Ecological Soil Screening Levels for Arsenic and Lead in the Tacoma Smelter Plume Footprint and Hanford Site Old Orchards

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Cover photos: Top: Hanford Old Orchards between H and F reactors.
Bottom: Colgate Park, University Place, WA

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by

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Abstract

This study assessed soil screening levels (SSLs) for arsenic and lead that are protective of plants, soil biota, and wildlife in the Tacoma Smelter Plume footprint and Hanford Old Orchards areas. Both areas have historic widespread arsenic and lead contamination greater than the current Washington Model Toxics Control Act (MTCA) SSLs for ecological protection. The main sources of the contamination in these two areas are: emissions from smelting operations at the American Smelting and Refining Company facility in Tacoma, Washington, and use of lead arsenate pesticides in Eastern Washington fruit orchards within the U.S. Department of Energy Hanford Site.

Twenty-five samples from the Tacoma Smelter Plume footprint and 11 samples from the Hanford Old Orchards area were collected in the spring and summer of 2010. Sampling followed current MTCA procedures for setting ecologically relevant cleanup levels. Sampling included both chemical analyses of soil, plants, and soil biota; and bioassay testing of soil using lettuce and earthworms.

Although different arsenic species have separate SSLs in MTCA, this study found that using total arsenic SSLs was reasonable for dry soils. This study also examined the effects of soil type on the uptake and accumulation of arsenic and lead in plants and soil biota. Only plant uptake of arsenic seemed to be influenced by soil type. Silt loam soils had greater uptake than sandy loam soils. However, arsenic and lead SSLs were evaluated on an area-wide basis until stronger evidence is available that soil type and particularly soil texture affects the bioaccumulation of arsenic and lead in plants and soil biota. SSLs were calculated for the two study areas based on data from this study for plants, soil biota, and wildlife receptors and compared to MTCA and Environmental Protection Agency SSL values.

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Introduction

Project Background

In Washington State, air emissions from metal smelters and the use of lead arsenate pesticides have resulted in widespread arsenic and lead contamination of soils. Elevated levels of these metals can pose a risk to plants, soil biota, and wildlife. These risks include decreased reproductive success, decreased growth, and behavioral changes (Eisler, 1988a,b; Efroymson et al., 1997a,b).

Study Areas

This study focused on two areas with historic arsenic and lead contamination:

1. Tacoma Smelter Plume footprint, a 1,000 square mile area surrounding Tacoma, WA contaminated by smelter stack emissions.
2. U.S. Department of Energy (USDOE) Hanford Site old orchards where lead arsenate pesticides were used, hereafter referred to as Hanford Old Orchards.

These two areas represent historic widespread arsenic and lead contamination from two different sources in the state of Washington. Figure 1 shows the study area locations, and Figure 2 is a timeline of each area's history.

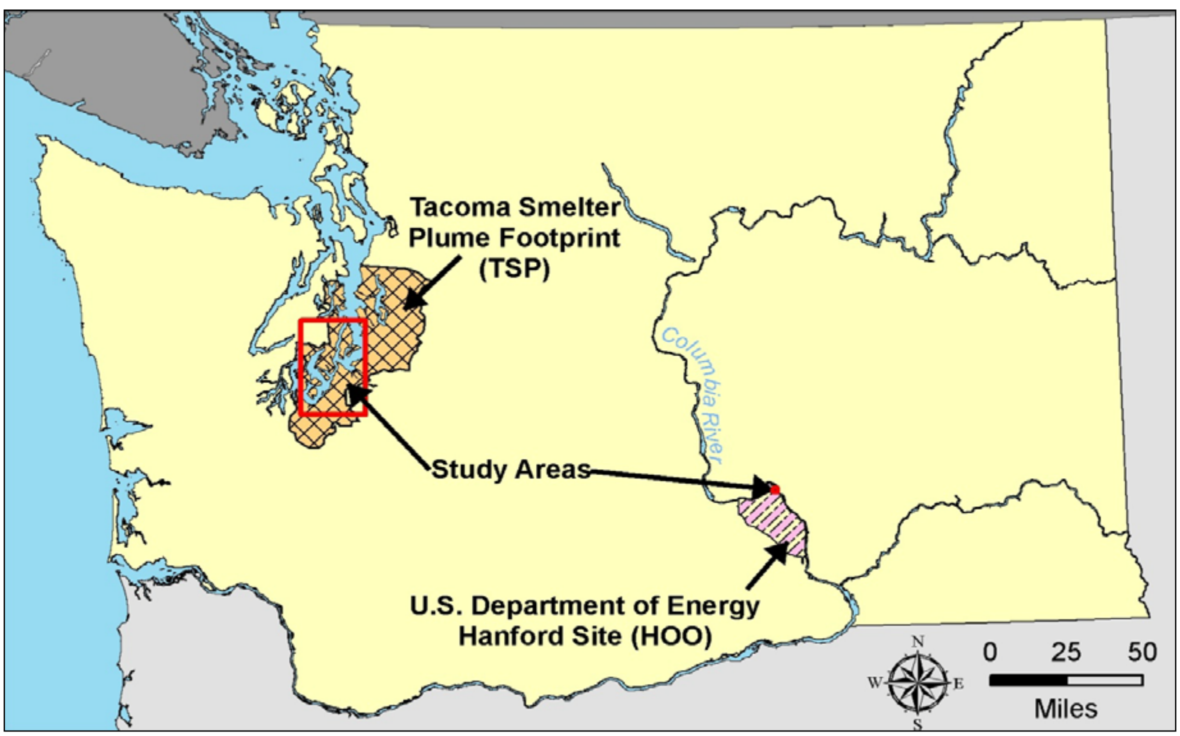

Figure 1. Map of Washington with sampling areas.

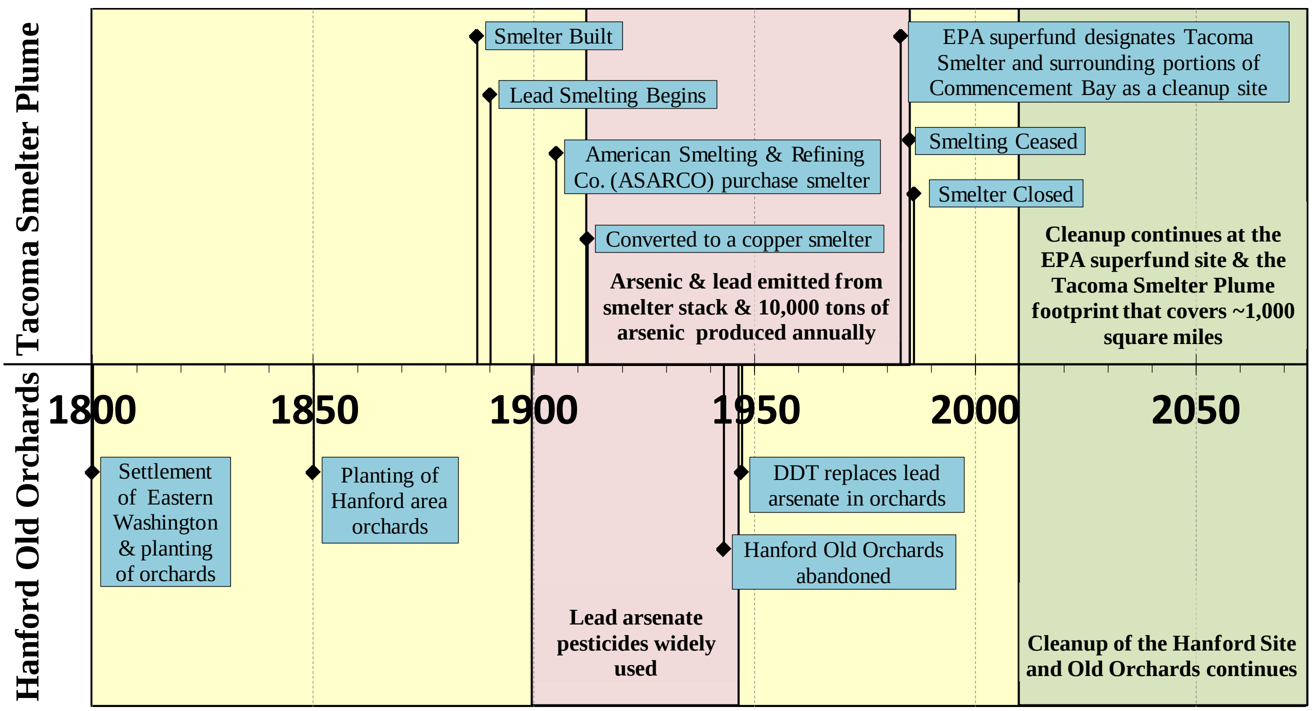

Figure 2. Timeline of arsenic and lead contamination for the Tacoma Smelter Plume footprint and Hanford Old Orchards.

