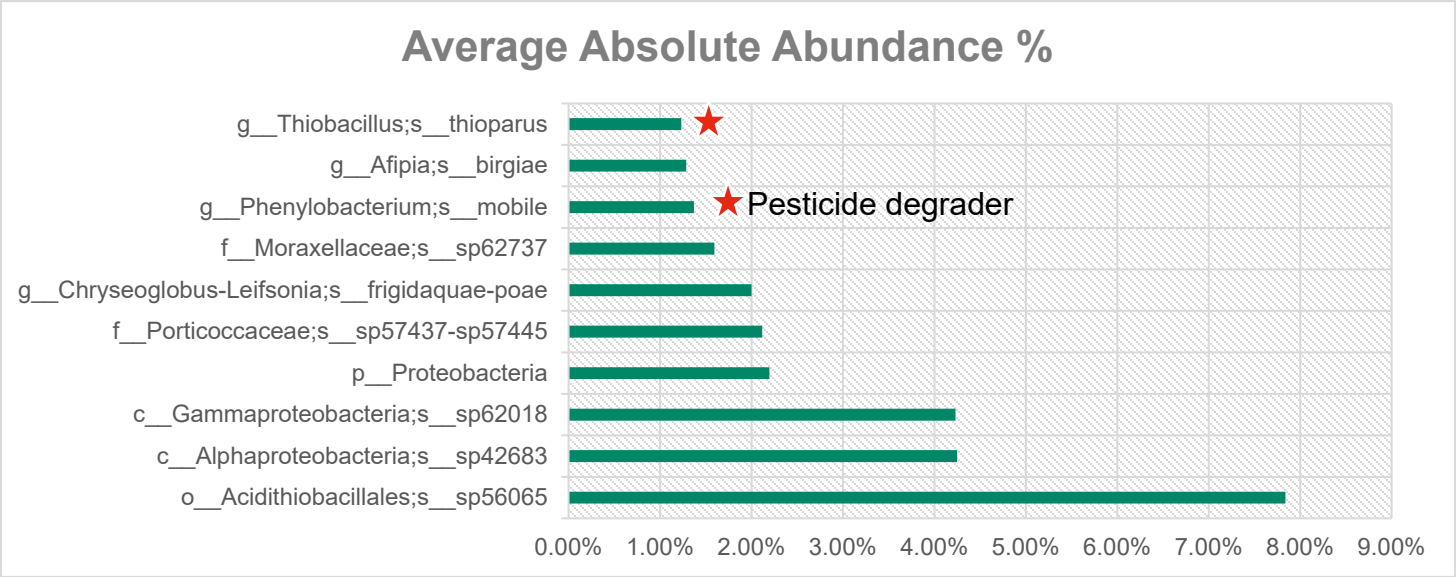
★ Endophyte

Baseline Sequencing of Bacteria in Sediments
(Showing top 10 identified taxa from Calumet Harbor & River)



