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Bioaccumulation of metals in Whatcom County estuaries by native tidal plants

By

Margaret Critchlow

Accepted in Partial Completion
of the Requirements for the Degree
Master of Science

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Master's Thesis

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Margaret D. Critchlow

July 7, 2022

Bioaccumulation of metals in Whatcom County estuaries by native tidal plants

A Thesis
Presented to
The Faculty of
Western Washington University

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

by
Margaret Critchlow
June 2022

Abstract

Estuaries are unique environments which provide many needed ecosystem services, but are threatened by anthropogenic activities. Contamination with metals represents a significant concern, as even small amounts can persist in the soil and affect biological functions. Phytoremediation, or the use of plants to take up contaminants from the soil, is one possible solution. There is a lack of research on estuarine phytoremediators native to the Pacific Northwest, as well as the ultimate fate of these metals following plant senescence. In this study, we evaluated the total metal concentration at three Whatcom County, WA estuaries and sampled four native plant species (*Atriplex patula*, *Distichlis spicata*, *Juncus balticus*, and *Plantago maritima*) at these sites repeatedly throughout the growing season. We used acid digestion of both plant tissue and soil, as well as a four-step sequential extraction of the soil, to measure metal concentrations with inductively coupled plasma mass spectrometry (ICP-MS). Elevated concentrations of metals were found at California and Padden Creek estuaries, while Chuckanut Village pocket estuary had metal concentrations aligned with the median background concentrations for the region. All four plant species were found to be hyperaccumulators of selenium. *Atriplex patula*, *P. maritima*, and *J. balticus* were found to accumulate magnesium, zinc, cadmium, and silver in appreciable quantities, with *J. balticus* also accumulating manganese. Metal concentrations in aboveground tissue declined over time, with the steepest decreases seen in *A. patula* and *P. maritima*. Numerous factors, such as pH and salinity, are known to affect the bioavailability of these metals in soil; site-specific measurements supported these influences. When choosing plants for phytoremediation, land managers should take care to consider the overall needs of their site and additional ecosystem services that vegetation can provide in conjunction with bioaccumulation abilities.

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1. Introduction

1.1 Ecosystem services of estuaries

Estuaries, which are the sheltered or protected areas where streams and rivers enter the ocean, represent a unique and essential ecosystem (NOAA Ocean Service Education Discovery Kits 2017). Estuaries fall under the larger category of tidal wetlands and 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 the completion of their life cycle. Due to the cyclic currents of both the tides and freshwater inputs, sediments and nutrients move effectively into the ecosystem, promoting soil accretion that can host a large number of both aquatic and terrestrial plant species (Tiner 2013). In turn, these plants form the base of an extensive food chain, supporting marine invertebrates, fish, and birds. Estuaries make up a key part of the habitat for over 75 percent of commercial fisheries in the United States, and even more for recreational fishing (NOAA Ocean Service Education Discovery Kits 2017). In the Pacific Northwest, juvenile salmonids utilize estuary habitats as they make the transition from freshwater to saltwater, and juveniles depend on protected estuaries such as pocket estuaries or deltas to evade predators (Beamer et al 2003).

Estuary structure is strongly influenced by tidal activity. The terrain is subject to both sediment deposition, as streams slow down and lose kinetic energy, and erosion from ocean waves and tidal movements, which ultimately causes increased habitat complexity and more biodiversity. In addition to terrain variation, there is also a salinity gradient in the soil, which influences the diversity of estuary plants (Tiner 2013; Cloern et al. 2017). The presence of tidal marsh plants strongly affects water passage in the soil, which is slowed due to higher

concentrations of smaller sediments, such as silt, and large root systems characteristic of estuary plants. Slowing water passage through this environment cools temperatures while reducing the number of limiting nutrients, such as nitrogen and phosphorous, that ultimately reach the ocean, thereby preventing harmful algal blooms (Barbier et al 2011). Because of their expansive root systems, salt marsh environments contribute to soil accretion, land formation, storm attenuation, erosion prevention, and improvements in water quality.

Despite these irreplaceable services that estuaries provide, habitat loss and pollutant accumulation present a significant threat to the health of these ecosystems. Historically, many estuaries within the Pacific Northwest have been anthropogenically modified, such as altering water flow by using dams or dikes, dredging channels, armoring to reduce shoreline erosion, and filling or leveling wetland sites for development, resulting in an 80% reduction in unmodified estuary habitat in Western Washington (Beamer et al. 2003). This impacts numerous functions and species, notably Pacific salmonids (*Oncorhynchus* sp.). 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 both juveniles and adults returning to spawn. Habitat loss, along with water pollution and marine traffic, have been identified as key drivers for the steep decline in salmon populations, which also impact their predators, particularly the critically endangered Southern Resident orca, *Orcinus orca* (Southern Resident Orca Task Force 2019). The Southern Resident Orcas Task Force, a collaboration of Washington State officials aiming to improve conditions that would improve orca wellbeing and population levels, has identified restoring salmon habitat and addressing aquatic contaminants as important steps to improve environmental conditions for orcas and the estuary food web.

1.2 Bioaccumulation of metals

Bioremediation using native estuary plants offers a promising method for both stabilization and extraction of pollutants from stormwater runoff contaminated soils. When bioaccumulation is applied in land management strategies, it is known as phytoremediation. Compared with traditional remediation techniques, such as excavation and chemical treatment, it 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: 1) phytostabilization, in which they can stabilize the metals and essentially sequester them in their roots and/or rhizosphere; 2) phytoexcretion or phytovolatilization, in which metals are translocated into plant tissue, moved through existing excretory pathways, and deposited on the leaf surface or released to the atmosphere; and 3) phytoextraction, which relocates and stores the metals into shoot tissue. The final location of metals ultimately defines the type of phytoremediation used, and each of these method requires different management strategies and presents different challenges.

Phytostabilization immobilizes the metals in the root area, preventing further movement and interaction in the soil. In long-lived species such as trees, phytostabilization into root tissue is preferred over translocation into leaf tissue because it reduces the risks of reintroducing the contaminant into soil and surrounding food webs (Mertens et al. 2004). Although the metals remain in the soil under phytostabilization if they are not absorbed into root tissue, they are either adsorbed to the surface of the root or formed into a precipitate with other ions in the soil, inhibiting the metal's bioavailability to other organisms. In estuaries, phytostabilization would help prevent the movement of contaminants into the marine environment by plant root architecture that can physically slow the movement of water and solutes within soils

(Schachtschneider et al. 2017). The issue of metal fate is compounded, however, when considering plant life cycles. Whigham and Simpson (1976) found that tidal wetland plants were taking up both organic and inorganic molecules from the soil, including metals, during growing season, but would release the metals back into the soil during decomposition (Tiner 2013). However, the rate at which this occurs seems to vary largely based on both the metal and plant species of interest (Březinová and Vymazal 2015).

In contrast to stabilization, phytoextractors transport soil metals into their vascular system and relocate them to the aboveground portion of the plant (leaf, stem, and inflorescence tissue). These species typically sequester these metals in the cellular vacuoles, and/or modify the ionization of these metals to improve their stability and reduce potential damage to plant tissues (Hryniewicz et al 2018). The metals' aboveground location allows for the removal and safe disposal of that contaminated tissue, although concentration in the aboveground tissue may change over time. Plants must be selected specifically for the metal of interest and vary in their rate of metal uptake and longevity. Certain plant species, termed hyperaccumulators, are able to take up significant quantities of metals from the soil. Hyperaccumulators generally have the following characteristics: when compared to other plants in the same conditions, hyperaccumulators' metal concentrations are 100 times higher; metal concentrations are elevated in leaves and stems compared to roots; and plants do not show any signs of ill health from metal uptake (Wei et al. 2004). Other beneficial traits of hyperaccumulators identified by these authors include the ability to take up multiple metals, rapid growth, significant aboveground biomass, and environmental stress tolerance.

On the surface, phytoextraction sounds like an ideal method for remediation, but it has potential issues. To prevent re-introduction of the contaminants back into the soil during

decomposition, land managers must rapidly remove the contaminated plant material before senescence. In addition, there are concerns of metal accumulation in the food web if primary consumers eat these phytoextractors. The best long-term method for stable and effective phytoremediation seems to include a variety of grasses, shrubs and trees that can offer an extensive root network to cover a wide surface area of soil, prevent erosion of contaminated soil, and uptake a wider range of potentially harmful heavy metals (Hryniewicz et al 2018; Mendez and Maier 2008). Fewer studies have investigated phytoremediation of metal contaminated estuarine water and soil. Common reed grass (*Phragmites australis*) has been used to phytoextract 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 effective phytostabilizers (Curado et al. 2014). However, both species are noxious weeds (Class A and B, respectively) in the Pacific Northwest, and therefore cannot be used for remediation in Washington State. Native plants are preferred because they are already well-suited to the growing environment and typically will complement long-term ecosystem management plans. Further studies to identify potential native species in the Pacific Northwest would be beneficial as phytoremediation is still a relatively new field.

1.3 Study objectives

This study will evaluate metal concentrations at three estuaries in Whatcom County with different anticipated levels and sources of metals. This study will measure and compare the change in metal concentration among common species at these three 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 are as follows: 1) to quantify the current metal concentration and distribution in the tidal marshes of estuary sites in

varying levels of restoration; 2) identify the native plant species that are efficient bioaccumulators of present soil metals; and 3) document the changes in metal concentration in plant tissue throughout the growing season and into plant senescence. This study could help develop additional remediation strategies for the Pacific Northwest through identifying phytostabilizing and phytoextracting species, along with developing an optimal timeline for maximizing contaminant removal while reducing the risk of reintroduction of the contaminant into the estuary soils.

2. Methods

2.1 Study Sites

Three pocket estuaries in Whatcom County were evaluated as field sites, which differ by amount of surrounding development and restoration timeline: Padden Creek, Chuckanut Village pocket estuary, and California Creek. Restoration projects have been ongoing at multiple sites in the county, including completed projects in the City of Bellingham at Chuckanut Village and Padden Creek, and planning underway at California Creek. These three pocket estuaries offered the chance to compare contaminant levels in an industrial, highly developed site (Padden Creek), a less developed, primarily residential area (Chuckanut Village), and a rural site (California Creek). In 1990, a buffer was added on the western side of Padden Creek estuary, and from 2014-15 the city completed several restoration goals, including trash and debris removal, disposal of creosote pilings, and modification of the topography of the site to increase geomorphological complexity (Clancy et al. 2012). Consultants for the feasibility report recommend follow-up soil testing at the estuary, especially with the risk of contaminants from the surrounding industrial sites, but no report of this testing can be found. Chuckanut Village pocket estuary was also restored to promote site complexity and maintain species health. A

culvert was replaced with a much larger one to allow for more water volume to pass through, parking lots were moved, vehicle access to wetlands was modified, and addition tidal marsh vegetation was planted closer to the shoreline, with the city completing these projects in 2009 (City of Bellingham 2009). California Creek, located in northern Whatcom County, has the benefit of being relatively undisturbed. In 2017, the non-profit Whatcom Land Trust bought 11.5 acres of land surrounding the mouth of the creek to protect this habitat for salmon and bird species. Long-term plans for the estuary include protection of habitat and public accessibility, although no specific goals are yet outlined (Whatcom Land Trust 2017).

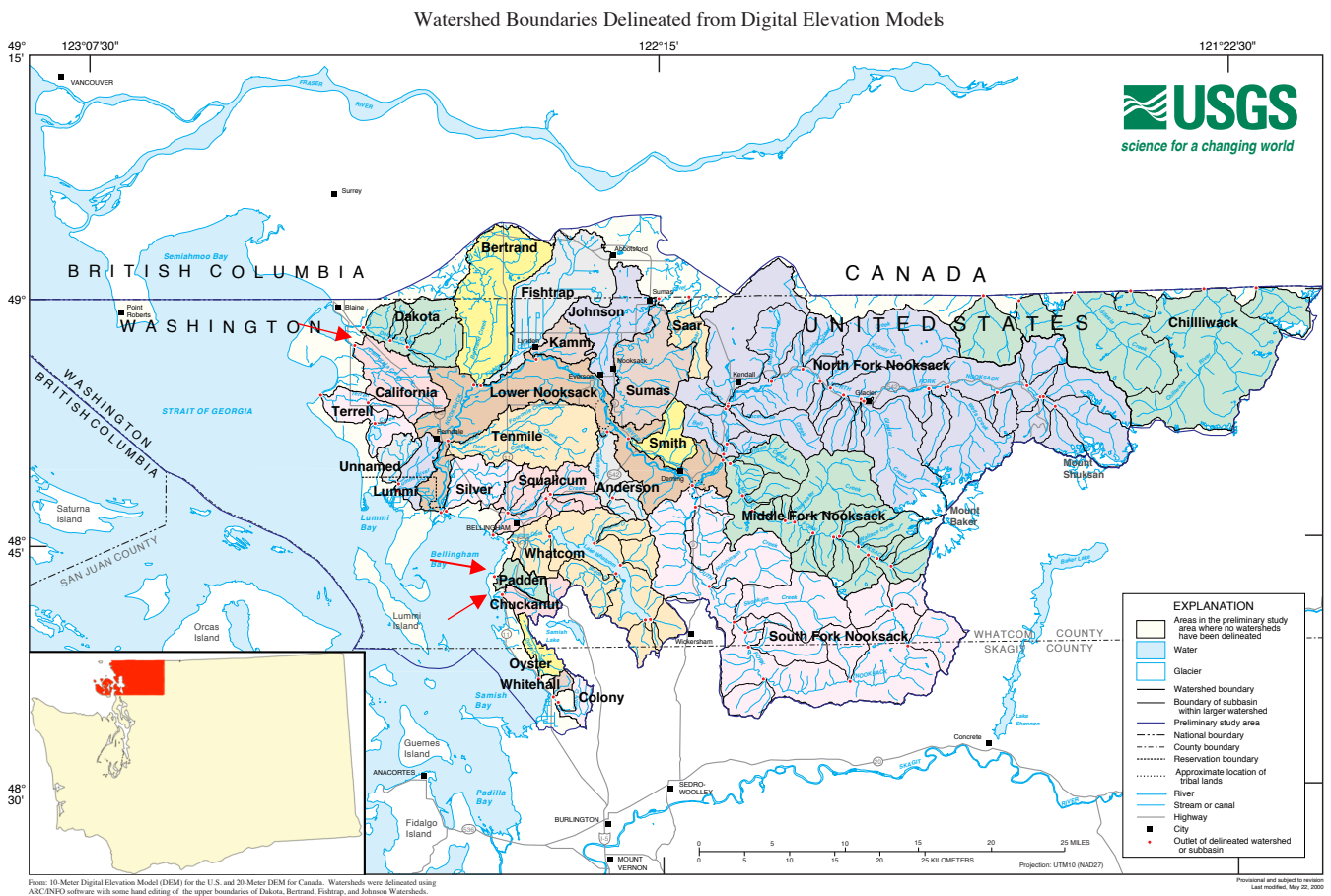


Figure 1: Map of watershed drainage areas in Whatcom County, with arrows showing study sites. California Creek (northernmost arrow) drains the California watershed in the north of the

county, while Padden Creek is part of the Padden Watershed. Chuckanut Village estuary (southernmost arrow) drains a small section of the Chuckanut watershed.

2.2 Preliminary Soil Testing

Prior to surveying and sampling the sites, preliminary testing was performed to validate that there were sufficient metal concentrations present at the sites for study. Three soil cores from each site were collected, dried and homogenized, and sent for analysis. Testing was conducted by Spectrum Analytic (Spectrum) of Washington Court House, Ohio. Spectrum Analytic used the Mehlich-3 method for evaluating soil metal concentrations. Because they are a soil testing company intended for agricultural purposes, not all of the metals of interest in this study were measured. Of the metals in this study, zinc, iron, copper, manganese, magnesium and aluminum were evaluated. Additional soil characteristics, such as pH, were also measured (Table 1).

Table 1: Soil characteristics and metal concentrations from preliminary testing performed by Spectrum Analytic. All elemental concentrations reported in parts per million (ppm). Elements extracted using Mechlich-3 method.

Site	California	Chuckanut	Padden
Soil pH	4.5	6.1	7.4
Organic Matter (%)	4.2	3.4	0.6
CEC	31.8	25.3	26.1
Phosphorous (P)	24	78	16
Potassium (K)	263	290	241
Magnesium (Mg)	856	1122	469
Calcium (Ca)	1297	994	12850
Sulfur (S)	1447	438	389
Zinc (Zn)	6.2	19.5	20.8
Iron (Fe)	631	183	136
Copper (Cu)	0.9	5.0	30.4
Manganese (Mn)	24	34	31
Aluminum (Al)	400	531	15

2.3 Vegetation Survey

To determine the most common shared species within these three estuary sites, two vegetation surveys were conducted in June and July of 2020. A line transect method was used to survey the estuaries, followed by randomly placed quadrats along the lines to determine percent cover. The lines were deliberately placed to try and capture the most area possible in the estuaries, but the starting points of each line were arbitrary. A 50-meter tape was used and the vegetation that the line intersected at every 50 cm was recorded, providing 100 data points per line. Plants were identified using Pojar-MacKinnon's *Plants of the Pacific Northwest Coast*, and Hitchcock's *Flora of the Pacific Northwest*. To determine percent cover by species, three 1-square meter quadrats were placed on each line. The location of these quadrats along the line was determined by a random number generator. For each location, data were collected on both sides of the line, so each transect had a total of 6 quadrat measurements. This allowed us to look at both the tidal and upland sides of the transect. To determine percent cover of each species, the Daubenmire method was employed (Daubenmire Method 2003) with six cover classes corresponding to a percentage range. After identifying each species, it was assigned a 1-6 cover class corresponding to its abundance in the quadrat. These cover class numbers were then converted into the midpoint of the percent range.

2.4 Field Sampling

Based on the results of the rank abundance calculation, four plant species were selected for further study due to their abundance in each site, native status, characteristics as a salt marsh plant, and their wide range of growth forms (Table 2; Figure 2). These species were: *Atriplex patula*, (marsh orache or fat hen); *Distichlis spicata*, (salt grass); *Juncus balticus* (Baltic rush); and *Plantago maritima* (sea plantain). *Atriplex patula* is an annual forb that can commonly grow

in disturbed sites. *Distichlis spicata* is a perennial grass that can tolerate highly saline soils, such as those found in estuaries. *Juncus balticus* is a mat-forming, perennial rush abundant in wetland environments and highly saturated soils. *Plantago maritima* is a salt-tolerant, perennial form with a white fleshy taproot (Pojar and Mackinnon 2014; Cooke 1997). All four of these species were present at the Chuckanut and California sites, but *Distichlis spicata* was not positively identified at Padden Creek, and so only three species were sampled from there.

Three replicates from each of the four species were sampled per site during low tide so sites were accessible. When possible, the replicates were selected deliberately to capture maximum area of the estuary. The GPS coordinates were recorded to resample the same plants later in the season. In the field, the aboveground tissue was removed to approximately 3 cm above the soil line using a pair of scissors and placed in a separate bag. To remove the crown and root tissue, a soil core, made of a PVC pipe of approximately 10 cm in diameter, was driven to a depth of 18 cm with the help a mallet, and used to extract both the belowground and remaining aboveground plant tissue (Figure 3). Only a portion of each plant sampled was taken, leaving most of it behind for repeated sampling later in the growing season. Samples were then transported to the lab where they were further separated by soil and tissue type. This sampling procedure was completed four times in July, August, October, and November of 2020, approximately six weeks apart.

Table 2: Rank abundance of top ten species at the three study sites chosen. Bolded species were chosen for analysis. Full list of species identified through vegetation surveys at sites listed in supplemental Table 7.

	Total rank	California Creek	Chuckanut Village	Padden Creek
1	<i>Juncus balticus</i>	<i>Salicornia depressa</i>	<i>Juncus balticus</i>	<i>Juncus balticus</i>
2	<i>Salicornia depressa</i>	<i>Distichlis spicata</i>	<i>Triglochin maritima</i>	<i>Triglochin maritima</i>
3	<i>Atriplex patula</i>	<i>Atriplex patula</i>	<i>Elymus mollis</i>	<i>Atriplex patula</i>
4	<i>Triglochin maritima</i>	<i>Rosa nutkana</i>	<i>Atriplex patula</i>	<i>Grindelia integrifolia</i>
5	<i>Distichlis spicata</i>	<i>Carex lyngbyei</i>	<i>Symphyotrichum subspicatum</i>	<i>Plantago maritima</i>
6	<i>Elymus mollis</i>	<i>Cuscuta salina</i>	<i>Convolvulus arvensis</i>	<i>Argentina egedii</i>
7	<i>Symphyotrichum subspicatum</i>	<i>Plantago maritima</i>	<i>Argentina egedii</i>	<i>Plantago lanceolata</i>
8	<i>Grindelia integrifolia</i>	<i>Elymus repens</i>	<i>Cirsium</i> sp.	<i>Salicornia depressa</i>
9	<i>Argentina egedii</i>	<i>Juncus balticus</i>	<i>Distichlis spicata</i>	<i>Spergularia canadensis</i>
10	<i>Plantago maritima</i>	<i>Grindelia integrifolia</i>	<i>Lonicera involucrata</i>	<i>Matricaria discoidea</i>



Figure 2: Plant species selected for bioaccumulation study. Clockwise from top left: *Atriplex patula* (marsh orache), *Distichlis spicata* (salt grass), *Plantago maritima* (sea plantain), and *Juncus balticus* (Baltic rush).



Figure 3: Removal of aboveground plant tissue (left) and driving soil corer into ground (right). Photos taken at California Creek estuary, October 2020.

2.5 Sample Preparation

2.5.1 Aboveground Tissue

Plants were separated into leaf/stem tissue and inflorescence/seeds using forceps and scissors. Visible dirt was rinsed off from the leaves using deionized water. All aboveground tissue was dried in a drying oven at 75°C for 72 hours. All plant samples, including leaves, roots, and inflorescence, were ground up in a Mueller Austria HyperGrind Precision coffee grinder mill. The grinder was thoroughly rinsed with deionized water and dried between each sample.