Old Orchards Timeline (Yokel and Delistraty, 2003).
Tacoma Smelter Timeline (Pacific Groundwater Group and TeraStat Inc., 2005; EPA, 2010; and Ecology, 2007).

Why Now?

In both the Tacoma Smelter Plume footprint and Hanford in general, cleanup has been focused on human health concerns. As cleanup progresses, ecological impacts are being considered. However, the ecological impacts of arsenic and lead contamination in these two areas are poorly understood. The data from this study will influence ecologically-relevant cleanup decisions for arsenic and lead contaminated soils in these two areas. In this study, plant, soil biota, and wildlife data were evaluated to determine appropriate, ecologically-relevant soil screening levels (SSL).

Why Was This Study Needed?

According to the Model Toxics Control Act (MTCA), sites with soil contamination that have the potential to impact wildlife must undergo a terrestrial ecological evaluation (TEE). However, the size of the two study areas makes it difficult to conduct a traditional TEE. This is due to needing site-specific data that is difficult to generalize over a large area. Gaining generalized knowledge about the effects of arsenic and lead in these areas informs targeted cleanup efforts and work to streamline the TEE process for each individual cleanup site.

TEE risk assessments use SSLs derived from bioassays and simple bioaccumulation models to evaluate ecological risk. If the SSLs are exceeded, then they may be used as a conservative cleanup level for the site. Therefore, it is important that SSLs adequately protect wildlife while considering the ecological and monetary expense of setting these values too low. The size of these areas prescribes an area-specific approach for setting arsenic and lead SSLs using the data from this study. Table 1 shows current SSLs used under MTCA and Environmental Protection Agency (EPA)-recommended Eco-SSLs.

Table 1. Arsenic and lead MTCA cleanup standards in mg/Kg dw.
The soil screening level (SSL) is the lowest screening concentration (bold).

<table>
<tr><th rowspan="2">Contaminant</th><th rowspan="2">Human Health[1]</th><th colspan="4">Ecological SSLs</th><th rowspan="2">Source of Ecological SSLs</th></tr>
<tr><th>Plants</th><th>Soil biota</th><th>Avian</th><th>Mammalian</th></tr>
<tr><td>Arsenic III</td><td>-</td><td>-</td><td>-</td><td>-</td><td>7</td><td>WAC 173-340-7493</td></tr>
<tr><td>Arsenic V</td><td>-</td><td>10</td><td>60</td><td>150</td><td>132</td><td>WAC 173-340-7493</td></tr>
<tr><td>Arsenic</td><td>20</td><td>18</td><td>-</td><td>43</td><td>46</td><td>EPA, 2005b</td></tr>
<tr><td rowspan="2">Lead</td><td rowspan="2">250</td><td>50</td><td>500</td><td>118</td><td>125</td><td>WAC 173-340-7493</td></tr>
<tr><td>120</td><td>1,700</td><td>11</td><td>56</td><td>EPA, 2005c</td></tr>
</table>

[1] MTCA Method A human health standards.

Study Objectives

The objectives of this study are to:

- Determine ecological SSLs for use in the Tacoma Smelter Plume footprint and Hanford Old Orchards.
- Collect and analyze data for risks to wildlife in the Tacoma Smelter Plume footprint and Hanford Old Orchards, based on current[1] and modified[2] wildlife exposure models.
- Increase knowledge of soil types and physical characteristics that influence arsenic and lead toxicity and speciation.

[1] The "current wildlife exposure model" is based on laboratory- derived toxicity and accumulation values.
[2] The "modified wildlife exposure model" will be based on field data collected as part of the study and literature values.

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Methods

This study evaluated the ecological risks of arsenic and lead in the Tacoma Smelter Plume footprint and Hanford Old Orchards areas. Soil, plants, and soil biota were collected from 25 locations in the Tacoma Smelter Plume footprint and 11 locations in the Hanford Old Orchards area. The majority of the sampling occurred in May and June of 2010 with additional soil biota collections in August of the same year.

Complete methods for this study are described in the final Quality Assurance Project Plan (QAPP), *Evaluating the Toxicity of Arsenic and Lead in the Soils of the Tacoma Smelter Plume Footprint and Hanford Area Old Orchards Areas* (Sloan, 2010).

Site Selection

It was important that a variety of locations were sampled to cover a range of environmental factors and metals concentrations. Sampling locations were selected because they:

- Are located within the Tacoma Smelter Plume footprint or Hanford Old Orchards.
- Represent a range of major soil types (Tacoma Smelter Plume footprint only).
- Represent a range of arsenic and lead concentrations.
- Are accessible for sampling.
- Are relevant to or are part of a cleanup site.
- Support or have the potential to support wildlife.

These criteria reflect the objectives of this project: to increase knowledge of soil types and physical characteristics that influence arsenic and lead toxicity and to address the lack of field data for arsenic and lead soil toxicity in the state of Washington. Locations were selected to meet these criteria and provide insight for soil screening levels, not for statistical characterization of the areas studied.

Figure 3 shows the selected locations. Table 2 shows the selected Tacoma Smelter Plume footprint locations and Table 3 shows the Hanford Old Orchards locations. Appendix A has additional information about the selected locations.

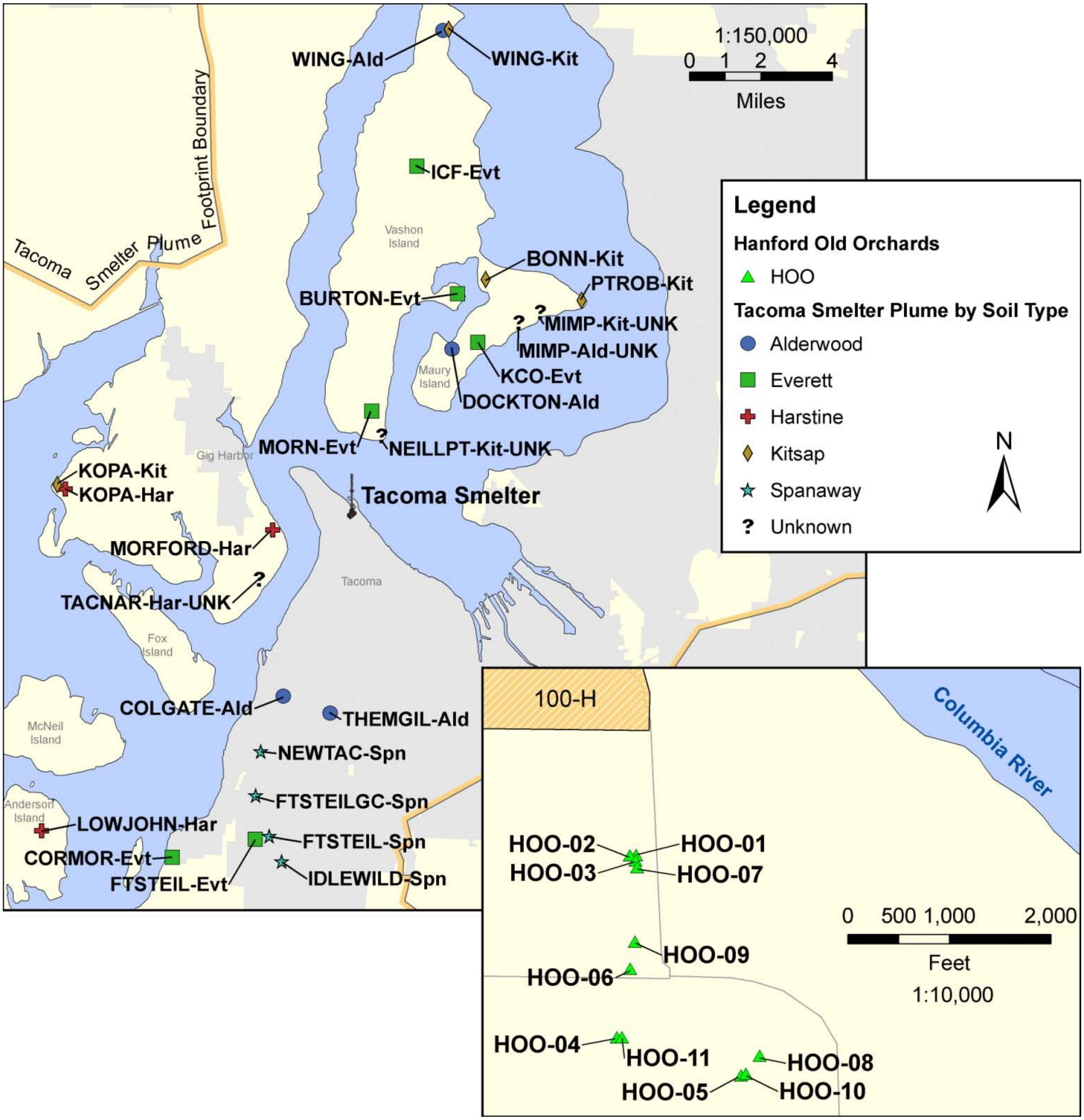

Figure 3. Sampling locations in the Tacoma Smelter Plume footprint and Hanford Old Orchards area.

