

**BIOACCUMULATION OF METALS BY ESTUARY PLANTS IN WHATCOM
COUNTY**

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To: Graduate Program Committees, Huxley College

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Applicant Signature

date

Approved by:

Dr. Jenise Bauman, chair
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Dr. Ruth Sofield

Background

Estuaries perform numerous direct and indirect functions for the benefit of the environment. By linking the terrestrial and marine environments, estuaries represent a transitional state from saltwater to freshwater, and as a result, have numerous species that depend on them for habitat and nutrients. Despite these irreplaceable services that estuaries provide, habitat loss and pollutant accumulation present a significant threat to the health of these ecosystems, including sites in the Pacific Northwest. For example, salmon are a keystone species in this region due to their central role within the marine and terrestrial food webs of the Pacific Northwest, and depend on the estuary environment as juveniles. The steep decline in salmon populations, particularly Chinook salmon, has been identified as a main cause of the Southern Resident orca decrease (Southern Resident Orca Task Force 2019). Restoring salmon habitat and addressing aquatic contaminants have been identified as important steps to improve environmental conditions for the orcas. Applied bioaccumulation of contaminants within plant tissue, known as phytoremediation, is one possible solution.

Compared with traditional remediation techniques, such as excavation and chemical treatment, phytoremediation is cost effective, adaptable to a wide range of situations, beneficial to the ecosystem's long-term health, and popular among public opinion (Hryniewicz et al. 2018). Plants can phytoremediate in three ways: phytostabilization, in which they can stabilize the metals and essentially sequester them in their roots; chemical conversion of the metals to less harmful and more stable forms either in root or aboveground tissue; and phytoextraction, which re-locates and stores the metals into shoot tissue. The final location of metals determines phytostabilization or phytoextraction, which require different management strategies and present different challenges. Phytoremediation of metal contaminated estuarine water and soil has been

evaluated at various field sites. Common reed grass (*Phragmites australis*) was used to extract copper, zinc, lead and chromium from water at field sites at the Yangtze Estuary (Huang et al 2017). Various cordgrass species (*Spartina spp.*), have also been studied as an effective phytostabilizer (Curado et al. 2014). However, both of these species are considered invasive in the Pacific Northwest. Native plants are preferred because they are already well-suited to the growing environment, and typically will complement long-term ecosystem management plans, while invasive species require more intensive management and can reduce overall species diversity in the area. Further studies to identify capable species, especially in the Pacific Northwest, would be beneficial as phytoremediation is still a relatively new field.

Purpose

This study will evaluate metal concentrations at four estuaries in Whatcom County in varying stages of restoration and with different anticipated levels and sources of metals. This study will measure and compare the change in metal concentration among common species at these four sites to evaluate their effectiveness as potential tools for soil metal removal and/or immobilization, as well as measuring seasonal changes in metal accumulation. This study aims to address i) the current heavy metal load and distribution in the tidal marshes of restored estuary sites; ii) identify the native plant species that are efficient at either stabilization or extraction of present metals; and iii) document the changes in metal concentration in plant tissue throughout the season and into plant senescence. This study will help develop additional estuary restoration strategies for the Pacific Northwest by identifying phytostabilizing and phytoextracting native plant species. In addition, this research will reduce the risk of reintroduction of the contaminant to the environment by developing an optimal timeline for contaminated tissue removal.

Methods

I will be initially testing the soil at four pocket estuaries in Whatcom County, with different levels of surrounding development and anticipated soil contamination: Padden Creek, Chuckanut Creek, Whatcom Creek, and California Creek. I will be collecting three soil cores for each of these sites using a 5cm diameter by 18cm depth soil corer, made of PVC pipe. After drying these samples and grinding them to a consistent size, I will then analyze them for concentrations of the following metals: lead, arsenic, cadmium, zinc, chromium, manganese and copper. Based on the results of the soil analysis, the estuary with the highest or most diverse range of metals will be selected for continued study. Also at this time, I will conduct a vegetation survey concurrently using a line-transect method. Randomly placed 1x1m quadrats along the transects will provide additional information about plant cover of these surveyed species.