2.5.2 Belowground Tissue and Soil

Soil cores containing roots were dried in the drying oven at 70°C for 72 hours. After drying, cores were placed into a sieve which allowed finer soil particles to filter through, and large roots were manually extracted using forceps. Larger chunks of soil were ground up using a

mortar and pestle so roots contained in the soil could be manually extracted and included with the large roots. Any rock present in the sample that was gravel-sized or larger was discarded. Care was taken to gently extract the roots to not disturb any metals sorbed to the exterior root tissue (phytostabilization).

2.6 Digestion

All samples underwent some form of digestion to prepare for analysis of metal concentrations via ICP-MS. Both plant and soil samples underwent a total digestion, which is intended to target all metals found within the samples and release them into solution. In addition, the soil samples underwent a four-step sequential extraction digestion, which exposes the samples to progressively stronger solutions which extract metals at different bioavailabilities.

2.6.1 Plant Total Digestion

Following grinding, the plant tissue was acid digested. Approximately 0.25 g of each sample was weighed using an analytic balance, and the exact weight was recorded to four significant figures. This sample was then combined with 9 mL of trace metal grade concentrated nitric acid (approximately 16 M). The samples were microwaved in a Milestone EZ Pro microwave digestion system. For the plant samples, the 'beech leaves' program was used, which consisted of raising the temperature to 180°C over 10 minutes, and then maintenance of the temperature at 180°C for 10 minutes. Between each round of sample digestion, a quick cleaning procedure was used on the vessels, consisting of 9 mL of nitric acid heated to 160°C over 10 minutes. After the rotors cooled, the inner vessels were rinsed thoroughly with deionized water and left to air dry.

2.6.2 Soil Sequential Extraction

To quantify the concentrations of metals found in each fraction of soil for analysis, a sequential extraction method was used. The sequential extraction procedure outlined in Tessier et al. (1979) was followed. The fractions analyzed were: exchangeable, bound to carbonates, bound to iron and manganese oxides, and bound to organic matter. The order of samples in the digestion process was randomized at each step. Approximately 1 gram of soil was weighed with an analytic balance, and the exact mass was recorded to four significant figures. Following each step of the sequential extraction process, samples were centrifuged at 4500 g for one hour. The supernatant was pipetted off using a plastic transfer pipette into a clean 50 mL centrifuge tube. 8 mL of deionized water was then added to the soil samples, briefly agitated, then centrifuged again at 4500 g for one hour. This step was performed as a rinse to ensure that reactions from the previous solution would not carry over into the next. The supernatant from this second rinse was pipetted off and discarded.

For the first step, 8 mL of 1 M magnesium chloride solution was used to free the exchangeable metals in the soil samples. The magnesium chloride solution was prepared using a trace metal grade magnesium chloride salt and deionized water. After the addition of the magnesium chloride, the samples were continuously agitated in 50 mL centrifuge tubes for one hour. The samples were then centrifuged following the above procedure. The first supernatant was diluted to 50 mL, acidified to 2% using trace metal grade nitric acid, and then a portion of this was diluted to a 1:20 dilution with deionized water and 2% nitric acid to keep total dissolved solids (TDS) under 0.5% for the ICP-MS.

To target the metals bound to carbonates, 8 mL of a 1 M sodium acetate solution was used. The 1 M sodium acetate solution was prepared from trace metal grade sodium acetate salt

and deionized water. The solution was brought to a pH of 5 with acetic acid. The samples were continuously agitated for five hours using a test tube mixer. Upon completion, the samples were centrifuged following the same process as above. The supernatant from the first round of centrifuging was diluted to 50 mL and acidified to 2% nitric acid, and diluted further to a 1:20 dilution prior to analysis with ICP-MS.

To release metals bound to iron and manganese oxides, a solution of 0.04 M hydroxylamine hydrochloride in 25% (v/v) acetic acid was used. The solution was prepared with trace metal grade glacial acetic acid, deionized water, and hydroxylamine hydrochloride salts. 20 mL of this solution was added to the soil samples. The samples were placed in a water bath maintained at 96°C for 6 hours. The samples were agitated approximately every 30 minutes. After centrifuging, the supernatant was diluted to 50 mL and acidified to 2% with nitric acid, and then diluted to 1:100 for ICP-MS.

To extract the metals bound to organic matter in the soil, 5 mL of trace metal grade 30% hydrogen peroxide, brought to a pH of 2 with nitric acid, and 3 mL of 0.02M nitric acid, were added to the samples. The samples were then placed in a water bath kept at 85°C for 2 hours and agitated every 30 minutes. After 2 hours, an additional 3 mL of the hydrogen peroxide solution was added to the samples, which were returned to the water bath for an additional 3 hours and agitated every 30 minutes. Following this process, the samples were removed from the water bath and allowed to cool to room temperature, which took approximately 90 minutes. Once samples were cooled, 5 mL of a 3.2 M ammonium acetate in 20% (v/v) nitric acid solution was added. The ammonium acetate solution prevented readsorption of the metals ions onto the soil, keeping them in solution (Tessier et al. 1979). The solution was brought to 20 mL with deionized water, and continuously agitated with a test tube mixer for 30 minutes. Afterwards, the samples

were centrifuged using the same process as above. The supernatant was diluted to 50 mL. The samples were diluted to a 1:100 ratio with deionized water and acidified to 2% prior to ICP-MS.

2.6.3 Total Soil Digestion

In addition to the sequential extraction, a total soil digestion on all samples was completed. For this purpose, *aqua regia* was used, consisting of 3 mL of hydrochloric acid (HCl) and 9 mL of nitric acid. Approximately 0.25 g of soil was weighed, and the exact weight was recorded to four significant figures. These soil samples were digested in the Milestone EZ Pro high-pressure rotors, which can accommodate the exothermic and volatile reaction of these two acids. The 'HP Soil Digest' microwave program was used, which raised the temperature to 220°C over the course of 5 minutes, then maintained the samples at 220°C for 10 minutes. Between each round of sample digestion, a quick cleaning procedure on the vessels was completed, consisting of 9 mL of nitric acid heated to 160°C over 10 minutes. After the rotors cooled, the inner vessels were rinsed thoroughly with deionized water and left to air dry.

2.7 ICP-MS Analysis and Calculations

All samples were analyzed for trace metals using inductively coupled plasma mass spectrometry (ICP-MS). The samples were analyzed for magnesium-25, aluminum-27, chromium-52, manganese-55, iron-56, iron-57, copper-63, zinc-66, arsenic-75, selenium-78, silver-107, cadmium-111, lead-207, and lead-208. Calibration standards were prepared from the ICP-MS-6020-CAL-R-1 standard produced by AccuStandard, containing 10 µg/mL of the above metals. The calibration standards encompassed concentrations from 0.1 ppb to 1000 ppb of each metal in a solution of 5% nitric acid, and for each sequential extraction step, an additional set of calibration standards were made using an identical matrix to the samples. Both quantification limits and method detection limits were measured and calculated for each analysis and with each

solution used in sequential extraction (“Definition and Procedure for the Determination of the Method Detection Limit, Revision 2” 2016). To mitigate common interferences with iron-56 and arsenic-75 (in the case of total soil digestion), these elements were analyzed in helium mode, which adds a helium reaction gas to the analysis (May and Wiedmeyer 1998). The purpose of this is to reduce the number of polyatomic ions with the same weight to charge ratio as the elements of interest from reaching the detector. Concentration measurements of each metal were converted from $\mu\text{g/L}$ into milligrams of metal per kilogram of plant tissue or soil (mg/kg), using the following equation:

$$C_{metal} \left(\frac{\text{mg}_{metal}}{\text{kg}_{plant\ or\ soil}} \right) = C_{sample} \left(\frac{\mu\text{g}_{metal}}{L_{solution}} \right) \times L_{sample} \times DF \left(\frac{L_{solution}}{L_{sample}} \right) \times \frac{1}{M_{plant\ or\ soil\ (g)}}$$

Where C_{metal} is the concentration of metal in the sample per kilogram of plant tissue or soil, C_{sample} is the concentration of metal measured in the sample by the ICP-MS; L_{sample} is the volume of acid or leachant used to digest the sample, DF is the dilution factor representing a ratio between $L_{solution}$ and L_{sample} , $L_{solution}$ is the final volume of the diluted sample, and $M_{plant\ or\ soil}$ is the initial mass of the plant or soil sample, weighed prior to digestion.

2.8 Statistical analysis

2.8.1 Metal load and distribution in sites

A simple one-way analysis of variance (ANOVA) was used to compare metal concentration, expressed by the metal concentrations from the total soil digestion, between sites. Prior to analysis, the assumption of normality was checked using a histogram and Shapiro-Wilk test on the response variable. The assumption of equal variance was verified using box plots and Levene’s test on the sites. If data exhibited significant non-normality or unequal variance, data transformations were applied and assumptions were re-checked. If ANOVA indicated significant

differences in soil, metal concentration by site, a Tukey's HSD post-hoc test was performed to identify which sites were the cause of this difference.

2.8.2 Metal accumulation by plant species

To compare species accumulation across sites, which had varying concentrations of metals in the soil, the bioaccumulation factor (BAF) of each sample replicate was calculated. The equation for BAF is as follows:

$$BAF = \frac{C_{plant}}{C_{soil}}$$

Where C_{plant} represents the total metal concentration within the plant (the sum of the concentration in the roots, leaves and stem, and inflorescence), in mg of metal per kg of plant tissue, and C_{soil} represents the metal concentration determined from the total soil digestion in mg of metal per kg of soil. Since this is a ratio, a BAF of greater than 1 indicates that the plant contains more metal compared to the surrounding soil, and a BAF less than 1 indicates less metal in the plant compared to the soil.

Data for the sampling events in July and August, when plant metal concentrations were highest during this study period, were used to compare BAFs of each metal by species. Species were compared using linear mixed models in an iterative fitting process, incorporating random intercepts for site or replicate if they improved model fit, using the “nlme” package in R (Pinheiro et al. 2021). Goodness of model fit was evaluated using Akaike information criterion (AIC), predicted vs fitted plots, and comparison of root mean square error (RMSE) and mean absolute error (MAE). Assumptions of linear mixed models were verified before selecting the final model, using histograms, Q-Q plots, and the Shapiro-Wilk test to evaluate normality of residuals, and residuals vs fitted plots to evaluate equal variance. Covariance structures (using “varIdent” function in “nlme”) were added to correct unequal variance, and the response variable

was transformed as needed to address non-normality. Post-hoc testing, using Tukey’s HSD, was also performed when species means showed significant difference from the intercept (*A. patula*). To determine if plant species met the criteria for a hyperaccumulator (see definition of a hyperaccumulator in introduction), total metal concentrations were compared to a “reference plant”, as determined by Makert (1991).

2.8.3 Change in metal concentration over time

To determine the fate of these metals from the end of the growing season into plant senescence, the translocation factor (TF) for each replicate was calculated as follows:

$$TF = \frac{C_{aboveground}}{C_{belowground}}$$

Where $C_{aboveground}$ represents the sum of the metal concentrations measured in leaf, stem, and inflorescence (if applicable), measured in mg of metal per kg of aboveground plant tissue, and $C_{belowground}$ represents the metal concentration measured in the root tissue, measured in mg of metal per kg of root tissue. A translocation factor above 1 indicates a net movement of metal aboveground, and a translocation factor below 1 indicates a greater proportion of metal in the root tissue.

Translocation factors across all four sampling events were compared for differences in date of sampling event and species using linear mixed models, in a similar iterative fitting process seen for BAFs. An interaction between date and species and random intercepts for site and replicate were included if they improved model fit. The optimal model was selected using a combination of AIC, predicted vs observed plots, and RMSE/MAE. Assumptions of normality and equal variance were verified for each model, and covariance structures and/or data transformations were applied as needed to meet assumptions.

To further understand different trends in metal accumulation between plant parts, date, and species, graphs using locally weighted smoothing (LOESS) were produced. The nonlinear nature of LOESS makes it difficult to evaluate these relationships statistically but allow for more nuance than the linear mixed model.

3. Results

3.1 Metal load in Whatcom County estuaries

With the exception of cadmium, there were significant differences in soil metal concentrations (mg/kg) for each metal analyzed across the three sites evaluated (Figure 4, Table 3). California Creek and Padden Creek had similar mean concentrations for all metals aside from chromium. Both sites typically had significantly higher metal concentrations than Chuckanut Village (Tukey HSD, $p < 0.05$; Table 3).

Sites also showed variation compared to the expected background concentrations found in the region (San Juan 1994). Both California and Padden Creek estuaries were measured above the median background concentration for chromium, manganese, copper, zinc, and arsenic, and above the 90th percentile of soils for cadmium and selenium. Chuckanut's medians were under the background levels except for lead, which was above the median background concentration in the region (San Juan 1994). Lead was also measured above the background concentration in the other two sites.

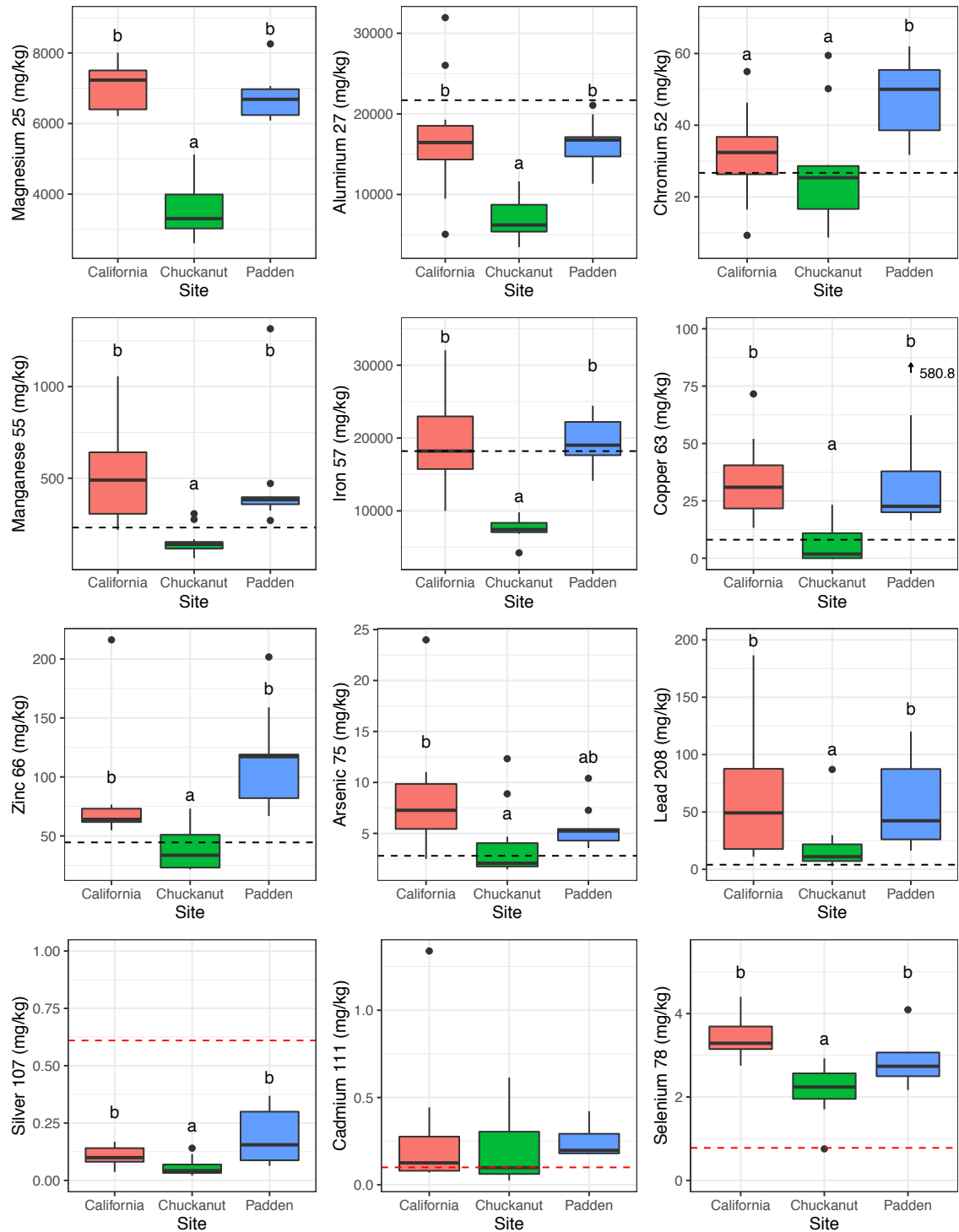


Figure 4: Boxplots of soil metal concentrations, measured via total digestion, at three estuary sites in Whatcom County. Letters indicate significant differences identified through post-hoc Tukey HSD ($\alpha = 0.05$). Black dashed line illustrates regional median background soil concentrations as reported in San Juan 1994, and red dashed line indicates regional 90th percentile background soil concentrations.

Table 3: Results of analysis of variance (ANOVA) of soil metal concentration by site, in mg of metal per kg of soil, along with site mean and standard error. Transformations of response variable applied as needed to meet assumptions of test. Significant results followed up with post hoc Tukey HSD, with superscript letters indicating statistically significant differences ($p < 0.05$). Full results reported in Table 7.

	California mean \pm SE	Chuckanut mean \pm SE	Padden mean \pm SE	F-value	p-value
Magnesium-25 [†]	7065.3 \pm 190.0 ^b	3392.3 \pm 250.5 ^a	6736.3 \pm 223.0 ^b	70.8 _(2,30)	<0.001
Aluminum-27	16959.2 \pm 2013.1 ^b	7029.8 \pm 699.7 ^a	16357.8 \pm 1016.6 ^b	15.9 _(2,30)	<0.001
Chromium-52	32.0 \pm 3.7 ^a	27.1 \pm 4.2 ^a	47.3 \pm 3.7 ^b	6.5 _(2,30)	0.0045
Manganese-55 ^γ	538.2 \pm 82.2 ^b	154.3 \pm 20.0 ^a	474.1 \pm 106.7 ^b	23.1 _(2,30)	<0.001
Iron-57 ^γ	19637.5 \pm 1860.6 ^b	7572.4 \pm 413.1 ^a	19464.8 \pm 1141.7 ^b	51.4 _(2,30)	<0.001
Copper-63 [°]	34.2 \pm 4.8 ^b	5.8 \pm 2.3 ^a	91.8 \pm 61.3 ^b	6.6 _(2,30)	0.004
Zinc-66 ^γ	78.1 \pm 12.7 ^b	40.2 \pm 5.4 ^a	115.0 \pm 14.3 ^b	20.7 _(2,30)	<0.001
Arsenic-75 ^γ	8.7 \pm 1.6 ^b	3.7 \pm 1.0 ^a	5.5 \pm 0.7 ^{ab}	8.8 _(2,30)	<0.001
Selenium-78	3.4 \pm 0.2 ^b	2.2 \pm 0.1 ^a	2.9 \pm 0.2 ^b	14.4 _(2,30)	<0.001
Silver-107 ^γ	0.1 \pm 0.01 ^b	0.06 \pm 0.01 ^a	0.2 \pm 0.04 ^b	12.2 _(2,30)	<0.001
Cadmium-111 ^γ	0.3 \pm 0.1	0.2 \pm 0.05	0.2 \pm 0.03	1.9 _(2,30)	0.168
Lead-208 ^γ	61.7 \pm 15.8 ^b	18.6 \pm 6.7 ^a	53.8 \pm 12.5 ^b	7.7 _(2,30)	0.002

[†] response variable squared to meet assumptions of ANOVA

^γ response variable log transformed to meet assumptions of ANOVA

[°] response variable square root transformed to meet assumptions of ANOVA

3.3 Differences in bioaccumulation by species

Some metals were not bioaccumulated in sizeable quantities (as defined by a BAF < 1.0), specifically aluminum, chromium, iron, and arsenic. Other metals, such as magnesium, manganese, copper, zinc, selenium, silver, and cadmium were bioaccumulated, and differences were identified between month and species (all statistics reported in Table 4; full model results in

Supplementary Table 9 and Supplementary Table 12). In July, *A. patula* and *P. maritima* were successful accumulators of magnesium, with both predicted and measured means of BAF greater than 2. Bioaccumulation of magnesium exhibited in these two species was significantly greater than *D. spicata* (Tukey HSD, $p < 0.05$; Table 4). Both *A. patula* and *P. maritima* also showed significantly higher BAFs for zinc and cadmium compared to the other plant species ($p < 0.05$). *Distichlis spicata* generally accumulated the lowest concentrations of metals among all four species, but did have a mean BAF greater than 4 for both selenium and silver. The greatest BAF values (>3.9) were found in *J. balticus* for selenium, silver, and cadmium in July, although due to high variation within the data, these differences were not statistically significant ($p > 0.05$ for all pairwise comparisons).

In August, *A. patula* and *P. maritima* remained the two species with the highest magnesium BAF, which was statistically significant (Tukey HSD, $p < 0.05$; Table 4; full statistics reported in Supplementary Table 10). These two plant species exhibited increased zinc and cadmium BAFs between July and August, which were significantly higher than *D. spicata* for cadmium, and significantly higher than both *D. spicata* and *J. balticus* for zinc (Tukey HSD, $p < 0.05$). *D. spicata* accumulation followed a similar pattern to July, exhibiting the lowest accumulation among species. This was particularly apparent for magnesium, zinc, and cadmium. *Juncus balticus*, which had stood out as an effective bioaccumulator in July, had a notable decrease for the month of August with all BAFs dropping to less than 2 for all metals. Due to the higher standard error present in the July data, most of the significant differences between species were identified in the August data, as seen in Table 4.

All four species had selenium concentrations 100 times larger than the reference plant (Table 5). While no other metal concentrations were quite this high, all plants had metal

concentrations greater than the reference plant except for manganese (*A. patula*, *D. spicata*, *P. maritima*) and silver (*A. patula* and *D. spicata*).

Table 4: Calculated mean and standard error of bioaccumulation factor (BAF) for each species and metal. Different letters indicate statistically significant differences in means ($p < 0.05$), identified through linear mixed model evaluation and post-hoc Tukey HSD analyses. All statistics reported in supplemental Tables 9 through 14.

