

# Characterizing Arsenic Contamination and its Effects on the Microbiomes of South Central Puget Sound Lakes.

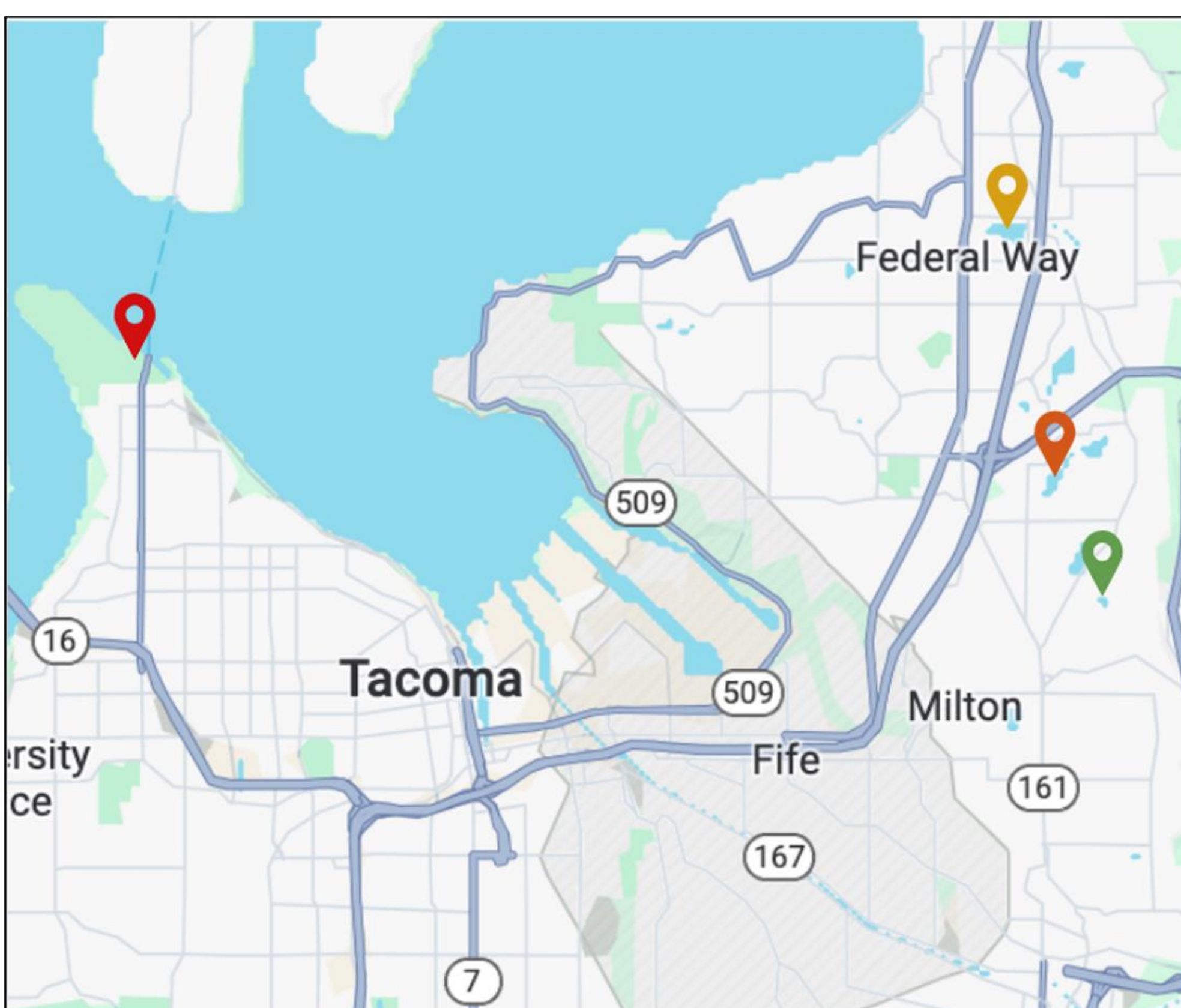
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## Background

Legacy arsenic (As) contamination in freshwater ecosystems in the south-central Puget Sound region (Figure 1) is a product of prolonged pollution from many sources, with the ASARCO copper smelter (1912-1993; Tacoma, WA) being one of the main contributors (Hull et al. 2021).