From the results of this survey, I will select the estuary with the largest metal load and three most common plant species for sampling, with three replicate plants per species collected. I will bisect these plants using a 10 cm diameter by 30 cm depth corer and transport the entire core back to the lab (Figure 1). This allows me to quantify changes over the growing season by repeatedly sampling the same individual plant colony while leaving the remaining plant colony intact and relatively undisturbed. During this same field visit, I will collect additional leaf tissue from each replicate, and secure it into leaf litter bags, which will be used to help quantify the release of metals during leaf composition. I will resample these species three more times, using the same procedure, and collect one leaf litter bag per field visit (June, August, September, December).

After returning to the lab with the plant-soil cores, I will carefully separate the soil and plant tissue. To prepare for analysis via inductively coupled plasma mass spectrometry (ICP-MS) both plant and soil tissue will be dried, ground to a consistent size, and undergo acid digestion with nitric acid to ensure all metals in the sample are available for analysis. I will combine the plant tissue with concentrated nitric acid and heat in a closed vessel microwave digester. For the soil, I will be using a sequential extraction method, outlined in Tessier 1979. This method uses a series of leachates to remove metal ions bound in various soil complexes, beginning with a solution of magnesium chloride to remove freely available metals. The leachates progressively get stronger, which allows me to quantify the fraction of total metals in the soil which are bioavailable to these plants, or could become bioavailable under various conditions, such as a change in pH or salinity. Following either acid digestion or sequential extraction, the samples will be diluted to 5% acid or less and stored in sealed bottles until analysis. ICP-MS will provide the concentrations of the above listed metals.



Figures 1 and 2. Demonstration of soil and plant coring process. Corers will be used to collect a section of the plant along with surrounding rhizosphere, which will be separated in the lab and analyzed individually.

Research Design

This project will collect a total of 147 samples: 12 soil samples for preliminary screening, 36 soil samples from subsequent sampling, 36 sets of aboveground plant tissue, 36 sets of belowground plant tissue, and 27 sets of leaf litter tissue. The results of ICP-MS analysis will allow me to compare metal amounts present in the soil in these Whatcom County estuaries, metal accumulation between species, between above and belowground tissue, and any changes in metal concentration over the duration of the study. Vegetation surveys will be useful in identifying estuary restoration progress, and help to select native, abundant species that could be beneficial in future phytoremediation projects. I will be using principle coordinates analysis to examine the species and abundances of plants collected through the vegetation surveys at all four sites. I will also use PCA to evaluate the metal elements and quantities in the soils. I will use an analysis of variance (ANOVA), followed by post hoc testing of means, to compare metal accumulation by plant species. In order to compare changes in metal accumulation over time by plant species, I will use an analysis of repeated variance (ANOVAR) to account for multiple measurements of the same individual, which will be followed by post-hoc testing of means to follow up on any statistically significant differences found.

Anticipated Results

I am anticipating that the estuaries found within the City of Bellingham will have higher levels of metals. Whatcom Creek and Padden Creek estuaries both have surrounding industrial sites, which could be a source of elevated metals in the soil, and drain more populated areas of the county, providing a nonpoint source of pollutants. Based on phytoextractors found in other studies (Curado et al. 2014, Huang et al. 2017), I expect that resilient, rapidly growing grasses will accumulate more metal contaminants from the soil than other species found at those sites.

However, plants that are capable of accumulating metals often do so as an evolutionary response to higher levels of specific elements found within the soil (Brooks 1998). This makes bioaccumulation highly species and contaminant specific, and so targeting a wide range of plants will help to address this issue. Previous research has shown that annual species, upon their death, release metals from their tissue back to the soil through decomposition (Tiner 2003).

