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Examining Variations in Microbial Communities and Establishing Biomarker Assays for Stress Induced Physiological Changes in Freshwater Snails in Arsenic Contaminated Ecosystems

Tacoma

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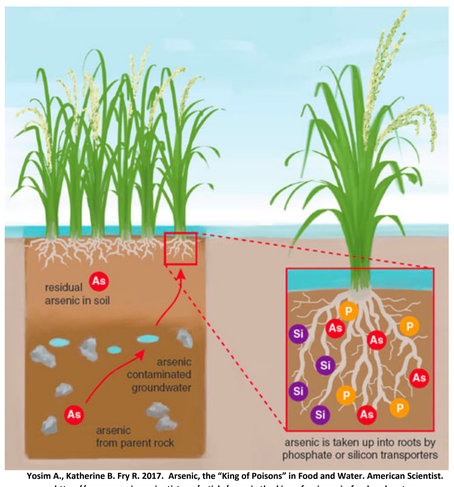
Introduction

It has been 38 years since the Asarco Company closed down it's copper smelter at the Ruston site in Tacoma, WA. Yet evidence of its operation still remains. As a result of the air pollution caused by the smelter, there are traces of heavy metals such as arsenic and lead found in the soil and lakes around the Puget Sound area. Our lab aims to observe variations in taxonomic diversity and abundance of bacteria in relation to variations of arsenic concentrations when comparing ecological compartments (sediment, water, plants, and Chinese Mystery Snails (CMS) visceral mass) of samples collected from lakes in Puget Sound: Pine Lake (little to no [As]: mean [As] in sediment is 6.09 ug/g dry weight), Steel Lake (moderate [As]: mean [As] in sediment is 51.1 ug/g dry weight), and Lake Killarney (high [As]: mean [As] in sediment is 153.4 ug/g dry weight). CMS, an invasive species of the Pacific Northwest, that resides in the contaminated lakes were used as a model organism to study their sensitivity to the condition of their new contaminated habitat.

Hypothesis I: We predict that we will observe variations in taxonomic diversity and abundance of bacteria when comparing ecological compartments (sediment, water, plants, and CMS snail visceral mass) between lakes.
Hypothesis II: We predict that we will observe variations in measured physiological stress on CMS between lakes utilizing Heat Shock Protein (HSP70) as a Biomarker.



Yosim A., Katherine B. Fry R. 2017. Arsenic, the "King of Poisons" in Food and Water. American Scientist. https://www.americanscientist.org/article/arsenic-the-king-of-poisons-in-food-and-water

Fig. 3 Illustration of contamination of Arsenic [As] entering into the ecosystem. Arsenic moving from rock/sediment, into the water, and eventually entering roots of plants. Primary consumers of these ecosystems eat these plants along with microbial biofilms (periphyton).

Methods/Results

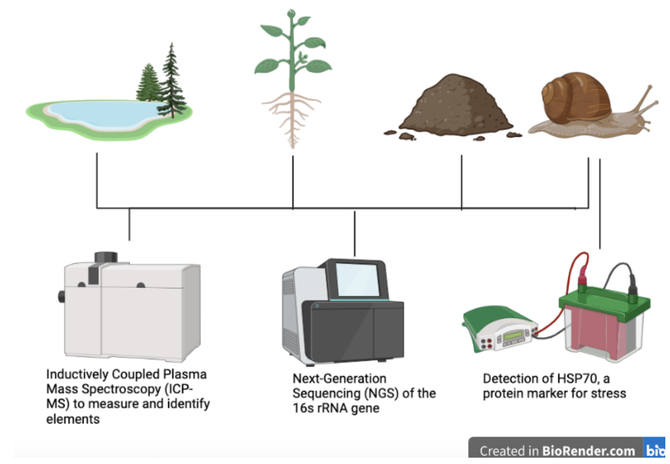


Fig. 4 Samples of water, CMS, sediment, and plant collected from Pine Lake, Steel Lake, and Lake Killarney were subject to ICP-MS and NGS, while CMS samples were subjected to immunoblotting of HSP70.

