ASARCO’s Legacy: Bioaccumulation of As, Cd, and Pb in *Mytilus trossulus and Mytilus californianus* of Puget Sound

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**ABSTRACT:** Mussels are a sentinel species used to assess hazards of pollutants in ecosystems. The concentrations of As, Cd, and Pb were measured in eight composite samples of mussel (Mytilus trossulus and Mytilus californianus). Samples were collected from four locations within five miles of the previous American Smelting and Refining Company (ASARCO) smelter in Tacoma, Washington during April and July of 2018. Concentrations of metals in individual specimens from Totten Inlet in July 2018 were also determined. Throughout each season, average concentrations of As were found to be the highest and average concentrations of Pb were found to be lowest. Concentrations of each metal were found to be the highest in the spring with As at 11 ± 2 μg $g^{-1}$, Cd at 2.8 ± 0.6 μg $g^{-1}$, and Pb at 2.2 ± 1.3 μg $g^{-1}$. The results are discussed with respect to the lasting anthropogenic impacts of legacy metals from ASARCO.

## INTRODUCTION

Estuaries are regions where freshwater from rivers mixes high salinity water from the ocean (Figure 1). These systems are influenced by tidal cycles, which result in mixing of marine and freshwater. Because of this continuous mixing between water sources, particulates and contaminants are recycled within the estuarine environment. Recycled matter includes nutrients, microplastics, and heavy metals.[7] Heavy metals exist in both particulate and dissolved phases in estuaries.[2] They can be found suspended throughout the water column, in the sea-surface microlayer, as well as in sediments. Various physicochemical processes influence the interaction and movement of heavy metals between sediment and the water column, and distributions vary individually with each estuary system.[3] The mobility and