Table 2. Tacoma Smelter Plume footprint locations and sample summary.

QAPP[1] Soil Type	Site Name	Soil Type Verified	Site Code Used in Report[2]	Samples Collected and Analyzed for Arsenic and Lead			Additional Soil Analyses			
				Plants	Soil Biota	Soil	TOC & % Solids	Bioassay	Arsenic Species	Copper
Alderwood (Ald)	Colgate Park	Yes	COLGATE-Ald	Salal[3]	Earthworm[3]	1[3]	1[3]	1	1[3]	1[3]
	Dockton Park	Yes	DOCKTON-Ald	Grass	Earthworm	1	1			
	Maury Island Marine Park	**No**	MIMP-Ald-UNK	Salal	Mix	1	1	1	1	1
	Thelma Gilmer Park	Yes	THEMGIL-Ald	Salal	Earthworm	1	1			
	Winghaven Park	Yes	WING-Ald	English Ivy & Nettles	Earthworm	1	1	1[4]		1
Everett (Evt)	Burton Acres Park	Yes	BURTON-Evt	Sweet Cicely	Mix	1	1	1	1	1
	Cormorant Park	Yes	CORMOR-Evt	Salal	Earthworm	1	1	1[4]		1
	Fort Steilacoom Park	Yes	FTSTEILP-Evt	English Ivy	Earthworm	1	1			
	Island Center Forest	Yes	ICF-Evt	Salal & Sweet Cicely	Earthworm	1	1			
	King County Owned	Yes	KCO-Evt	Salal & Oregon Grape	Earthworm	1	1	1		1
	Morningside Farm	Yes	MORN-Evt	Salal & Evergreen Huckleberry	Earthworm	1	1			
Harstine (Har)	Kopachuck State Park	Yes	KOPA-Har	Salal	Earthworm	1	1	1[4]		1
	Lowell Johnson Park	Yes	LOWJOHN-Har	Salal	Earthworm	1	1			
	Morford's Open Space	Yes	MORFORD-Har	*Rubus sp.*	Earthworm	1	1	1	1	1
	Tacoma Narrows Park	**No**	TACNAR-Har-UNK	*Rubus sp.*	Earthworm	1	1	1	1	1
Kitsap (Kit)	Bonneville International	Yes	BONN-Kit	Grass & *Rubus sp.*	Earthworm	1	1			
	Kopachuck State Park	Yes	KOPA-Kit	Nettles & *Rubus sp.*	Earthworm	1	1			
	Maury Island Marine Park	**No**	MIMP-Kit-UNK	Salal	Earthworm	1	1	1	1	1
	Neill Point Natural Area	**No**	NEILLPT-Kit-UNK	Nettles	Earthworm	1	1			
	Point Robinson Park	Yes	PTROB-Kit	Nettles	Earthworm	1	1	1	1	1
	Winghaven Park	Yes	WING-Kit	Grass & Unknown	Earthworm	1	1	1[4]		1
Spanaway (Spn)	Fort Steilacoom Golf Course	Yes	FTSTEILGC-Spn	Grass[3]	Earthworm	1[3]	1[3]	1	1	1[3]
	Fort Steilacoom Park	Yes	FTSTEILP-Spn	Grass	Earthworm	1	1			
	Idlewild School	Yes	IDLEWILD-Spn	English Ivy	Earthworm	1	1	1[4]		1
	New Tacoma Cemetery	Yes	NEWTAC-Spn	Grass	Earthworm	1	1	1	1	1

[1]Expected soil type for each location based on Natural Resource Conservation Service – U.S. Department of Agriculture soil survey maps.
[2]Abbreviation of the location name – soil type abbreviation – UNK added if soil type was not verified.
[3]Field replicate collected.
[4]Reference location for bioassay comparisons, pre-selected based on data from previous studies.
Mix: Variety of different invertebrates, e.g., centipedes and grubs.

Table 3. Hanford Old Orchards locations and sample summary.

Site Code Used in Report	Orchard[1]	Samples Collected and Analyzed for Arsenic and Lead			Additional Soil Analyses			
		Plants	Soil Biota[2]	Soil	TOC & % Solids	Bioassay	Arsenic Species	Copper
HOO-01	North, closest to 100-H	Cheat Grass	Beetle[AE] & Mix[B]	1	1	1	1	1
HOO-02	North, closest to 100-H	Cheat Grass	Beetle[A] & Mix[B]	1	1	1	1	1
HOO-03	North, closest to 100-H	Cheat Grass	Beetle[AD] & Mix[B]	1	1	1	1	1
HOO-04	Southwest	Cheat Grass	Beetle[C]	1	1	1	1	1
HOO-05	Southeast	Cheat Grass[3]	Beetle	1[3]	1[3]	1[4]	1	1[3]
HOO-06	Central, at road intersection	Cheat Grass	Beetle[AF] & Mix[B]	1	1	1	1	1
HOO-07	North, closest to 100-H	Cheat Grass	Beetle[D]	1	1			1
HOO-08	Southeast	Cheat Grass		1	1			1
HOO-09	Central, at road intersection	Cheat Grass	Beetle[AG] & Mix[B]	1	1			1
HOO-10	Southeast	Cheat Grass		1	1			1
HOO-11	Southwest	Cheat Grass	Beetle[C]	1	1			1
HOO-General[5]	All four orchards		Beetle & Mix					

[1]Four individual orchards were sampled. Names are given for each orchard's relative location.
[2]Due to low sample masses for soil biota, samples from several locations were combined to conduct the metals analysis. The letters indicate samples that were combined, e.g., HOO-04 Beetles and HOO-11 Beetles were combined into one sample, indicated with the letter C.
[3]Field replicate collected.
[4]Reference location for bioassay comparisons, pre-selected location based on initial XRF readings.
[5]HOO-General represents beetles and other invertebrates collected within the four orchards sampled but not necessarily associated with any particular locations.
Mix: Variety of different invertebrates, e.g., crickets, spiders, and grubs.

Tacoma Smelter Plume Footprint Soil Series

Soil type was used to group the various factors that may influence the toxicity of arsenic and lead. Grouping areas by soil type provided a foundation for assessing locations not sampled as part of this project. In the Tacoma Smelter Plume footprint, the Alderwood, Everett, Harstine, Spanaway, and Kitsap soil series were selected. All of these series were originally formed by glacial activity. The selected series and characteristics in the Tacoma Smelter Plume footprint are listed in Table 4 and a few examples are shown in Figure 4.

Soil series were verified in the top six inches of soil for most sampling locations. At DOCKTON-Ald, THEMGIL-Ald, WING-Ald, BURTON-Evt, ICF-Evt, KCO-Evt, MORN-Evt, KOPA-Har, and IDLEWILD-Spn more detailed soil series verifications were conducted because it was not immediately evident what series was present. The soil series could not be verified at MIMP-Ald-UNK, TACNAR-Har-UNK, MIMP-Kit-UNK, and NEILLPT-Kit-UNK as indicated by the UNK in the location abbreviation codes (Table 2).

Table 4. Tacoma Smelter Plume footprint soil series characteristics.

