Assessing Arsenic Accumulation by Periphyton Bacteria from Lake Killarney

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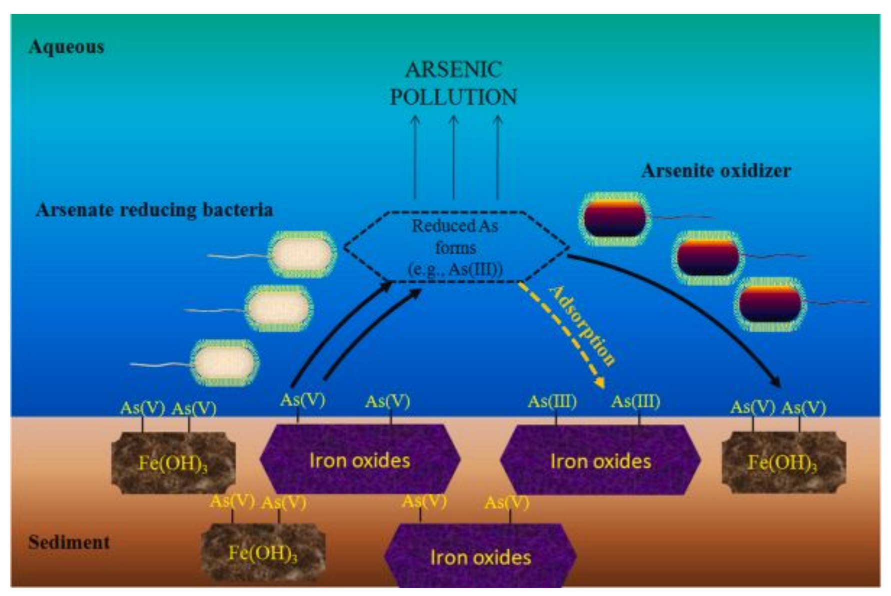
Introduction

Arsenic (As) contamination is a worldwide problem that affects millions of people and ecosystems, especially because it's known to be one of the world's most hazardous chemicals due to its carcinogenic properties[3]. Research done by our colleagues through the UW Superfund Research Project showed that several lakes in the Puget Sound Region have high levels of As contamination as well as significant arsenic bioaccumulation in various aquatic organisms from these lakes[1]. These findings led us to believe that periphyton, a rich community of algae, bacteria, and other microorganisms that adhere to open surfaces in aquatic environments, might be arsenic's entry point into the food chain.

Hypothesis I: Isolated arsenic tolerant bacteria of the periphyton collected from Lake Killarney are responsible for arsenic uptake.

Hypothesis II: Periphyton grown in controlled conditions with minimal media over a longer period will demonstrate higher arsenic bioaccumulation.

Hypothesis III: Periphyton grown in controlled environmental conditions will demonstrate higher arsenic bioaccumulation.



(Hussain et al. 2021)

Methods/Results

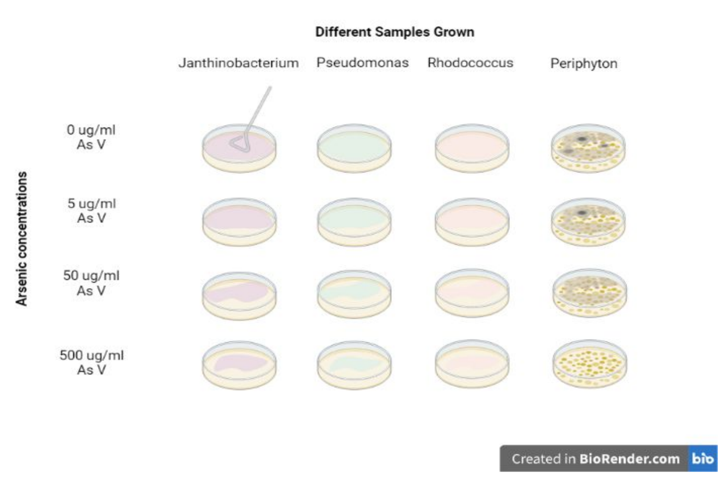


Figure 1. Periphyton and arsenic tolerant bacterial isolates, *Janthinobacterium sp, Rhodococcus sp,* and *Pseudomonas sp,* were grown in LB with different Arsenic V concentrations (0 ug/ml, 5 ug/ml, 50 ug/ml, and 500 ug/ml).