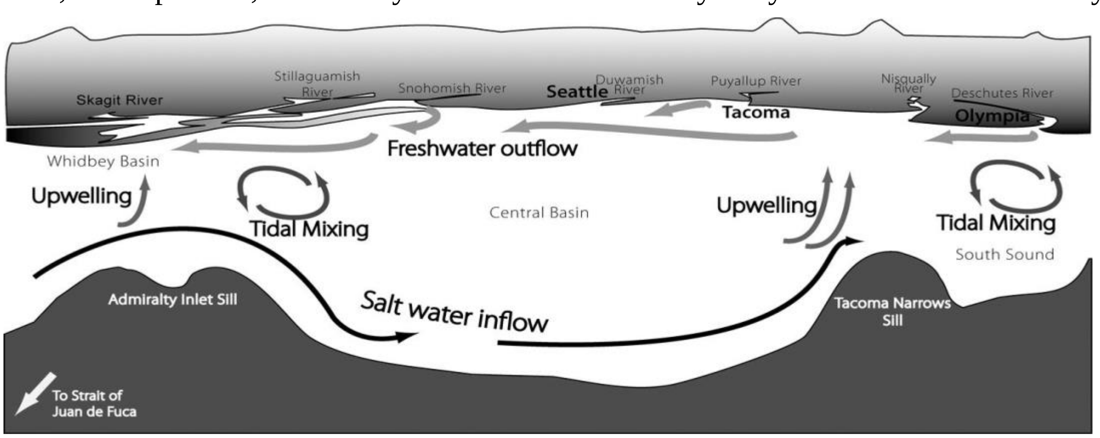


**Figure 1.** Longitudinal diagram of the Puget Sound estuary’s circulation patterns and characteristic bathymetry. Rivers are they key sources of freshwater and the Strait of Juan de Fuca is the key source of seawater. Mixing of these sources of fresh and saltwater are what make Puget Sound an estuary system. It reaches its deepest point 298-m in the central basin of the fjord.
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partitioning of heavy metals in estuaries is a function of pH, salinity, temperature, redox conditions, baseline heavy metal concentrations, sediment re-suspension, and organic matter (OM).[3, 4, 6] Binding affinities of heavy metals can also influence their bioavailability. Heavy metals with different binding affinities may compete for binding sites in sediment or to suspended particulate matter (SPM).[2, 3] The majority of heavy metals in estuaries are typically found in SPM and sediment.[1] Porewater, the water that surrounds sediment particles, is thought to be an important pathway of exposure to filter feeders.[2] The complex distributions of heavy metals between dissolved and particulate forms for a system can be described by the distribution coefficient ($K_d$ or $K_p$).[3] This is a useful parameter for the analysis of the bioavailability heavy metals to filter feeders, since the distributions fluctuate with changes in physicochemical parameters of the system.

Estuarine environments disperse trace elements such as heavy metals, which can complicate tracing sources of contamination. Bivalves such as mussels have been used as biomonitors of contaminants since the 20th century.[8] Biomonitors are useful to assess levels of contamination because of their high rates of bioaccumulation. Mussels and other bivalves have high rates of bioaccumulation because they are filter feeders. Filtering up to 50 L of sea water each day, mussels are exposed to a variety of contaminants that may be accumulated in their tissue. These high rates of bioaccumulation allow for heavy metal analysis that would otherwise not be possible due to largely variable concentrations and forms of heavy metals in estuarine systems.[8] Mussels are also useful biomonitors because they are stationary organisms, and their bioaccumulation of contaminants is thought to be representative of the local pollution in a given region. While there is some dispute over whether biomonitors provide an entirely accurate measure of local environmental contamination, they are accepted for their representation of the bioavailability of contaminants in a marine system.[8]

The American Smelting and Refining Company (ASARCO) in Tacoma operated for nearly 100 years smelting copper ore that was high in arsenic and lead until 1911 when they reportedly stopped smelting ore that contained lead. High arsenic ore was ideal for the smelter because of the precious metals that are also found within it. These metals include silver, gold, platinum, palladium, and often cadmium which was a contaminant.

The air pollution from the smelter spanned over 1,000 square miles of the Puget Sound basin. Commencement Bay, the region where the smelter was located, was the most heavily polluted region (Figure 2). Legacy pollution and contaminants still exist in Commencement Bay as a result of the smelter's operation.

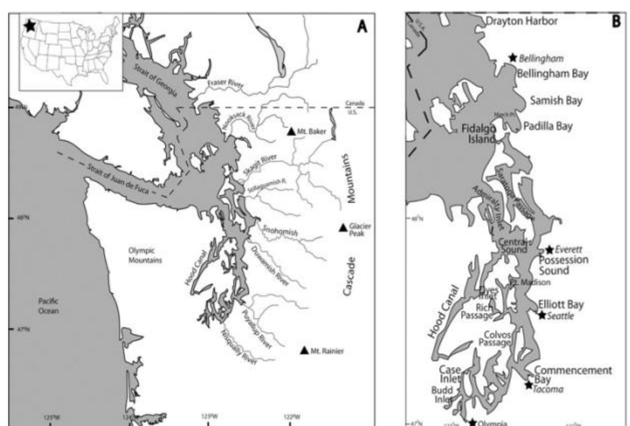

**Figure 2.** (A) Map of Washington state's coastal line and exposure to the Pacific Ocean. Seawater from the Pacific Ocean enters the Puget Sound through the Strait of Juan de Fuca. (B) Magnified view of the Puget Sound and its various straits and inlets. Tacoma and Commencement Bay are shown in the South Sound towards the bottom of the image.

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This study focuses on the bioaccumulation of arsenic (As), cadmium

(Cd), and lead (Pb) in mussel tissue. While certain metals can be toxic at higher levels of accumulation, As, Cd, and Pb are all toxic in trace amounts. These are three of the heavy metals that ASARCO smelted and processed when in operation. The objective of this study was to assess the legacy pollution in the environment around ASARCO and develop a method to digest and analyze mussel tissue for heavy metals using the new Perkin Elmer Titan MPS microwave digestion system and NexION 350 inductively coupled plasma mass spectrometer (ICP-MS). It is hypothesized that these metals will be highest in mussel tissue of organisms sampled nearest the previous smelting site.