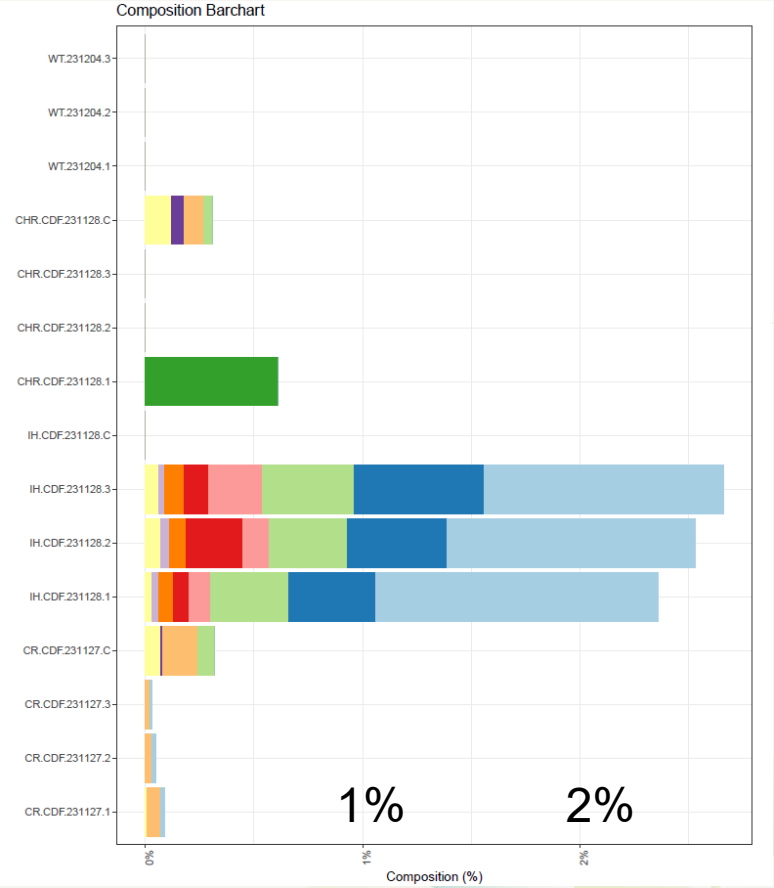
★ Specific substrate dweller

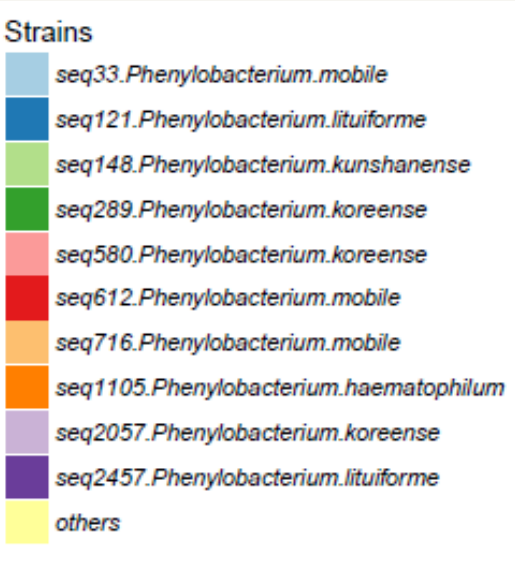
Baseline Sequencing of Bacteria in Sediments
(Showing top 10 identified taxa from Indiana Harbor)