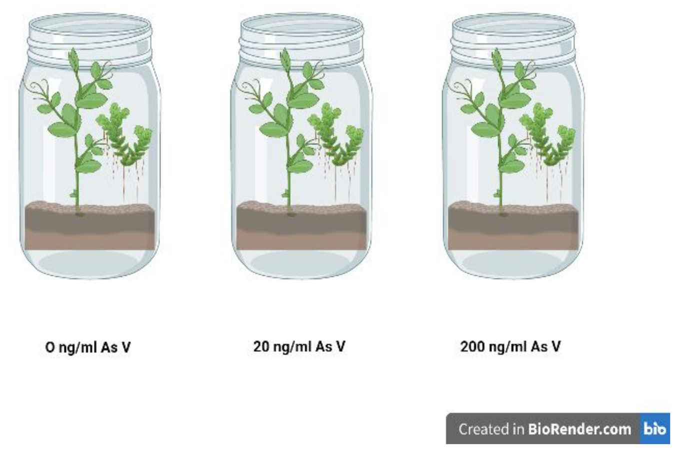


Figure 2. Environmental replicates for periphyton cultivation. A set of twelve jars consisted of different combinations of water, sediment, and plant sources collected from Lake Killarney, Steel Lake, and Angle Lake; and adding different arsenic V concentrations.

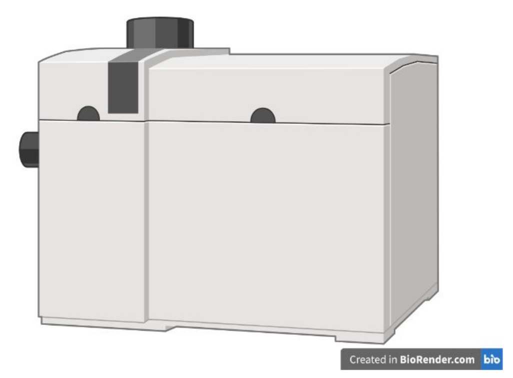


Figure 3. ICP-MS procedure and protocol followed for trace element analysis and quantification of Arsenic uptake from samples.