## MATERIALS AND METHODS

**Mussel Collection and Preparation.** Mussels were collected from four sites within a five-mile radius of the original ASARCO smelter location. Site locations were chosen along the shoreline of Commencement Bay such that mussel specimens could be collected on land at low tide (Figure S7). The 17 samples from Totten Inlet were obtained through store purchase in the summer of 2017. Samples from Commencement Bay were collected during the spring and summer of 2018 in April and July. In April, 55 mussels were collected from the four sites (n = 9 at Site 1, n = 14 at Site 2, n = 18 at Site 3, and n = 14 at Site 4). In July, 69 samples were collected (n = 16 at Site 1, n = 24 at Site 2, n = 9 at Site 3, and n = 20 at Site 4).

Mussels were frozen at -80 °C after collection. Samples were removed and allowed to thaw at room temperature before being rinsed in nanopure water. The exterior of the shells were scrubbed and byssus threads were removed during this process. Tissues were extracted and using ceramic blades to minimize metal contamination. Ceramic blades were rinsed with dilute high-purity nitric acid between mussel extractions. The tissues were immediately added to a ZipLock bag for freeze-drying. Prior to freeze-drying, the samples were frozen at -80 °C. After freeze-drying, the samples were homogenized inside the ZipLock bags by physical force.

Mussels from Totten Inlet in 2017 were digested and analyzed by individual specimens in this experiment. These data were compared to those found by the Mussel Watch program, which digested and analyzed mussels as composite samples. The Mussel Watch program suspended mussels in cages for two-month periods at each location. [11] Composite samples were used for the mussels collected from Commencement Bay in 2018 for this experiment. Composite samples are beneficial in that the method removes variation between individual specimens and provides a broader estimate of the mussel population.

**Instrumentation**. Mussel samples were dried in ZipLock bags using a Labconco Lyph-Lock 6 lyophilizer and Labconco fast-freeze flasks and silicone rubber top fits. Upon completion of drying, each sample was digested in the PerkinElmer Titan MPS™ Microwave Sample Preparation System using the programmed EPA3052 method. The samples were digested in PerkinElmer standard 75-mL TFM™ vessels and caps. Method blanks were digested in standard pressure vessels with DPC™ Direct Pressure Control caps. Digested samples were analyzed using a PerkinElmer NexION 350 ICP-MS in standard mode. This instrument uses a glass concentric, glass self-aspirating MEINHARD® nebulizer; glass cyclonic spray chamber, demountable quartz, and triple cone interface composed of a Ni sampler, skimmer, and hyper skimmer cone. The samples were introduced to the nebulizer from the Elemental Scientific 2DX autosampler through a peristaltic pump in PerkinElmer Flared PVC peristaltic pump tubing (0.38-mm i.d.). The instrument was operated under the conditions listed in Table 1.

**Table 1. Instrument parameters and operation conditions for PerkinElmer NexION 350 ICP-MS and PerkinElmer Titan MPS running under EPA-3052 method**

| | |
|---|---|
| ICP-MS Parameters | |
| Plasma gas flow | 18.00 L $min^{-1}$ |
| Auxiliary gas flow | |
| Nebulizer gas flow | 1.0 L $min^{-1}$ |
| Plasma power | 25 V |
| Titan MPS parameters | |
| Peak pressure | 30 bar |
| Peak temperature | 180 ºC |

An example of the standard performance check method criterion is listed in Table 2.