	<i>Atriplex patula</i> Mean \pm SE	<i>Distichlis spicata</i> Mean \pm SE	<i>Juncus balticus</i> Mean \pm SE	<i>Plantago maritima</i> Mean \pm SE
Magnesium 25				
July	2.36 \pm 0.38 ^b	1.11 \pm 0.18 ^a	1.67 \pm 0.19 ^a	2.59 \pm 0.47 ^b
August	3.56 \pm 0.75 ^c	1.02 \pm 0.19 ^a	1.05 \pm 0.11 ^a	2.40 \pm 0.42 ^b
Aluminum 27				
July	0.39 \pm 0.15 ^a	0.23 \pm 0.07 ^{bc}	0.35 \pm 0.04 ^c	0.19 \pm 0.05 ^b
August	0.19 \pm 0.05 ^{ab}	0.14 \pm 0.02 ^a	0.25 \pm 0.05 ^b	0.22 \pm 0.02 ^b
Chromium 52				
July	0.83 \pm 0.34 ^a	0.77 \pm 0.11 ^c	0.67 \pm 0.07 ^c	0.32 \pm 0.07 ^b
August	0.24 \pm 0.04 ^a	0.39 \pm 0.07 ^a	0.35 \pm 0.09 ^a	0.38 \pm 0.08 ^a
Manganese 55				
July	0.77 \pm 0.30 ^{ab}	0.48 \pm 0.16 ^a	1.10 \pm 0.17 ^b	0.40 \pm 0.05 ^a
August	0.64 \pm 0.24 ^{ab}	0.81 \pm 0.50 ^a	1.30 \pm 0.46 ^b	0.42 \pm 0.06 ^{ab}
Iron 57				
July	0.49 \pm 0.18 ^a	0.29 \pm 0.08 ^{bc}	0.38 \pm 0.03 ^c	0.23 \pm 0.05 ^b
August	0.19 \pm 0.04 ^{ab}	0.18 \pm 0.04 ^a	0.27 \pm 0.05 ^b	0.23 \pm 0.03 ^b
Copper 63				
July	1.94 \pm 1.03 ^a	2.56 \pm 1.07 ^{ab}	1.86 \pm 0.31 ^{ab}	1.29 \pm 0.24 ^b
August	0.70 \pm 0.18 ^a	0.96 \pm 0.54 ^a	0.71 \pm 0.13 ^a	0.99 \pm 0.28 ^b
Zinc 66				
July	2.54 \pm 0.84 ^a	2.45 \pm 0.68 ^a	2.85 \pm 0.41 ^a	1.94 \pm 0.26 ^a
August	3.02 \pm 1.07 ^{ab}	2.11 \pm 0.81 ^a	1.65 \pm 0.35 ^a	3.45 \pm 0.70 ^b
Arsenic 75				
July	0.92 \pm 0.37 ^a	0.47 \pm 0.17 ^b	0.61 \pm 0.07 ^b	0.49 \pm 0.04 ^b
August	0.31 \pm 0.06 ^{ab}	0.24 \pm 0.06 ^a	0.46 \pm 0.09 ^a	0.59 \pm 0.15 ^b
Selenium 78				
July	4.25 \pm 1.10 ^a	5.97 \pm 2.17 ^a	13.30 \pm 7.34 ^a	6.04 \pm 1.72 ^a
August	4.09 \pm 3.67 ^{ab}	1.36 \pm 0.94 ^a	1.52 \pm 0.90 ^a	2.68 \pm 1.22 ^b
Silver 107				
July	2.66 \pm 1.06 ^a	3.04 \pm 0.94 ^{ab}	11.17 \pm 6.04 ^b	3.67 \pm 0.99 ^{ab}
August	2.00 \pm 0.42 ^b	1.14 \pm 0.28 ^a	1.22 \pm 0.38 ^a	2.66 \pm 1.13 ^{ab}
Cadmium 111				
July	3.48 \pm 1.42 ^a	2.53 \pm 0.99 ^a	9.34 \pm 4.07 ^b	3.68 \pm 1.17 ^{ab}
August	9.11 \pm 6.22 ^{bc}	1.64 \pm 0.93 ^a	4.40 \pm 2.64 ^{ab}	3.41 \pm 0.70 ^c
Lead 208				
July	1.66 \pm 1.11 ^a	0.79 \pm 0.43 ^{ab}	1.11 \pm 0.27 ^b	0.22 \pm 0.05 ^b
August	0.29 \pm 0.14 ^a	0.37 \pm 0.22 ^a	0.29 \pm 0.06 ^a	0.34 \pm 0.08 ^a

Table 5: Mean and standard error of metal concentrations (mg/kg) of study species compared to reference plant concentrations, as established in Makert 1991. A concentration greater than one hundred times the reference plant is a criterion of a hyperaccumulator (Wei et al. 2004).

Metal	<i>A. patula</i>	<i>D. spicata</i>	<i>J. balticus</i>	<i>P. maritima</i>	Ref.
Mg-25	24,591.1 ± 3,926.8	6,223.3 ± 1,024.3	10,725.7 ± 1,195.7	21,996.0 ± 2,271.6	2000
Shoots	18,677.1 ± 1,740.1	3,100.6 ± 497.3	6,066.6 ± 1,017.9	15,497.6 ± 2,466.6	
Roots	4,440.6 ± 453.0	3,685.2 ± 572.5	4,996.1 ± 319.0	6,5098.8 ± 475.3	
Al-27	2,669.1 ± 674.4	2,335.5 ± 558.1	5,009.9 ± 1,091.9	3,528.5 ± 593.9	80
Shoots	199.2 ± 27.2	267.4 ± 73.1	1,089.2 ± 510.2	784.9 ± 259.2	
Roots	2,673.3 ± 700.3	2,163.7 ± 565.0	3,981.3 ± 700.5	2,832.4 ± 445.2	
Cr-52	11.5 ± 3.0	16.6 ± 3.1	16.7 ± 3.2	14.6 ± 1.4	1.5
Shoots	2.0 ± 0.2	8.6 ± 1.5	5.7 ± 1.7	4.4 ± 0.9	
Roots	10.2 ± 3.0	7.9 ± 1.8	11.3 ± 1.8	10.4 ± 1.1	
Mn-55	207.2 ± 43.7	186.6 ± 36.9	412.8 ± 89.4	197.5 ± 27.0	200
Shoots	69.5 ± 16.44	53.6 ± 13.5	218.1 ± 47.4	65.4 ± 14.9	
Roots	131.2 ± 34.4	147.2 ± 28.8	206.9 ± 52.6	137.4 ± 20.8	
Fe-57	2,821.4 ± 693.5	3,004.8 ± 569.7	6,353.0 ± 1,499.4	4,068.2 ± 581.2	150
Shoots	297.0 ± 32.0	424.6 ± 95.9	1,476.5 ± 687.7	1,076.0 ± 328.7	
Roots	2,727.0 ± 716.8	2,670.5 ± 574.7	4,958.5 ± 941.6	3,085.5 ± 380.6	
Cu-63	22.1 ± 3.2	18.2 ± 2.6	29.7 ± 4.1	56.4 ± 5.5	10
Shoots	11.0 ± 1.9	6.7 ± 1.5	11.5 ± 2.6	33.0 ± 5.3	
Roots	12.5 ± 2.2	12.5 ± 1.5	18.8 ± 2.3	24.1 ± 2.8	
Zn-66	214.5 ± 55.7	119.4 ± 12.7	169.3 ± 18.8	216.5 ± 10.2	50
Shoots	136.9 ± 49.2	46.3 ± 7.6	98.1 ± 14.1	99.5 ± 13.0	
Roots	78.6 ± 13.0	79.8 ± 8.0	76.7 ± 6.3	117.2 ± 13.4	
As-75	1.5 ± 0.4	1.7 ± 0.3	6.0 ± 1.1	2.9 ± 0.3	0.1
Shoots	0.2 ± 0.1	0.3 ± 0.1	1.2 ± 0.5	1.1 ± 0.2	
Roots	1.4 ± 0.4	1.5 ± 0.3	4.8 ± 0.7	1.8 ± 0.2	
Se-78	4.3 ± 0.8	6.1 ± 1.6	6.8 ± 1.4	8.2 ± 1.6	0.02
Shoots	3.3 ± 0.7	6.2 ± 1.7	3.7 ± 0.8	7.4 ± 1.6	
Roots	1.3 ± 0.8	0.8 ± 0.2	3.3 ± 0.8	0.7 ± 0.2	
Ag-107	0.3 ± 0.04	0.2 ± 0.03	0.3 ± 0.1	0.3 ± 0.04	0.2
Shoots	0.1 ± 0.02	0.1 ± 0.02	0.2 ± 0.04	0.2 ± 0.03	
Roots	0.1 ± 0.02	0.1 ± 0.02	0.2 ± 0.03	0.1 ± 0.02	
Cd-111	0.7 ± 0.1	0.3 ± 0.1	0.9 ± 0.2	0.5 ± 0.1	0.05
Shoots	0.5 ± 0.2	0.1 ± 0.04	0.3 ± 0.1	0.2 ± 0.1	
Roots	0.3 ± 0.1	0.2 ± 0.03	0.7 ± 0.2	0.3 ± 0.03	
Pb-208	6.6 ± 2.3	6.7 ± 1.1	26.3 ± 6.8	6.3 ± 0.9	1
Shoots	0.8 ± 0.2	0.9 ± 0.3	4.1 ± 2.2	1.7 ± 0.4	
Roots	6.1 ± 2.4	5.6 ± 1.0	22.3 ± 5.6	4.8 ± 0.8	

3.4 Translocation Factor (TF)

There were significant differences in translocation factor (TF) rate of change over time for most metals and plant species (Table 6; Figure 5). *Atriplex patula* and *P. maritima* illustrated a significant decrease in TF for cadmium, copper, magnesium, selenium, silver, and zinc, indicating that metals were being translocated from the aboveground tissue and into the roots from July to November (Figure 5; Panels A-H). Other plant species such as *J. balticus* illustrated an increased TF over time, indicating metals allocated to the aboveground tissues for copper, magnesium, silver, zinc, manganese, iron, and selenium (Figure 5; Panels A-H). The pattern of change in TF was not as clear in either direction or strength for *D. spicata* as it was for the other species. As noted in Section 3.3, *D. spicata* also had lower overall BAFs across the metals studied (Table 4).

The rate of change per month for aluminum, arsenic, chromium, and lead was minimal ($<0.01/\text{month}$), though significant ($p < 0.05$) regardless of plant species, (Figure 5; Panels I-L). These metals were also reported to have low BAFs in the previous section, indicating low concentrations of metals in the plant (Table 4).

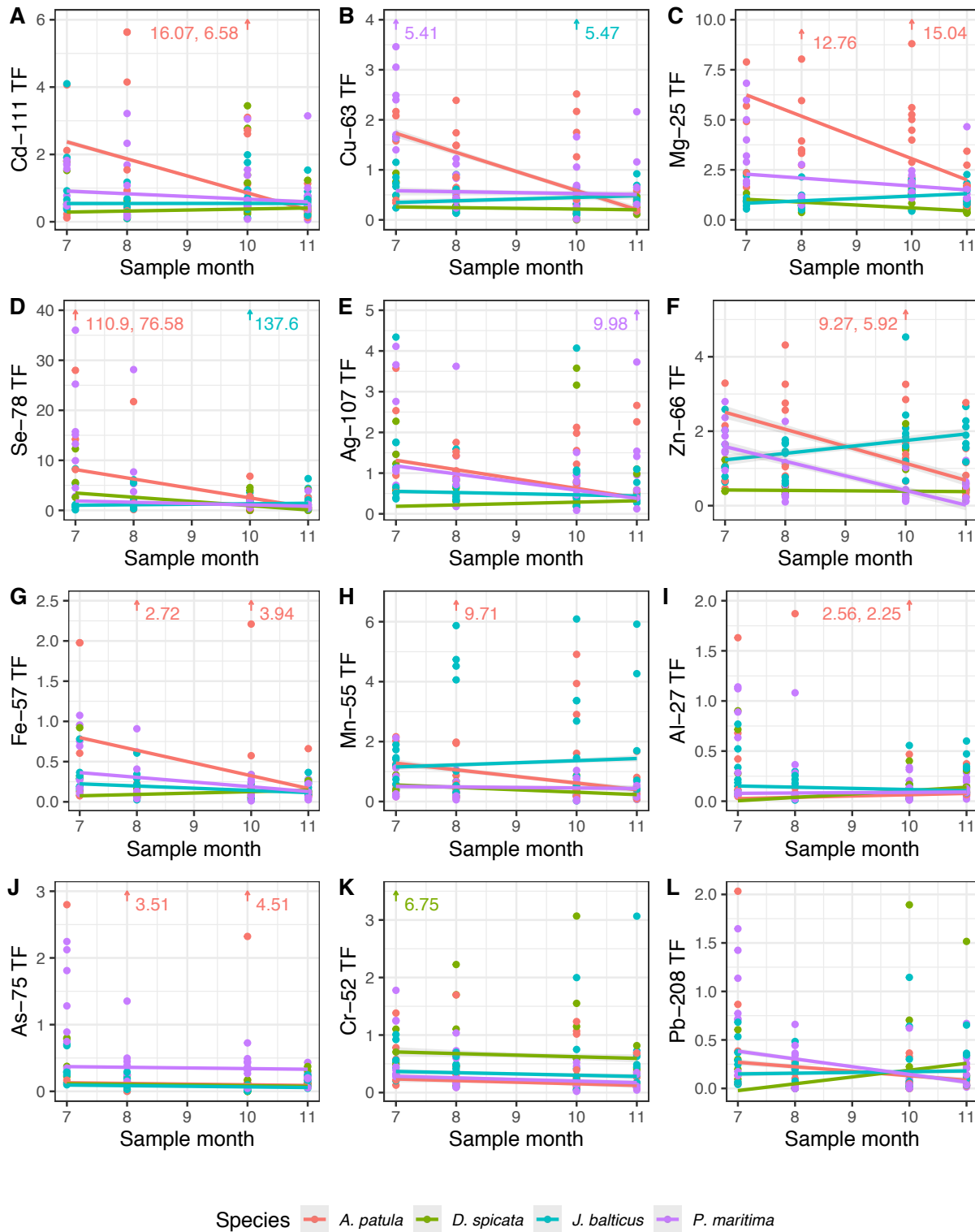


Figure 5: Translocation factor (TF) change over time by date and species. Points show sample translocation factors and lines show predicted change. Slopes of these lines are displayed in Table 6 and the full analysis results reported in Supplemental Table 15.

Table 6: Results of linear mixed model evaluation of translocation factor by date and species, including the interaction of these terms. Asterisks indicate statistical significance ($p < 0.05$) when compared to intercept of model (*A. patula* TF for month of July). Full results available in Table 15.

	<i>A. patula</i> Date	<i>D. spicata</i> Date * <i>D. spicata</i>	<i>J. balticus</i> Date * <i>J. balticus</i>	<i>P. maritima</i> Date * <i>P. maritima</i>
Cadmium-111	2.36 ± 0.20 *	-2.14 ± 0.51 *	-1.82 ± 0.50 *	-1.44 ± 0.53 *
	-0.50 ± 0.13 *	0.53 ± 0.13 *	0.50 ± 0.13 *	0.42 ± 0.14 *
Copper-63	1.71 ± 0.15 *	-1.54 ± 0.13 *	-1.38 ± 0.11 *	-1.13 ± 0.13 *
	-0.37 ± 0.03 *	0.36 ± 0.04 *	0.42 ± 0.04 *	0.35 ± 0.05 *
Magnesium-25	5.59 ± 0.96 *	-4.69 ± 0.96 *	-4.47 ± 0.97 *	-4.45 ± 0.99 *
	-0.93 ± 0.25 *	0.80 ± 0.26 *	0.99 ± 0.26 *	1.13 ± 0.27 *
Selenium-78	8.15 ± 0.09 *	-4.68 ± 0.98 *	-7.13 ± 0.10 *	-6.27 ± 0.89 *
	-1.89 ± 0.03 *	1.04 ± 0.25 *	1.98 ± 0.06 *	1.62 ± 0.26 *
Silver-107	1.31 ± 0.10 *	-1.18 ± 0.12 *	-0.77 ± 0.09 *	-0.12 ± 0.08
	-0.23 ± 0.03 *	0.26 ± 0.03 *	0.21 ± 0.03 *	0.02 ± 0.03
Zinc-66	2.48 ± 0.40 *	-2.23 ± 0.37 *	-1.28 ± 0.37 *	-0.88 ± 0.37 *
	-0.44 ± 0.09 *	0.43 ± 0.09 *	0.64 ± 0.10 *	0.05 ± 0.10
Iron-57	0.79 ± 0.29 *	-0.73 ± 0.29 *	-0.57 ± 0.30	-0.43 ± 0.30
	-0.16 ± 0.08 *	0.17 ± 0.08 *	0.13 ± 0.08	0.10 ± 0.08
Manganese-55	1.26 ± 0.25 *	-0.23 ± 0.24 *	-0.13 ± 0.42	-0.74 ± 0.23 *
	-0.21 ± 0.08 *	0.13 ± 0.09	0.30 ± 0.16	0.19 ± 0.08 *
Aluminum-27	0.02 ± 0.007 *	-0.02 ± 0.001 *	0.13 ± 0.03 *	0.05 ± 0.01 *
	0.01 ± 0.001 *	0.02 ± 0.005 *	-0.02 ± 0.01	-0.01 ± 0.005
Arsenic-75	0.13 ± 0.01 *	-0.02 ± 0.01	-0.03 ± 0.01 *	0.25 ± 0.02 **
	-0.009 ± 0.003 *	-	-	-
Chromium-52	0.22 ± 0.06 *	0.47 ± 0.06 *	0.15 ± 0.02 *	0.05 ± 0.02 *
	-0.03 ± 0.004 *	-	-	-
Lead-208	0.30 ± 0.09 *	-0.31 ± 0.12 *	-0.10 ± 0.11	0.13 ± 0.11
	-0.07 ± 0.03 *	0.14 ± 0.05 *	0.05 ± 0.04	-0.02 ± 0.04

3.5 Change over time

To better understand where in the plant metals are being stored, total concentrations were compared across metals and plant species using locally weighted smoothing (LOESS). For *A. patula* concentrations of magnesium, selenium, and silver, the primary cause of the decreasing TF was a decline in leaf tissue with no increase in other tissues between the months of July and August (Figure 6, panels C, D, E). The negative slope of copper, zinc, iron, and manganese was primarily driven by increasing concentration in the roots, while the leaf concentration did not

change (Figure 6, panels B and F, and Figure 6, panels G and H). Cadmium concentration decreased over time in the inflorescence but stayed relatively constant in other tissues (Figure 6, panel A).

When analyzing *D. spicata* a few patterns became apparent: root, leaf, and inflorescence tissue were generally stable over time with cadmium, magnesium, silver, and zinc concentrations (Figure 6, panels A, C, E, F). Of the elements, selenium seemed to be in greatest concentration in the leaf and flower tissue in July, then dropped significantly into August, then moderately increased from August to November in all parts of the plant (Figure 6, panel D).

Juncus balticus exhibited the lowest metal concentrations in aboveground tissue for the month of August for all metals, dropping steeply after July, with slight to moderate increases in subsequent months. The exception was selenium concentration in inflorescence, which increased from July to November. Silver and copper root concentrations were highest in both July and October (Figure 6, panels B and E). Cadmium peaked in the root tissue in August and was at its highest in leaf tissue in October (Figure 6, panel A). Magnesium, selenium, and zinc concentration in the roots decreased over time (Figure 6, panels C, D and F).

In *P. maritima* tissue, aboveground concentrations of copper, magnesium, selenium, silver, and zinc were highest in July and typically declined significantly in August (Figure 6, panels B-F). Root tissue concentrations of copper, selenium and iron increased over time, while cadmium, silver and manganese stayed relatively consistent during the study period (Figure 6, panels B-E; Figure 6, panels G and H). Magnesium and zinc root concentrations both reached their maximum in August, and mostly plateaued in subsequent months (Figure 6, panels C and F).

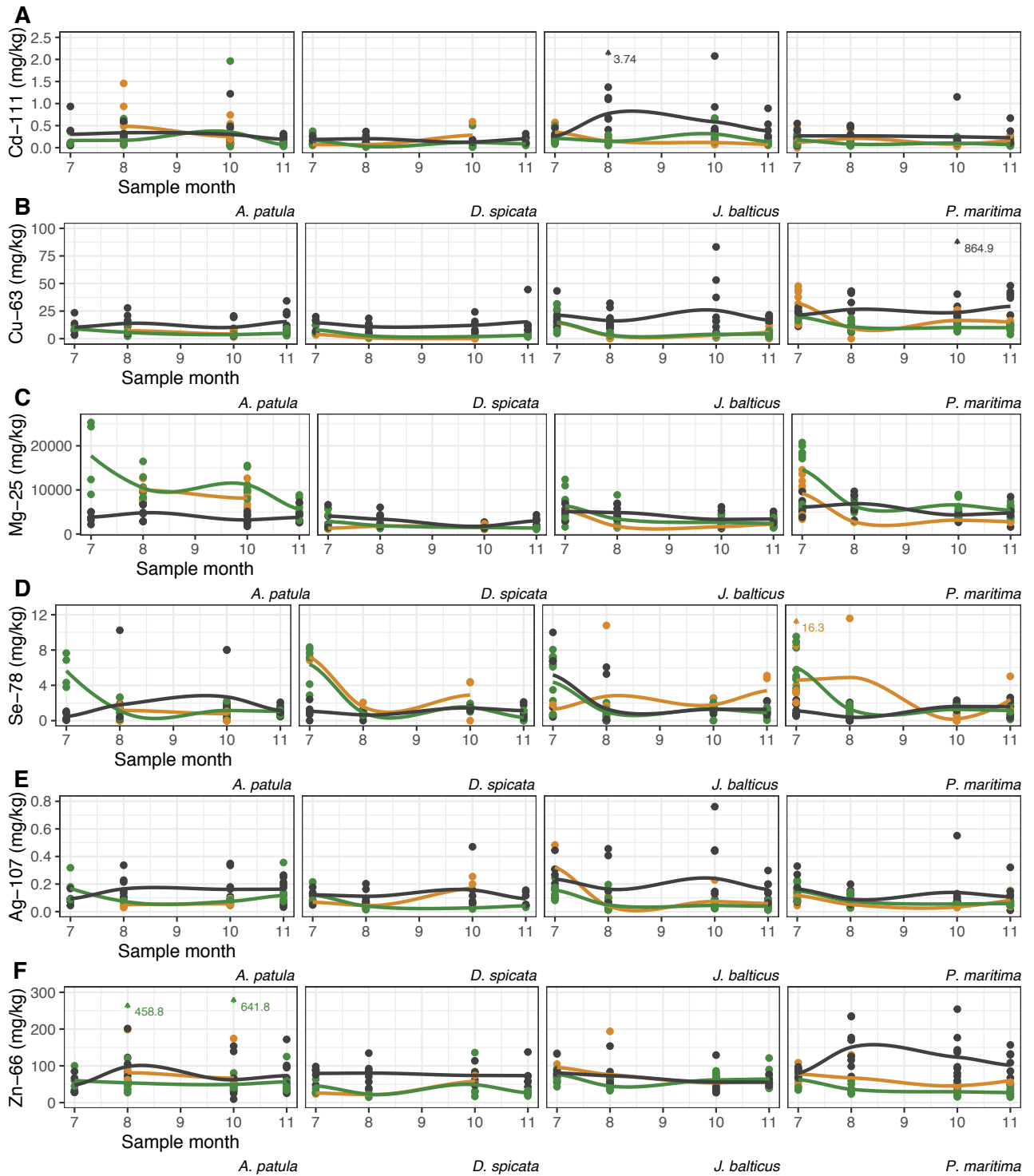


Figure 6: Change in metal concentration by plant part over time, modeled by LOESS (locally weighted smoothing) fit. Outliers indicated with arrows and text.