Arsenic Content of Collected Materials

Lake	Sample Description	As[ug/g] Dry Weight Average
Pine	Visceral Mass	4.15
	Plant	3.70
	Sediment	6.09
Steel	Visceral Mass	4.34
	Plant	6.18
	Sediment	51.1
Killarney	Visceral Mass	10.9
	Plant	92.4
	Sediment	153.4

Fig. 5 Arsenic concentration [ug/g] dry weight per mass for the visceral mass of CMS, plants, and sediment. Samples were collected from Pine, Killarney, and Steel Lakes on Sept. 2022 and underwent ICP-MS.

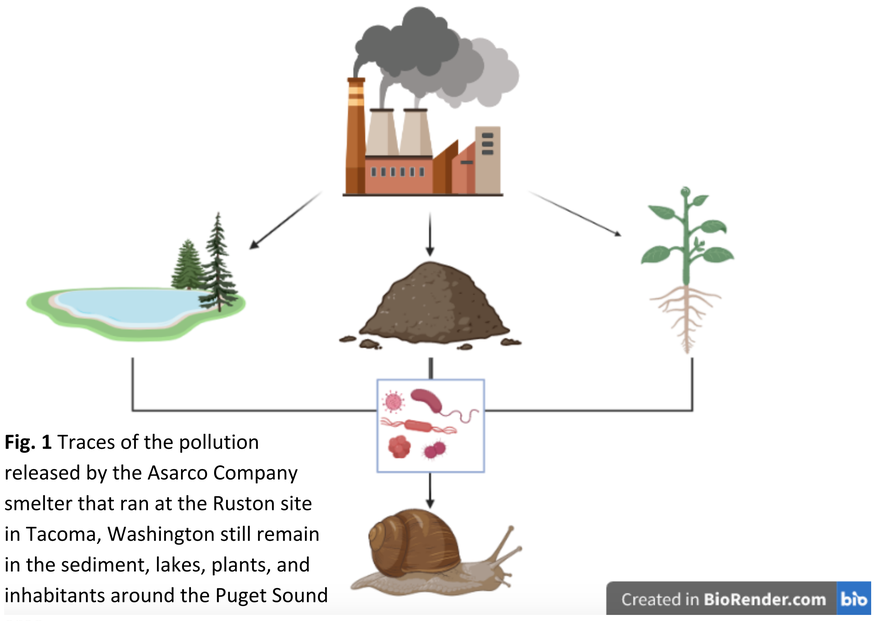
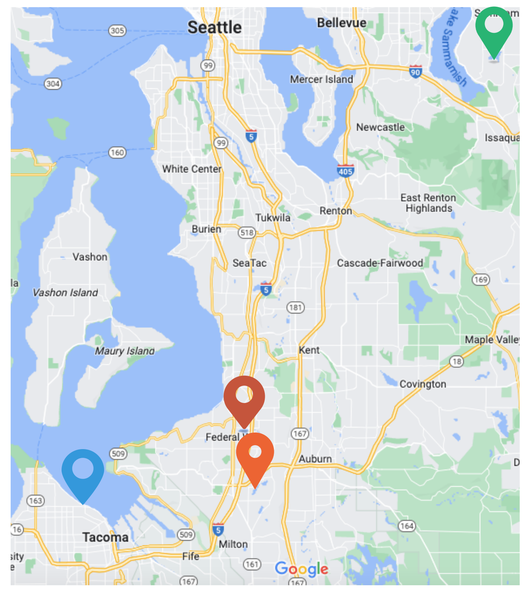


Fig. 1 Traces of the pollution released by the Asarco Company smelter that ran at the Ruston site in Tacoma, Washington still remain in the sediment, lakes, plants, and inhabitants around the Puget Sound area.



Pine Lake

Lake Killarney

Steel Lake

Asarco Copper Smelter

Fig. 2 Map of the relevant sites in this study. Green indicates Pine Lake (little to no [As]). Red indicates Steel Lake (moderate [As]). Orange indicates Lake Killarney (high [As]). Blue indicates the Asarco Copper Smelter at the Ruston Site where arsenic contamination began.