Soil Series	Origin	Drainage	Texture	Friable?	Sticky?	Plastic	Other Characteristics	% of TSP footprint [1]
Alderwood (Ald)	Glacial Till	Moderate	Gravelly Ashy Sandy Loam	Very	Slightly	Slightly	Prone to high water table due to cemented layer	25% +1% Everett[2] +3% Kitsap[2]
Everett (Evt)	Glacial Outwash	Excessive	Very Gravelly Sandy Loam	Very	No	No	Rocks clean & arranged in layers	8%
Harstine (Har)	Glacial Till	Moderate	Gravelly Ashy Sandy Loam	Very	-	Slightly		8%
Spanaway (Spn)	Glacial Outwash	Excessive	Gravelly Sandy Loam	Very	No	No	High organic matter content	4% +5%[3]
Kitsap (Kit)	Glacial Lakebed	Moderate	Silt Loam	Moderate	Slightly	Slightly		2%

Un-mapped area of the Tacoma Smelter Plume footprint: 25%.
[1]TSP: Tacoma Smelter Plume footprint. Areas do not include water.
[2]Mapping was not detailed enough to distinguish between these series.
[3]Similar series.
Soil Survey Staff, 2008, and personal communication with Chuck Natsuhara at the Natural Resource Conservation Service.

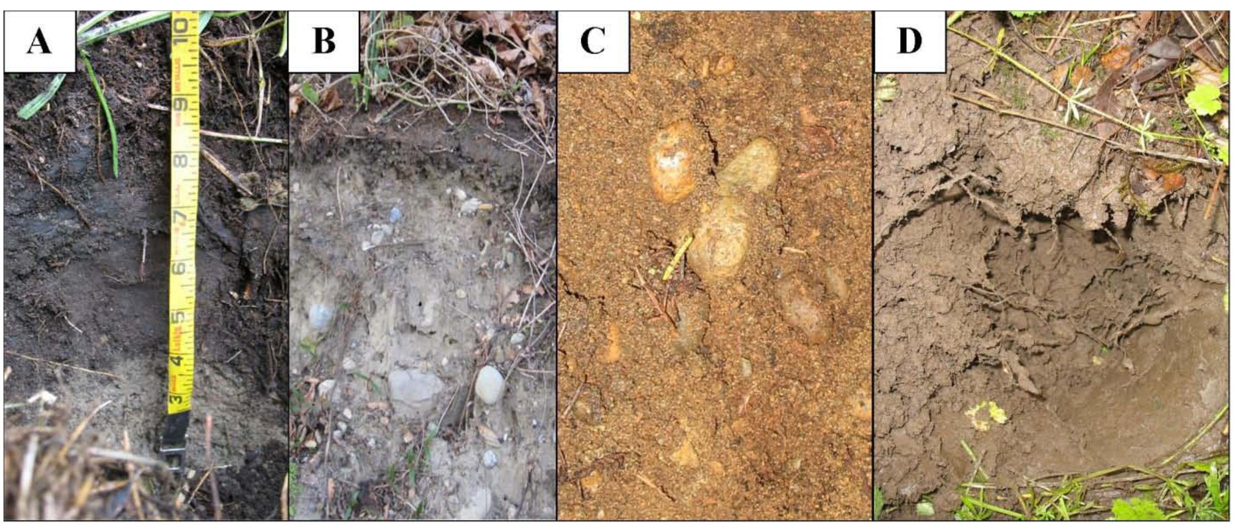

Figure 4. Pictures of soil series.

A) Spanaway series from IDLEWILD-Spn.
B) Example of the Alderwood series.
C) Everett series from KCO-Evt.
D) Kitsap series from KOPA-Kit.

Hanford Old Orchards Soil Series

Specific soil series were not selected in the Hanford Old Orchards area because of the following logistical difficulties:

- Location of individual orchards relative to the mapped soil series was unknown.
- Orchards were selected based on minimizing contamination risks from Hanford Site operations.
- Old orchards within culturally or biologically significant areas were not considered.

These constraints led to the selection of four orchards between H and F reactors located in the northern portion of the Hanford site near the Columbia River. Multiple samples were collected in each orchard to obtain 11 samples.

Site Characterization

Upon arrival at a location, staff noted any wildlife or any signs of wildlife (e.g., droppings, prints, hair). Staff also noted general habitat descriptions and weather - temperature, general wind speed, cloudiness, and precipitation. Figure 5 shows field work activities.

Figure 5. Field work pictures.

A: XRF and GPS at a Hanford Old Orchards location.
B: Making notes about the location and setting up field gear.
C: Searching for earthworms.
D: Large earthworm from CORMOR-Evt.
E: Weighing earthworms in the field.

Soil Field Analysis

An Innov-X Systems X-ray Fluorescence Instrument (XRF) was used to measure the arsenic and lead concentration in the soil at each location. It was difficult to find the appropriate concentrations at BURTON-Evt, LOWJOHN-Har, MIMP-Kit, and PTROB-Kit; sampling at these locations was conducted regardless of the XRF results. XRF readings were not taken at ICF-Evt, KOPA-Har, and MORFORD-Har due to rain. TACNAR-Har-UNK and KCO-Evt readings were taken from the homogenized soil sample. Additional XRF readings were also taken on dried and re-wetted soil samples from each location.

pH was evaluated after sampling instead of before sampling because it took a significant amount of time to settle and filter the samples.

Soil Collection

Soil samples were collected from the 0-6 inch depth horizon. Five sub-samples were collected at each location and composited into one sample. Only four sub-samples were collected at PTROB-Kit and TACNAR-Har-UNK due to a change in soil type or appearance, and at MIMP-Kit-UNK due to low XRF readings.

Soil was collected using a pre-cleaned stainless steel hand trowel or pre-cleaned stainless steel spoon, then placed in a pre-cleaned stainless steel bowl. Roots and other debris such as large rocks were carefully removed from the sample. The soil was homogenized in the stainless steel bowl with a stainless steel spoon at the location. Once homogenized the sample was split in the field for analysis of pH; grain size; total arsenic and lead; total copper; arsenic species As(III) and As(V); total organic carbon content; percent solids; or bioassays. Parameters analyzed varied by location and are described in Tables 2, 3, and 5.

Table 5. Number of locations analyzed for each parameter.

<table>
<tr><th rowspan="2">Parameter</th><th colspan="2">Soil</th><th colspan="2">Soil Biota</th><th colspan="2">Plant Tissue</th></tr>
<tr><th>TSP</th><th>HOO</th><th>TSP</th><th>HOO</th><th>TSP</th><th>HOO</th></tr>
<tr><td>pH</td><td>25</td><td>9</td><td colspan="4"></td></tr>
<tr><td>Arsenic</td><td rowspan="2">25</td><td rowspan="2">11</td><td rowspan="2">24</td><td rowspan="2">9</td><td rowspan="2">25</td><td rowspan="2">11</td></tr>
<tr><td>Lead</td></tr>
<tr><td>Copper</td><td>15</td><td>11</td><td colspan="4" rowspan="3"></td></tr>
<tr><td>Arsenic (III)</td><td rowspan="2">10</td><td rowspan="2">6</td></tr>
<tr><td>Arsenic (V)</td></tr>
<tr><td>Solids</td><td>25</td><td>11</td><td>24</td><td>9</td><td>25</td><td>11</td></tr>
<tr><td>Total Organic Carbon</td><td>25</td><td>11</td><td colspan="4" rowspan="3"></td></tr>
<tr><td>Lettuce Bioassay</td><td>15</td><td>6</td></tr>
<tr><td>Earthworm Bioassay</td><td>15</td><td>6</td></tr>
</table>

TSP: Tacoma Smelter Plume footprint.
HOO: Hanford Old Orchards.

Sites that were tested with bioassays also had copper analyzed in the soil to aid in the interpretation of the bioassays. Copper was accidently analyzed in soil samples HOO 07-11. In addition, one location from each soil type and the Hanford Old Orchards area were pre-selected as reference locations based on low expected arsenic and lead for bioassay comparisons. Data from previous studies and preliminary XRF results were used to predict arsenic and lead concentrations at each location. See Table 2 and Table 3 for locations selected for additional soil analyses.

Plant Collection

The leaves, stems, and roots of plants were collected from each location. Plants were collected within 10 feet of a soil sub-sample. Cheatgrass, English ivy, evergreen huckleberry, grass, nettles, Oregon grape, *Rubus sp.*, sweet cicely, salal, and one unknown plant were collected during the study (Table 2 and 3). None of the plants exhibited obvious abnormalities or deformities.

After collection, plants were placed in plastic bags and transported back to the Washington State Department of Ecology (Ecology). In the lab, dust and soil particles were rinsed from the surface of each plant with de-ionized or tap water (Walsh et al., 1977). After being rinsed, plants were cut up, placed in 8-ounce jars, and sent to Ecology's Manchester Environmental Laboratory (MEL). At MEL, they were freeze-dried, ground, homogenized, and analyzed for arsenic and lead.