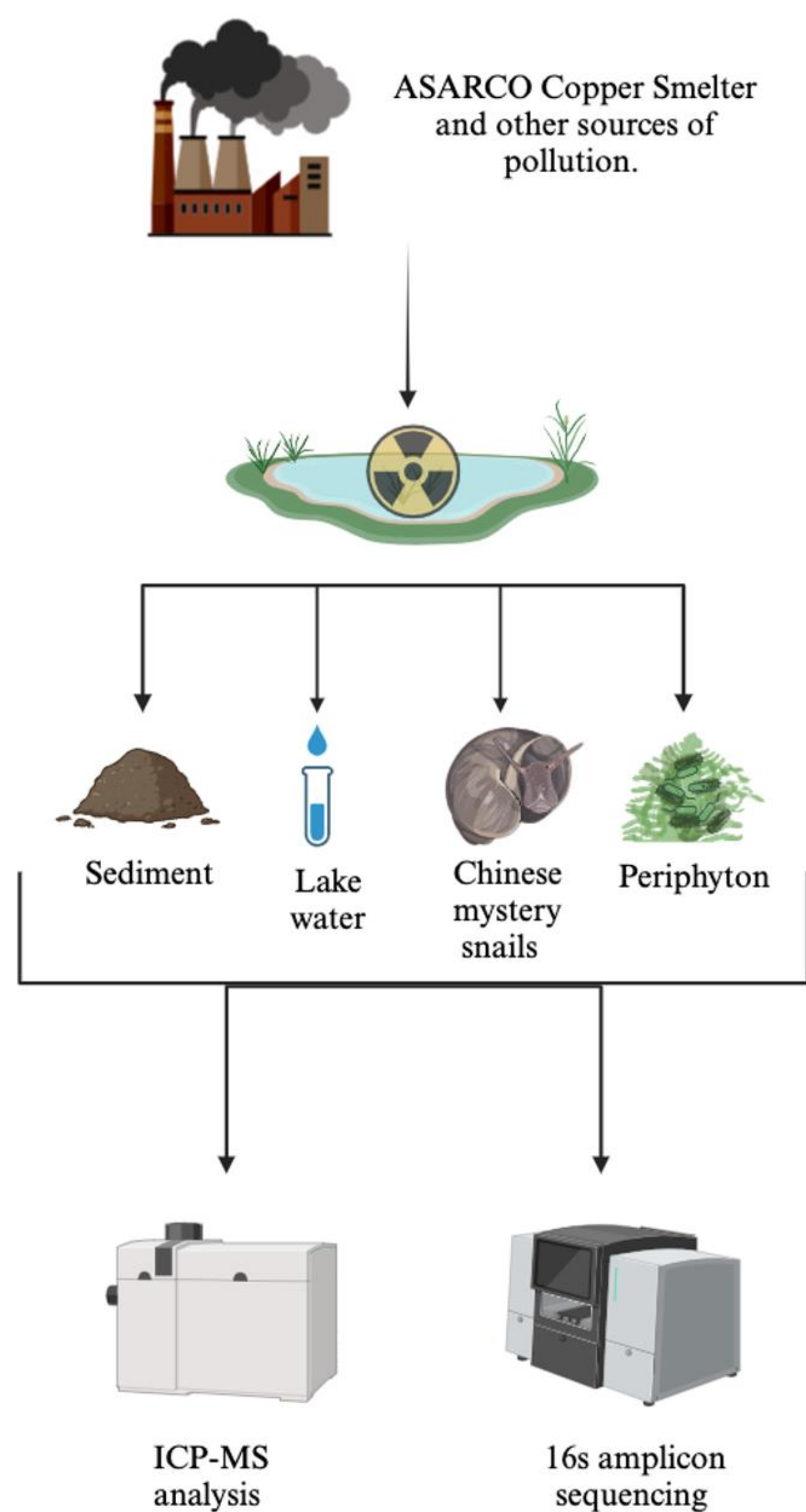
Preliminary experiments comparing As-contaminated and reference lakes showed that the bacterial community composition in periphyton differs, suggesting that there is selection for As-tolerant microbe species in contaminated lakes.