Therefore, I am predicting that metal concentrations will increase until plant death or senescence, when the accumulated metals will be returned to the soil without intervention.

Schedule

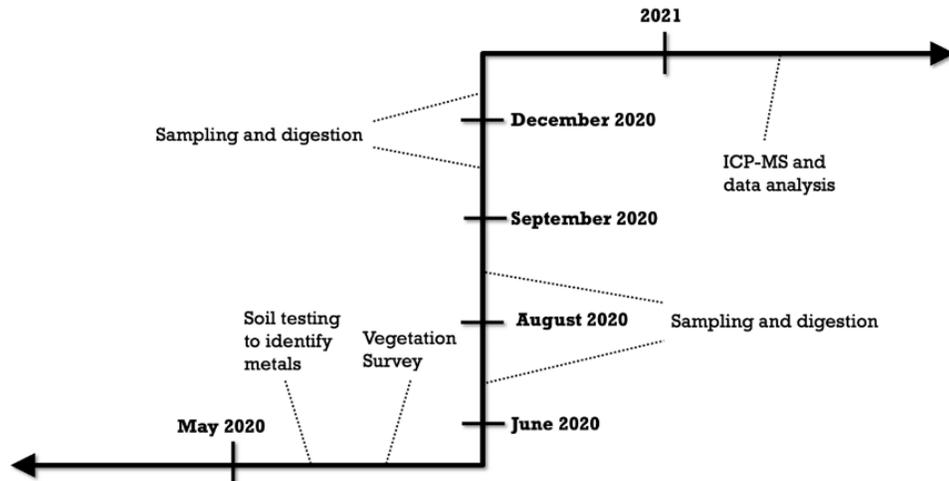


Figure 2. Proposed timeline of thesis research. For each sampling event after the first, a leaf litter bag will be collected along with replicate samples.

Budget

a. Proposed Budget and Supplies

These supplies will cover testing resources for 147 soil and plant samples.

Item	Cost	Supplier	Justification
Concentrated nitric acid, trace metal grade, 5L	\$340.00	VWR	Acid digestion of plant tissue and sequential extraction of soil
Magnesium chloride, trace metal grade, 20g	\$120.00	VWR	Used to make 1M solution for sequential extraction of exchangeable metals
Sodium acetate, trace metal grade, 250g	\$54.00	VWR	Used to make 1M solution for sequential extraction of carbonate bound metals

25% acetic acid, trace metal grade, 1L	\$26.00	VWR	Solvent for sequential extraction to ensure appropriate pH
Ammonium acetate, trace metal grade, 50g	\$83.00	VWR	Used to stabilize extracted metals previously bound to organic matter
Hydroxylamine hydrochloride, 2g	\$60.00	VWR	Used to make 0.04M solution for sequential extraction of iron and manganese bound metals
Gloves, 2 boxes	\$16.00	VWR	Personal protective equipment for work with chemicals
ICP-MS usage	\$310	WWU AMSEC	Used to analyze concentrations and identities of metals in samples. \$5/hour charge for internal users. Cost calculated using AMSEC's Usage Estimator page. Includes time allotted for training on equipment.
Total	\$1079.54		Includes tax, shipping and handling

Table 1: Itemized budget including all items for thesis proposal.

b. I will be borrowing items, such as transect measuring tapes, glassware, and acid digestion equipment, from Huxley lab services. I already own additional protective equipment, such as a lab coat and goggles.

c. I have also applied for the Research Sponsored Programs (RSP) spring quarter grant from Western Washington University for the same items listed in section A.

d. If I am not selected for the Huxley Small Grant, and do not obtain the RSP grant in spring quarter, I will reapply for both in the fall quarter. I can modify my project plans to composite samples, thereby reducing the total number of samples and using less chemicals. In addition, I can research ways to preserve my samples and run ICP-MS analysis in fewer trips, reducing the usage fee.

e. The total amount for my project is the same as the requested amount from this Huxley Small Grants.

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