Soil Biota Collection

In the Tacoma Smelter Plume footprint and Hanford Old Orchards earthworms and darkling beetles, were targeted respectively to represent the soil biota. Soil biota were collected within 10 feet of a soil sub-sample. Collection methods included grunting[3] or digging for the Tacoma Smelter Plume footprint locations and searching and pitfall traps for the Hanford Old Orchards locations. No obvious deformities, tumors, or lack of response to stimuli was noted.

After collection of soil biota, dust and soil particles were rinsed from the surface of each individual with de-ionized water. The invertebrates were then placed in a jar containing a moistened Kim Wipe and transported back to Ecology.

Collected earthworms and any other invertebrates were kept alive for 48 hours in jars containing moistened Kim Wipes stored at 4°C, to evacuate soil in the gut (Button et al., 2009; Ma et al., 2009; Langdon et al., 2005). This procedure ensured that the arsenic and lead being measured was associated with tissue, not soil, so an accurate bioaccumulation factor (BAF) could be calculated. However, it was noted that some soil remained in the earthworm guts even after 48 hours of depuration.

[3] Grunting is a worm collection technique where a wooden stake is driven into the ground, and then a piece of wood or metal is rubbed on the top of the stake to create vibrations. The vibrations cause the worms to come to the surface of the soil.

After rinsing and holding was complete, the invertebrates were frozen on dry ice and sent to MEL in jars. At MEL they were freeze-dried, ground, homogenized, and analyzed for arsenic and lead.

Additional biomarker analyses were performed on earthworms collected in the field and from bioassays during this study. This additional work was conducted by Josh Sullivan and Jim Gawel at the University of Washington – Tacoma. See Appendix G for the detailed report.

Tacoma Smelter Plume Footprint Soil Biota

After searching for up to three hours at BURTON-Evt and MIMP-Ald-UNK, very few earthworms were found; therefore, alternative invertebrates were collected. After more than one hour of extensive searching - including outside the planned sampling area - no soil biota were found at KOPA-Har.

Hanford Old Orchards Area Soil Biota

During the initial sampling event, very few beetles and other invertebrates were collected despite extensive searching and use of pit traps. Invertebrates were found at locations HOO-01, 02, 03, 06, and 09. These were combined into a sample with only darkling beetles and a sample with a mixture of invertebrates. A second outing to look for beetles on August 25, 2010 was more successful than the first attempt but invertebrates were still scarce. Darkling beetle samples were collected from locations HOO-01, 03, 04, 05, 06, 07, 09, and 11. Darkling beetles from locations HOO-03 and 07 were combined into one sample; darkling beetles from locations HOO-04 and 11 were also combined to provide enough mass for analysis. Other invertebrates were collected from the general Hanford Old Orchards area. See Table 3 for details.

Analysis Methods

All of the methods used in this study follow those outlined in the approved Quality Assurance Project Plan (QAPP) except for the arsenic speciation and grain size analyses (Sloan, 2010). Tables 6 and 7 list the analyses that were conducted and the associated method.

Due to instrument problems, the HPLC-HG-ICP-MS w/DRC[4] method specified in the QAPP for arsenic speciation could not be performed. Therefore EPA Method 1632 modified was substituted. This method change does not influence the interpretation of the results.

Grain size analyses followed an Ecology standard operating procedure based on ASTM D6913-04 standard test methods for measuring grain size in sands. Due to the nature of the soils being investigated the grain size procedure produced unreliable results; therefore, these results were not used for this study. Soil texture, a method commonly used to approximate grain size distribution in soils, was used as an acceptable substitute for the omitted grain size data.

[4] High-Pressure Liquid Chromatography system coupled to an Inductively Coupled Plasma-Mass Spectrometer using Hydride Generation post-column and dynamic reaction cell technology.

Table 6. Parameters measured in this study and their associated methods.

Analysis	Laboratory	Instrument/ Technique	Analytical Method	Reporting Limits/ Resolution
Field Measurements				
pH		Orion pH meter	EPA Method 9045D	0.1 SU
Arsenic		XRF	EPA Method 6200 & Instrument Manual	10 ppm
Lead				10 ppm
Laboratory Analyses				
Total Organic Carbon	MEL	-	PSEP, 1997	0.1%
Total Solids	MEL	-	SM 2540G	1%
Arsenic Copper Lead	MEL	ICP/MS	EPA Method 200.8	0.1 mg/Kg dw
Arsenic Speciation	BRL	HG-QFAAS	EPA Method 1632 modified	0.1 mg/Kg dw

PSEP: Puget Sound Estuary Program.
ICP/MS: Inductively coupled plasma mass spectrometry.
XRF: X-ray Fluorescence Instrument.
HG-QFAAS: Hydride generation quartz furnace atomic absorption spectrometry.
Dw: dry weight.
MEL: Manchester Environmental Laboratory.
BRL: Brooks Rand Laboratory.
Arsenic Speciation parameters: total Arsenic and Arsenic (III).

Table 7. Laboratory procedures for bioassay analyses.

Bioassay	Laboratory	Endpoints Measured	Method
Lettuce	Nautilus Environmental Laboratory	Mortality, Biomass	Norton, 1996a
Earthworm		Mortality, Morphological and Behavioral Alterations	Norton, 1996b

Data Quality

All bioassay and chemistry data have been reviewed for completeness, accuracy, and usability. Appendix B and D show additional quality control details.

Chemistry Data Quality

Below is a summary of the quality assurance results for chemistry; detailed results are in Appendix B. All data were evaluated for adherence to measurement quality objectives specified in the QAPP (Sloan, 2010). All chemistry data collected for this project is considered usable as qualified.

Metals Analyses Data Quality

Manchester Environmental Laboratory (MEL) Metals Data Quality

The relative percent difference for the duplicate nettles sample at NEILLPT-Kit-UNK was higher than the acceptance criteria for arsenic and lead. MIMP-Kit-UNK also had a high relative percent difference for the earthworm laboratory duplicate for arsenic. These samples were qualified as estimates, "J". No laboratory duplicates were performed for the Hanford Old Orchards soil biota samples collected on August 25, 2010, nine samples total.

The matrix spike level for lead was insufficient for the HOO-04 and THEMGIL-Ald soil samples; therefore, recoveries were not calculated. Sample heterogeneity caused the matrix spike and matrix spike duplicate recoveries for arsenic in soil from THEMGIL-Ald and lead in earthworms from TACNAR-Har-UNK to be outside acceptable limits. These two results were qualified as estimates, "J".

All other quality control samples for metals at MEL met the acceptance criteria.

Brooks Rand Laboratory (BRL) Metals Data Quality

The total arsenic matrix spike and the matrix spike duplicate were under-spiked; therefore, recoveries were not calculated. Post-spike samples were prepared and met the acceptance criteria; therefore, all total arsenic data were reported without qualification.

The arsenic (III) matrix spike for HOO-01 had an elevated recovery while the duplicate had an acceptable recovery. Due to the elevated matrix spike and the high relative percent difference between the two spikes, arsenic (III) results for HOO-01 were qualified as estimates, "J".

All other quality control samples for metals at BRL met the acceptance criteria.

General Chemistry Analyses Data Quality

Grain Size Data Quality

Grain size analysis was conducted using an Ecology standard operating procedure based on ASTM D6913-04 standard test methods. The amount of silts in the soils collected from this project made it difficult to disaggregate the particles without using a mortar and pestle for most soils once dried. Given this and an inability to determine if the mortar and pestle process changed the native grain sizes, these data has not been used or reported. Instead, soil texture was used as an approximate grain size estimator. It is recommended that future studies use a wet sieving grain size method for soils containing silts or clays.

pH Data Quality

Both locations in Maury Island Marine Park were filtered using a disposable Nalgene® filter instead of the reusable filter used for the remaining locations. Unusually low pH values from these two locations may be from residual nitric acid in the Nalgene® filters from the cleaning process as these filters are typically used for metals analysis. Therefore, using the relationship between pH values from Nautilus Environmental Laboratory (conducted bioassays) and those collected in the field, corrected field pH values have been calculated for these two locations (Table 8).

Table 8. Corrected pH values summary.

Site	Original Field pH	Nautilus Corrected pH	Calculated Field pH
MIMP-Ald-UNK	4.5	5.12	5.99
MIMP-Kit-UNK	4.9	5.79	6.60

Calculated field pH = (0.8904 * Corrected Nautilus pH) + 1.4398, R^2 = 0.663

pH was taken for HOO-08, 09 and 10, but was not recorded in the field notebook. It is reasonable to assume that these three locations would have similar pH values to the rest of the Hanford Old Orchards locations because a pH reading was recorded at another location within the same orchard, and the Hanford Old Orchards locations had a relatively small pH range (6.65-7.68) and a small standard deviation (0.35).