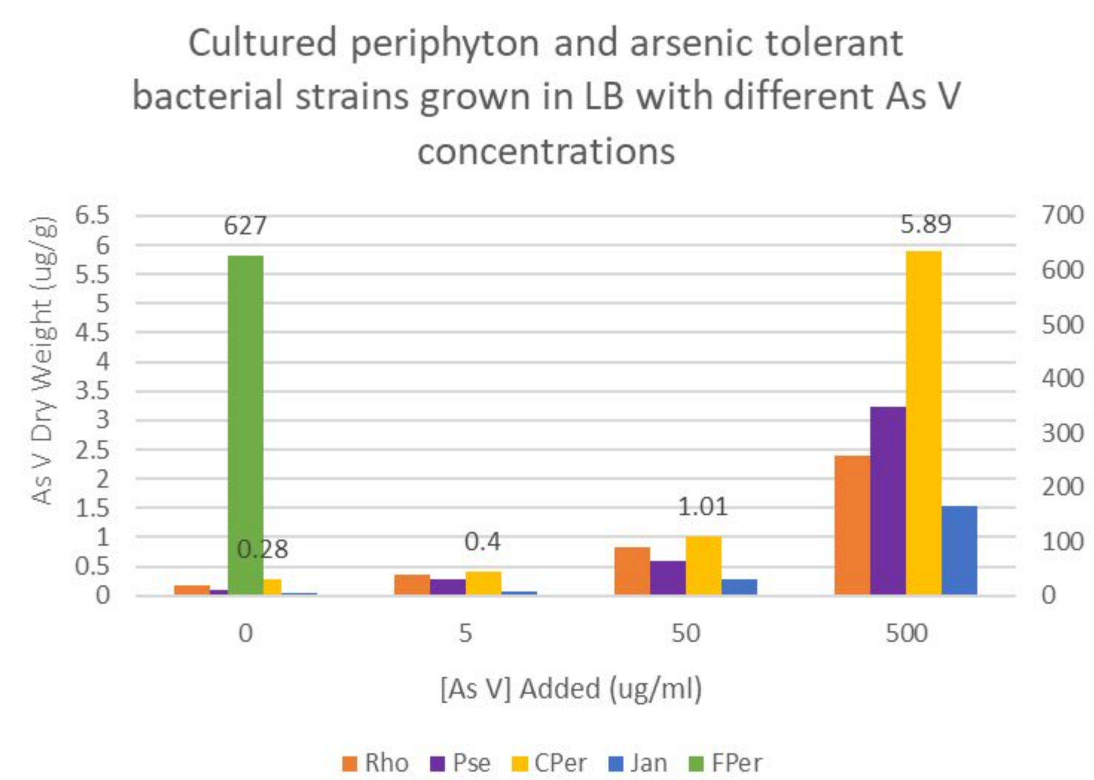


Figure 4. Cultured periphyton bacteria show a substantially low uptake of Arsenic compared to periphyton collected from Lake Killarney. Although there is a huge difference between cultured Periphyton compared to the other 3 cultures, it still has a 100x difference in comparison to the field Periphyton. Results were impacted by the growth on LB agar plates in concentrations of (0 ug/mL, 5ug/mL, 50 ug/mL, and 500 ug/mL) which introduced several limitations on the overall bacterial community growth.

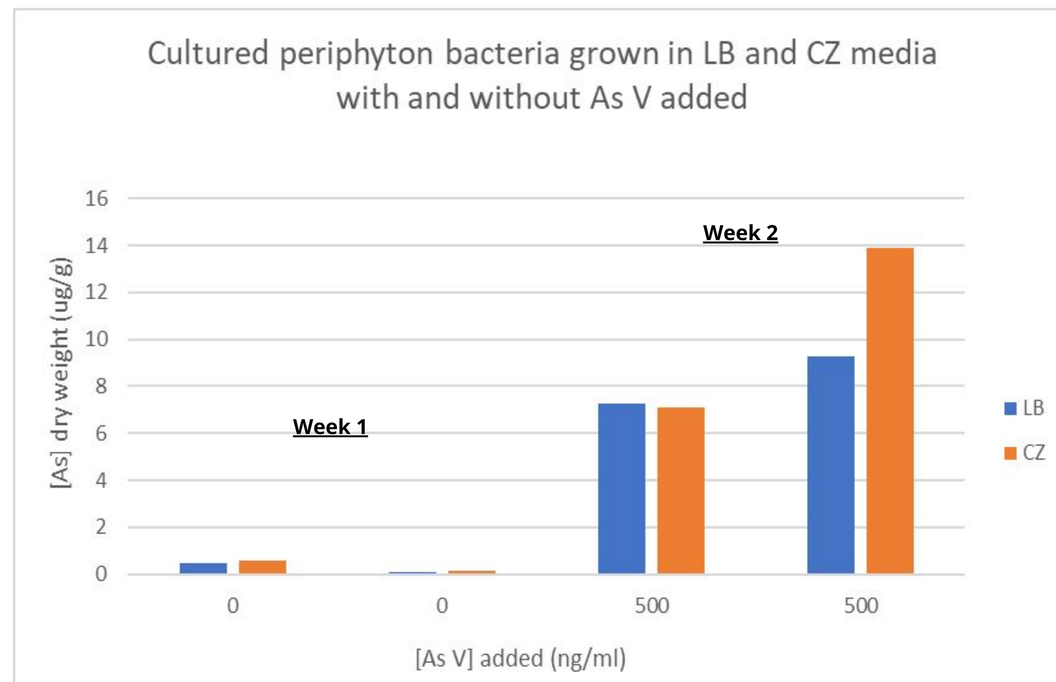


Figure 5. Periphyton bacteria cultured in minimal medium (CZ) take up slightly more arsenic than in complex medium (LB) but are still substantially lower than periphyton collected from Lake Killarney. We tested two different time intervals (1 week vs 2 weeks) and different media (LB vs CZ) to determine if arsenic uptake by periphyton derived bacterial cultures could increase after our initial experiments showed minimal uptake.

