Arsenic Toxicity in Lake Ecosystems: Periphyton Biotransformation and Chinese Mystery Snail Bioaccumulation

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Introduction

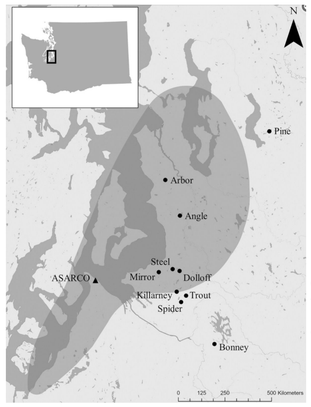


Figure 1. Map of polluted regions from the ASARCO plume adapted from Hull et al.

- Trout Lake
  - Sediment: 7 µg As g^{-1}
  - Periphyton: 25±3.2 µg As g^{-1}
- Steel Lake
  - Sediment: 50±26.5 µg As g^{-1}
  - Periphyton: 128±6.7 µg As g^{-1}
- Lake Killarney
  - Sediment: 213±84 µg As g^{-1}
  - Periphyton: 627±92 µg As g^{-1}

Impacts of arsenic on these lake ecosystems are currently unknown. The Chinese Mystery Snail (CMS) is a ubiquitous lake species that is utilized to research possible impacts.

Objective: Identify: (1) arsenic bioaccumulation in different CMS tissue types, including gut, mantle, head and foot, and other 'remaining viscera', and (2) arsenic biotransformation genes in periphyton

Hypothesis: Bioaccumulation of arsenic will be higher in gut tissue, and biotransformation genes will be present in periphyton due to its high accumulation of arsenic.

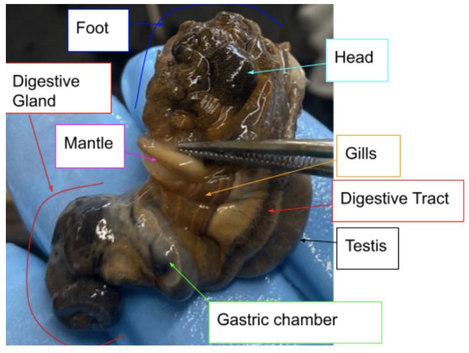


Image of intact CMS anatomy, taken by Renee Warren and Jules Milovich.

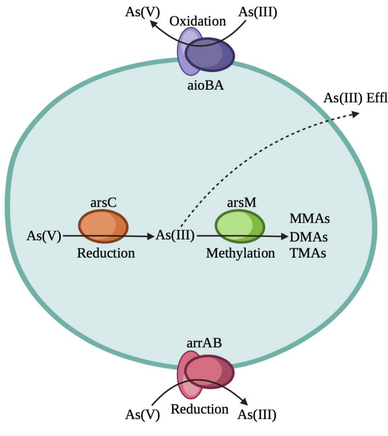
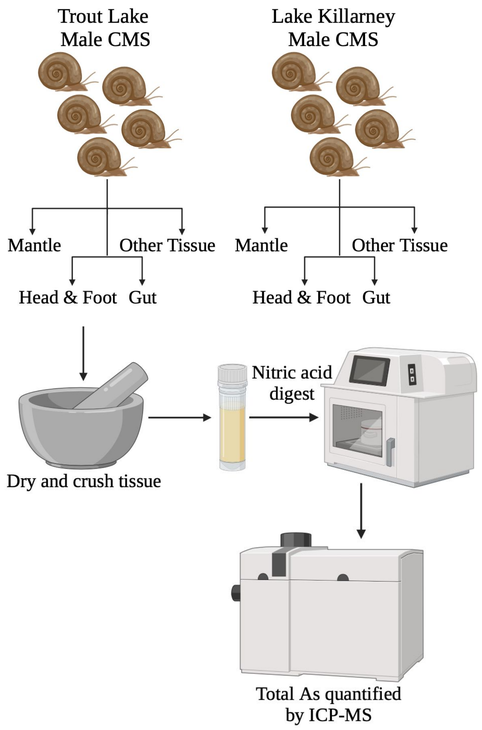