The overall goal of this project is to determine how varying levels of As contamination in freshwater lakes influences the existing compartmental microbiota communities in the water, sediment, periphyton, and guts of an invertebrate primary consumer, the Chinese Mystery snail (CMS).



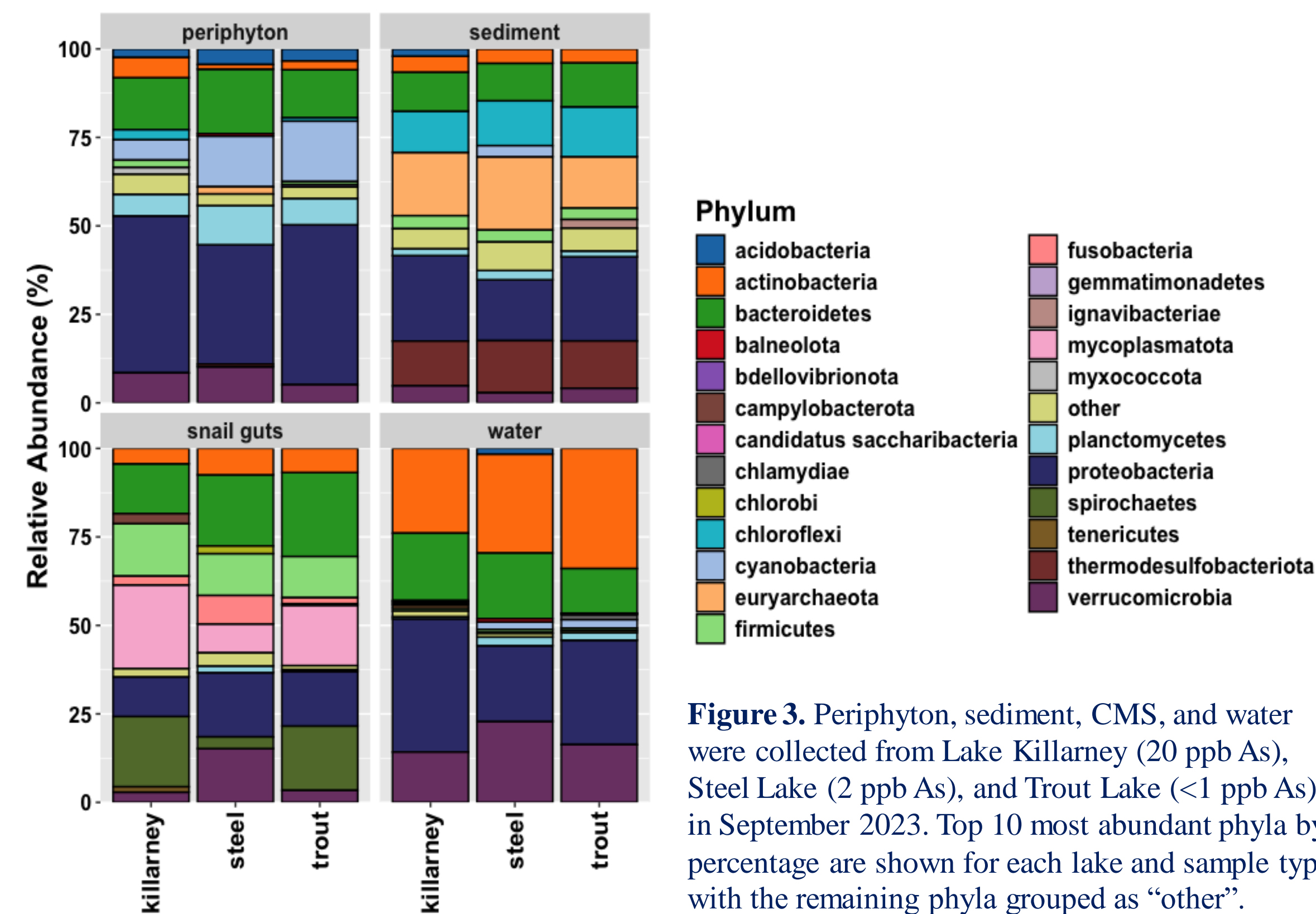
**Figure 1.** Map indicating location of three lakes of interest and their proximity to the ASARCO copper smelter (Red pin). Orange pin is for Lake Killarney (20 parts per billion (ppb) As in water), yellow pin for Steel Lake (2 ppb As in water), and green pin for Trout Lake (< 1 ppb As in water) (Hull et al. 2021). Biorender. 2024