Table 1. Periphyton collected from laboratory-based aquatic plant cultivations from Lake Killarney, Angle, and Steel Lakes with added Arsenic (As) concentrations of 0 ng/mL, 20 ng/mL, and 200 ng/mL show accumulation of Arsenic. Plants, lake water, and sediments were collected from indicated lakes and incubated in an environmental chamber with a diurnal cycle at 25℃ for ten weeks, with or without arsenic supplementation. Periphyton was collected and arsenic content was determined by ICP-MS.

Sample #	Plant Source	Water/Sediment Source	Concentration of Arsenate Added (ng/ml)	[As] dry weight (ug/g)
1	Steel	Steel	0	2.496517
2	Steel	Killarney	0	3.380008
3	Steel	Steel	20	17.84063
4	Steel	Killarney	20	4.685251
5	Steel	Steel	200	**29.69709**
6	**Steel**	**Killarney**	**200**	**75.8992**
7	Angle	Angle	0	**17.50367**
8	Angle	Killarney	0	4.738072
9	Angle	Angle	20	4.920307
10	Angle	Killarney	20	12.16515
11	Angle	Angle	200	19.30548
12	Angle	Killarney	200	**23.81661**

Conclusions/More Research

Conclusion I: Isolated arsenic tolerant bacteria; *Janthinobacteria sp*, *Rhodoccocus sp*, and *Pseudomonas sp* are not responsible for the bioaccumulation of arsenic. We conclude that these bacteria might not have the mechanisms to uptake arsenic.

Conclusion II: Periphyton grown in CZ minimal media showed slightly higher arsenic accumulation than when grown in LB media. Results suggest that arsenic uptake increases when phosphate levels in the medium are limited. No substantial difference was observed between periods of growth.

Conclusion III: Periphyton collected from laboratory-based aquatic plant cultivations showed higher arsenic uptake than when collected from plates. These results suggest that cultivating plants collected from local lakes may be useful for periphyton cultivation to use in future experiments.

More Research:

- Develop the ideal environment for periphyton cultivation with high arsenic accumulation.
- Use cultivated periphyton to feed aquatic organisms and test the physiological effects of high arsenic uptake.

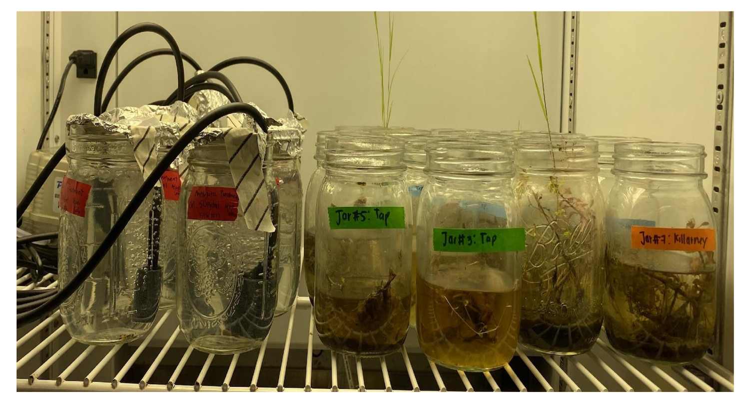


Figure 7. Environmental replicate jars for periphyton cultivation and jars containing juvenile *Pomacea diffusa* snails treated with antibiotics for initial inflammation biomarker analysis.

Figure 8. *Pomacea diffusa* breeding aquatic tank.

Acknowledgments
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References

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