**Table 2. Standard performance check method settings and results (in counts per second) used to optimize the ICP-MS prior to data collection.**

| | Optimization Criterion | Optimization Results |
|---|---|---|
| Intensity Criterion (cps) | Be 9 > 2000 | 5350.02 |
| | In 115 > 40000 | 46752.16 |
| | U 238 > 30000 | 41143.71 |
| | Bkgd 220 ≤ 1 | 0.10 |
| Formula Criterion (cps) | CeO 156 / Ce 140 ≤ 0.025 | 0.020 (= 861.17 / 43676.95) |
| | Ce++ 70 / Ce 140 ≤ 0.03 | 0.022 (= 949.77 / 43676.95) |

**Chemicals**. PerkinElmer Pure Plus standards for As, Cd, Pb, and the internal standard were in trace metal grade 2% $HNO_3$. The internal standard contained 50 µg $mL^{-1}$ of Ge and Sc, and 10 µg $mL^{-1}$of In, Rh, and Tb. Ultrapure trace metal grade hydrogen peroxide (30%) was purchased from VWR Chemicals BDH® and stored in a cabinet. Stock solutions of As (10,000 µg $L^{-1}$), Cd (1,000 µg $L^{-1}$), and Pb (1,000 µg $L^{-1}$) were prepared in a 2% $HNO_3$ matrix in 1-L Type A KIMAX® volumetric flasks. These stock solutions were stored in dark cabinets. They were diluted to the concentrations that were used for the external standards (5, 25, 50, 100, and 200 µg $L^{-1}$As, Cd, and Pb). Optimizations were performed each day before data collection using PerkinElmer NexION Setup Solution (1 µg $L^{-1}$ Be, Ce, Fe, In, Li, Mg, Pb, and U in 1% $HNO_3$).

**Heavy metal determination**. The EPA 3052 and 3051A methods were adapted and modified for this experiment. Dried mussel tissue (0.5 g) was combined with 6 mL of nanopore water (18-22 MΩ), 1 mL of 30% trace-metal grade hydrogen peroxide, and 2 mL of trace-metal grade concentrated nitric acid. After cooling, digested samples were diluted up to 50 mL with nanopore water in 50-mL Corning® polystyrene centrifuge tubes. These solutions were filtered through 25-mm Pall Corporation Acrodisc® syringe filters with 0.45-µm HT Tuffryn® membranes. Henke Sass Wolf Norm-Ject® 10-mL syringes were used. Aliquots of 7 mL diluted sample were further diluted up to 14 mL with nanopore water (~2% $HNO_3$). The internal standard (0.10 µL) was added to the 14 mL dilutions, external standards, and blanks before analysis on the ICP-MS. Concentrations of target analytes in dry mussel tissue were calculated using Equation 1.

$$[M_{d/w}] = \frac{M_{ICP} * 0.1}{m} \quad (1)$$

In Equation 1, $M_{d/w}$ represents the calculated concentration of metal in µg $g^{-1}$, $M_{ICP}$ is the concentration in µg $L^{-1}$ reported by the ICP-MS software, m is the sample mass introduced into the digestion in grams (~0.5g), and 0.1 is a constant in L of liters to account for the dilutions after digestion.

The elements listed in Table 3 include the internal standards used for each metal of interest. The Ge (73.9219 amu) was used as an internal standard for As (74.9216 amu), In (114.904 amu) for Cd (110.904 amu), and Tb

(158.925 amu) was used for Pb (207.977 amu). The corrections performed by the instrument to account for any potential interferences are listed alongside the corresponding analytes.