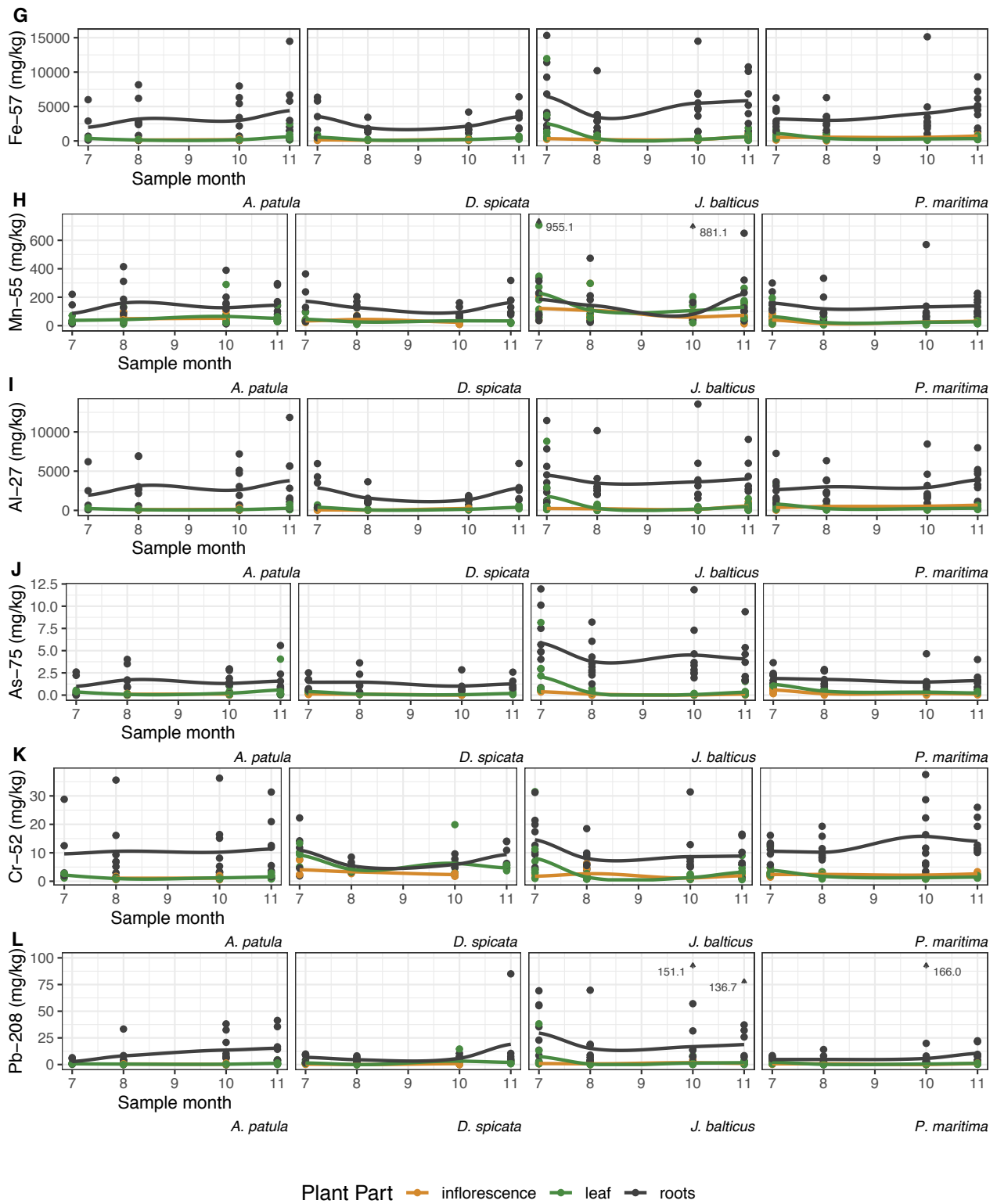


Figure 6 (continued): Change in metal concentration by plant part over time, modeled by LOESS (locally weighted smoothing) fit. Outliers indicated with arrows and text.

4. Discussion

The aims of this study were three-fold: 1) to explore differences in metal concentrations between study sites, 2) compare the bioaccumulation factors of four common native estuary species, and 3) determine changes in translocation factor and plant part concentration over time. I found high levels of several metals in soils at California and Padden Creek estuaries, which were significantly higher than metals at Chuckanut Village estuary. Measured by bioaccumulation factor, I identified significant differences in metal accumulation between the species studied. Finally, my study showed significant differences in translocation factor over the growing season, which was used to measure movement of metals between above and belowground plant tissue. I discuss my findings in detail below.

4.1. Differences in soil metal concentrations between study sites

The analysis demonstrated that, except for cadmium, total metal concentrations in soils significantly differed between sites. Although cadmium was not significantly different by site, all three sites displayed concentrations that were higher than the 90th percentile of expected background concentration (San Juan 1994). Selenium was also measured above the 90th percentile of expected background concentration at all three sites, and lead above the median at all three sites (San Juan 1994).

California and Padden Creek estuaries had similar metal concentrations in the soil, which were above the median background soil concentrations in the region for many elements. Higher metal concentrations for Padden Creek may be due to the surrounding urbanized environment, including some industrial sites. Historically, this site housed sawmills, lumber manufacturing companies, and railroad tracks, all of which could be potential sources of soil contamination (Herrenkohl 2012). Although California Creek, which empties into Drayton Harbor, is more

rural than either of the other two estuaries studied, its watershed is significant and encompasses land with multiple uses, including roadways (such as I-5 which is a major highway), residential properties, forests, and agriculture, which makes up the majority of land in the watershed. No major point sources for metals were identified, but agriculture can be a sizeable generator of nonpoint pollution of metals (Kelepertzis 2014).

Chuckanut Village pocket estuary showed significantly lower concentrations of soil metals that tended to match the median backgrounds for many elements. This estuary has a much smaller watershed than the other sites, and is primarily residential (Figure 1). No major point sources of metal pollution are identified in this area, but it is expected that similar vehicle nonpoint sources of pollution affect this site as in Padden Creek. In 2009, the bridge and culvert from the access road were replaced with a pedestrian-only bridge over a channel, which eliminated vehicle disturbance and facilitated tidal exchange between the salt marsh and the bay. This site also contains a salt marsh, unlike the others studied, with heavy vegetation cover that was present in pre-restoration site characterizations (Wetland Characterization: Chuckanut Village Marsh 2008). This salt marsh area had the lowest proportion of bare ground across all sites when we surveyed in 2020 (Table 6). Pre-restoration vegetation surveys noted the significance of the salt marsh, both for its dense perennial plant cover, seasonal flooding cycles, and organic soils. This all contributes to the improvement of water quality by preventing pollutant runoff, which is considered an invaluable ecosystem service provided by tidal marshes (Wetland Characterization: Chuckanut Village Marsh 2008). This reinforces the importance of vegetation in managing pollutants; the roots of salt marsh plants physically prevent the movement of contaminants through the soil, regardless of their ability to uptake metals, and should be considered when planning estuary remediation and conservation projects.

4.2. Bioaccumulation factor by species

Species' abilities to uptake metals were compared for the months of July and August using their bioaccumulation factors (BAF) to standardize soil conditions of the various sites. Of the metals studied, selenium was the only element that was hyperaccumulated by all four plant species: all had selenium concentrations 100 times greater than the reference species, higher concentrations in shoots compared to roots, and no visible signs of stress. It is worth noting that an ICP-MS interference exists with selenium that could contribute to inaccurately high measurements, but there is no evidence to support that this was the cause of the high selenium identified in this study, rather than the plants hyperaccumulating this element (May and Wiedmeyer 1998). Although no other metals were hyperaccumulated, several metals were taken up by plants in significant concentrations ($BAF > 1$) and could therefore be useful for remediation of these elements. Differences in BAF were identified across species and months, with July typically having higher BAFs than August, and for some metals and species, this difference was large.

4.2.1 *Distichlis spicata*

Distichlis spicata had BAFs greater than 1 for magnesium, copper (July only), zinc, selenium, silver, and cadmium; however, this species had the lower BAFs across metals when compared to *J. balticus*, *A. patula*, and *P. maritima*. Among the species in this study, *D. spicata* stands out for its ability to tolerate highly saline soils by sequestering salt in its vacuoles and using high osmotic pressure to excrete salt onto the surface of its leaves, inflorescences, and stems (Pojar and Mackinnon 2014; Producers and Inskeep 1991). It was notable that *D. spicata* was not present at Padden Creek, likely due to lower sodium concentrations and competition by other plant species (Wisheu and Keddy 1990; Mitsch 2007). Based on the results of my study, *D.*

spicata could be used in the phytoextraction (metal uptake into aboveground tissue) of magnesium, zinc, selenium, silver, and cadmium, as these metals were taken up in modest quantities into the aboveground tissue, although the other species studied were more effective. However, it is significant ecologically for its salt tolerance and an extensive root system that would aid in stabilizing the soil and should be considered for these specific needs.

4.2.2 *Atriplex patula*

Atriplex patula had BAFs greater than 1 for magnesium, copper (July only), zinc, selenium, silver, lead (July only), and cadmium. Along with *P. maritima*, *A. patula* had the highest magnesium, zinc, selenium, and cadmium BAFs compared to the other species. This estuary species is a fast-growing annual, which can colonize a wide variety of soils as a pioneer species and can tolerate a range of environmental stressors (Pojar and Mackinnon 2014). In this study, it was observed growing along the margins of sites and closer to potential inflows of metal contamination such as roadways and railroad tracks. Like other members of the *Atriplex* genus, it is a halophyte, and has been effectively used in remediating salt-contaminated sites (Young et al. 2011). Additional studies on *Atriplex* species support uptake of zinc, copper, cadmium, nickel and lead, primarily into root tissue (Amer et al. 2012; Eissa et al. 2016; Kachout et al. 2009). Based on my results, high concentration noted in aboveground tissue indicates *A. patula* as an effective plant for phytoextraction of selenium, silver, zinc, and magnesium. While uptake was modest for many of the metals studied, its ability to tolerate unfavorable soil conditions in the estuary and rapid growth could meet an important need for land managers.

4.2.3 *Plantago maritima*

Plantago maritima had BAFs greater than 1 for magnesium, copper, zinc, selenium, silver, and cadmium. Studies have identified other members of the *Plantago* genus as metal

hyperaccumulators, particularly for aluminum, zinc, copper, and lead, with evidence that aluminum hyperaccumulation is a genetic trait common among several *Plantago* species (Serrano et al. 2016). However, that was not found in my study; *P. maritima* did not bioaccumulate aluminum. *Plantago maritima* stood out for its ability to accumulate zinc, copper, silver, selenium, and cadmium in its roots, meriting consideration for phytostabilization (metals sequestered in belowground soil and root tissue). It also shows promise in phytoextraction of magnesium, selenium, silver, and cadmium. *Plantago maritima* is a perennial that grows well in sandy, coarse soils, and can tolerate high levels of moisture, which could indicate its use in these specific environments.

4.2.4 *Juncus balticus*

Juncus balticus had BAFs greater than 1 for magnesium, manganese, copper (July only), zinc, selenium, silver, cadmium, and lead (July only) but was most efficient at accumulating manganese and cadmium when compared to the other plant species. Other studies have documented elevated metal concentrations in the roots and rhizosphere of *J. maritimus* and *J. usitatus* compared to the surrounding soil and other species studied (Alimeda et al. 2004; Archer and Caldwell 2004). Further, spatial evaluation of soils showed decreased concentrations of aluminum downstream from *J. effusus*, despite no significant amounts of aluminum in its tissue, which also supports its effectiveness for phytostabilization (Schachtschneider et al. 2017). From the results of my study, *J. balticus* can be considered for phytostabilization of copper and lead. In addition, my results illustrated high tissue concentrations of selenium, silver, cadmium, zinc, manganese, and magnesium, which also makes *J. balticus* an efficient phytoextractor of those elements. The Chuckanut Village salt marsh is dominated by *J. balticus*, which has an extensive root system. Root systems of estuary species, like *J. balticus*, slows the movement of water from

land to ocean, reducing the amount of nutrients and contaminants carried through the soil, and is one strategy for mitigating excess nutrient runoff into waterways (Barbier et al. 2011).

4.2.5 Site Factors influencing BAF

Although differences in BAF between species were observed, there was also a high amount of variability between sites that could be explained by other soil factors beyond metal concentration. Preliminary soil evaluation of the sites showed pH ranging from slightly alkaline at Padden to acidic in California soils. As pH increases and the soil environment becomes more basic, the availability of free metal ions (the most bioavailable form) in solution decreases as metal cations become bound to anions abundant in soil, such as carbonates, which removes them from solution (Liang et al. 2017; Oxtoby et al. 2019). Chuckanut, with a slightly acidic pH (6.1), falls within both the optimal plant tolerance range and increased bioavailability of metal ions in soil, which is likely an important factor in explaining the higher BAFs identified at this site (Petelka et al. 2019).

There are additional factors influencing metal uptake that could explain differences in BAFs between sample replicates, such as salinity. Increased salinity decreases the concentration of free metal ions in solution (Fritioff et al. 2008). As estuarine soils have salinity gradients based on their distance from the intertidal zone, this could explain the variability of metals within the same site and species (Cloern et al. 2017). The presence of other elements can also influence behavior of metals, as studies on *A. undulata* and *A. lentiformis* found that higher levels of phosphorous and chloride, which would be abundant in estuarine soils, reduced translocation of zinc and lead from roots into aboveground tissue due to the precipitation of lead into an insoluble complex that prevents plant uptake (Eissa 2015). There is also likely a relationship between these factors, and interaction between them may also play a role in metal and plant behavior.

Differences in metal uptake could also be due to metal speciation and bioavailability across sites. Although bioavailability of metals may differ between sites due to the above factors, in previous studies not all metals found to be readily available to the plant were accumulated (Alimeda et al. 2004). The different uptake patterns of metals despite bioavailability could be due to the function of these metals in plant metabolism, or similarities to metals that do perform necessary functions. Alimeda et al. (2004) theorized that the significant accumulation of cadmium by *J. maritimus* identified in their study is due to the similar elemental properties and behaviors of cadmium and zinc, and that zinc uptake mechanisms would also collect cadmium. Other metals accumulated in my study, such as selenium, would be taken up via sulfur and oxygen pathways, and silver can be linked to copper.

4.3. Changes in translocation factor and plant part concentration over time

To determine the movement of metals within plant tissue over the course of the study, the translocation factor (TF) was compared between sampling dates and species. The TF represents the ratio of metals within the plant, with higher TF values indicating a larger proportion of metals in the aboveground tissue, and a lower TF corresponding to a higher proportion of metals in the root tissue. Knowing the location of metals within the plant tissue, and how concentrations change within plant parts over the course of the growing season, allows for better planning. If metal removal, or phytoextraction, is the goal, removing the aboveground tissue when TF is highest is most efficient. In my study, changes in translocation factor were evident across both species and sampling dates. *Atriplex patula* and *P. maritima* showed similar patterns in steeply decreasing TFs for metals over time, indicating rapid movement of metals from shoots to roots between each sampling date. In contrast, the decrease in metal TFs for *J. balticus* was less evident indicating slower movement of metals from shoots to roots over time. The TFs of *D.*

spicata stayed relatively consistent over time, as did metal concentrations in roots, shoots, and inflorescence indicating minimal initial uptake and subsequent translocation.

Connecting TF with BAF illustrated some similarities in the patterns across species and date. *Distichlis spicata* had both the lowest BAFs and TFs. Arsenic, aluminum, chromium, and lead showed minimal changes in TF and BAF across all species and dates. By November, average TFs by species were at their lowest, and evaluation by plant part shows that aboveground tissue concentrations were also at their lowest. When considered along with root tissue concentrations, this would suggest that metals were lost to the environment. Root tissue concentrations stayed largely consistent for metals that had both higher BAFs and TFs, or were primarily accumulated aboveground (such as magnesium, selenium, silver, and zinc). This suggests either that metals in the aboveground tissue were lost to the environment as shoot tissue underwent senescence, and that metals in the root tissue remained there, without additional accumulation or translocation. For the metals with lower TFs, root concentrations appeared to increase slightly, but when considered with TF as context, the amount of metals accumulated in root tissue was minimal and this change over time is likely insignificant.

4.3.1 Phytoextraction versus phytostabilization and additional factors influencing fate of metals

Like results seen in prior studies, I found that metals were accumulated differently over time. These variations are thought to be mainly caused by differences in plant physiology across seasons, as well as differences in metal bioavailability by season driven by environmental fluctuations, such as pH and water availability (Galal et al. 2020; Galal et al. 2017). Other studies have noted that peak metal accumulation in aboveground tissue does not always correspond with maximum biomass, concluding that plant removal early in the growing season may be the most efficient way to meet phytoextraction goals (Březinová and Vymazal 2015). Therefore,

identifying a single optimal time and method for metal removal is challenging and depends on remediation priorities, but my results support that removal of aboveground tissue in July for phytoextraction would be effective. Future studies could measure metal concentrations in these plants for the first part of the growing season to see if earlier removal would be warranted.

Benefits of phytoextraction include the physical removal of the metal from the site once metal-contaminated tissue is harvested. Metals collected in this way can even be repurposed, such as through the phytomining of valuable metals (including nickel, gold, or cobalt), or the supplementation of selenium in animal feed (“Introduction to Phytoremediation” 2000; Morse 2020). In cases where plant tissue can only be disposed of as biowaste, phytoextraction is still much less disruptive and expensive than traditional soil remediation methods. However, it does require more active management compared to phytostabilization, as without aboveground tissue removal and disposal, the contaminant will be re-released upon plant senescence (“Introduction to Phytoremediation” 2000). Of additional concern is introducing toxic levels of metals into the food web, so it is recommended to avoid plants that are also significantly consumed or plant species that store metals into inflorescence that could impact pollinators.

Heavily contaminated soils may necessitate the use of phytostabilization over phytoextraction, as toxic levels of metals slow or inhibit plant growth, particularly in aboveground tissue, unless the plants are able to sequester metals in belowground tissue or vacuoles, which improves tolerance (Galal 2020). Furthermore, when contaminants need to be corrected in a shorter timeline, phytostabilization improves measurable soil conditions much more quickly than phytoextraction, as immobilization by plant roots is a faster process than the growth and uptake of phytoextraction (Epelde et al. 2013). Although phytostabilization effectively immobilizes metals, they are not removed from the environment like phytoextraction

would. Current guidelines from the EPA recommend that phytostabilization only be used as a temporary measure, while more permanent removal methods are undertaken (“Introduction to Phytoremediation” 2000). To date, the long-term immobilization of metals in the soil is still relatively unknown, as many studies on the effectiveness of phytostabilization are only one or two years long (Mendez and Maier 2008).

Estuarine soils, however, exhibit regular flooding through tidal activity, which has a sizeable effect on soil oxygen, pH, and salinity, all of which would influence metal behavior as described above (Mitsch 2007). Because of this, we would expect the release of metals bound in organic matter, such as decomposing plant tissue to occur much more slowly under wetland conditions when compared to other soils. It seems reasonable to expect that phytostabilization would be effective for a much longer timeline in an estuarine environment, but this exact timeline remains uncertain, and any remediation projects should include monitoring soil conditions over the long-term to ensure that target metals remain immobile in soil. When considered in conjunction with the physical barriers to contaminant movement provided by plant roots, phytostabilization would offer an important benefit to water quality by lessening the concentration of contaminant reaching the marine environment.

4.4. Conclusions

This study demonstrates that *A. patula*, *J. balticus*, and *P. maritima* are effective for the bioaccumulation of magnesium, selenium, silver, and cadmium, and modestly effective at accumulating copper. *Juncus balticus* was also moderately effective at accumulating manganese. For metals where phytoextraction is the goal, optimal removal time is highly dependent on the metal, but earlier in the growing season would be effective to remove a majority of metals.

Juncus balticus and *P. maritima* also show promise for phytostabilization of selenium, silver, cadmium, zinc, manganese, and magnesium.

The results also support the need for plant community diversity, both for rebuilding healthy estuaries with emphasis on salt marshes and targeting a wider range of metals. Plant community diversity is an essential part of phytostabilization, as the differing niches occupied, root depths and life cycles stabilize the soil and contaminants over time, as well as promoting soil microbiota, some of which can more directly detoxify the soil (Mendez and Maier 2008, Baycu et al. 2015). A balance of different life cycles, such as annuals and perennials, can also benefit these goals. Plant diversity improves the ability of the estuary to meet ecosystem services, including water filtration, animal habitat, and carbon storage through offering different root structures to contact and stabilize a larger area of the soil, varying aboveground structures to offer habitat, and a range of life cycles to offer both short- and long-term solutions.