## Field Sampling and Processing Methods



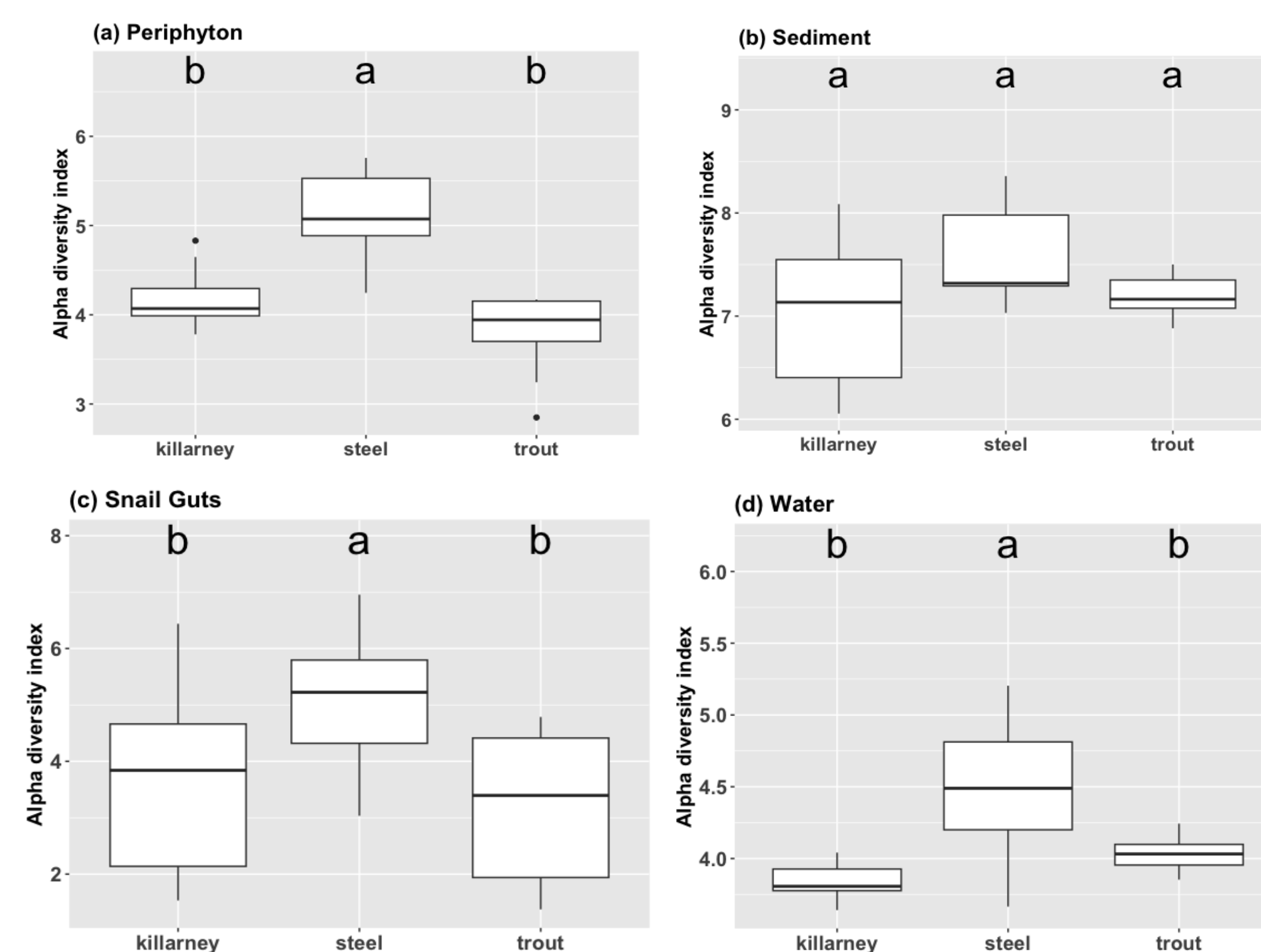
**Figure 2.** Schematic detailing the heavy metal pollution found in some freshwater lakes. In September 2023, water, sediment, periphyton, and CMS gut samples were collected from three lakes with differing concentrations of As: Lake Killarney, Steel Lake, and Trout Lake. Inductively coupled plasma-mass spectrometry (ICP-MS) and 16S ribosomal (rRNA) amplicon sequencing was performed on all four sample types to quantify the total As levels and identify the microbial composition within each sample type and lake, respectively. Made with Biorender, 2024.