**Table 3. Metals of interest and their corresponding internal standards are listed alongside corresponding interferences and corrections made**

| Analyte | Corrections | Potential Interferences |
|---|---|---|
| Ge | -0.116645 * Se 77 | Se, ArS, Nd++, Sm++ |
| As | | ArCl, Sm++, Nd++, Eu++ |
| In | -0.014038 * Sn 118 | Sn, MoO |
| Cd | | MoO |
| Tb | | NdO, PrO |
| Pb | | |

## RESULTS AND DISCUSSION

The analyte found highest in concentration was As with a mean concentration of 10 ± 2 μg $g^{-1}$, while Pb was found to have the lowest average concentration at 1.8 ± 1 μg $g^{-1}$. The Cd concentrations were found to be closer to those of Pb than As at 2.4 ± 0.7 μg $g^{-1}$. The average concentrations for spring, summer, and Totten Inlet samples were all found to have As in the highest concentration and Pb in the lowest concentration. Concentrations for each metal were greater in the spring than in the summer samples. Significance in these differences between seasons were compared through F-Tests. The standard deviations between seasons for As and Pb were found to be significantly different above the 95% confidence interval. The difference in standard deviations between spring and summer for Cd was not significant above the 95% confidence interval (Table 4, Figure S6).

**Table 4. Seasonal averages for each metal (μg $g^{-1}$) at the Commencement Bay (ASARCO) sites and Totten Inlet.**

| Collection Site | As (μg $g^{-1}$) | Cd (μg $g^{-1}$) | Pb (μg $g^{-1}$) |
|---|---|---|---|
| ASARCO (April 2018) | 11 ± 2 | 2.8 ± 0.6 | 2.2 ± 1.3 |
| ASARCO (July 2018) | 9.3 ± 0.4 | 2.0 ± 0.5 | 1.3 ± 0.6 |
| Totten Inlet (July 2017) | 6.6 ± 1.0 | 2.7 ± 0.7 | 0.72 ± 0.05 |

For the concentrations reported during each season, the error is calculated as the standard deviation of all samples taken at all four sites. Differences between replicate composite samples were also calculated using Equation 2 where $M_{diff}$ is the percent difference between replicate samples, $M_{max}$ is the higher of the two concentrations (μg $g^{-1}$), $M_{min}$ is the lower of the two (μg $g^{-1}$), and $M_{avg}$ is the average between the two (μg $g^{-1}$).

$$M_{diff} = \frac{M_{max} - M_{min}}{M_{avg}} * 100\% \qquad (2)$$

Concentrations of As, Cd, and Pb were also analyzed to determine if there was a correlation between concentration and distance to the previous ASARCO smelter location. To do so, the concentration of metal found at each site was plotted against the distance from the smelter. Corresponding $R^2$ values were used to determine whether there was a strong correlation between these factors. The highest $R^2$ value was 0.47 and the lowest was 0.0, which demonstrates that there is no strong trend between these two factors (Figure S4, Figure S5).

Mussel size was tested against the distance from the ASARCO smelter to determine if any spatial trends existed. The $R^2$ value found in April was 0.03 and in July was 0.17, both of which demonstrate that there was no direct correlation between mussel size and distance to the ASARCO smelter. Sites 1 and 2 had noticeable changes in the variation of size between the mussel samples from spring to summer. The F-Test was used again to determine whether changes in size variation between season at each site were significant. Sites 1, 2, and 4 were all found to have significantly different standard deviations from spring to summer above the 95% confidence interval. The average between all four sites in spring was found to be 41 ± 7 mm. The same overall average was found for the mussels collected in summer, so no significant difference was found when comparing the seasons overall.

Mussel size and concentration were examined to determine whether a relationship between the two existed. Each average metal concentration per site was plotted as a function of the average mussel size at that site. These graphs were produced separately for each season (Figure S4, Figure S5). The highest $R^2$ of these graphs was 0.78 for As in the spring, and the lowest was 0.05 for As in the summer. Average sizes of mussels were used to plot against average concentrations of duplicate samples because the homogenized tissues were composite samples of all mussels collected at each corresponding location.