In considering the endangered species of the Salish Sea, salmon smolt deaths were strongly influenced by the concentrations of cadmium, zinc, and copper present in the water, with researchers finding cumulative effects with combinations of these metals compared to individual metals alone (Lorz et al. 1978). I would hypothesize that phytoextraction and phytostabilization would assist in reducing the amount of these metals in runoff entering water systems, offering benefits throughout the lifecycles of aquatic species that are targeted for conservation. Estuaries with intact tidal marshes may be more efficient in lessening the influx of metals from non-point pollution sources through these pathways, protecting aquatic species during migration. Therefore, lessening the concentrations of metals introduced into the riparian and marine environments, specifically in times of migration, would not only benefit salmon

populations, but would also improve conditions of the estuarine food web and the health of the ecosystem at large.

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6. Supplemental Tables and Figures

Table 7: Full list of plant species identified in vegetation surveys of four Whatcom county pocket estuaries (Ca = California, ChM = Chuckanut marsh, ChB = Chuckanut beach, P = Padden, W = Whatcom). Whatcom Creek estuary was surveyed but dropped from further study. Native status key: N = native, I = introduced, NO = noxious. Plants identified using Pojar and Mackinnon 2014, Hitchcock 1973, and Cooke 1997. Native status found using Pojar and Mackinnon 2014 and USDA Plants Database. Wetland indicator found using Cooke 1997 and USDA Plants database.

Scientific name	Common name	Present at Sites	Native status	Wetland indicator	Rank abundance
-	Bare ground	Ca, ChM, ChB, P, W	-	-	25.3
<i>Juncus balticus</i>	Baltic rush	Ca, ChB, ChM, P	N	FACW	19.9
<i>Atriplex patula</i>	Marsh orache	Ca, ChB, ChM, P	N	FACW	7.1
<i>Salicornia depressa</i>	Pickleweed	Ca, ChB, P	N	OBL	6.6
<i>Distichlis spicata</i>	Salt grass	Ca, ChB	N	FACW	6.3
<i>Argentina ededii</i>	Pacific silverweed	ChB, ChM, P, W	N	OBL	4.8
<i>Triglochin maritima</i>	Seaside arrowgrass	Ca, ChB, ChM, P, W	N	OBL	3.3
<i>Grindelia integrifolia</i>	Puget Sound gumweed	Ca, ChM, P	N	FACW	3.2
<i>Elymus mollis</i>	American dunegrass	Ca, ChB, P	N	FACU	3.1
<i>Puccinellia</i> sp.	Alkali grass	ChM	-	-	2.0
<i>Dactylis glomerata</i>	Orchardgrass	ChM, P	I	FACU	1.8
<i>Holcus lanatus</i>	Common velvetgrass	ChB, ChM	I	FAC	1.7
<i>Agrostis capillaris</i>	Colonial bentgrass	ChM, P	I	FAC	1.6
<i>Elymus repens</i>	Quackgrass	Ca, ChM, P	I	FAC	1.3
<i>Symphotrichum subspicatum</i>	Douglas aster	Ca, ChM, W	N	FACW	1.3
<i>Plantago maritima</i>	Sea plantain	Ca, ChB, P	N	FACW	1.1
<i>Achillea millefolium</i>	Common yarrow	ChB, P	N	FACU	0.7
<i>Asteraceae</i> sp.	Unknown aster	ChB	-	-	0.7
<i>Convolvulus arvensis</i>	Field bindweed	ChB, P	NO	-	0.7
<i>Trifolium dubium</i>	Small hop clover	P	I	FACU	0.6
<i>Cuscuta salina</i>	Salt marsh dodder	Ca	N	-	0.6
<i>Poaceae</i> sp.	Unknown grass	P	-	-	0.5
<i>Cirsium</i> sp.	Thistle species	ChB, W	NO	-	0.5
<i>Rosa nutkana</i>	Nootka rose	Ca, ChB, W	N	FAC	0.5
<i>Plantago lanceolata</i>	Narrowleaf plantain	ChB, P	I	FACU	0.5

<i>Carex lyngbyei</i>	Lyngbye's sedge	Ca, W	N	OBL	0.4
<i>Melilotus officinalis</i>	Sweetclover	Ca, P	I	FACU	0.4
<i>Ambrosia chamissonis</i>	Silver bur ragweed	ChB	N	-	0.3
<i>Asteraceae</i> sp.	Unknown aster	Ca	-	-	0.3
<i>Deschampsia cespitosa</i>	Tufted hairgrass	Ca	N	FACW	0.3
<i>Juncus</i> sp.	Unknown rush	P	-	-	0.3
<i>Sonchus asper</i>	Spiny sowthistle	ChB	I	FACU	0.2
<i>Spergularia canadensis</i>	Canadian sandspurry	Ca, P	N	FACW	0.2
<i>Poaceae</i> sp.	Unknown grass	Ca	-	-	0.2
<i>Cytisus scoparius</i>	Scotch broom	Ca	NO	-	0.2
<i>Typha latifolia</i>	Broadleaf cattail	ChM	N	OBL	0.1
<i>Matricaria discoidea</i>	Pineapple weed	ChB, P	I	FACU	0.1
<i>Phalaris arundinacea</i>	Reed canarygrass	Ca, P	NO	FACW	0.1
<i>Lonicera involucrata</i>	Twinberry	ChM	N	FAC	0.1
<i>Carex pluriflora</i>	Manyflower sedge	ChM	N	OBL	0.1
<i>Poaceae</i> sp.	Unknown grass	P	-	-	0.1
<i>Bolboschoenus maritimus</i>	Cosmopolitan bullrush	P	N	OBL	0.1
<i>Spergularia salina</i>	Salt sandspurry	P	N	OBL	0.1
<i>Camissonia contorta</i>	Evening primrose	Ca	N	-	0.1
<i>Poaceae</i> sp.		P	-	-	0.1
<i>Agrostis exarata</i>	Spike bentgrass	Ca,	N	FACW	0.1
<i>Schoenoplectus acutus var. acutus</i>	Hardstem bulrush	P	N	OBL	0.1
<i>Ammophila arenaria</i>	European beachgrass	Ca,	I	FACU	0.1
<i>Phacelia procera</i>	Tall phacelia	Ca	N	FAC	0.1
<i>Poaceae</i> sp.	Unknown grass	Ch	-	-	0.1
<i>Juncus</i> sp.	Unknown rush	P	-	-	0.1
<i>Hordeum barchyantherum</i>	Meadow barley	Ca, Ch	N	FACW	<0.1
<i>Festuca occidentalis</i>	Western fescue	Ca,	N		<0.1
<i>Galium trifidum</i>	Threepetal bedstraw	ChM	N	FACW	<0.1
<i>Cakile edentula</i>	American searocket	Ca	N	FACU	<0.1
<i>Ribes triste</i>	Red currant	Ca	N	FAC	<0.1
<i>Conioselinum pacificum</i>	Pacific hemlock- parsley	Ca	N	FACW	<0.1
<i>Vicia americana</i>	American vetch	Ca	N	FAC	<0.1
<i>Equisetum arvense</i>	Field horsetail	Ca, P	N	FAC	<0.1
<i>Heracleum maximum</i>	Cow parsnip	ChB	N	FAC	<0.1
<i>Agrostis gigantea</i>	Redtop	Ca, W	I	FAC	<0.1
<i>Epilobium ciliatum</i>	Fringed willowherb	P	N	FACW	<0.1
<i>Holcus lanatus</i>	Common velvetgrass	Ca	I	FAC	<0.1

<i>Alnus rubra</i>	Red alder	P	N	FAC	<0.1
<i>Lilaeopsis occidentalis</i>	Western grasswort	P, W	N	OBL	<0.1
<i>Bromus</i> sp.	Brome species	P	-	-	<0.1
<i>Iris pseudacorus</i>	Yellowflag iris	ChM, W	NO	OBL	<0.1
<i>Rumex crispus</i>	Curly dock	Ca, ChB	I	FAC	<0.1
<i>Schoenoplectus americanus</i>	Chairmaker's bulrush	P, W	N	OBL	<0.1
<i>Rubus armeniacus</i>	Himalayan blackberry	Ca	NO	FAC	<0.1
<i>Dipsacus</i> sp.	Teasel	W	I	-	-
<i>Poaceae</i> sp.	Unknown grass	W	-	-	-
<i>Poaceae</i> sp.	Unknown grass	W	-	-	-
<i>Poaceae</i> sp.	Unknown grass	W	-	-	-
<i>Poaceae</i> sp.	Unknown grass	W	-	-	-
<i>Carex</i> sp.	Unknown sedge	W	-	-	-
<i>Daucus carota</i>	Queen Anne's lace	W	I	FACU	-
<i>Asteraceae</i> sp.	Unknown aster	W	-	-	-
<i>Salix scouleriana</i>	Scouler's willow	W	N	FAC	-
<i>Juncus</i> sp.	Rush sp.	W	-	-	-
<i>Juncus effusus</i>	Common rush	W	N	FACW	-

Table 8: Results of post-hoc Tukey HSD on total metal concentration by site. Symbols denote transformation of dependent variable to fit assumptions of ANOVA. Differences reported in transformed units.

	Difference	Lower, upper CI	P-adjusted
Magnesium-25 ^γ			
Chuckanut-California	-38116996	-46617365, -29616627	< 0.001
Padden-California	-4539141	-13720588, 4642307	0.45
Padden-Chuckanut	33577855	24396408, 42759303	< 0.001
Aluminum-27			
Chuckanut-California	-9929.3880	-14699.633, -5159.143	< 0.001
Padden-California	-601.3972	-5753.851, 4551.056	0.96
Padden-Chuckanut	9327.9908	4175.537, 14480.444	< 0.001
Chromium-52			
Chuckanut-California	-4.96439	-18.079393, 8.150613	0.62
Padden-California	15.26483	1.099010, 29.430655	0.03
Padden-Chuckanut	20.22922	6.063401, 34.395045	0.003
Manganese-55 *			
Chuckanut-California	-1.2029012	-1.6751014, -0.7307010	< 0.001
Padden-California	-0.1194191	-0.6294537, 0.3906154	0.83
Padden-Chuckanut	1.0834821	0.5734476, 1.5935166	< 0.001
Iron-57 *			

Chuckanut-California	-0.92291146	-1.1792562, -0.6665667	< 0.001
Padden-California	0.02605427	-0.2508297, 0.3029382	0.97
Padden-Chuckanut	0.94896573	0.6720818, 1.2258497	< 0.001
Copper-63 ⁺			
Chuckanut-California	-3.579481	-7.089663, -0.06929906	0.04
Padden-California	1.763325	-2.028105, 5.55475495	0.49
Padden-Chuckanut	5.342806	1.551376, 9.13423613	0.004
Zinc-66 *			
Chuckanut-California	-0.6814171	-1.07990834, -0.2829259	< 0.001
Padden-California	0.4104066	-0.02001316, 0.8408263	0.06
Padden-Chuckanut	1.0918237	0.66140397, 1.5222434	< 0.001
Arsenic-75 *			
Chuckanut-California	-0.9721739	-1.54710079, -0.3972471	< 0.001
Padden-California	-0.3647741	-0.98576604, 0.2562179	0.33
Padden-Chuckanut	0.6073999	-0.01359212, 1.2283919	0.06
Selenium-78			
Chuckanut-California	-1.2405863	-1.81033961, -0.67083297	< 0.001
Padden-California	-0.5714669	-1.18687080, 0.04393705	0.07
Padden-Chuckanut	0.6691194	0.05371549, 1.28452334	0.03
Silver-107 *			
Chuckanut-California	-0.6949700	-1.2614295, -0.1285105	0.01
Padden-California	0.5114073	-0.1004388, 1.1232535	0.12
Padden-Chuckanut	1.2063773	0.5945312, 1.8182235	< 0.001
Lead-208 *			
Chuckanut-California	-1.280287349	-2.1916933, -0.3688814	0.004
Padden-California	0.009671943	-0.9747590, 0.9941029	0.99
Padden-Chuckanut	1.289959292	0.3055283, 2.2743902	0.008

^γ response variable squared to meet assumptions of ANOVA

* response variable log transformed to meet assumptions of ANOVA

⁺ response variable square root + 1 transformed to meet assumptions of ANOVA

Table 9: Results of linear mixed model evaluation of bioaccumulation by species for month of July.

	BAF (calc.)	BAF (pred.)	Value	Standard error	t-value	p-value
Magnesium 25						
<i>A. patula</i>	2.36 + 0.38	2.49	2.4919773	0.2744471	9.079992	<0.001
<i>D. spicata</i>	1.11 + 0.18	1.07	-1.4214623	0.3071293	-4.628222	<0.001
<i>J. balticus</i>	1.67 + 0.19	1.61	-0.8771324	0.2975585	-2.947764	<0.01
<i>P. maritima</i>	2.59 + 0.47	2.90	0.4103093	0.4540988	0.903569	0.37

Aluminum 27							
<i>A. patula</i>	0.39 + 0.15	0.03	0.03153733	0.00874128	3.607861	<0.01	
<i>D. spicata</i>	0.23 + 0.07	0.22	0.18509359	0.06417298	2.884292	<0.01	
<i>J. balticus</i>	0.35 + 0.04	0.34	0.30545028	0.03490993	8.749668	<0.001	
<i>P. maritima</i>	0.19 + 0.05	0.15	0.11554656	0.02033126	5.683198	<0.001	
Chromium 52							
<i>A. patula</i>	0.83 + 0.34	0.14	0.1372961	0.10420237	1.317591	0.20	
<i>D. spicata</i>	0.77 + 0.11	0.62	0.4842369	0.03377757	14.336049	<0.001	
<i>J. balticus</i>	0.67 + 0.07	0.67	0.5307672	0.06814916	7.788315	<0.001	
<i>P. maritima</i>	0.32 + 0.07	0.38	0.2454678	0.06880595	3.567537	<0.01	
Manganese 55							
<i>A. patula</i>	0.77 + 0.30	0.78	0.7671644	0.2985907	2.5692841	0.02	
<i>D. spicata</i>	0.48 + 0.16	0.42	-0.2829310	0.3371627	-0.8391529	0.41	
<i>J. balticus</i>	1.10 + 0.17	1.10	0.3371359	0.3437126	0.9808658	0.34	
<i>P. maritima</i>	0.40 + 0.05	0.46	-0.3716521	0.3025631	-1.2283458	0.23	
Iron 57							
<i>A. patula</i>	0.49 + 0.18	0.04	0.0397777	0.00982007	4.050653	<0.001	
<i>D. spicata</i>	0.29 + 0.08	0.26	0.2250853	0.05163768	4.358935	<0.001	
<i>J. balticus</i>	0.38 + 0.03	0.38	0.3438997	0.03321190	10.354712	<0.001	
<i>P. maritima</i>	0.23 + 0.05	0.21	0.1671858	0.03938329	4.245095	<0.001	
Copper 63							
<i>A. patula</i>	1.94 + 1.03	0.90	0.8971405	0.3951888	2.270157	0.03	
<i>D. spicata</i>	2.56 + 1.07	1.53	0.6329432	0.5701703	1.110095	0.28	
<i>J. balticus</i>	1.86 + 0.31	1.78	0.8786677	0.3743706	2.347053	0.03	
<i>P. maritima</i>	1.29 + 0.24	2.30	1.4079796	0.3875499	3.633028	<0.01	
Zinc 66							
<i>A. patula</i>	2.54 + 0.84	2.54	2.5439664	0.8361378	3.0425203	<0.01	
<i>D. spicata</i>	2.45 + 0.68	2.44	-0.0961317	1.0790074	-0.0890927	0.93	
<i>J. balticus</i>	2.85 + 0.41	2.85	0.3075197	0.9333254	0.3294882	0.74	
<i>P. maritima</i>	1.94 + 0.26	1.94	-0.6031960	0.8752823	-0.6891445	0.50	
Arsenic 75							
<i>A. patula</i>	0.92 + 0.37	0.12	0.1225310	0.05053057	2.424888	0.02	
<i>D. spicata</i>	0.47 + 0.17	0.37	0.2523761	0.05827302	4.330925	<0.001	
<i>J. balticus</i>	0.61 + 0.07	0.54	0.4194044	0.04394274	9.544340	<0.001	
<i>P. maritima</i>	0.49 + 0.04	0.52	0.3933342	0.03437183	11.443506	<0.001	
Selenium 78							
<i>A. patula</i>	4.25 + 1.10	4.32	4.320449	1.4467552	2.9863027	<0.01	
<i>D. spicata</i>	5.97 + 2.17	6.81	2.485950	1.7015873	1.4609595	0.16	
<i>J. balticus</i>	13.30 + 7.34	4.00	-0.318396	0.8604723	-0.3700245	0.71	
<i>P. maritima</i>	6.04 + 1.72	5.70	1.377735	1.0114109	1.3621912	0.19	
Silver 107							
<i>A. patula</i>	2.66 + 1.06	2.59	2.5869273	0.9414998	2.747666	0.01	
<i>D. spicata</i>	3.04 + 0.94	3.00	0.4101327	0.6274261	0.653675	0.52	
<i>J. balticus</i>	11.17 + 6.04	4.15	1.5605313	0.5938542	2.627802	0.02	
<i>P. maritima</i>	3.67 + 0.99	3.20	0.6157920	0.4962857	1.240801	0.23	
Cadmium 111							

<i>A. patula</i>	3.48 + 1.42	2.84	2.835764	1.0561970	2.6848815	0.01
<i>D. spicata</i>	2.53 + 0.99	2.92	0.081998	0.5243369	0.1563841	0.88
<i>J. balticus</i>	9.34 + 4.07	7.07	4.235522	1.4805853	2.8607077	0.01
<i>P. maritima</i>	3.68 + 1.17	3.30	0.464990	0.7069918	0.6577026	0.52
Lead 208						
<i>A. patula</i>	1.66 + 1.11	0.502	0.5020429	0.4273358	1.174821	0.25
<i>D. spicata</i>	0.79 + 0.43	0.706	0.2035236	0.0947190	2.148710	0.04
<i>J. balticus</i>	1.11 + 0.27	0.974	0.4721905	0.1845159	2.559078	0.02
<i>P. maritima</i>	0.22 + 0.04	0.653	0.1509194	0.0585416	2.577985	0.02

Table 10: Post-hoc Tukey HSD testing on July linear mixed model of bioaccumulation by species. Species key: ATPA4 = *A. patula*, DISP = *D. spicata*, JUBA = *J. balticus*, PLMA3 = *P. maritima*

	Estimate	Standard error	z-value	p-value
Magnesium-25				
DISP - ATPA4 = 0	-1.4215	0.3071	-4.628	<0.001
JUBA - ATPA4 = 0	-0.8771	0.2976	-2.948	0.02
PLMA3 - ATPA4 = 0	0.4103	0.4541	0.904	0.79
JUBA - DISP = 0	0.5443	0.2876	1.893	0.22
PLMA3 - DISP = 0	1.8318	0.4584	3.996	<0.001
PLMA3 - JUBA = 0	1.2874	0.4473	2.878	0.01
Aluminum-27				
DISP - ATPA4 = 0	0.18509	0.06417	2.884	0.02
JUBA - ATPA4 = 0	0.30545	0.03491	8.750	<0.001
PLMA3 - ATPA4 = 0	0.11555	0.02033	5.683	<0.001
JUBA - DISP = 0	0.12036	0.07200	1.672	0.31
PLMA3 - DISP = 0	-0.06955	0.06617	-1.051	0.69
PLMA3 - JUBA = 0	-0.18990	0.03846	-4.938	<0.001
Chromium-52				
DISP - ATPA4 = 0	0.48424	0.03378	14.336	<0.001
JUBA - ATPA4 = 0	0.53077	0.06815	7.788	<0.001
PLMA3 - ATPA4 = 0	0.24547	0.06881	3.568	<0.01
JUBA - DISP = 0	0.04653	0.06544	0.711	0.88
PLMA3 - DISP = 0	-0.23877	0.06619	-3.608	<0.01
PLMA3 - JUBA = 0	-0.28530	0.04770	-5.981	<0.001
Manganese-55				
DISP - ATPA4 = 0	-0.28293	0.33716	-0.839	0.82
JUBA - ATPA4 = 0	0.33714	0.34371	0.981	0.74
PLMA3 - ATPA4 = 0	-0.37165	0.30256	-1.228	0.59
JUBA - DISP = 0	0.62007	0.23131	2.681	0.03
PLMA3 - DISP = 0	-0.08872	0.16404	-0.541	0.94
PLMA3 - JUBA = 0	-0.70879	0.17712	-4.002	<0.001
Iron-57				
DISP - ATPA4 = 0	0.22509	0.05164	4.359	<0.001
JUBA - ATPA4 = 0	0.34390	0.03321	10.355	<0.001
PLMA3 - ATPA4 = 0	0.16719	0.03938	4.245	<0.001

JUBA - DISP = 0	0.11881	0.05980	1.987	0.18
PLMA3 - DISP = 0	-0.05790	0.06344	-0.913	0.78
PLMA3 - JUBA = 0	-0.17671	0.04961	-3.562	<0.01
Copper-63				
DISP - ATPA4 = 0	0.6329	0.5702	1.110	0.67
JUBA - ATPA4 = 0	0.8787	0.3744	2.347	0.08
PLMA3 - ATPA4 = 0	1.4080	0.3875	3.633	<0.01
JUBA - DISP = 0	0.2457	0.5697	0.431	0.97
PLMA3 - DISP = 0	0.7750	0.5871	1.320	0.54
PLMA3 - JUBA = 0	0.5293	0.3871	1.367	0.51
Zinc-66				
DISP - ATPA4 = 0	-0.09613	1.07901	-0.089	1
JUBA - ATPA4 = 0	0.30752	0.93333	0.329	0.99
PLMA3 - ATPA4 = 0	-0.60320	0.87528	-0.689	0.90
JUBA - DISP = 0	0.40365	0.79819	0.506	0.96
PLMA3 - DISP = 0	-0.50706	0.72947	-0.695	0.89
PLMA3 - JUBA = 0	-0.91072	0.48884	-1.863	0.23
Arsenic-75				
DISP - ATPA4 = 0	0.25238	0.05827	4.331	<0.001
JUBA - ATPA4 = 0	0.41940	0.04394	9.544	<0.001
PLMA3 - ATPA4 = 0	0.39333	0.03437	11.444	<0.001
JUBA - DISP = 0	0.16703	0.06743	2.477	0.06
PLMA3 - DISP = 0	0.14096	0.06231	2.262	0.10
PLMA3 - JUBA = 0	-0.02607	0.04780	-0.545	0.95
Selenium-78				
DISP - ATPA4 = 0	2.4860	1.7016	1.461	0.45
JUBA - ATPA4 = 0	-0.3184	0.8605	-0.370	0.98
PLMA3 - ATPA4 = 0	1.3777	1.0114	1.362	0.51
JUBA - DISP = 0	-2.8043	1.6376	-1.713	0.30
PLMA3 - DISP = 0	-1.1082	1.7329	-0.640	0.92
PLMA3 - JUBA = 0	1.6961	0.8854	1.916	0.21
Silver-107				
DISP - ATPA4 = 0	0.4101	0.6274	0.654	0.91
JUBA - ATPA4 = 0	1.5605	0.5939	2.628	0.04
PLMA3 - ATPA4 = 0	0.6158	0.4963	1.241	0.59
JUBA - DISP = 0	1.1504	0.5641	2.039	0.17
PLMA3 - DISP = 0	0.2057	0.4612	0.446	0.97
PLMA3 - JUBA = 0	-0.9447	0.4015	-2.353	0.08
Cadmium-111				
DISP - ATPA4 = 0	0.0820	0.5243	0.156	0.99
JUBA - ATPA4 = 0	4.2355	1.4806	2.861	0.02
PLMA3 - ATPA4 = 0	0.4650	0.7070	0.658	0.91
JUBA - DISP = 0	4.1535	1.4908	2.786	0.02
PLMA3 - DISP = 0	0.3830	0.6754	0.567	0.94
PLMA3 - JUBA = 0	-3.7705	1.5618	-2.414	0.07
Lead-208				

DISP - ATPA4 = 0	0.20352	0.09472	2.149	0.12
JUBA - ATPA4 = 0	0.47219	0.18452	2.559	0.04
PLMA3 - ATPA4 = 0	0.15092	0.05854	2.578	0.04
JUBA - DISP = 0	0.26867	0.20475	1.312	0.52
PLMA3 - DISP = 0	-0.05260	0.10537	-0.499	0.95
PLMA3 - JUBA = 0	-0.32127	0.18925	-1.698	0.30

Table 11: Goodness-of-fit measurements for linear mixed models of BAF by species in the month of July.

