UNIVERSITY of WASHINGTON TACOMA

# Looking at the Physiological Stress in Snails from Arsenic Contaminated Lakes

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## Background Information:

The Asarco Company operated a copper smelter in Tacoma, WA that created pollution consisting of arsenic, lead, and other heavy metal contaminants that settled in the soil and water sediments around Pierce and King County areas. Our research focuses on the physiological effects of arsenic contamination in snails and the impact seen if they express any arsenic in their body. Previous research noted that snails from Lake Steel and Killarney contained the highest levels of inorganic arsenic within their tissues due to their ability to filter phytoplankton from the water.

**Figure 1.** ASARCO co. in Tacoma WA

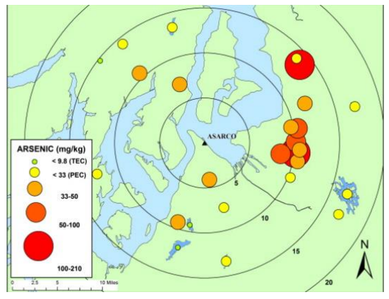


**Figure 2.** Map of arsenic contamination in Puget Sound area

## Objective:

Our study was to determine if snails that were fed periphyton from Steel Lake, Lake Killarney, and Lake Meridian would have different expression of Heat Shock Protein 70 (Hsp70) due to their arsenic levels in body.

## Hypothesis:

If feeding on arsenic exposed plants & microbes induces physiological stress, then Hsp70 expression will be detected in Lake Killarney snails.

**Secondary Hypothesis:** If there is Hsp70 expression in the foot region, then we can mark it as a cause for physiological stress and an appearance of arsenic in the foot region.

## METHODS AND MATERIALS

To assess the physiological stress in arsenic contaminated organisms in the lake, HSPs were utilized since they have a response to high stress stimuli

Objective was to determine if feeding periphyton from each lake to the Chinese Mystery Snails would have a difference in HSP70 expression due to the different levels of arsenic

Apple snails were exchanged from Chinese Mystery Snails because using these snails gave us more control of how much Arsenic is entering its body by feeding it periphyton from the lakes instead

Looked at their antibiotic resistance/sensitivity for future lab studies in this research

We used frozen parts of the snail, my group using the foot region and then crushing it to conduct an SDS-PAGE gel electrophoresis.

We would then use the process to make a Western Blot and Coomassie Blue to see if there are any results of HSP70 expression from the different arsenic levels

This gave us the conclusion that the foot region of the snail has little to no expression of Hsp70 in response to As exposure through lake water and plants.

## RESULTS

No indication of Hsp70 expression was noted for any of the foot treatment groups. Bacterial sensitivity towards arsenic concentration and antibiotic resistance were examined in bacterial colonies present in different tissues isolated from Apple snails. Bacterial colonies were observed to be sensitive to certain antibiotics but resistant to As(V) plates. Overall results indicated that Hsp70 expression was seen in tissue samples consisting of the head or gut region of Apple Snails fed with plants and associated microbes from Lake Meridian plants [low As].

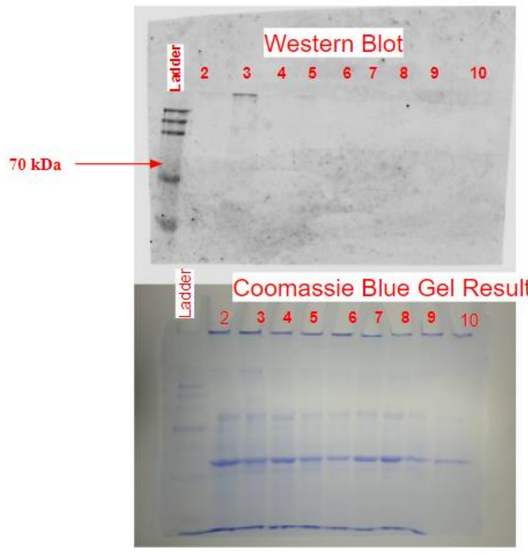


**Figure 3.** Hsp70 biomarker results were examined for presence of bands at 70 kDa. No Hsp70 expression was seen in the foot region. Western blot loading order of each sample; lane 1 contained the ladder, lanes 2-4 contained Steel Lake fed samples, lanes 5-7 contained Lake Killarney fed samples, and lanes 8-10 contained Lake Meridian fed samples. Coomassie Blue gel results visualized total protein loaded per well for each sample to establish a comparison to Western blot gel. Protein bands were present at approximately 20 kDa for each lane. Coomassie gel load order followed load order present in Western blot analysis.

| Periphyton location | Relative Hsp70 expression in snails (0, *, ***) |
|---|---|
| Lake Meridian (low arsenic) | 0 (foot, mantle), * (gut), *** (head) |
| Lake Killarney (high arsenic) | 0 (foot, gut, mantle), * (head) |
| Steel Lake (medium arsenic) | 0 (foot, head, mantle), *** (gut) |

**Table 1.** Complete data of Hsp70 expression in researched areas of snail. Snails were fed periphyton from lakes containing different levels of arsenic; 0 = little to no As expression, * = medium As expression, *** = high As expression

**REFERENCES**

Image of ASARCO building:
https://apps.ecology.wa.gov/cleanupsearch/site/3657

Arsenic map:
https://pubmed.ncbi.nlm.nih.gov/24317160/

**ACKNOWLEDGEMENTS**

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