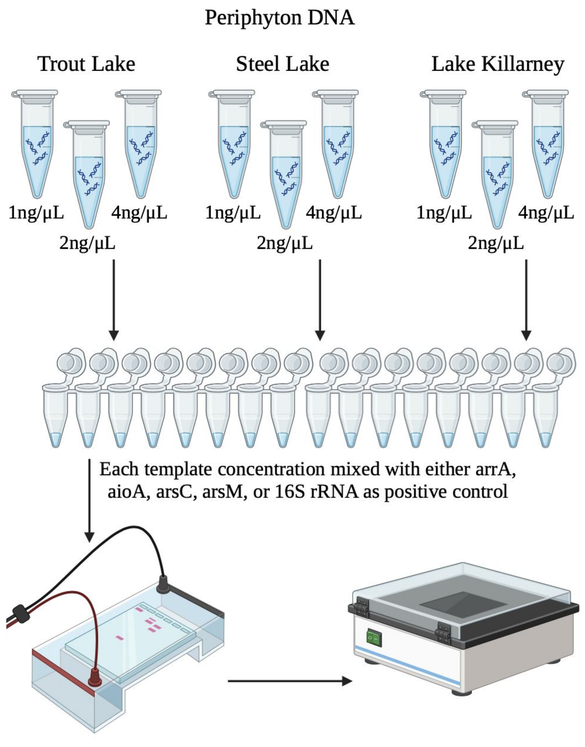
Figure 2. Arsenic biotransformation genes studied in this research and their pathways.[2 3 4]

- Concentration of arsenic in periphyton is about triple the amount as found in sediment across each lake.
- Periphyton consists of microbial life that may contribute to such an elevated concentration through. biotransformation and detoxification.
- A few genes that encode for this are aioBA, arsC, arsM, and arrAB.

Bioaccumulation Analysis



Biotransformation Analysis



Arsenic Bioaccumulates in Snail Gut

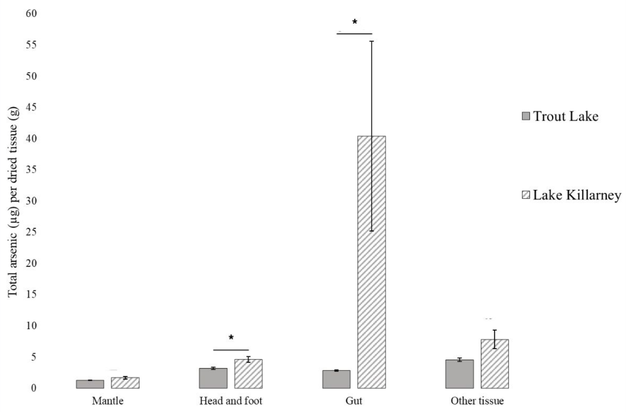


Figure 3. Mean total arsenic concentration of Chinese Mystery Snail tissues compared between lakes. Ten male adult Chinese Mystery snails were collected in total from two lakes: Trout Lake (low arsenic: < 1 ppb Arsenic concentration; n = 5) and Lake Killarney (~ 20 ppb Arsenic concentration; n = 5). Their tissues were dissected into 4 regions: mantle, head and foot, gut, and the remaining "other tissue". Their tissue samples were dehydrated, digested with nitric acid, and then underwent ICP-MS to quantify the total arsenic (all species) per dry mass of each sample. This graph displays mean total arsenic concentration, standard error bars, and p-values of one-tailed Welch's t-tests for the comparison of total arsenic concentration between Trout Lake and Lake Killarney for each tissue type.