Metal	Root means square error (RMSE)	Mean average error (MAE)
Magnesium-25	0.713	0.547
Aluminum-27	0.305	0.174
Chromium-52	0.637	0.292
Manganese-55	0.522	0.384
Iron-57	0.370	0.197
Copper-63	1.98	1.04
Zinc-66	1.53	1.26
Arsenic-75	0.69	0.33
Selenium-78	12.98	5.03
Silver-107	10.43	3.61
Cadmium-111	6.77	3.18
Lead-208	1.84	0.69

Table 12: Statistics resulting from linear mixed models used to evaluate the bioaccumulation by species for the month of August.

	BAF (actual)	BAF (pred.)	Value	Standard error	t-value	p-value
Magnesium 25						
<i>A. patula</i>	3.56 + 0.75	2.87	2.8672660	0.4998229	5.736564	<0.001
<i>D. spicata</i>	1.02 + 0.19	1.21	-1.6550601	0.2060128	-8.033772	<0.001
<i>J. balticus</i>	1.05 + 0.11	1.34	-1.5249813	0.1808248	-8.433475	<0.001
<i>P. maritima</i>	2.40 + 0.42	2.09	-0.7806353	0.1703757	-4.581847	<0.001
Aluminum 27						
<i>A. patula</i>	0.19 + 0.05	0.18	0.18490142	0.04752686	3.890462	<0.01
<i>D. spicata</i>	0.14 + 0.02	0.15	-0.03956676	0.04829360	-0.819296	0.42
<i>J. balticus</i>	0.25 + 0.05	0.19	0.00459542	0.04835698	0.095031	0.93
<i>P. maritima</i>	0.22 + 0.02	0.19	0.00200534	0.04813329	0.041662	0.97
Chromium 52						
<i>A. patula</i>	0.24 + 0.04	0.28	0.28437704	0.09010533	3.1560514	<0.01
<i>D. spicata</i>	0.39 + 0.07	0.33	0.04326068	0.10187162	0.4246588	0.67
<i>J. balticus</i>	0.35 + 0.09	0.32	0.03608760	0.06539970	0.5518008	0.59
<i>P. maritima</i>	0.38 + 0.08	0.35	0.06152169	0.06419370	0.9583757	0.35

Manganese 55							
<i>A. patula</i>	0.64 + 0.24	0.34	0.3405305	0.06584239	5.171903	<0.001	
<i>D. spicata</i>	0.81 + 0.50	0.47	-0.0765629	0.05530279	-1.384431	0.18	
<i>J. balticus</i>	1.30 + 0.46	0.98	0.1520063	0.06500365	2.338427	0.03	
<i>P. maritima</i>	0.42 + 0.06	0.11	0.0894470	0.06855393	1.304768	0.20	
Iron 57							
<i>A. patula</i>	0.19 + 0.04	0.19	0.18604601	0.06929442	2.6848629	0.01	
<i>D. spicata</i>	0.18 + 0.04	0.19	0.00747919	0.04703021	0.1590294	0.87	
<i>J. balticus</i>	0.27 + 0.05	0.25	0.06109764	0.04656624	1.3120586	0.20	
<i>P. maritima</i>	0.23 + 0.03	0.23	0.04243343	0.04636674	0.9151696	0.37	
Copper 63							
<i>A. patula</i>	0.70 + 0.18	0.57	0.5708311	0.02519326	22.658088	<0.001	
<i>D. spicata</i>	0.96 + 0.54	0.57	0.0045600	0.02764690	0.164936	0.87	
<i>J. balticus</i>	0.71 + 0.13	0.69	0.0774970	0.10567645	0.733342	0.47	
<i>P. maritima</i>	0.99 + 0.28	1.40	0.8604103	0.16126332	5.335437	<0.001	
Zinc 66							
<i>A. patula</i>	3.02 + 1.07	2.45	2.4530127	0.9195083	2.667744	0.01	
<i>D. spicata</i>	2.11 + 0.81	1.95	-0.5021123	0.2562897	-1.959159	0.06	
<i>J. balticus</i>	1.65 + 0.35	2.01	-0.4434157	0.2238509	-1.980852	0.06	
<i>P. maritima</i>	3.45 + 0.70	2.96	0.5105446	0.2109413	2.420316	0.02	
Arsenic 75							
<i>A. patula</i>	0.31 + 0.06	0.31	0.31295433	0.06814810	4.592268	<0.001	
<i>D. spicata</i>	0.24 + 0.06	0.25	-0.06588075	0.06672529	-0.987343	0.33	
<i>J. balticus</i>	0.46 + 0.09	0.31	-0.00353255	0.06782731	-0.052081	0.96	
<i>P. maritima</i>	0.59 + 0.15	0.34	0.02397000	0.07061163	0.339462	0.73	
Selenium 78							
<i>A. patula</i>	4.09 + 3.67	1.25	1.2468863	0.9656482	1.291243	0.21	
<i>D. spicata</i>	1.36 + 0.94	1.00	-0.2466743	0.1648124	-1.496698	0.15	
<i>J. balticus</i>	1.52 + 0.90	0.98	-0.2693560	0.1664128	-1.618602	0.12	
<i>P. maritima</i>	2.68 + 1.22	1.49	0.2440603	0.1962598	1.243557	0.22	
Silver 107							
<i>A. patula</i>	2.00 + 0.42	1.33	1.3299960	0.1115903	11.918567	<0.001	
<i>D. spicata</i>	1.14 + 0.28	0.87	-0.4635150	0.1642039	-2.822802	<0.01	
<i>J. balticus</i>	1.22 + 0.38	0.72	-0.6060088	0.1658750	-3.653405	<0.01	
<i>P. maritima</i>	2.66 + 1.13	1.35	0.0217581	0.3844650	0.056593	0.96	
Cadmium 111							
<i>A. patula</i>	9.11 + 6.22	2.95	2.9457961	0.4502423	6.542690	<0.001	
<i>D. spicata</i>	1.64 + 0.93	1.04	-1.9104196	0.4526312	-4.220698	<0.01	
<i>J. balticus</i>	4.40 + 2.64	1.60	-1.3451725	0.5889346	-2.284078	0.03	
<i>P. maritima</i>	3.41 + 0.70	3.61	0.6647686	0.6960343	0.955080	0.35	
Lead 208							
<i>A. patula</i>	0.29 + 0.07	0.36	0.3646478	0.11582515	3.148261	<0.01	
<i>D. spicata</i>	0.37 + 0.22	0.21	-0.1455203	0.06131896	-2.373170	0.03	
<i>J. balticus</i>	0.29 + 0.06	0.27	-0.0904967	0.06101854	-1.483102	0.15	
<i>P. maritima</i>	0.34 + 0.08	0.28	-0.0833693	0.05635158	-1.479450	0.15	

Table 13: Post-hoc Tukey HSD testing on August linear mixed model of bioaccumulation by species. ATPA4 = *A. patula*, DISP = *D. spicata*, JUBA = *J. balticus*, PLMA3 = *P. maritima*

	Estimate	Standard error	z-value	p-value
Magnesium-25				
DISP - ATPA4 = 0	-1.6551	0.2060	-8.034	<0.001
JUBA - ATPA4 = 0	-1.5250	0.1808	-8.433	<0.001
PLMA3 - ATPA4 = 0	-0.7806	0.1704	-4.582	<0.001
JUBA - DISP = 0	0.1301	0.2183	0.596	0.93
PLMA3 - DISP = 0	0.8744	0.2058	4.249	<0.001
PLMA3 - JUBA = 0	0.7443	0.1806	4.121	<0.001
Aluminum-27				
DISP - ATPA4 = 0	-0.039567	0.048294	-0.819	0.83
JUBA - ATPA4 = 0	0.004595	0.048357	0.095	0.99
PLMA3 - ATPA4 = 0	0.002005	0.048133	0.042	0.99
JUBA - DISP = 0	0.044162	0.012372	3.570	<0.01
PLMA3 - DISP = 0	0.041572	0.011466	3.626	<0.01
PLMA3 - JUBA = 0	-0.002590	0.011731	-0.221	0.99
Chromium-52				
DISP - ATPA4 = 0	0.043261	0.101872	0.425	0.97
JUBA - ATPA4 = 0	0.036088	0.065400	0.552	0.94
PLMA3 - ATPA4 = 0	0.061522	0.064194	0.958	0.77
JUBA - DISP = 0	-0.007173	0.102833	-0.070	0.99
PLMA3 - DISP = 0	0.018261	0.101001	0.181	0.99
PLMA3 - JUBA = 0	0.025434	0.064813	0.392	0.98
Manganese-55				
DISP - ATPA4 = 0	-0.07656	0.05530	-1.384	0.50
JUBA - ATPA4 = 0	0.15201	0.06500	2.338	0.09
PLMA3 - ATPA4 = 0	0.08945	0.06855	1.305	0.55
JUBA - DISP = 0	0.22857	0.07021	3.256	<0.01
PLMA3 - DISP = 0	0.16601	0.07729	2.148	0.13
PLMA3 - JUBA = 0	-0.06256	0.08639	-0.724	0.88
Iron-57				
DISP - ATPA4 = 0	0.007479	0.047030	0.159	0.99
JUBA - ATPA4 = 0	0.061098	0.046566	1.312	0.52
PLMA3 - ATPA4 = 0	0.042433	0.046367	0.915	0.77
JUBA - DISP = 0	0.053618	0.008544	6.275	<0.001
PLMA3 - DISP = 0	0.034954	0.012963	2.696	0.03
PLMA3 - JUBA = 0	-0.018664	0.011510	-1.622	0.33
Copper-63				
DISP - ATPA4 = 0	0.00456	0.02765	0.165	0.99
JUBA - ATPA4 = 0	0.07750	0.10568	0.733	0.86
PLMA3 - ATPA4 = 0	0.86041	0.16126	5.335	<0.001
JUBA - DISP = 0	0.07294	0.10326	0.706	0.88
PLMA3 - DISP = 0	0.85585	0.15969	5.359	<0.001
PLMA3 - JUBA = 0	0.78291	0.18948	4.132	<0.001
Zinc-66				

DISP - ATPA4 = 0	-0.5021	0.2563	-1.959	0.20
JUBA - ATPA4 = 0	-0.4434	0.2238	-1.981	0.19
PLMA3 - ATPA4 = 0	0.5105	0.2109	2.420	0.07
JUBA - DISP = 0	0.0587	0.2717	0.216	0.99
PLMA3 - DISP = 0	1.0127	0.2561	3.954	<0.001
PLMA3 - JUBA = 0	0.9540	0.2237	4.265	<0.001
Arsenic-75				
DISP - ATPA4 = 0	-0.065881	0.066725	-0.987	0.74
JUBA - ATPA4 = 0	-0.003533	0.067827	-0.052	0.99
PLMA3 - ATPA4 = 0	0.023970	0.070612	0.339	0.99
JUBA - DISP = 0	0.062348	0.032280	1.931	0.20
PLMA3 - DISP = 0	0.089851	0.035470	2.533	0.05
PLMA3 - JUBA = 0	0.027503	0.041244	0.667	0.90
Selenium-78				
DISP - ATPA4 = 0	-0.24667	0.16481	-1.497	0.39
JUBA - ATPA4 = 0	-0.26936	0.16641	-1.619	0.33
PLMA3 - ATPA4 = 0	0.24406	0.19626	1.244	0.56
JUBA - DISP = 0	-0.02268	0.03007	-0.754	0.86
PLMA3 - DISP = 0	0.49073	0.11453	4.285	<0.001
PLMA3 - JUBA = 0	0.51342	0.11071	4.637	<0.001
Silver-107				
DISP - ATPA4 = 0	-0.46352	0.16420	-2.823	0.02
JUBA - ATPA4 = 0	-0.60601	0.16588	-3.653	<0.01
PLMA3 - ATPA4 = 0	0.02176	0.38447	0.057	0.99
JUBA - DISP = 0	-0.14249	0.17197	-0.829	0.84
PLMA3 - DISP = 0	0.48527	0.38713	1.254	0.57
PLMA3 - JUBA = 0	0.62777	0.38784	1.619	0.35
Cadmium-111				
DISP - ATPA4 = 0	-1.9104	0.4526	-4.221	<0.001
JUBA - ATPA4 = 0	-1.3452	0.5889	-2.284	0.09
PLMA3 - ATPA4 = 0	0.6648	0.6960	0.955	0.76
JUBA - DISP = 0	0.5652	0.3825	1.478	0.43
PLMA3 - DISP = 0	2.5752	0.5328	4.833	<0.001
PLMA3 - JUBA = 0	2.0099	0.6526	3.080	<0.01
Lead-208				
DISP - ATPA4 = 0	-0.145520	0.061319	-2.373	0.08
JUBA - ATPA4 = 0	-0.090497	0.061019	-1.483	0.45
PLMA3 - ATPA4 = 0	-0.083369	0.056352	-1.479	0.45
JUBA - DISP = 0	0.055024	0.066204	0.831	0.84
PLMA3 - DISP = 0	0.062151	0.061160	1.016	0.74
PLMA3 - JUBA = 0	0.007127	0.060851	0.117	0.99

Table 14: Goodness-of-fit measurements for linear mixed models of BAF by species in the month of August.

Metal	Root mean square error (RMSE)	Mean average error (MAE)
Magnesium-25	1.02	0.627
Aluminum-27	0.11	0.078
Chromium-52	0.171	0.131
Manganese-55	0.958	0.464
Iron-57	0.093	0.063
Copper-63	0.806	0.486
Zinc-66	1.525	0.876
Arsenic-75	0.304	0.186
Selenium-78	5.299	2.047
Silver-107	2.080	1.001
Cadmium-111	9.835	3.408
Lead-208	0.237	0.142

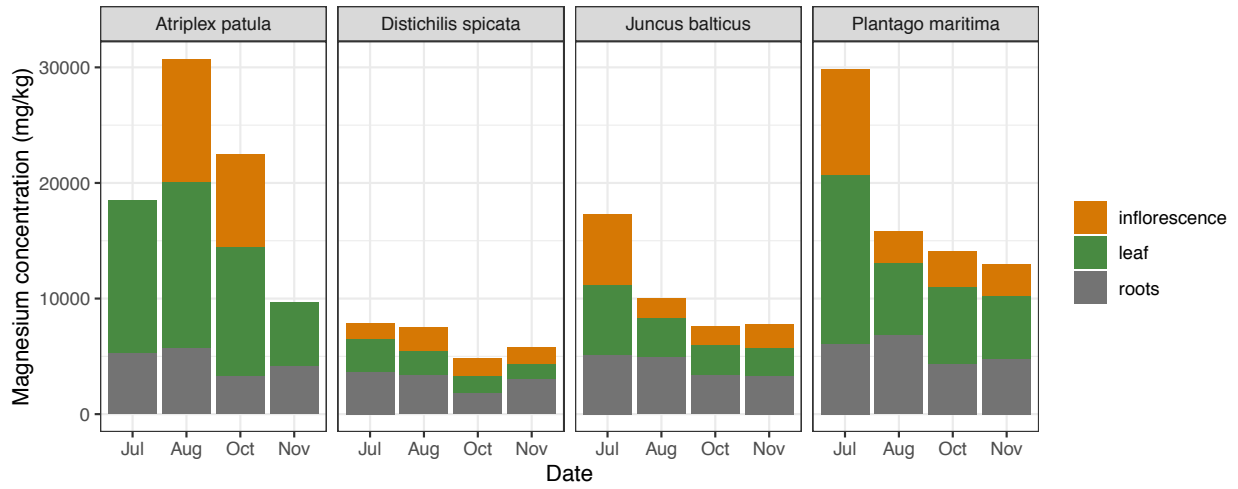
Table 15: Statistics resulting from linear mixed models used to evaluate translocation factor (TF) by month of sample collection, expressed as “Date”, and species. Value represents the change in mean TF relative to the intercept (*A. patula* TF in the month of July). ATPA4 = *A. patula*, DISP = *D. spicata*, JUBA = *J. balticus*, PLMA3 = *P. maritima*

	Value	Std. error	t-value	p-value	RMSE	MAE
Magnesium-25					1.94	1.04
Intercept	5.591532	0.9647065	5.796097	<0.001		
Date	-0.932313	0.2545706	-3.662294	<0.001		
DISP	-4.695715	0.9572492	-4.905426	<0.001		
JUBA	-4.470145	0.9749878	-4.584821	<0.001		
PLMA3	-4.452570	0.9915554	-4.490490	<0.001		
Date:Disp	0.802887	0.2554124	3.143495	<0.01		
Date:JUBA	0.993942	0.2636805	3.769493	<0.001		
Date:PLMA3	1.131322	0.2747111	4.118225	<0.001		
Aluminum-27					0.462	0.208
Intercept	0.02491414	0.006638689	3.752871	<0.001		
Date	0.01325385	0.001450821	9.135413	<0.001		
DISP	-0.02487527	0.011886830	-2.092675	0.04		
JUBA	0.12602405	0.028871390	4.365015	<0.001		
PLMA3	0.05372387	0.011052420	4.860824	<0.001		
Date:Disp	0.02060774	0.005364706	3.841354	<0.001		
Date:JUBA	-0.02464402	0.012963544	-1.901025	0.06		
Date:PLMA3	-0.00999764	0.005348090	-1.869385	0.06		
Chromium-52					0.784	0.350
Intercept	0.2242389	0.05802792	3.864329	<0.001		
Date	-0.0253510	0.00437500	-5.794510	<0.001		
DISP	0.4686343	0.05509578	8.505810	<0.001		

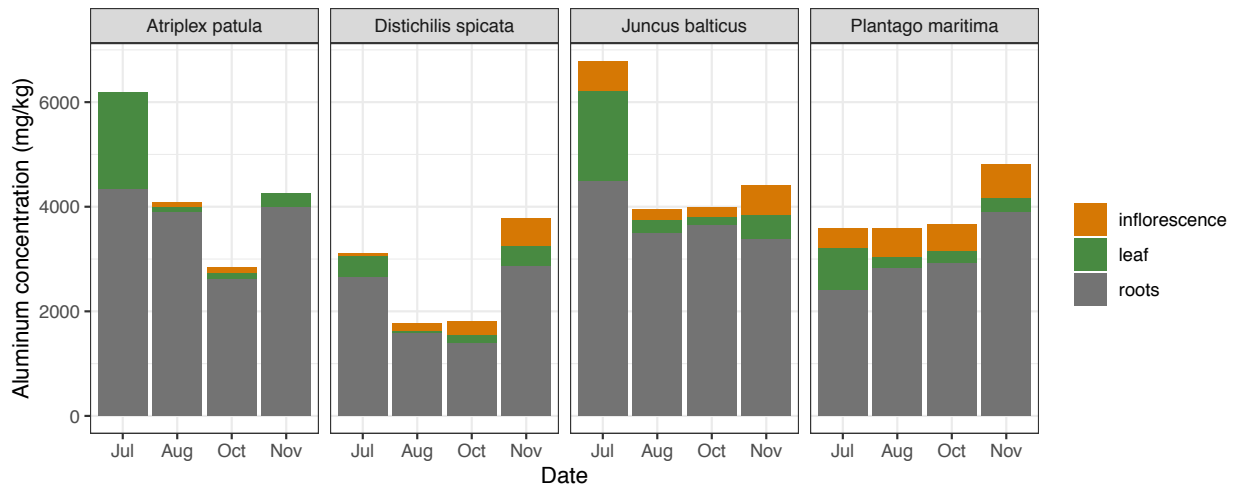
	JUBA	0.1471529	0.02198737	6.692609	<0.001		
	PLMA3	0.0515872	0.01928301	2.675267	0.008		
Manganese-55						1.40	0.776
	Intercept	1.2559701	0.2501189	5.021492	<0.001		
	Date	-0.2111610	0.0775979	-2.721221	0.008		
	DISP	-0.8298915	0.2409631	-3.444060	<0.001		
	JUBA	-0.1269182	0.4187629	-0.303079	0.76		
	PLMA3	-0.7438877	0.2258732	-3.293387	<0.001		
	Date:Disp	0.1310592	0.0864863	1.515376	0.13		
	Date:JUBA	0.2955595	0.1583146	1.866913	0.06		
	Date:PLMA3	0.1913615	0.0836989	2.286308	0.02		
Iron-57						0.515	0.256
	Intercept	0.7945202	0.29353678	2.706714	0.008		
	Date	-0.1568036	0.07641869	-2.051901	0.04		
	DISP	-0.7337285	0.29398117	-2.495835	0.01		
	JUBA	-0.5721549	0.29853889	-1.916517	0.06		
	PLMA3	-0.4318216	0.30386523	-1.421096	0.16		
	Date:Disp	0.1745080	0.07718962	2.260771	0.03		
	Date:JUBA	0.1300622	0.07891245	1.648183	0.10		
	Date:PLMA3	0.0983064	0.07981521	1.231675	0.22		
Copper-63						0.916	0.484
	Intercept	1.7143304	0.14591714	11.748657	<0.001		
	Date	-0.3747865	0.03409530	-10.992321	<0.001		
	DISP	-1.5425751	0.12919965	-11.939469	<0.001		
	JUBA	-1.3826123	0.11307530	-12.227359	<0.001		
	PLMA3	-1.1269827	0.13321295	-8.460008	<0.001		
	Date:Disp	0.3609888	0.03841527	9.397015	<0.001		
	Date:JUBA	0.4179410	0.03704135	11.283093	<0.001		
	Date:PLMA3	0.3545632	0.04615775	7.681552	<0.001		
Zinc-66						1.14	0.638
	Intercept	2.4762975	0.4038045	6.132416	<0.001		
	Date	-0.4431978	0.0925956	-4.786384	<0.001		
	DISP	-2.2346212	0.3639130	-6.140536	<0.001		
	JUBA	-1.2791876	0.3673946	-3.481781	<0.001		
	PLMA3	-0.8787035	0.3701895	-2.373658	0.02		
	Date:Disp	0.4330087	0.0935272	4.629763	<0.001		
	Date:JUBA	0.6366410	0.0953557	6.676485	<0.001		
	Date:PLMA3	0.0470530	0.0958325	0.490992	0.62		
Arsenic-75						0.943	0.352
	Intercept	0.12573756	0.013169355	9.547739	<0.001		
	Date	-0.00932467	0.003081902	-3.025621	<0.01		
	DISP	-0.01877661	0.014626121	-1.283772	0.20		
	JUBA	-0.03175892	0.012318598	-2.578128	0.01		
	PLMA3	0.24295975	0.015406377	15.770077	<0.001		
Selenium-78						35.92	15.24
	Intercept	8.154851	0.0901074	90.50143	<0.001		