Spatial trends were analyzed using the four sites in Commencement Bay. The data from Totten Inlet were used as a general comparison of other mussels found in Puget Sound, and were not included in spatiotemporal trend analyses. Further comparison to the experimental results can be made to data accessible from the National Oceanic and Atmospheric Administration's (NOAA's) Mussel Watch program. To make these comparisons, the average concentrations of As, Cd, and Pb were calculated from the four Mussel Watch sites at Point Defiance Park, Commencement Bay (Skookum Wuldge), Tacoma (Ruston Way, Puget Creek), and the Tacoma Ruston Waterfront.[12] The average values calculated from these sites are collectively referred to as the NOAA Commencement Bay site in Table 5.

**Table 5. The overall average concentration (μg $g^{-1}$) of each metal is listed for each site in comparison to similar sites studied by NOAA**

| Collection Site | As (μg $g^{-1}$) | Cd (μg $g^{-1}$) | Pb (μg $g^{-1}$) |
|---|---|---|---|
| Sites 1-4 | 10 ± 2 | 2.4 ± 0.7 | 1.8 ± 1.1 |
| Commencement Bay (NOAA) | 5.7 | 2.2 | 0.4 |
| Totten Inlet | 6.6 ± 1.0 | 2.7 ± 0.7 | 0.72 ± 0.05 |
| Totten Inlet (NOAA) | 6.6 | 2.1 | 0.21 |

Data collected from the NOAA Mussel Watch program at Totten Inlet were also compared to the values found in this experiment (Table 5). Values at Totten Inlet are more similar between the two data sets than the data taken from Commencement Bay.

Variation between the data collected in this experiment and that reported by NOAA is expected to vary given the four-year gap between the two studies and differences between methods. Sample sizes at each of NOAA's sites (n = 32) were larger than those collected for this study. The variations in years that samples were collected, how many samples were collected at each site, and whether they were processed as composites or individuals could all contribute to variation between the datasets.

The FDA sets action levels for metals found in mussels, oysters, and clams. These limits are 86 ppm, 4 ppm, and 1.9 ppm for As, Cd, and Pb respectively. However, these values are based on wet weight concentrations, which were not recorded for individual mussels in this due to the sample preparation method. Given that the mussels were frozen and then rinsed in nanopure water before tissue extraction, there would be difficulty in measuring true wet weight. The amount of water within mussel tissue after rinsing in nanopure water as compared to seawater would result in mass changes that do not accurately portray the wet weight.

## CONCLUSION

Mussels were collected at four sites in Commencement Bay during July 2018 and April 2018. These mussels, as well as the samples obtained from Totten Inlet in July 2017, were homogenized and digested on the PerkinElmer Titan-MPS microwave digestion system. Following digestion, samples were diluted and analyzed for metal content on the PerkinElmer NexION 350 ICP-MS. This method was developed as a modified version of the EPA 3051A and 3052 methods for organic tissue analysis of As, Cd, and Pb. Overall, no trend seen between metal concentration and sample site proximity from the ASARCO smelter for the four sites located within Commencement Bay. This result is likely due to the small sampling radius (< 5 miles) and due to the location being a highly dynamic system situated within an estuary. There was significant variation seen between seasons for As and Pb between the four sites in Commencement Bay. Significant seasonal variation in mussel sizes was also detected at three of the four sampling locations, but overall seasonal averages had no significant difference in size. After comparing corresponding sizes of mussels collected the concentrations of metals detected, there was no correlation found for any metal during either season.

In future studies, samples should be collected from sites of interest throughout the year in order to obtain information on whether there is significant seasonal variation in metal uptake by mussels. It is also recommended that at minimum of 32 mussels be collected at each site to comply with NOAA's Mussel Watch program.[12] Additionally, mussels of similar size should be collected at each site and during each season. This will minimize variation in size that was seen in this current experiment. Minimizing size variation should increase the likelihood that mussels are of the same age group. Mussels of the same age in the same location would be exposed to the same chemical environment for the same period of time and should therefore bioaccumulate similar concentrations of metals. It is also proposed that samples from additional locations around the Sound be collected in future experiments for better assessment of the localized metal profile in Commencement Bay.

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