

Metallothionein as a Bioindicator of Metal Stress in Mussels

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Abstract

ASARCO (American Smelting And Refining Company) was a metal smelter located in the town of Ruston, near Point Defiance which created a peninsula of slag and spread metals throughout Puget Sound. Mussels are a good bioindicator species for marine pollution as they are found throughout Puget sound. They produce metallothionein (MT) when stressed by metal pollution and provide a direct indicator of metal toxicity. Our study measures MT in mussels from the native populations of south Puget Sound. We sampled mussels in two locations, Point Defiance and Thea Foss (chosen as an indicator of urban runoff). Our results show that the MT concentration in mussels in Point Defiance remain fairly consistent. Metal concentration in these mussels show a seasonal fluctuation. ASARCO may be a source of pollution in Point Defiance. After a major replacement of substrate in Thea Foss waterway MT production is increasing. This study may show that there continues to be a deposition of metal pollutants and that the historic metal pollutants don't have as large of an impact as once thought. The study may also be used to decide how we should spend our clean up money more wisely.

Introduction

- From 1890 to 1985, ASARCO deposited an estimated 15 million tons of slag on the property and in Commencement Bay. In the 1940s, ASARCO poured molten slag into the bay creating a 23-acre peninsula. Although the operations stopped in 1985, this did not prevent the release of metals such as arsenic, cadmium, copper, lead, and zinc into the soil, air, and the bay. (EPA 2010)
- Metallothionein (MT) is a family of cysteine-rich, low molecular weight proteins. MT in cells helps to regulate zinc and copper levels and to rid the cell of cadmium and mercury. (EPA 2004).

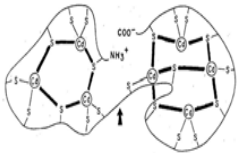


Figure 1 . Chemical structure of MT

- Being filter feeders, mussels are an important indicator species and offer insight into the biological condition of the water they are in. They are affected by pollution and habitat alteration.
- Bioaccumulation of heavy metals in the food chain places stress on the Puget Sound ecosystem. Therefore, the effect on the fishing industry can be detrimental.

Methods

Field Methods:

In 2010:

- We placed plastic mesh bags containing 40-50 mussels (*Mytilus* sp.) in Point Defiance Boat launch, each month removing five to add to the samples. For the months March – May, 20 random mussels were gathered and used in the protein analysis.
- All samples were stored on ice for transportation and dissected within an hour.

In 2011:

- Twenty mussels were gathered monthly from December to April. We dissected the samples of mussels after recording height and width of shell, along with the mass of the soft tissue. Samples were stored at -80 degrees Centigrade and a protein extraction protocol was performed (Viarengo et al. 1997).
- Each month we collected three bags of 20 periwinkle snails off rocks in the intertidal zone by the Point Defiance Boat launch.
- All samples treated the same as in 2010.



Figure 2 . Arrow indicates location of field study

Lab methods at SAM:

- Gathered our dissection tools/materials
- Opened mussel
- Cut out the digestive track which is located on the hinge, the color is black
- Weighed and zeroed plastic bag
- Added the mussel to the bag, then weighed it, recording the mass of the mussel
- Stored digestive track in plastic vial
- Stored vial in liquid nitrogen

Lab methods at UWT:

- Homogenized mussels and extracted MT (Viarengo et al. 1997)
- Constructed a calibration curve using glutathione
- Resuspended MT and measured using DTNB at 412 nm in the spectrophotometer (Viarengo et al. 1997)

Results

Point Defiance Concentration of Zinc and Copper over 9 months

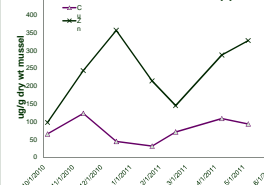


Figure 3 shows the variation in zinc and copper concentration levels at Point Defiance over the study period (mg/g of dry mass)

Point Defiance Concentration of Arsenic and Cadmium over 9 months

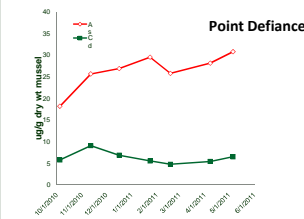


Figure 4 shows the variation in Arsenic and Cadmium concentration levels at Point Defiance over a 9 month period (mg/g of dry mass)

Average mol MT/g fr wt Point Defiance

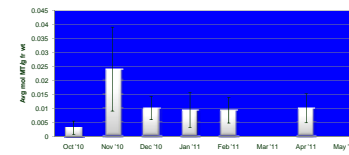


Figure 5 shows the variation in MT concentration levels at Point Defiance over a 9 month period (mol MT/g fr wt)

Avg mol MT/g fr weight by Month PD and TF

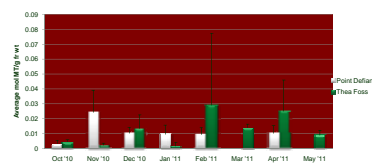


Figure 6 shows the variation in MT concentration levels at Point Defiance and Thea Foss over a 9 month period (mol MT/g fr wt)

Discussion

Natural occurring mussels:

- Recorded 9 months of info.
- Mytilus edulis* reproduce all year round with major peaks in the spring and further "opportunistic spawning in the summer" (Seed and Suchanek 1992) possibly correlating with metal increases in spring.
- There are observable concentration levels spikes in the metals during the study period in winter and spring.
- Fig 6, TF shows high concentrations of MT in calm water while PD shows lower and stable production of MT in seasonal unstable water over the study period.
- If the mussels are bioindicators, then are other organisms experiencing similar stresses?

MT= Metallothionein, PD=Point Defiance ,TF= Thea Foss

Point Defiance:

- Some areas of PD are observably cleaner or less polluted than the others.
- Point Defiance Marina was once the site of a large smelter operation, which is the source for the heavy metals found in slag piles.
- The seasonal change in the presence metals could be due to storms in the wintertime disturbing sediments in PD.

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Details:

- Amount of MT is an indicator of metal stress in mussels.
- Compare metal concentrations to other studies.
- These mussels could be bioindicators of the health in the PD marine ecosystem.
- Two to three years ago the test site was dredged and covered over with gravel sprawls; the area was scraped, replaced, and cleaned up.
- Mussels that are living the TF environment have higher levels of MT.
- The amount of MT infers that metal stress is less in PD from the slag/sediments leachate than in TF due to stormwater possibly.
- Since mussels are a keystone species, they can tell us that the other species could be experiencing the same stresses caused by production.

Future Work

- Look at the MT in worms found in sediment, plankton, etc.
- Look at the levels of MT production in correlation to spawning cycles and seasonal weather.
- Move onto organisms higher in the food chain such as sea stars to see if the metal/stress moves up the food chain
- Look at other cities with metal pollution.
- Look at areas such as rivers and lakes that are near paper mills or other pollution sources.

Work Cited

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