Date	-1.886587	0.0343791	-54.87600	<0.001		
DISP	-4.683714	0.9815716	-4.77165	<0.001		
JUBA	-7.129732	0.0985211	-72.36756	<0.001		
PLMA3	-6.272188	0.8859012	-7.08001	<0.001		
Date:Disp	1.043299	0.2484174	4.19978	<0.001		
Date:JUBA	1.984743	0.0603194	32.90387	<0.001		
Date:PLMA3	1.624311	0.2642211	6.14754	<0.001		
Silver-107					1.339	0.663
Intercept	1.3092472	0.10464683	12.511103	<0.001		
Date	-0.2265046	0.02653329	-8.536619	<0.001		
DISP	-1.1761393	0.12139671	-9.688395	<0.001		
JUBA	-0.7684884	0.09027068	-8.513156	<0.001		
PLMA3	-0.1248732	0.07906540	-1.579365	0.12		
Date:Disp	0.2601918	0.03401363	7.649634	<0.001		
Date:JUBA	0.2050412	0.03026066	6.775834	<0.001		
Date:PLMA3	0.0249932	0.03140407	0.795858	0.43		
Cadmium-111					1.842	0.861
Intercept	2.3556399	0.5049535	4.238066	<0.001		
Date	-0.4991443	0.1260186	-3.960878	<0.001		
DISP	-2.1432302	0.5087657	-4.094934	<0.001		
JUBA	-1.8242916	0.5038483	-3.841215	<0.001		
PLMA3	-1.4427501	0.5342385	-2.899365	<0.01		
Date:Disp	0.5309369	0.1328991	3.995037	<0.001		
Date:JUBA	0.5061994	0.1282588	3.946702	<0.001		
Date:PLMA3	0.4187857	0.1406122	2.978303	<0.01		
Lead-208					0.576	0.264
Intercept	0.2980254	0.08560772	3.481291	<0.01		
Date	-0.0656641	0.02774221	-2.366938	0.02		
DISP	-0.3189760	0.12397858	-2.572831	0.01		
JUBA	-0.0932866	0.11273946	-0.827453	0.41		
PLMA3	0.1263503	0.11207472	1.127376	0.26		
Date:Disp	0.1373000	0.04755259	2.8873284	<0.01		
Date:JUBA	0.0535830	0.03738044	1.4334514	0.15		
Date:PLMA3	-0.0243249	0.03685004	-0.6601041	0.51		

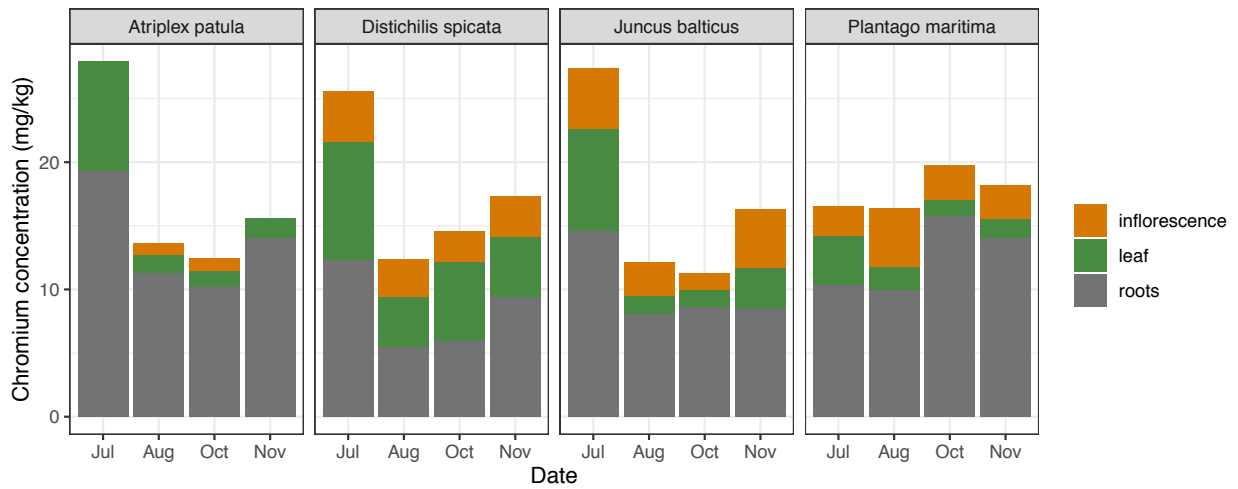
Magnesium-25 Change Over Time



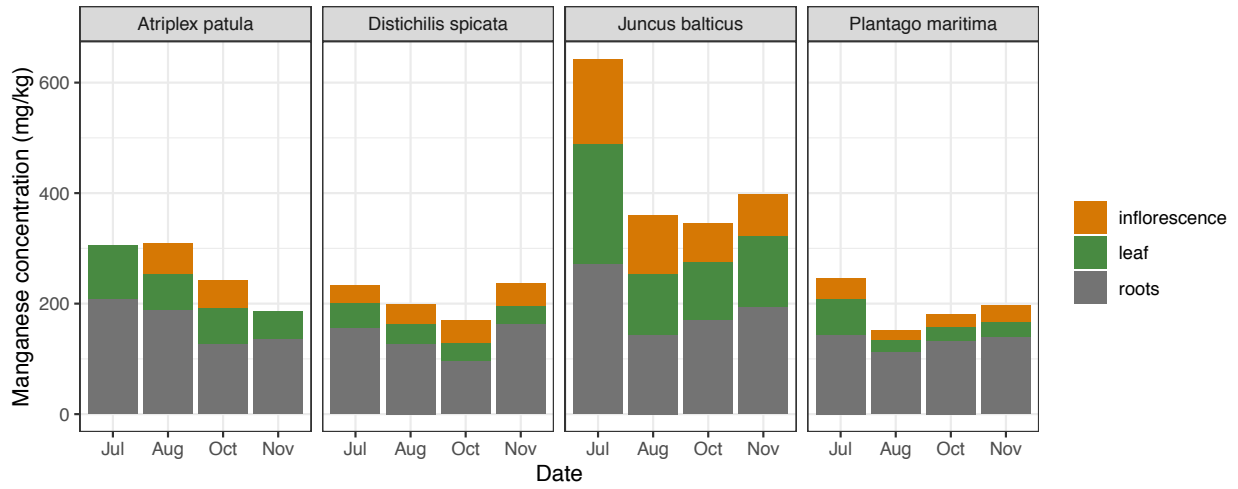
Aluminum-27 Change Over Time



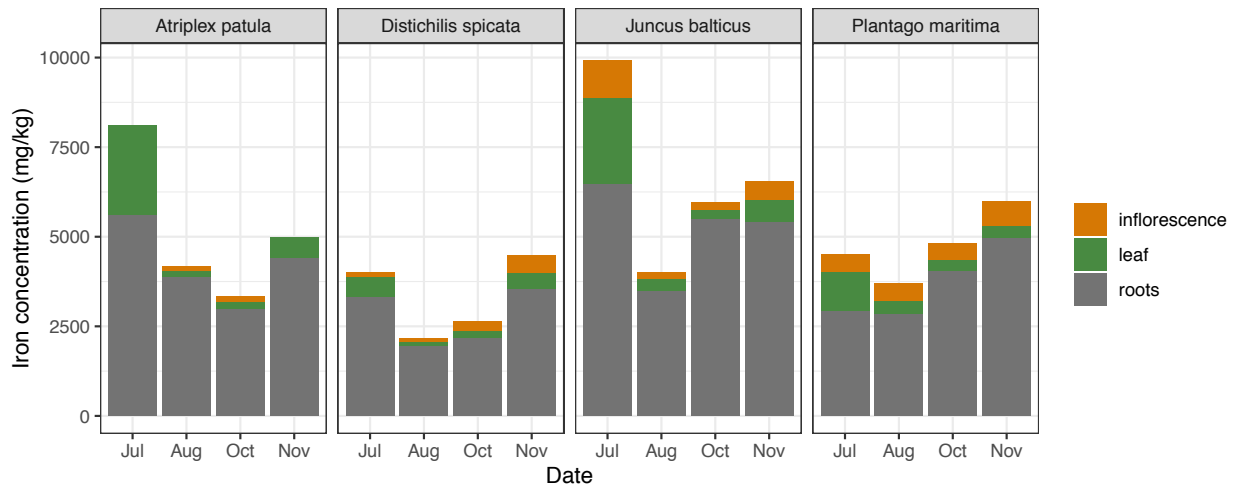
Chromium-52 Change Over Time



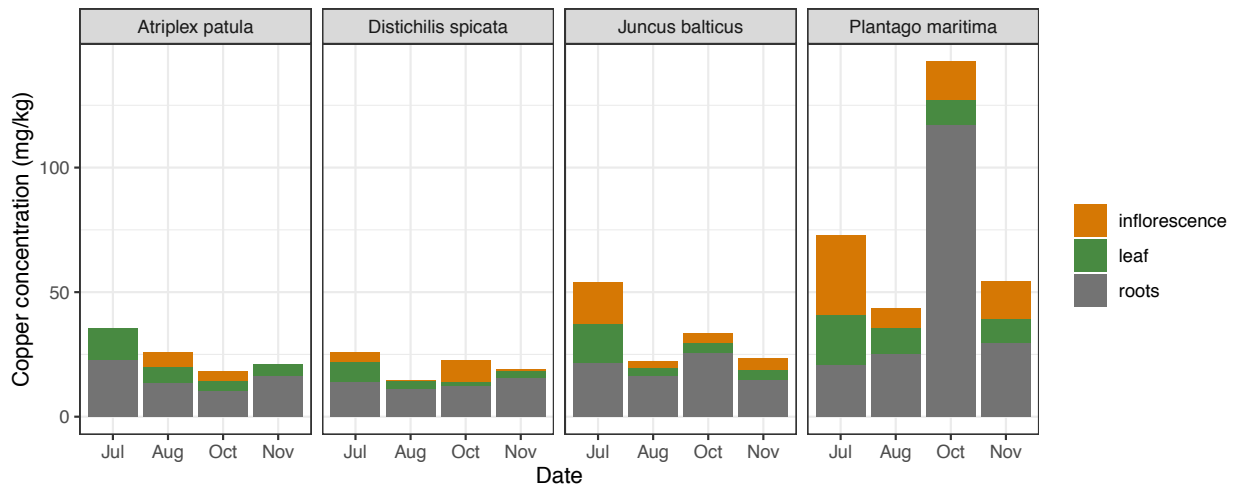
Manganese-55 Change Over Time



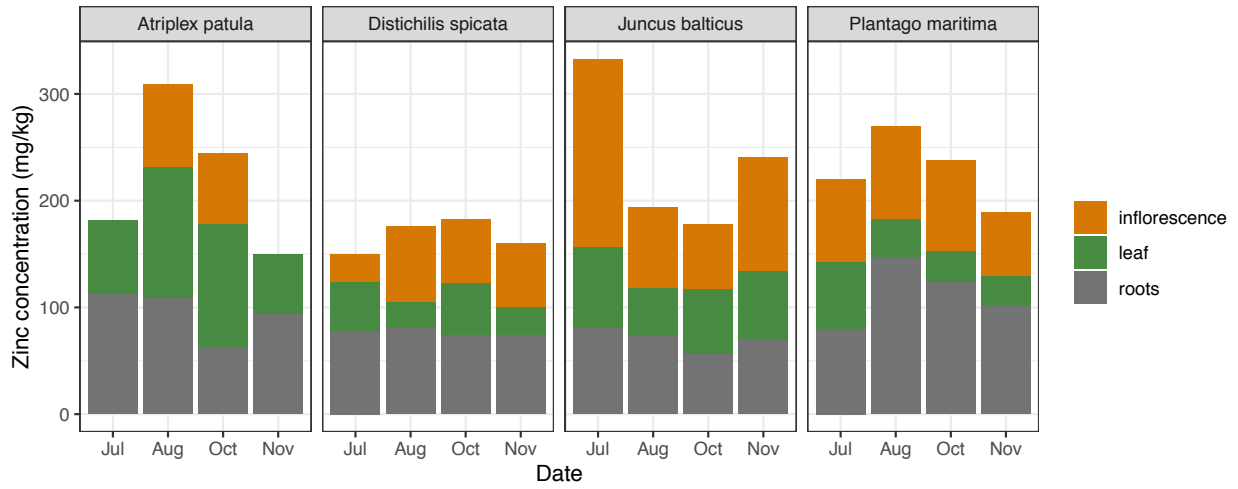
Iron-57 Change Over Time



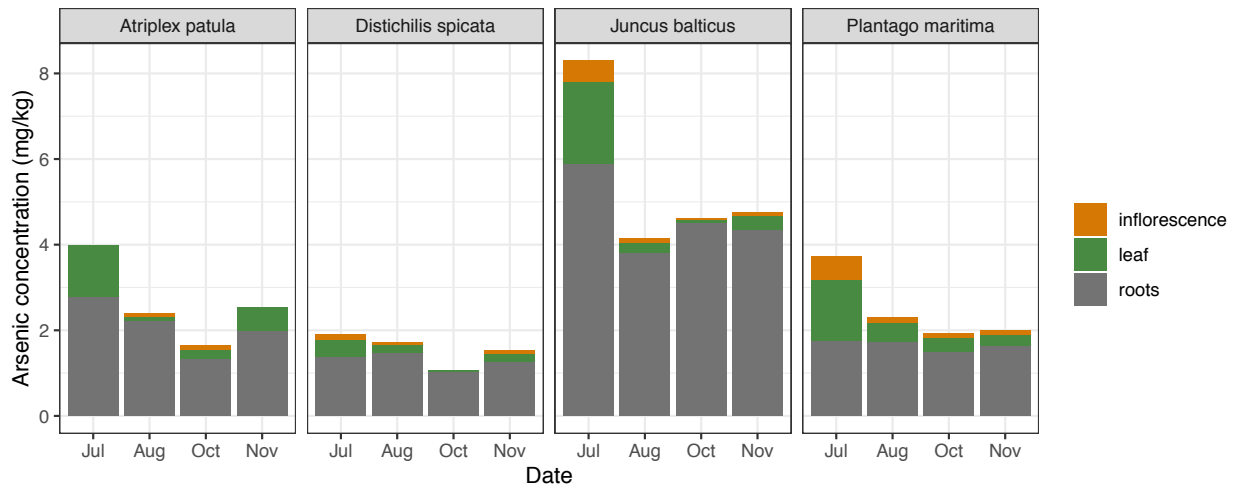
Copper-63 Change Over Time



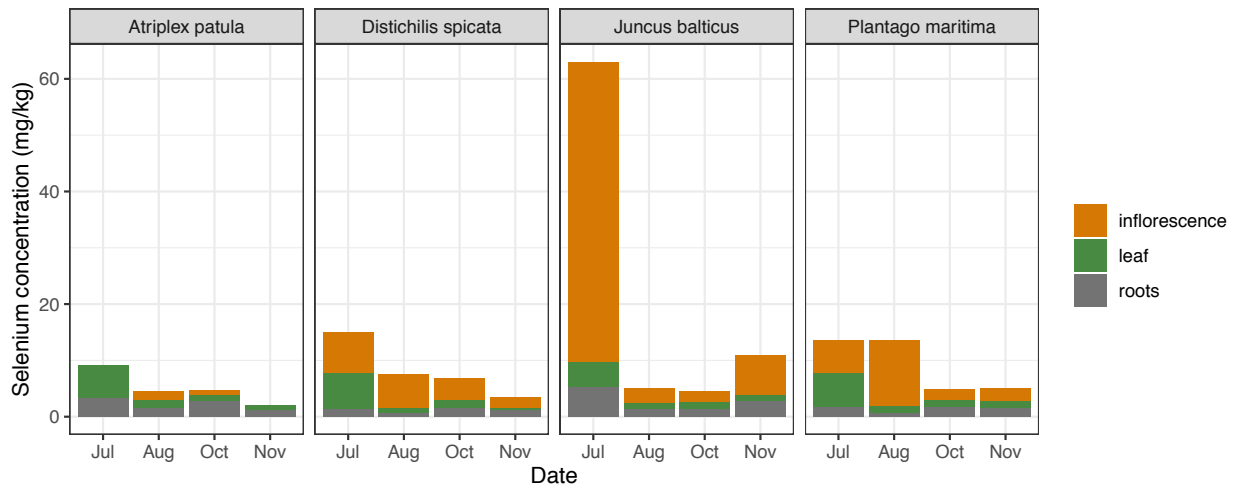
Zinc-66 Change Over Time



Arsenic-75 Change Over Time



Selenium-78 Change Over Time



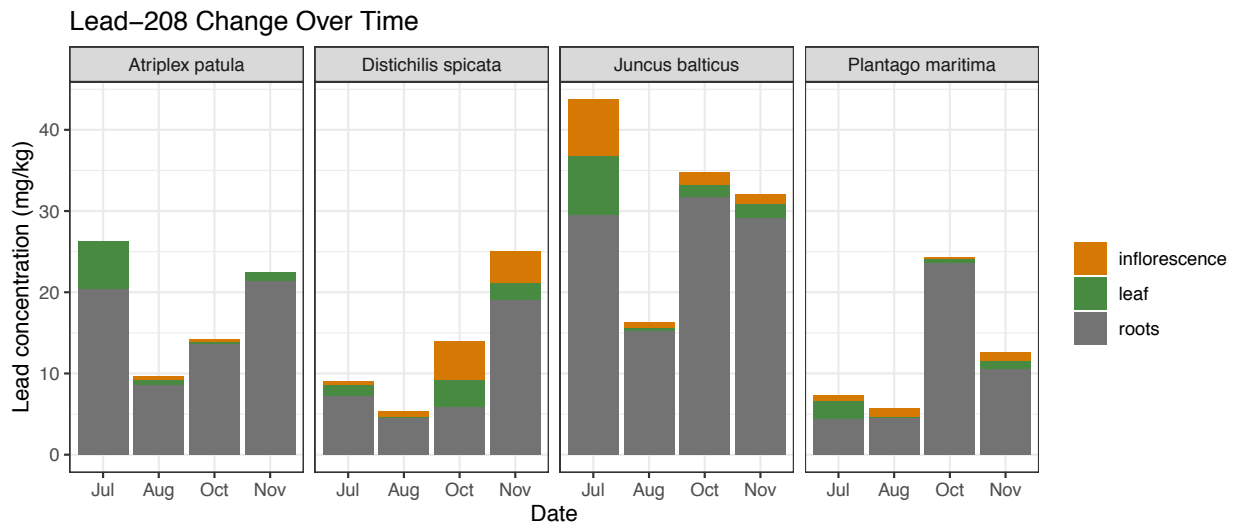
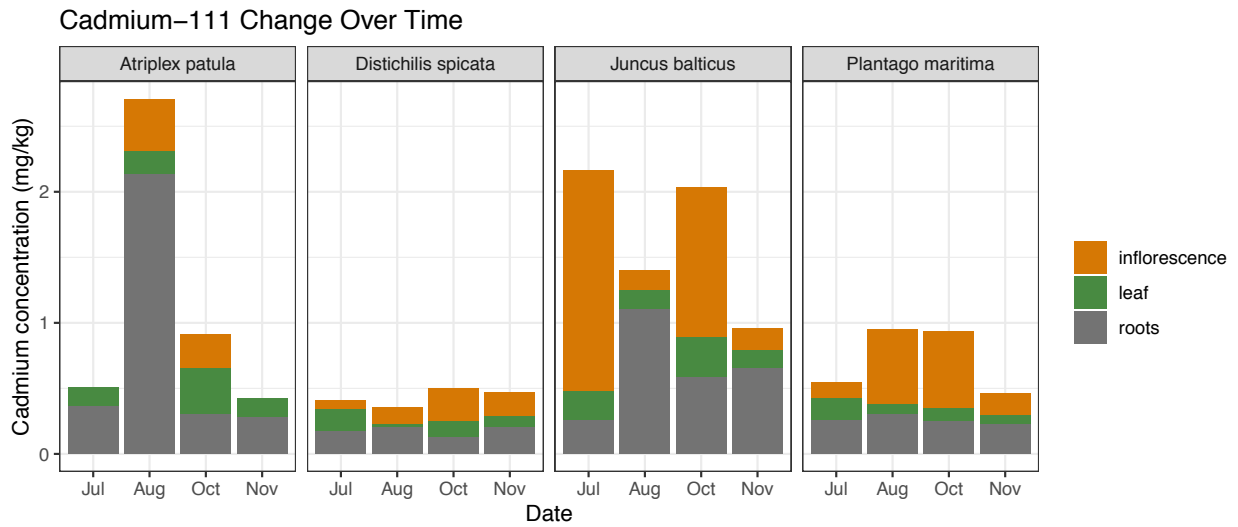
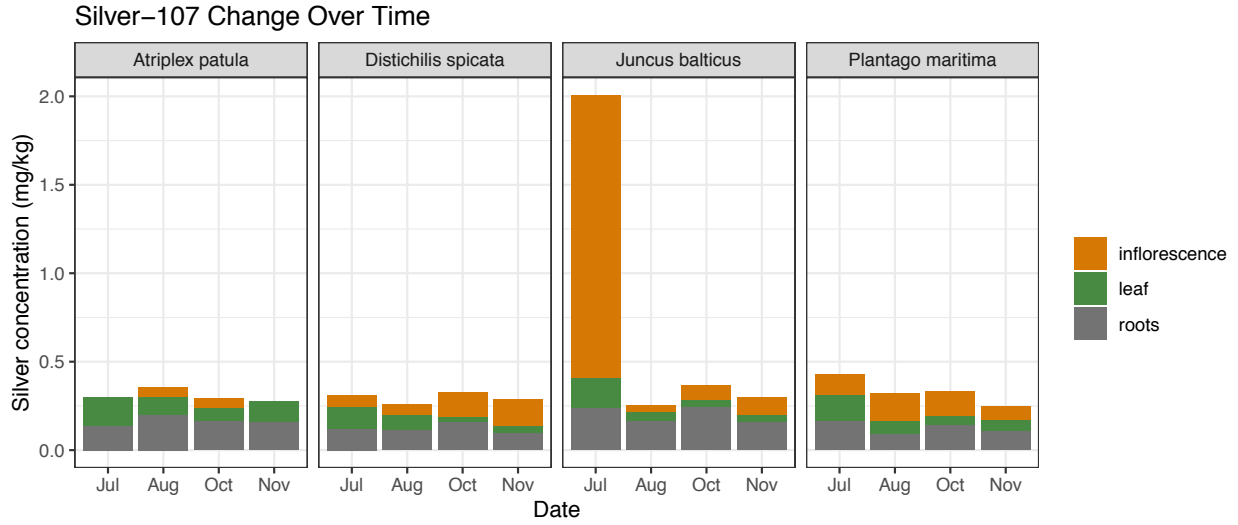


Figure 7: Stacked barplots demonstrating mean change over time in metal concentration between plant parts and species.