Taxonomic Composition of Microbial Communities Varies Between Lakes and Compartments

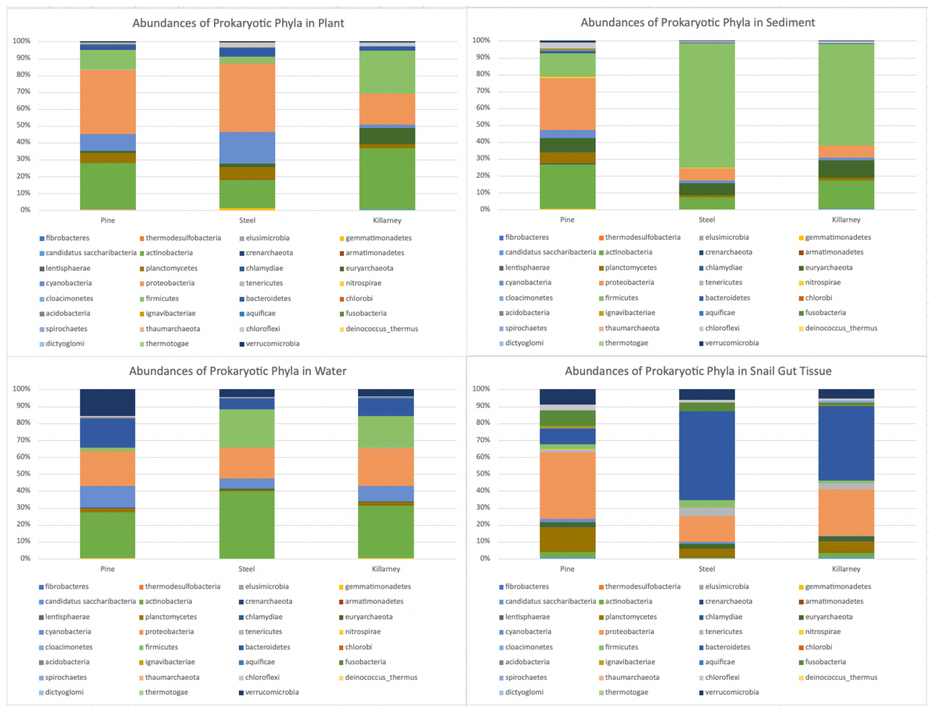


Fig. 6 Plant, Water, Sediment and CMS Gut samples were field collected from Pine Lake, Steel Lake, and Lake Killarney on Sept. 2022. (from left to right) Pine denoted as Pine Lake, Steel denoted as Steel Lake, and Killarney denoted as Lake Killarney. All samples were subjected to 16srRNA Next Generation Sequencing to determine Phylum Percentages present in each sample set collection from each lake.

