# Tacoma Smelter Plume: An opportunity to determine environmental risk using earthworms as bioindicators

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## Introduction

Earthworms have been increasingly studied as many correlations between high levels of metals in soil and worms have been found (Suthar & Singh, 2009). Earthworms ingest soil to obtain food which leads to an increased exposure to metals in soils, some of which have negative biological reactions. One biological effect is an increase in proteins known as metallothioneins (MT). MT proteins in earthworms can be measured in the laboratory and have been correlated with high metal content in soils (Carpenèa et al., 2006). MT analysis was used in the present study to help quantify the metal levels at which earthworms begin to exhibit physiological effects. Current Model Toxic Control Act (MTCA) ecological clean-up levels are based on laboratory tests using spiked soils to measure metal stress tolerance in earthworms. These laboratory tests increase metal stressors incrementally until mortality and do not represent long term exposure. Physiological tolerance of these organisms in the field to these stressors may be greater than exposure to spiked soils in the laboratory.

This study focuses on earthworms collected in soils from the Tacoma Smelter Plume footprint to determine stress responses in the form of MT production under different known arsenic and lead soil concentrations.

## MT Analysis

Field collected and bioassay earthworms were analyzed for MT at University of Washington Tacoma using the Viarengo et al. (1997) spectrophotometric method (Fig. 1). The method was changed slightly to adjust for sample weight and consistency. Six ml of homogenizing solution was used instead of 5ml per 0.5g dry weight. The samples were not kept on ice during homogenation. The bioassay worms were already frozen and field collected earthworms had been freeze dried.

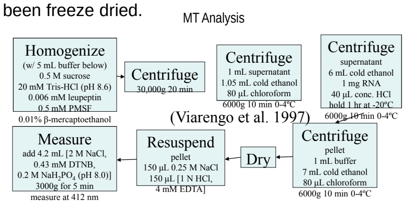


Fig. 1: MT Analysis Method

## Sampling

Worms were collected, weighed, and put into pre-cleaned glass jars. After collection, the worms were put on ice and transported back to Ecology headquarters where they were held for 48 hours at 4°C to evacuate the gut. After holding time the worms were euthanized and sent to MEL where they were freeze dried and homogenized for analysis of arsenic and lead by EPA method 200.8. Soil was sampled within 0-6 in. of the surface because it is the most biologically active zone (Suthar & Singh, 2009). The soil sample was put on ice and taken back to Ecology headquarters where it was placed into individually labeled glass jars and stored at -4°C until processing. Soil samples were sent to the Ecology Manchester Environmental Laboratory (MEL) for metal analysis by EPA method 200.8 (inductively coupled plasma/mass spectrometry).

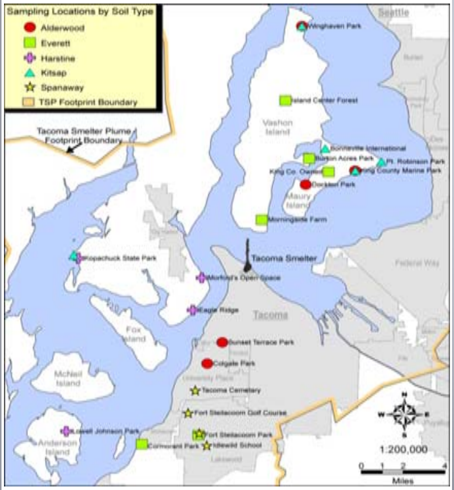


Fig. 2: Sampling locations

## Results & Discussion

Metal:

Metal concentrations in the worms were correlated with metal concentrations in the soils (Fig. 3).

| | MEAN mol-SH/g dry wt | As Soil Biota (mg/kg dry weight) | As Soil (mg/kg dry weight) | Pb soil biota (mg/kg dry weight) | Pb soil (mg/kg dry weight) |
|---|---|---|---|---|---|
| MEAN mol-SH/g dry wt | - | 0.570 | 0.213 | 0.790 | 0.422 |
| As Soil Biota (mg/kg dry weight) | | - | 0.000 | 0.000 | 0.000 |
| As Soil (mg/kg dry weight) | | | - | 0.401 | 0.000 |
| Pb soil biota (mg/kg dry weight) | | | | - | 0.296 |
| Pb soil (mg/kg dry weight) | | | | | - |

Fig. 3: Pearson Probability matrix

## Results & Discussion Cntd.

Arsenic (As) Results:

Arsenic was highly correlated (p<0.01) between soil and worms (Fig. 4). This finding is consistent with a number of studies that have shown arsenic readily accumulates in the tissue of earthworms (Suthar & Sushma 2009; Carpenèa et Al;. 2006; Meharg et al.; 1998).