Arsenic Methyltransferase Gene is Detected in Periphyton

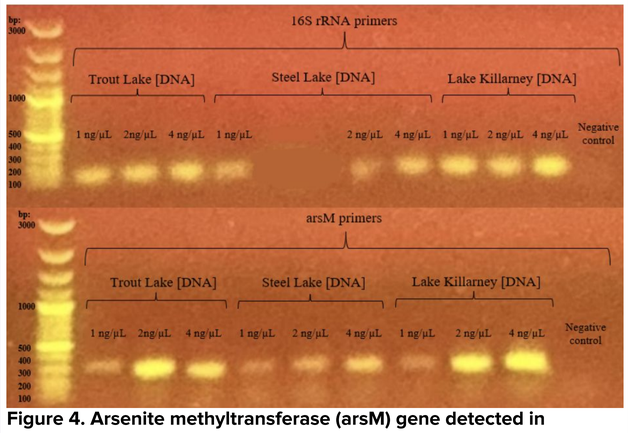


Figure 4. Arsenite methyltransferase (arsM) gene detected in periphyton DNA from Trout Lake, Steel Lake, and Lake Killarney by PCR using degenerate primers targeting a conserved region of the gene. Periphyton was collected from three south Puget Sound Lakes with varying levels of arsenic: Trout Lake (< 1 ppb), Steel Lake (~ 2 ppb), and Lake Killarney (~ 20 ppb). DNA was extracted from periphyton samples and used as template in polymerase chain reaction (PCR) at varying concentrations: 1 ng/uL, 2 ng/uL, and 4 ng/uL. PCR was performed with one of two primer pairs: complementary to either 16S rRNA or arsM genes. Agarose gel electrophoresis was performed with the PCR products. This figure displays the gels visualized with fluorescent dye, molecular weight (MW) ladder, and variable labels. The 16S rRNA primers were expected to lead to a PCR product of 111 base pairs (bp), and the arsM primers (MF1 and MR2) were expected to lead to a PCR product between 302 and 346 bp.

Conclusions

With higher concentrations of arsenic in lake ecosystems, there is an increase of accumulation in the Chinese Mystery Snail across all tissue types. Gut tissue is the most impacted by arsenic toxicity as there is a 20 times increase from Trout Lake samples to Lake Killarney samples.

The expected positive result of arsM gene around 400 bp did appear across all samples from Trout Lake, Steel Lake, and Lake Killarney. This confirms the presence of genetic material needed for the periphyton to produce arsenite methyltransferase.

Continued Research

Utilizing different primers for the genes previously tested and repeating this portion of the experiment would confirm or deny current PCR results. After this point, a qualitative PCR test will provide information on the possible genetic expression of arsM. Analyzing the activation of arsM in periphyton from varying environmental arsenic concentrations is critical to understanding how this metal contaminate interacts with these lake ecosystems.

Additionally, investigating factors that contribute to the increase of arsenic in CMS gut tissue would provide a deeper understanding on how arsenic toxicity impacts this aquatic species. Two factors to consider are interactions of the snails microbiome with arsenic and snails intaking arsenic through filter feeding.

Acknowledgements

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Citations

[1]Hull EA, Stiling RR, Barajas M, Neumann RB, Olden JD, Gawel JE. 2023. Littoral sediment arsenic concentrations predict arsenic trophic transfer and human health risk in contaminated lakes. PLoS One. doi:10.1371/journal.pone.0293214.

[2]Shi K, Wang Q, Wang G. 2020 Sep 15. Microbial Oxidation of Arsenite: Regulation, Chemotaxis, Phosphate Metabolism and Energy Generation. Front Microbiol. doi:10.3389/fmicb.2020.569282.

[3]William VU and Magpantay HD. 2023 Dec 30. Arsenic and Microorganisms: Genes, Molecular Mechanisms, and Recent Advances in Microbial Arsenic Bioremediation. Microorganisms, vol. 12, no. 1, pp. 74–74, doi:10.3390/microorganisms12010074.

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