Table 16: Mean and standard errors of concentrations of metals, in mg/kg, and respective percentage of total concentration, separated by month. Total digestion percentage indicates percent recovery of metals from sequential extraction digestions.

	Exchangeable (Fraction 1)	Carbonates (Fraction 2)	Fe/Mn oxides (Fraction 3)	Organic Matter (Fraction 4)	Total (Total digestion)
Magnesium-25	n/a				
July		13039 ± 2006 (154.16%)	2539 ± 380 (30.01%)	826.7 ± 80.60 (9.77%)	8458 ± 548 (193.95%)
August		13230 ± 1421 (161.16%)	2666 ± 331 (32.47%)	902.1 ± 93.18 (10.99%)	8209 ± 578 (204.62%)
October		11205 ± 1444 (149.01%)	2214 ± 326 (29.44%)	798.6 ± 82.34 (10.62%)	7520 ± 435 (189.07%)
November		11420 ± 1365 (139.54%)	2818 ± 336 (34.43%)	840.38 ± 79.47 (10.27%)	81.84 ± 425 (184.24%)
Aluminum-27					
July	0.45 ± 0.12 (0.0025%)	14.40 ± 2.28 (0.08%)	733.4 ± 56.93 (4.09%)	2036 ± 180 (11.36%)	17928 ± 1809 (15.53%)
August	0.66 ± 0.33 (0.0039%)	20.82 ± 3.99 (0.12%)	785.1 ± 74.49 (4.61%)	2421 ± 213 (14.23%)	17014 ± 1563 (18.97%)
October	0.39 ± 0.20 (0.0022%)	24.89 ± 4.80 (0.14%)	807.8 ± 64.20 (4.60%)	2217 ± 252 (12.61%)	17579 ± 1530 (17.35%)
November	0.004 ± 0.004 (0.0028%)	24.66 ± 5.04 (0.16%)	824.3 ± 65.19 (5.31%)	2186 ± 238 (14.07%)	15532 ± 1301 (19.54%)
Chromium-52					
July	0.76 ± 0.03 (1.77%)	1.40 ± 0.05 (3.29%)	9.91 ± 0.27 (23.26%)	5.42 ± 0.53 (12.73%)	42.59 ± 4.96 (41.06%)
August	0.69 ± 0.03 (1.59%)	1.45 ± 0.04 (3.33%)	10.10 ± 0.29 (23.18%)	6.45 ± 0.78 (14.80%)	43.56 ± 3.46 (42.90%)
October	0.60 ± 0.02 (1.32%)	1.43 ± 0.05 (3.12%)	10.08 ± 0.34 (21.98%)	5.47 ± 0.77 (11.93%)	45.87 ± 3.51 (38.35%)
November	0.89 ± 0.03 (1.86%)	1.40 ± 0.06 (2.91%)	10.06 ± 0.34 (20.86%)	5.93 ± 0.90 (12.29%)	48.22 ± 5.18 (37.91%)
Manganese-55					
July	50.37 ± 9.64 (7.74%)	138.8 ± 30.19 (21.34%)	178.7 ± 29.88 (27.47%)	28.06 ± 4.60 (4.31%)	650.4 ± 90.44 (60.87%)
August	44.33 ± 9.36 (7.99%)	119.1 ± 24.98 (21.46%)	158.4 ± 24.55 (28.54%)	31.33 ± 5.25 (5.64%)	555.0 ± 80.84 (63.63%)
October	18.27 ± 3.60 (3.60%)	114.4 ± 32.40 (22.51%)	176.5 ± 31.71 (34.73%)	29.69 ± 5.93 (5.84%)	508.2 ± 68.11 (66.68%)
November	23.64 ± 5.06 (3.57%)	93.98 ± 16.54 (14.19%)	288.0 ± 109.6 (43.48%)	40.69 ± 13.47 (6.14%)	662.4 ± 129.9 (67.38%)
Iron-57					
July	80.59 ± 3.08 (0.38%)	39.94 ± 5.13 (0.19%)	3455 ± 307 (16.31%)	2065 ± 366 (9.75%)	21185 ± 2937 (26.63%)
August					

	October	65.76 ± 1.98 (0.38%)	42.50 ± 9.38 (0.25%)	3211 ± 323 (18.79%)	2304 ± 423 (13.48%)	17090 ± 1603 (32.90%)
	November	56.49 ± 1.61 (0.28%)	36.10 ± 6.76 (0.19%)	3564 ± 401 (17.58%)	1997 ± 404 (9.85%)	20271 ± 1677 (27.89%)
		80.95 ± 1.84 (0.43%)	51.24 ± 9.89 (0.27%)	4002 ± 356 (21.15%)	2115 ± 392 (11.18%)	18920 ± 1318 (33.03%)
Copper-63						
	July	0.60 ± 0.06 (2.21%)	1.55 ± 0.20 (5.71%)	1.68 ± 0.47 (6.20%)	1.49 ± 0.92 (5.51%)	27.08 ± 4.33 (19.62%)
	August	0.31 ± 0.05 (1.04%)	2.13 ± 0.17 (7.07%)	0.57 ± 0.24 (1.89%)	35.34 ± 34.70 (117.4%)	30.10 ± 4.29 (127.44%)
	October	0.10 ± 0.07 (0.18%)	5.74 ± 3.47 (10.87%)	10.27 ± 8.06 (19.45%)	0.98 ± 0.98 (1.85%)	52.80 ± 22.96 (32.36%)
	November	0.04 ± 0.02 (0.11%)	1.95 ± 0.28 (6.14%)	1.33 ± 0.75 (4.19%)	0.41 ± 0.34 (1.28%)	31.75 ± 4.54 (11.73%)
Zinc-66						
	July	1.26 ± 0.12 (1.28%)	14.31 ± 0.87 (14.53%)	40.97 ± 3.10 (41.60%)	12.28 ± 1.91 (12.46%)	98.49 ± 8.74 (69.88%)
	August	1.34 ± 0.22 (1.32%)	14.90 ± 1.05 (14.67%)	41.25 ± 3.71 (40.62%)	19.25 ± 3.51 (18.95%)	101.56 ± 8.24 (75.57%)
	October	0.71 ± 0.15 (0.71%)	15.33 ± 1.35 (15.47%)	43.06 ± 4.53 (43.43%)	3.09 ± 0.75 (3.12%)	99.16 ± 10.77 (62.72%)
	November	0.98 ± 0.19 (0.96%)	15.42 ± 0.96 (15.16%)	43.59 ± 3.18 (42.86%)	5.05 ± 1.34 (4.97%)	101.71 ± 7.04 (63.95%)
Arsenic-75						
	July	5.54 ± 0.34 (64.16%)	0.31 ± 0.06 (3.57%)	1.30 ± 0.20 (15.07%)	None detected	
	August	5.94 ± 0.39 (85.72%)	0.30 ± 0.05 (4.31%)	1.26 ± 0.18 (18.19%)	8.63 ± 1.03 (82.79%)	
	October	4.27 ± 0.22 (53.17%)	0.22 ± 0.05 (2.71%)	1.24 ± 0.23 (15.41%)	6.93 ± 0.60 (108.22%)	
	November	7.56 ± 0.39 (102.45%)	0.23 ± 0.05 (3.13%)	1.51 ± 0.25 (20.41%)	8.02 ± 1.04 (71.30%)	
Selenium-78						
	July	4.85 ± 0.17 (185.92%)	0.84 ± 0.30 (32.31%)	3.32 ± 0.31 (127.31%)	14.60 ± 1.74 (559.7%)	2.61 ± 0.20 (905.22%)
	August	6.00 ± 0.22 (54.26%)	0.56 ± 0.05 (5.07%)	2.70 ± 0.42 (24.43%)	10.73 ± 2.30 (97.04%)	11.06 ± 3.41 (180.80%)
	October	5.58 ± 0.23 (148.01%)	0.79 ± 0.06 (20.96%)	8.30 ± 0.30 (220.34%)	17.16 ± 1.74 (455.3%)	3.77 ± 0.18 (844.62%)
	November	9.03 ± 0.18 (177.14%)	0.75 ± 0.11 (14.62%)	4.22 ± 0.73 (82.77%)	2.55 ± 0.86 (50.05%)	5.10 ± 0.23 (324.58%)
Silver-107						
	July	1.20 ± 0.17 (250.86%)	0.01 ± 0.001 (1.73%)	0.22 ± 0.04 (45.71%)	167.0 ± 167 (3490%)	0.48 ± 0.33 (35198%)

August	1.08 ± 0.16 (539.21%)	11.43 ± 8.17 (5718%)	2.50 ± 1.61 (1249%)	366.9 ± 366 (18358%)	0.20 ± 0.02 (191089%)
October	1.05 ± 0.15 (689.89%)	0.01 ± 0.002 (4.43%)	0.23 ± 0.04 (153.25%)	0.15 ± 0.08 (101.5%)	0.15 ± 0.02 (949.04%)
November	0.76 ± 0.13 (96.57%)	0.02 ± 0.002 2.05%	0.20 ± 0.03 (82.77%)	0.06 ± 0.05 (8.07%)	0.79 ± 0.64 (131.51%)
Cadmium-111					
July	0.09 ± 0.01 (45.92%)	30.79 ± 17.47 (15561%)	16.98 ± 5.50 (8581%)	0.0 ± 0.00 (0%)	0.20 ± 0.02 (24189%)
August	0.08 ± 0.01 (17.72%)	102.2 ± 83.4 (22118%)	25.78 ± 25.38 (5577%)	0.27 ± 0.27 (58.66%)	0.46 ± 0.08 (27772%)
October	0.06 ± 0.01 (19.35%)	230.9 ± 124.5 (75868%)	2.99 ± 1.64 (983.25%)	33.31 ± 33.27 (10946%)	0.30 ± 0.06 (87818%)
November	0.10 ± 0.01 (30.57%)	0.09 ± 0.02 (28.70%)	0.32 ± 0.09 (100.30%)	0.22 ± 0.21 (70.64%)	0.32 ± 0.04 (229.31%)
Lead-208					
July	0.23 ± 0.02 (0.44%)	3.10 ± 0.57 (5.88%)	21.04 ± 4.50 (39.87%)	6.67 ± 3.22 (12.63%)	52.78 ± 12.64 (58.81%)
August	0.39 ± 0.14 (0.66%)	4.13 ± 2.02 (7.04%)	19.02 ± 7.73 (32.37%)	20.75 ± 14.91 (35.32%)	58.74 ± 29.95 (75.38%)
October	0.17 ± 0.08 (0.27%)	6.79 ± 0.70 (6.16%)	22.78 ± 3.43 (37.07%)	8.43 ± 1.91 (13.72%)	61.46 ± 10.36 (57.22%)
November	0.21 ± 0.11 (0.27%)	8.12 ± 1.93 (10.26%)	27.02 ± 6.42 (34.14%)	10.09 ± 2.98 (12.75%)	79.15 ± 20.48 (57.42%)

Table 17: Mean and standard errors of concentrations of metals, in mg/kg, and respective percentage of total concentration, separated by estuary site. Total digestion percentage indicates percent recovery of metals from sequential extraction digestions.

	Exchangeable (Fraction 1)	Carbonates (Fraction 2)	Fe/Mn oxides (Fraction 3)	Organic Matter (Fraction 4)	Total (Total Digestion)
Magnesium-25	n/a				
California		16566 ± 1403 (167.53%)	3783 ± 276 (38.25%)	1150 ± 57.51 (11.63%)	9889 ± 176 (217.41%)
Chuckanut		10136 ± 1296 (202.62%)	1886 ± 301 (37.71%)	455.0 ± 48.23 (9.10%)	5003 ± 260 (249.42%)
Padden		9216 ± 883 (96.32%)	1824 ± 118 (19.06%)	947.1 ± 64.23 (9.90%)	9568 ± 309 (125.28%)
Aluminum-27					
California	0.48 ± 0.20 (0.002%)	21.03 ± 4.00 (0.11%)	842.5 ± 64.52 (4.33%)	3236 ± 129 (16.62%)	19469 ± 1244 (21.06%)
Chuckanut	0.49 ± 0.20 (0.005%)	17.23 ± 3.33 (0.18%)	562.5 ± 33.74 (5.93%)	1225 ± 126 (12.91%)	9492 ± 554 (19.02%)
Padden	0.08 ± 0.04 (0.004%)	26.69 ± 3.21 (0.12%)	1015 ± 38.64 (4.40%)	2173 ± 179 (9.43%)	23051 ± 1067 (13.95%)
Chromium-52					

California	0.63 ± 0.02 (1.71%)	1.29 ± 0.04 (3.50%)	9.31 ± 0.20 (25.26%)	7.23 ± 0.38 (19.60%)	36.87 ± 2.31 (50.07%)
Chuckanut	0.78 ± 0.03 (2.21%)	1.47 ± 0.04 (4.19%)	9.53 ± 0.19 (27.10%)	3.77 ± 0.69 (10.73%)	35.15 ± 3.45 (44.23%)
Padden	0.83 ± 0.04 (1.22%)	1.53 ± 0.04 (2.26%)	11.68 ± 0.28 (17.25%)	6.67 ± 0.77 (9.85%)	67.60 ± 3.05 (30.57%)
Manganese-55					
California	60.82 ± 7.22 (6.98%)	158.7 ± 19.06 (18.21%)	333.4 ± 75.20 (38.27%)	59.48 ± 9.63 (6.83%)	871.2 ± 92.65 (70.29%)
Chuckanut	10.46 ± 1.96 (4.90%)	37.42 ± 5.30 (17.53%)	59.29 ± 7.62 (27.77%)	8.43 ± 0.92 (3.95%)	213.5 ± 17.44 (54.15%)
Padden	30.18 ± 7.33 (4.29%)	166.0 ± 36.68 (23.58%)	211.3 ± 27.74 (30.01%)	28.41 ± 2.65 (4.04%)	704.0 ± 74.47 (61.91%)
Iron-57					
California	66.81 ± 2.27 (0.31%)	41.44 ± 5.88 (0.19%)	4548 ± 296 (20.84%)	3780 ± 376 (17.32%)	21822 ± 1169 (38.66%)
Chuckanut	73.81 ± 2.30 (0.70%)	35.40 ± 6.32 (0.34%)	1860 ± 146 (17.66%)	1224 ± 211 (10.67%)	10532 ± 603 (29.37%)
Padden	72.64 ± 2.79 (0.27%)	53.18 ± 8.98 (0.20%)	4501 ± 220 (16.72%)	1237 ± 178 (4.59%)	26918 ± 1972 (21.78%)
Copper-63					
California	0.23 ± 0.05 (0.62%)	1.52 ± 0.13 (4.11%)	0.80 ± 0.19 (2.15%)	0.89 ± 0.43 (2.41%)	36.99 ± 3.26 (9.29%)
Chuckanut	0.19 ± 0.04 (1.75%)	1.77 ± 0.12 (16.35%)	0.47 ± 0.29 (4.34%)	0.07 ± 0.07 (0.69%)	10.80 ± 1.77 (23.14%)
Padden	0.39 ± 0.08 (0.60%)	6.04 ± 3.17 (9.29%)	11.01 ± 7.37 (16.94%)	33.75 ± 31.79 (51.92%)	65.00 ± 20.66 (78.76%)
Zinc-66					
California	1.04 ± 0.08 (1.04%)	12.20 ± 0.58 (12.11%)	40.29 ± 2.00 (39.99%)	9.23 ± 1.50 (9.17%)	100.7 ± 4.81 (62.30%)
Chuckanut	1.21 ± 0.19 (1.80%)	15.66 ± 0.81 (23.37%)	31.50 ± 2.32 (47.01%)	10.90 ± 2.43 (16.27%)	67.01 ± 4.83 (88.45%)
Padden	0.93 ± 0.16 (0.66%)	17.83 ± 1.24 (12.70%)	59.08 ± 4.00 (42.10%)	9.51 ± 2.21 (6.78%)	140.35 ± 8.93 (62.24%)
Arsenic-75					
California	4.29 ± 0.19 (46.65%)	0.27 ± 0.03 (2.93%)	1.58 ± 0.14 (17.13%)	None detected	
Chuckanut	6.30 ± 0.29 (111.18%)	0.34 ± 0.07 (6.04%)	1.29 ± 0.25 (22.73%)	9.20 ± 0.69 (66.71%)	
Padden	7.24 ± 0.40 (86.57%)	0.15 ± 0.02 (1.81%)	1.04 ± 0.09 (12.46%)	5.67 ± 0.72 (139.96%)	
Selenium-78					
California	5.69 ± 0.24 (55.67%)	1.01 ± 0.21 (9.92%)	5.16 ± 0.54 (50.43%)	11.10 ± 1.73 (108.52%)	10.23 ± 2.40 (224.55%)
Chuckanut	6.43 ± 0.27	0.47 ± 0.06	4.19 ± 0.47	11.29 ± 1.52	3.05 ± 0.24

		(210.67%)	(15.27%)	(137.18%)	(369.74%)	(732.86%)
	Padden	7.17 ± 0.37	0.72 ± 0.07	4.54 ± 0.56	11.44 ± 1.95	3.33 ± 0.20
		(215.29%)	(21.61%)	(136.29%)	(343.47%)	(716.65%)
Silver-107						
	California	0.67 ± 0.11	1.83 ± 1.82	1.08 ± 1.10	0.07 ± 0.04	0.19 ± 0.01
		(354.94%)	(974.7%)	(785.65%)	(37.98%)	(2153.3%)
	Chuckanut	1.16 ± 0.13	6.04 ± 5.39	0.17 ± 0.03	252.3 ± 252	0.32 ± 0.21
		(361.21%)	(1882%)	(54.21%)	(78611%)	(80908%)
	Padden	1.31 ± 0.14	0.01 ± 0.001	0.68 ± 0.28	153.1 ± 153	0.76 ± 0.57
		(171.81%)	(1.30%)	(89.43%)	(20064%)	(20327%)
Cadmium-111						
	California	0.09 ± 0.01	142.4 ± 77.53	7.34 ± 3.84	23.07 ± 22.87	0.34 ± 0.06
		(26.02%)	(41661%)	(2146%)	(6749%)	(50583%)
	Chuckanut	0.08 ± 0.01	43.24 ± 42.90	20.25 ± 17.43	0.002 ± 0.002	0.31 ± 0.04
		(25.83%)	(13799%)	(6462%)	(0.66%)	(20288%)
	Padden	0.07 ± 0.01	86.15 ± 75.22	5.45 ± 1.87	0.23 ± 0.20	0.32 ± 0.05
		(23.67%)	(27337%)	(1730%)	(72.48%)	(29163%)
Lead-208						
	California	0.28 ± 0.09	6.28 ± 1.93	30.40 ± 7.27	23.91 ± 10.44	97.38 ± 25.71
		(0.29%)	(6.45%)	(31.21%)	(24.55%)	(62.51%)
	Chuckanut	0.32 ± 0.10	3.50 ± 0.48	10.60 ± 1.17	5.31 ± 1.44	25.18 ± 4.04
		(1.29%)	(13.91%)	(42.11%)	(21.10%)	(78.41%)
	Padden	0.10 ± 0.02	4.50 ± 0.63	27.71 ± 2.58	3.15 ± 0.71	67.46 ± 8.70
		(0.15%)	(6.67%)	(41.08%)	(4.67%)	(52.57%)