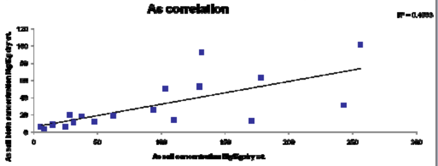


Fig. 4: As soil correlation to worm concentration

Lead (Pb) Results:

Lead did not have a significant correlation in worm tissue (Fig. 5). This is to be expected as studies have shown that there is high variability between lead availability to earthworms (Smith et al., 2010).

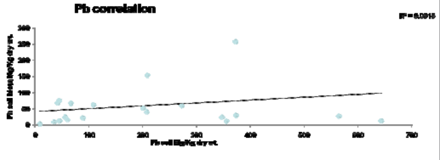


Fig. 5: Pb soil vs. Pb worm concentrations

MT Results:

MT in the worms did not have a statistically significant correlation to soil metal concentrations (Fig 6).

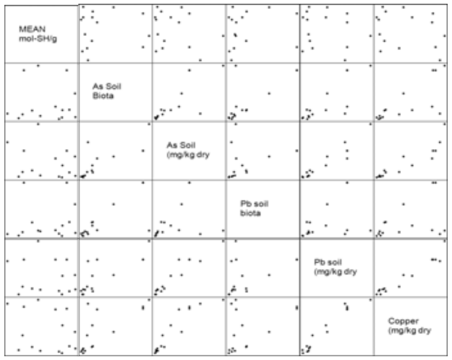


Fig 6: Scatter plots

## Results & Discussion Cntd.

Bioassay Results:

The bioassay earthworms showed a difference in MT concentrations compared to the field collected earthworms (Fig. 7). This is most likely because the in-situ worms had a longer exposure to soil metals than the bioassay worms which were exposed for a much shorter time.

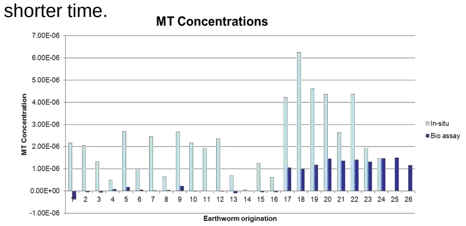


Fig. 7: Bioassay MT concentration Vs. Insitu MT

## Conclusion

- Laboratory tests using spiked soils are unable to recreate natural variables in soils which may affect the speciation of metals. Therefore, they can be ineffective in determining ecologically relevant clean up levels.
- arsenic and lead are poor inducers of MT in earthworms. More studies should be done using other metals such as Cu to determine MT concentrations in earthworms as an effective biomarker for soil metal concentration. More relevant studies for ecological responses of *in situ* worms (i.e. mortality rates, reproduction etc.) should also be conducted before any policy change.

## Acknowledgements:

I want to thank Jim Gawel of the University of Washington, Tacoma for help in guiding the project as well as fellow student Jolene Spencer for her help in analyzing the samples. I would also like to thank Janice Sloan for the opportunity to work with the Washington State Deptartment of Ecology.

## References

CARPENE, E., ANDREANI, g, MONARI, M., CASTELLANI, G. & ISANI, G. 2006. Distribution of Cd, Zn, Cu and Fe among selected tissues of the earthworm (*Allolobophora caliginosa*) and Eurasian woodcock (*Scolopax rusticola*). Science of the Total Environment, 363, 126-135.

SUTHAR, S. & SINGH, S. 2009. Bioconcentrations of Metals (Fe, Cu, Zn, Pb) in Earthworms (*Eisenia fetida*), Inoculated in Municipal Sewage Sludge: Do Earthworms Pose a Possible Risk of Terrestrial Food Chain Contamination? Environmental Toxicology, 24, 25-32.

VIARENGO, A., PONZANO, E., DONDERO, F. & FABBRI, R. 1997. A simple spectrophotometric method for metallothionein evaluation in marine organisms: An application to Mediterranean and Antarctic molluscs. Marine Environmental Research, 44, 69-84.