Total Solids and Total Organic Carbon Data Quality

The relative percent difference for the soil total organic carbon laboratory duplicate for ICF-Evt was above the acceptance criteria; thus, the result was qualified as an estimate, “J”. There were no data quality issues with the total solids from MEL. BRL total solids had a detected quantity of solids in the blank; however, the blank still met acceptance criteria.

Bioassay Data Quality

Positive controls were acceptable for both the earthworm and lettuce bioassays. The locations BURTON-Evt, WING-Ald, and WING-Kit were two days past the 14-day holding time for both tests. This exceedance of holding time is not expected to have influenced the results. Below is a summary of the quality assurance results for bioassays; detailed results are in Appendix D.

Earthworm Bioassay Data Quality

Deviations from the hydration protocol and pH criteria occurred during the earthworm tests. Details of these deviations are below. In addition, unexpected toxicity was observed for CORMOR-Evt resulting in the omission of this location from the data analysis.

Earthworm Bioassay Test pH

The initial pH values were below the acceptable range, pH 5.0-9.0, for the following locations:

- BURTON-Evt
- COLGATE-Ald
- CORMOR-Evt
- FTSTEILGC-Spn
- IDLEWILD-Spn
- KCO-Evt
- KOPA – Harstine
- MIMP-Ald-UNK
- MORFORD-Har
- TACNAR-Har-UNK
- WING-Ald

While pH may have been a factor in the toxicity exhibited in the earthworm bioassays, there is evidence that this may not have been the case. Two of the locations with low pH had complete survival, and a few of the locations exhibited survival and sublethal effects. Given a mixture of toxicity at low pH, pH may not have been the sole cause of toxicity.

Due to low pH, BURTON-Evt, CORMOR-Evt, KOPA-Har, and MORFORD-Har samples were re-run. Additional pH testing was conducted to ensure a more accurate assessment of the pH of these samples. The re-run samples were past the acceptable holding time of two weeks.

During analysis of the original test results, it was suspected that there may have been an error with the original pH readings so additional pH measurements were taken during the re-run tests. The pH results in Table 9 show that Nautilus Environmental pH readings were consistently lower than Ecology pH readings. Both were measured using EPA Method 9045D, which involves adding water to create a soil slurry and the pH of the water is measured. The results also had many readings below the bottom of the acceptable range of 5.0. However, the Kelway soil pH tester had no readings below the bottom of the acceptable range. The Kelway pH soil tester is placed in direct contact with the soil without the addition of water (unlike the EPA Method

9045D) to obtain a pH reading. Due to these results, bioassay tests with low pH were considered usable.

Table 9. pH results from bioassay re-runs.

Bioassay Test	Sample	Nautilus Environmental Laboratory pH meter				Ecology Field pH meter				Kelway pH Tester	
	Settling Time - >	5	30	5	30	5	30	5	30	-	-
	When pH Taken->	Before	Before	After	After	Before	Before	After	After	Before	After
Lettuce	Control	6.98	6.85	7.04	7.58	7.10	7.04	7.75	7.70	5.6	-
	BURTON-Evt	3.58	3.59	2.93	3.65	3.79	3.80	3.63	3.61	6.8	-
	CORMOR-Evt	5.10	5.05	4.46	5.14	5.21	5.24	5.11	5.06	6.8	-
Earth-worm	Control	6.98	6.85	7.16	7.70	7.10	7.04	7.84	7.74	5.6	6.6
	CORMOR-Evt	5.10	5.05	4.59	5.31	5.21	5.24	5.25	5.26	6.8	6.8
	KOPA-Har	4.76	4.63	3.98	5.10	4.75	4.74	4.52	4.56	6.9	6.8
	KCO-Evt	4.58	4.57	4.07	4.55	4.89	4.54	4.70	4.70	7.0	6.6
	MORFORD-Har	4.80	4.76	4.03	4.77	4.92	4.89	4.73	4.74	6.4	6.8

Settling Time: According to the bioassay test protocols, pH is read after 5 minutes of stirring in the soil slurry and then in the supernatant after 30 minutes of settling.
When pH Taken: pH is measured before the bioassay test begins and after the test is completed.

Earthworm Bioassay Test Hydration

The Hanford Old Orchards locations HOO-01, 02, 03, and 06 were hydrated to 20-25% rather than the 35% recommended in the earthworm protocol. Further hydration of these soils would have created standing water in the test chamber that could have caused mortality. Samples that contained more moisture than recommended in the protocol, 45%, were not manipulated. These deviations are not expected to have influenced the results of the test.

Earthworm Test Unexpected Toxicity

CORMOR-Evt was a reference location yet exhibited toxicity both in the initial test and in the re-run test. It is unlikely that arsenic, lead, or copper produced this toxic effect given that KCO-Evt had more than twice the concentration of each metal and exhibited less toxicity. In addition, dissipating toxicity was observed for CORMOR-Evt, where the initial test had zero worms survive and the re-run test had a mean survival of 50%. Dissipating toxicity is not usually a characteristic of metals effects but is more often seen for volatile compounds such as polycyclic aromatic hydrocarbons or compounds that degrade in the environment such as pesticides or pathogens. Therefore this particular location was not considered an appropriate reference or a representation of toxic effects due to arsenic and lead.

KCO-Evt, KOPA-Har, and MORFORD-Har all showed some dissipating toxicity in the re-run results, but not to the same extent as CORMOR-Evt. There was not a significant change in pH from the original test to the re-run tests to account for decreased metals toxicity. These locations have been kept in the analyses because the differences between the two tests were not large and the metals concentrations at KCO-Evt and MORFORD-Har may have been adequate to cause toxicity.

Lettuce Bioassay Data Quality

Similar to the earthworm bioassays, the initial pH values for the lettuce bioassays were below the acceptable range of 5.0-10.0 for the locations in Table 10. BURTON-Evt is the only location that may have been affected by low pH levels. This location had the lowest pH level of 3.65 and had significantly lower growth when compared to the control. Due to the low pH, BURTON-Evt and CORMOR-Evt were re-run. Additional pH testing was conducted to ensure a more accurate assessment of the pH of these samples. The re-run samples were past the acceptable holding time of two weeks.

More than the 12 seeds recommended in the protocol were added to the replicates listed in Table 10. The initial count was adjusted for the additional seeds and is not expected to influence the results. In addition, native seedlings were found in several samples, see Table 10. These seedlings were removed when they became apparent. The presence of native plants is not expected to have influenced results.

Table 10. Sites with lettuce protocol deviations.

Site	pH	Extra Seeds Added	Native Seedlings Present
BURTON-Evt	Yes[1]		
COLGATE-Ald	Yes		
CORMOR-Evt			Yes
FTSTEILGC-Spn	Yes	Rep 4	Yes
IDLEWILD-Spn			Yes
KCO-Evt	Yes		
KOPA-Har	Yes		
MIMP-Ald-UNK			Yes
MORFORD-Har	Yes	Reps 3 and 4	
PTROB-Kit	Yes		Yes
WING-Ald	Yes		Yes
WING-Kit		Rep 1	Yes
HOO-02		Reps 3 and 5	
HOO-03		Rep 2	
HOO-06		Reps 2 and 3	

Only locations with deviations are listed. Blank boxes indicate that deviation did not apply to that location.
Unless noted, the deviation is not expected to have influenced the results.
[1]Deviation may have influenced the results of the test.

Data Analysis

Bioassay Data Analysis

Bioassay data were analyzed using Microsoft Excel, SPSS 14.0, and U.S. Army Corps of Engineers Biostat software. Bioassay results were evaluated by comparing results with the control for statistical significance at the $p \leq 0.05$ level. Results were also compared to pre-selected reference locations for statistical significance at the $p \leq 0.05$ level.

Chemistry Data Analysis

Chemistry data were analyzed using Microsoft Excel and SPSS 14.0 software.