## Relative Abundances of Prokaryotic Phyla Vary by Lake and Environmental Compartment



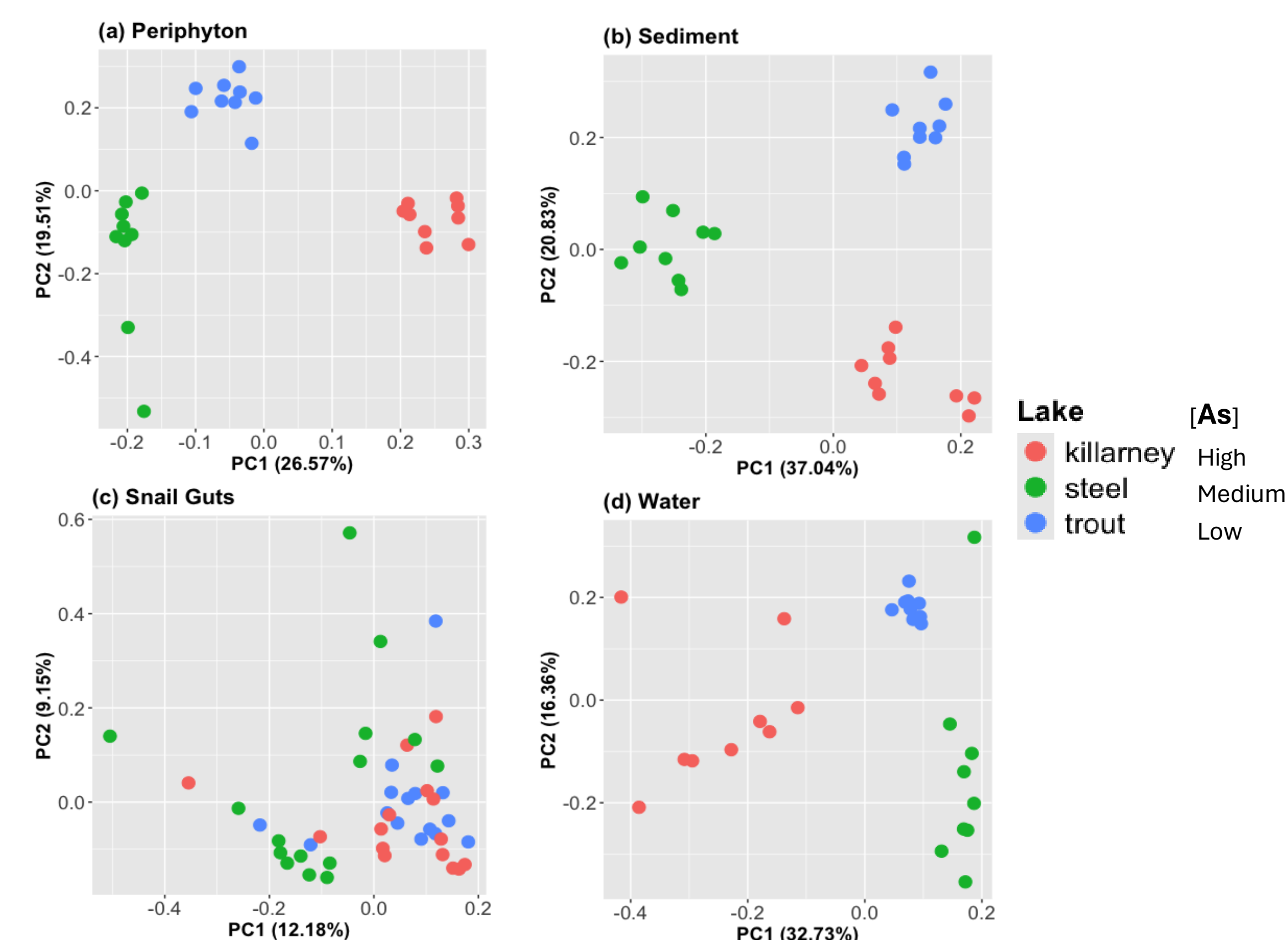
**Figure 3.** Periphyton, sediment, CMS, and water were collected from Lake Killarney (20 ppb As), Steel Lake (2 ppb As), and Trout Lake (<1 ppb As) in September 2023. Top 10 most abundant phyla by percentage are shown for each lake and sample type, with the remaining phyla grouped as "other".