★ Specific substrate dweller

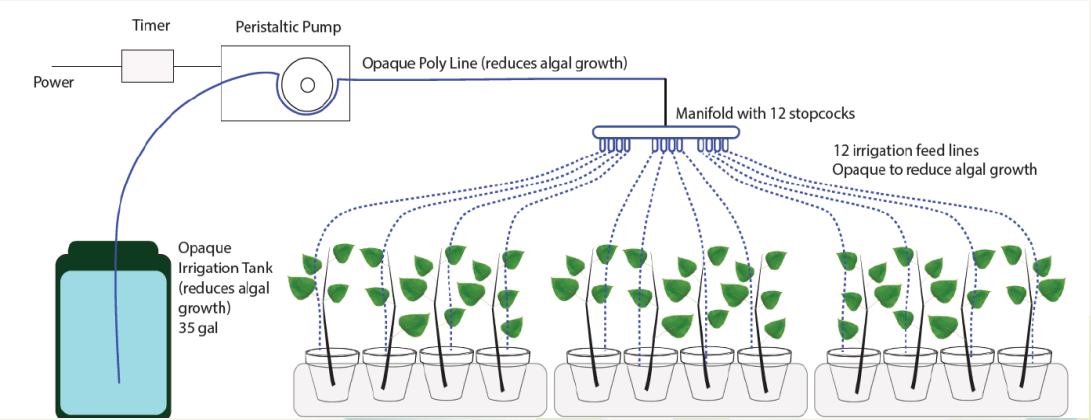
Phenylobacterium sp. at Indiana Harbor

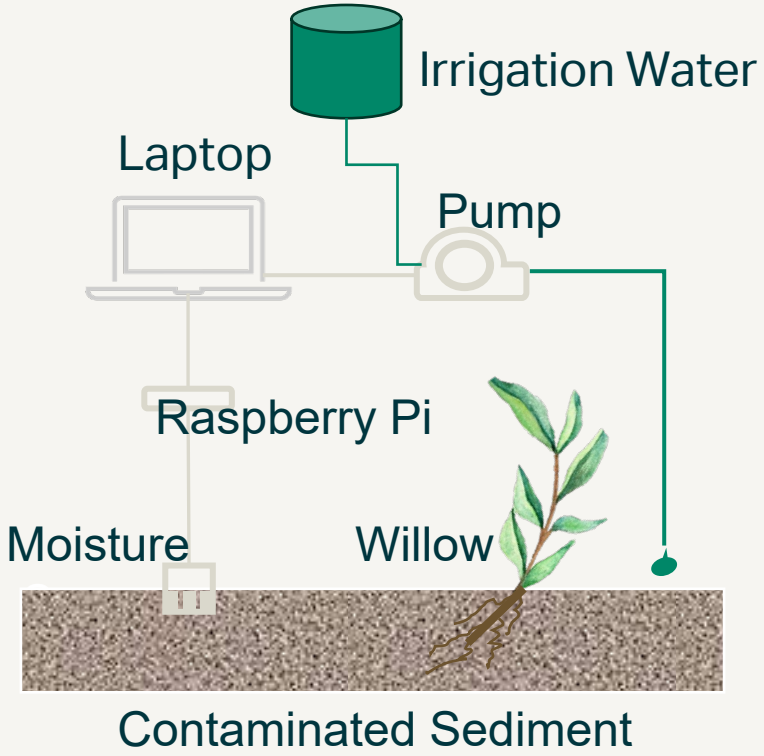




Automated Irrigation

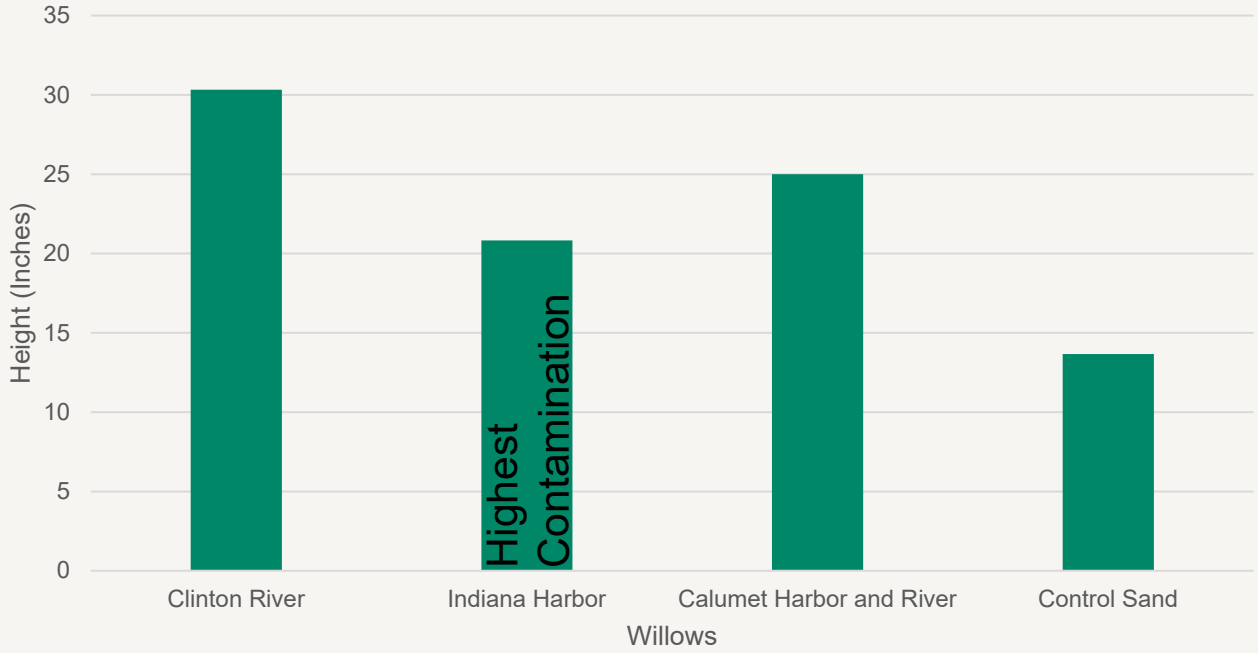
- Automated Irrigation using programmed code
- Moisture probe is a “capacitance touch pad” which senses interstitial pore-water in sediment.
- Using ChatGPT, AECOM prompted a code development that mimics software “Seesaw” which probe uses.
- Triggers for watering included a moisture value <30%



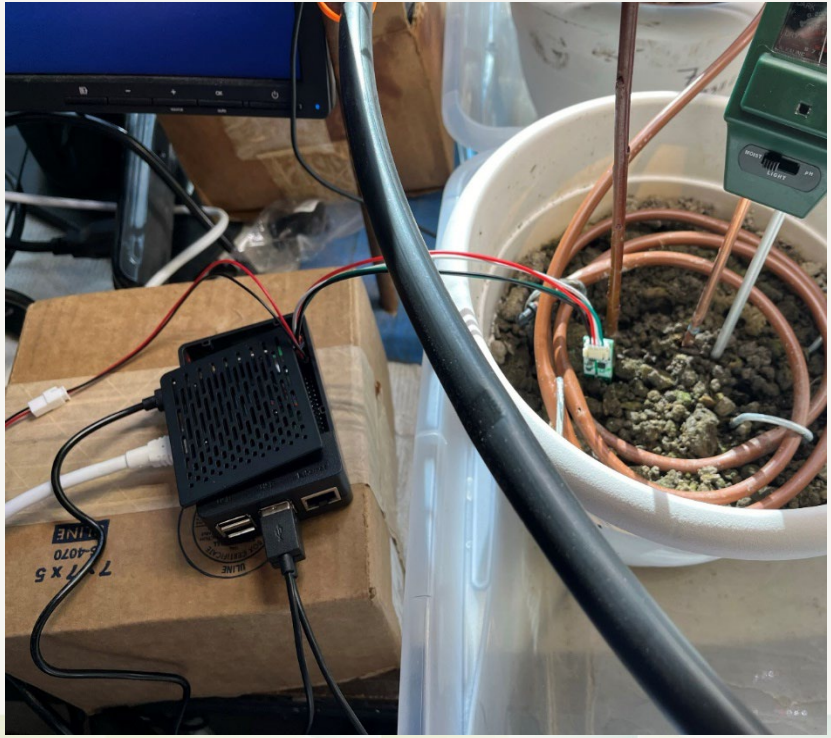


Willows Growth (3 months)

- Tallest tree is over 3 ft
- Will need to evaluate growth restrictions and root binding as the study continues
- Plant NGS planned for early Spring



Plant Growth Study



Next Steps

- Let potted trees grow through April 2024
- Collect root and tree tissue samples for analysis of bacteria using NGS
- Compare bacteria in sediment to those in plant tissues
- Use of AI to compare differences and commonality of bacteria
- Prepare report of findings, later 2024.

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