Results and Discussion

Chemistry Results

Soil, plants, and soil biota were analyzed for arsenic and lead. Soil arsenic and lead concentrations span the historical distribution of concentrations observed in the Tacoma Smelter Plume footprint as shown in Figure 6. Yokel and Delistraty (2003) sampled a larger concentration range in the Hanford Old Orchards area than represented by this study. Their range for arsenic was 2.9-270 mg/Kg, while this study found 6.1-128 mg/Kg. Lead concentrations for this study ranged from 35-390 mg/Kg while Yokel and Delistraty found 6.5-1,900 mg/Kg. Despite this, there is Hanford Old Orchards data for each targeted concentration range specified in the QAPP. Table 11 shows a basic statistical summary of the Tacoma Smelter Plume footprint and Hanford Old Orchards metals data for this study. For more details see Appendix C.

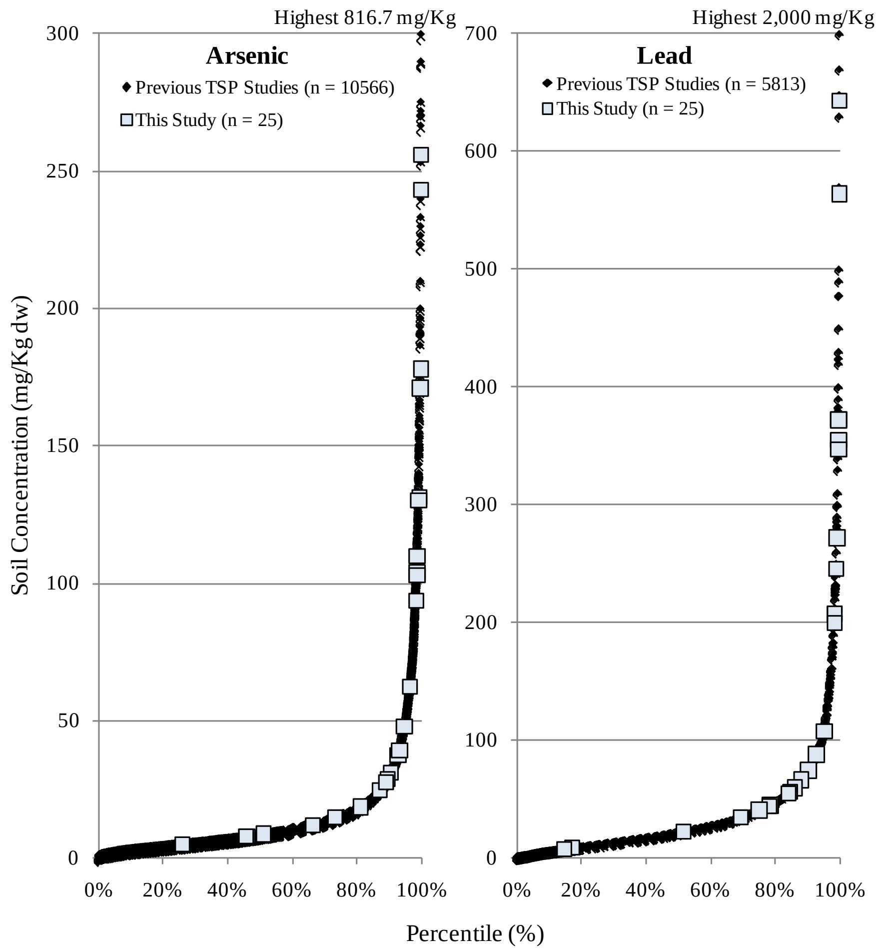

Figure 6. Distribution plots for arsenic and lead in the Tacoma Smelter Plume footprint.
Results from this study compared with other Tacoma Smelter Plume footprint studies compiled from the Ecology's Environmental Information Management database.
Only detected results are shown.

Table 11. Basic metals statistics in mg/Kg dw.

Matrix	Area	N	Arsenic				Lead			
			Min	Max	Mean	Std Dev	Min	Max	Mean	Std Dev
Soil	TSP	27	5.18	256	76.4	72.4	8.06	643	183	176
	HOO	12	6.08	128	37.2	39.6	35.2	390	201	137
Soil Biota Tissue	TSP	25	3.12	104	29.6	29.5	2.19	286	54.9	69.7
	HOO	11	1.85	38.6	9.13	10.3	0.7	47.8	11.4	14.7
Plant Tissue	TSP	34	0.1	23.8	1.93	4.25	0.1	32.6	5.35	8.85
	HOO	12	0.64	27.5	3.65	7.58	2.41	71.2	11.6	19.3

TSP: Tacoma Smelter Plume footprint.
HOO: Hanford Old Orchards.

Arsenic in Soil

Total arsenic was measured by two traditional laboratory methods and XRF. Arsenic results from MEL using EPA method 200.8 have been used here unless noted.

A large range of arsenic concentrations in soil were found in this study, from 5.18 mg/Kg at KOPA-Kit to 256 mg/Kg at KCO-Evt. Figure 7 shows the arsenic concentrations at each location. Arsenic species were also measured for a sub-set of the sampling locations in this study.

Arsenic species, important to consider in terrestrial ecological risk?

The two arsenic species of concern are arsenic (V) and the more toxic arsenic (III). Due to the higher toxicity of arsenic (III), separate MTCA SSL values were established for each species rather than using total arsenic. However, total arsenic data are more readily available at cleanup locations, due to its use in human health assessments. Also total arsenic is a cheaper analysis method. Therefore this study evaluated the need for separate SSLs based on arsenic species versus an SSL for total arsenic.

Arsenic species results

Arsenic (III) concentrations ranged from a minimum of 0.086 mg/Kg dw at HOO-06 to 1.93 mg/Kg at KCO-Evt (Figure 8). The MORFORD-Har arsenic (III) concentration represented the greatest proportion of the total arsenic at 12.3%, while at the remaining locations arsenic (III) represented less than 1.1% of the total. None of the arsenic (III) concentrations exceed the MTCA SSL of 7 mg/Kg dw for the protection of wildlife.

Based on EPA Method 1632, arsenic (V) concentrations were calculated:

Arsenic (V) = Total Arsenic[5] – Arsenic (III)

[5] Total arsenic analyzed by Brooks Rand Laboratory using EPA Method 1632 modified.

Since only small amounts of arsenic (III) were detected in soil samples, the arsenic (V) concentration was only slightly less than the total arsenic concentration. The minimum arsenic (V) concentration was 7.140 mg/Kg dw at MORFORD-Har and the maximum was 282 mg/Kg dw at KCO-Evt. MORFORD-Har was the only location that did not exceed the MTCA SSL of 10 mg/Kg dw arsenic (V) for ecological risk. Table 12 summarizes the arsenic species data and Figure 8 shows the arsenic speciation results graphically.