## Prokaryotic Diversity is Highest in the Lake with Moderate As Concentration



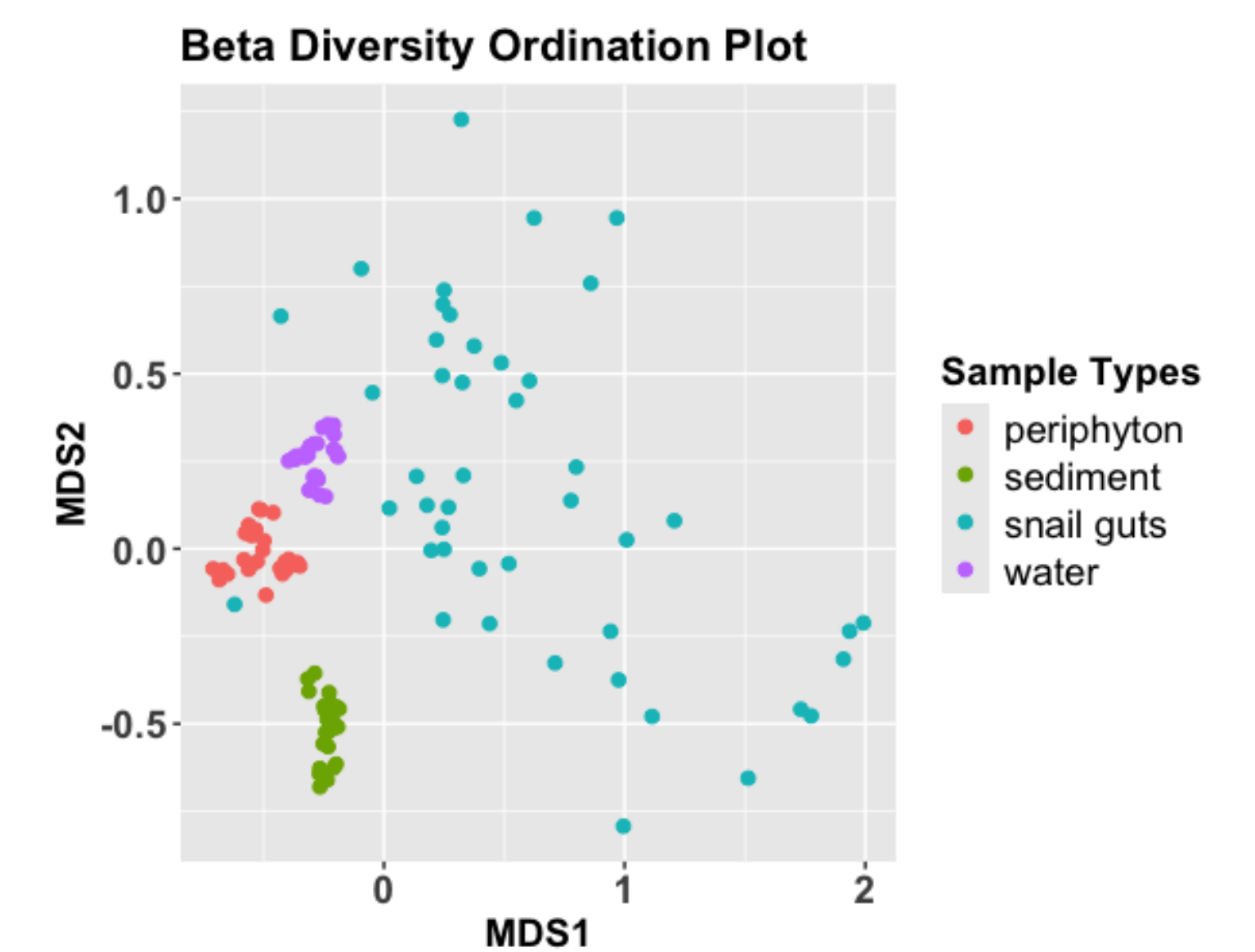
**Figure 4A-D.** (a) Periphyton, (b) sediment, (c) CMS snail guts, and (d) lake water were collected from Lake Killarney (20 ppb As, high), Steel Lake (2 ppb As, mid), and Trout Lake (<1 ppb As, low) in September 2023. Alpha diversity analysis was derived by using inverse Simpson index, where a lower index value is equal to lesser diversity within the group.