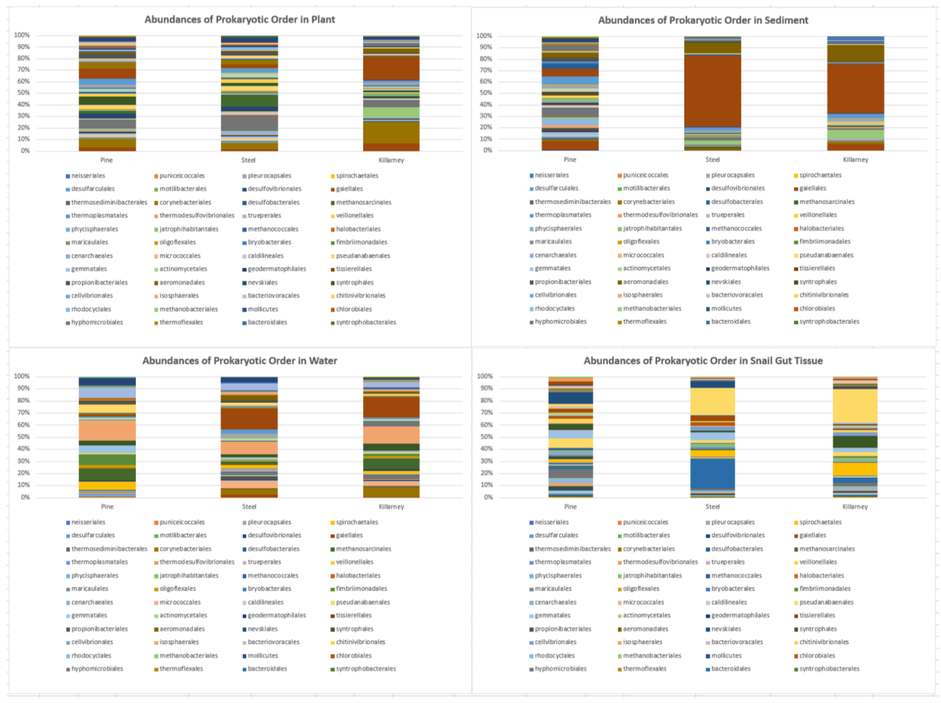


Fig. 7 Plant, Water, Sediment and CMS Gut samples were field collected from Pine Lake, Steel Lake, and Lake Killarney on Sept. 2022. (from left to right) Pine denoted as Pine Lake, Steel denoted as Steel Lake, and Killarney denoted as Lake Killarney. All samples were subjected to 16srRNA Next Generation Sequencing to determine Order Percentages present in each sample set collection from each lake.

Testing Potential Biomarkers of As Induced Physiological Stress

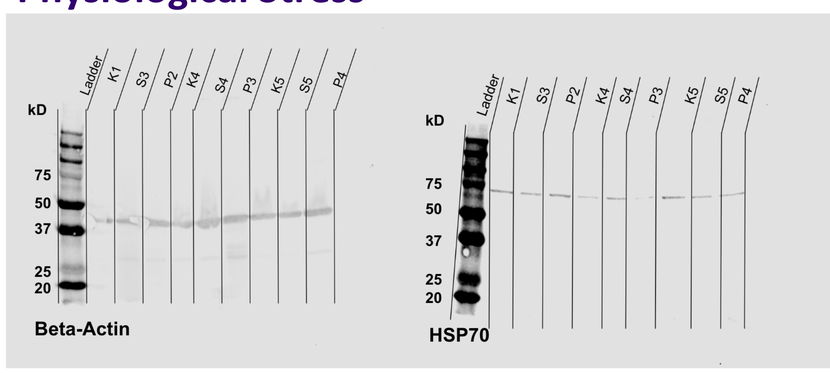


Fig. 8 Chinese Mystery Snails (CMS) gut samples field collected from Pine Lake, Steel Lake, and Lake Killarney were assessed for physiological stress via biomarker Heat Shock Protein (HSP70). Samples were prepped and ran through Western Blot, and later probed with Beta-Actin (House Keeping Gene and Loading Control) and HSP70. Samples labeled from left to right (K: Killarney, S: Steel, P: Pine), each had 3 replicates to correctly assess physiologically induced stress. Results indicate low expression of HSP70 from Lake Killarney Samples, which concludes HSP70 is not a good indicator of physiological stress

Conclusions

- ICPMS results illustrate high arsenic concentrations in Lake Killarney throughout all samples, compared to Steel Lake and Pine Lake
- The abundance of Phyla and Order Of Microbial Communities across samples show similarities of composition between Lake Killarney and Steel Lake rather than Pine Lake
- HSP70 is not a good indicator of stress resulting from chronic As exposure in CMS

Future Directions

- Determining a more efficient biomarker to assess physiological stress induced on CMS
- Assessing hemocyte(s) as a potential biomarker to measure correlated impacts of As concentrations in cell morphology
- Perform a Principal Component Analysis on the taxonomic data to reduce the dimensionality of the large data set
- Establishing a Gnotobiotic Snail Model to induce various concentrations of arsenic to determine physiological stress and microbiome alteration

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References