## Environmental Compartment and Source Lake Contribute to Microbiome Structure in Periphyton, Sediment, and Water



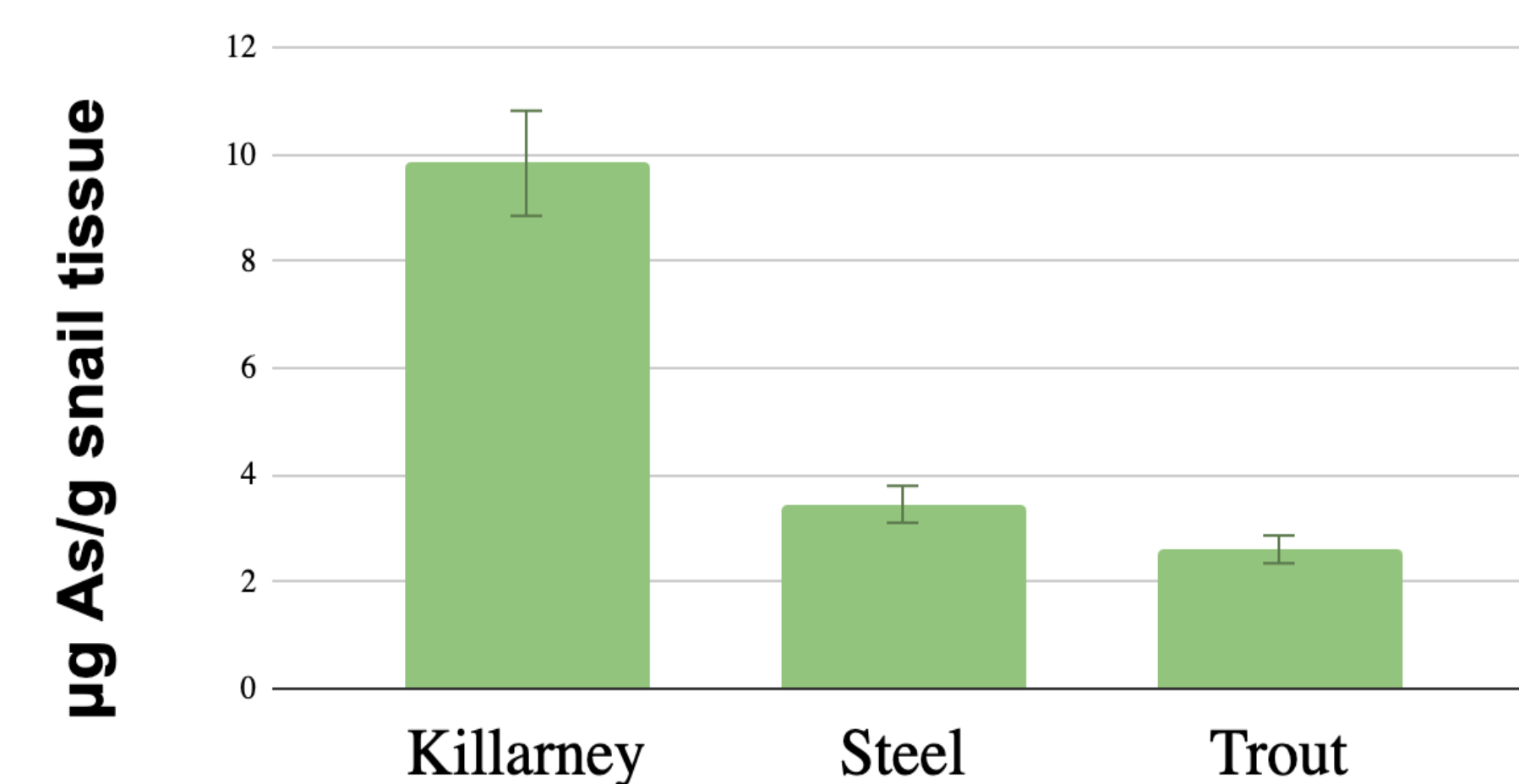
**Figure 5A-D.** Principal component analysis of (a) periphyton, (b) sediment, (c) CMS guts, and (d) lake water were collected from Lake Killarney (20 ppb As, high), Steel Lake (2 ppb As, medium), and Trout Lake (<1 ppb As, low) in September 2023.

## Distinct Microbiomes Are Found Within Lake Environmental Compartments



**Figure 5.** Beta diversity analysis (ordination plot with Bray-Curtis Dissimilarity and Multi-Dimensional Scaling) of different sample types across all lakes. Periphyton, sediment, CMS snail guts, and lake water were collected from Lake Killarney (20 ppb As, high), Steel Lake (2 ppb As, mid), and Trout Lake (<1 ppb As, low) in September 2023.

## As Accumulates in Lake Killarney CMS Tissues



## Field Samples by Lakes

**Figure 6.** CMS collected in the summer of 2023 from three lakes were analyzed for total As concentration using ICP-MS. Tissues, excluding the guts from 15 samples from each lake (Lake Killarney, Steel Lake, and Trout Lake) revealed a significant variation in As concentration between the lakes.

## Future Directions

- Identification of prokaryotic taxa that consistently change in abundance for environmental compartment microbiomes within lakes of varying As levels.
- Quantification of prokaryotic As metabolism genes present in lake environmental compartment microbiomes.
- Multi-season sampling of lakes to determine if/how microbiome structure changes seasonally and over several years.

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