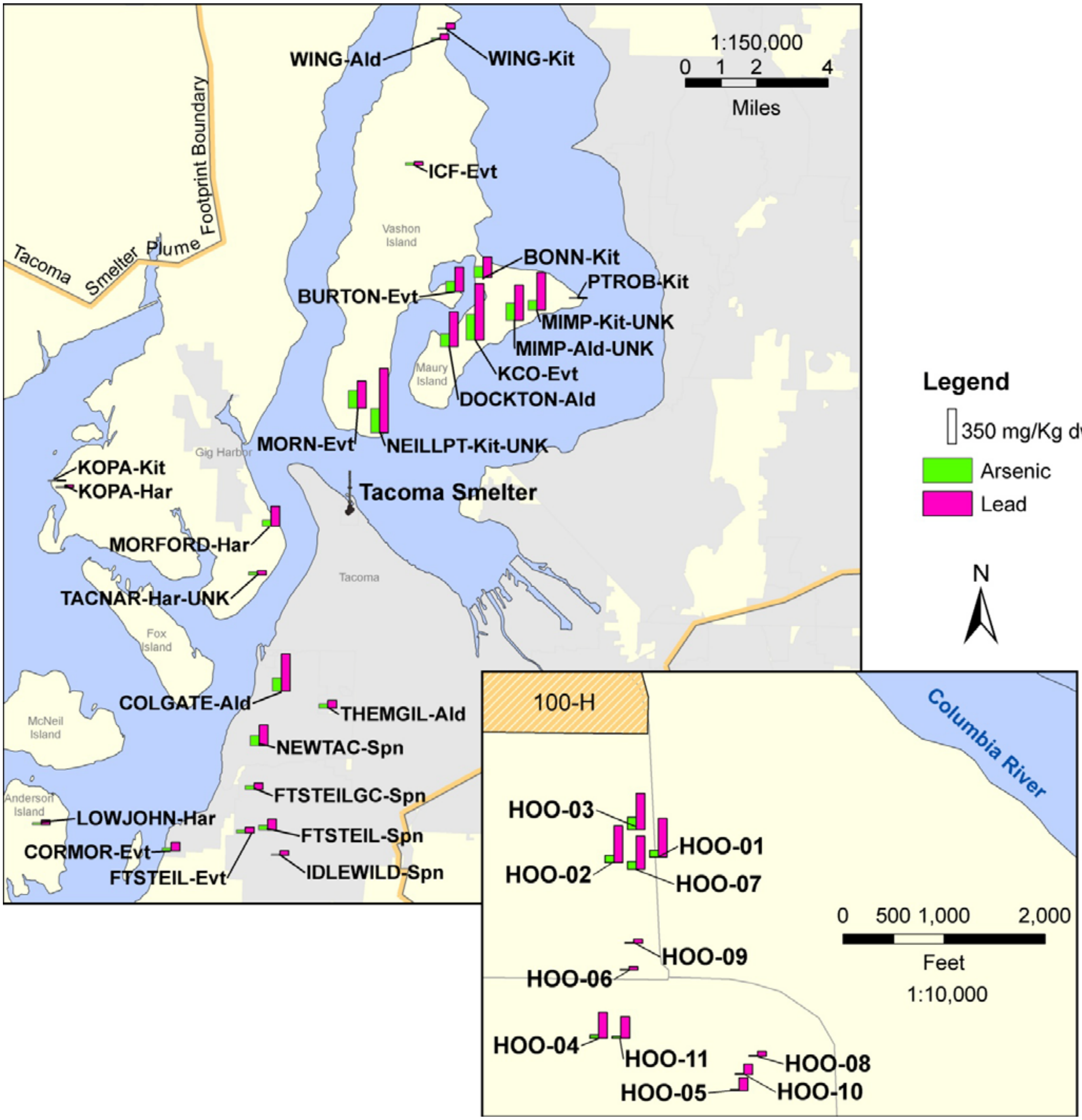

Figure 7. Arsenic and lead soil concentrations.

Table 12. Arsenic species summary statistics in mg/Kg dw.

Area	N	Arsenic(III)				Arsenic(V)				Total Arsenic[1]			
		Min	Max	Mean	StDev	Min	Max	Mean	StDev	Min	Max	Mean	StDev
HOO	7	0.086	0.77	0.368	0.26	12.1	117	59.1	37.8	12.2	118	59.5	38.0
TSP	11	0.258	1.93	0.937	0.551	7.14	282	112	75.1	8.14	284	113	75.5
Total	18	0.086	1.93	0.716	0.533	7.14	282	91.5	67.3	8.14	284	92.3	67.7

[1] Total arsenic analyzed by Brooks Rand Laboratory using EPA Method 1632 modified.
TSP: Tacoma Smelter Plume footprint.
HOO: Hanford Old Orchards.

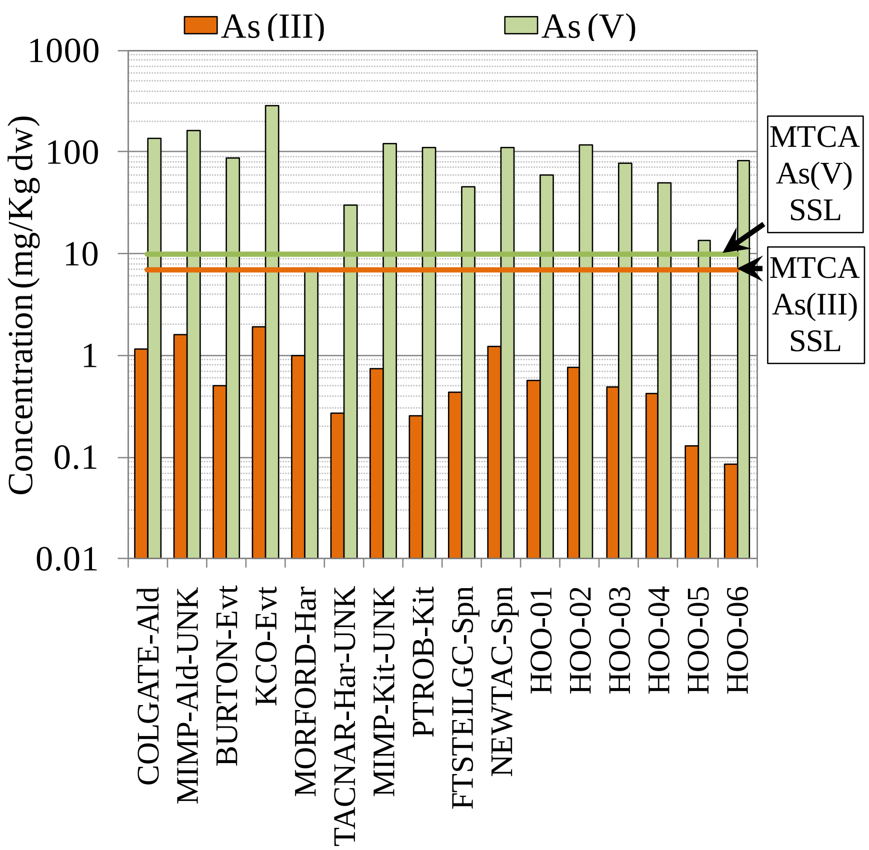

Figure 8. Arsenic speciation results.
Note the log scale for concentration.
As(III) + As (V) = Total Arsenic

Arsenic species versus Total arsenic

Due to the lack of arsenic (III) in the samples, use of total arsenic values for the protection of ecological receptors is justified. However, this only applies to dry soils, not saturated or inundated soils. Arsenic (III) may be more prevalent in saturated soils with reducing conditions such as wetland soils.

Lead in Soil

Total lead was measured by a traditional laboratory method and XRF. The highest lead concentration in the soil was 643 mg/Kg at NEILLPT-KIT-UNK, and the lowest was 8.06 mg/Kg at PTROB-Kit. Figure 7 shows the lead concentration at each location.

XRF results compared to traditional laboratory results

The ease of use and quickness of the results makes the XRF a useful tool for screening soils for metal contamination on location. Ecology uses XRFs to screen soils for arsenic and lead contamination in both the Tacoma Smelter Plume footprint and Eastern Washington old orchards including the Hanford Old Orchards. This project provided an opportunity to compare XRF results to laboratory results for a wide range of concentrations and soil factors. The Tacoma Smelter Plume footprint results are shown here, and the Hanford Old Orchards XRF results are in Appendix F.

Three conditions were tested with the XRF:

1. Direct measurement in the field of soils before collection for laboratory analysis (XRF field).
2. Measurement of a sample dried at 60°F from the same jar as the laboratory metals analysis (XRF dry).
3. Measurement of the dried sample after the addition of water (XRF wet).

XRF Field Measurements vs. Laboratory Analyses

The XRF field measurement represents the common usage of the XRF by Ecology staff. MEL provided both wet-weight and dry-weight metals results for comparison purposes (MEL wet and MEL dry). Figure 9 shows the relationship between the average field reading and the laboratory result. Ideally the relationship between the XRF field and laboratory results should be strong with a coefficient of determination[6] (R^2) close to 1.0. In addition the trendline representing the relationship should have a slope close to 1.0, indicating that the XRF results tend to match or approximate the laboratory value.

The XRF field and the MEL wet results have R^2 values and slopes closer to 1.0 than the XRF field and MEL dry results for both arsenic and lead. The slopes for each comparison are less than 1.0 indicating that the XRF field result tended to be lower than the MEL results.

Using the XRF in the field to screen soils is meant to represent the laboratory dry result even though the wet laboratory results have a stronger relationship. To illustrate how this could be an issue, the MTCA human health level for lead (250 ppm) has been delineated on Figure 9 for the XRF field vs. MEL dry graph. The values that fall in the dark gray box and the white space have been correctly screened for human health. While, the values that fall in the light gray boxes have been incorrectly screened. In this example the results that fall in the light gray box would be

[6] Coefficient of determination, R^2, indicates the portion of the variation in a variable (Y) that is explained by a linear function of variable (X), i.e. how well a trendline approximates the real data points.

considered acceptable by the XRF but not acceptable if the soil was sent to the laboratory for analysis.

This result poses a problem if the XRF is used without laboratory verification samples to make cleanup decisions (i.e., if the XRF is used to determine if soil samples should be sent to the laboratory or to determine the progress of cleanup at a site). Soil samples above the human health criteria would be missed if only the XRF were used. Thus, contaminated areas may not be addressed during cleanup. One way to correct for the bias is to develop an equation that represents the relationship between the XRF and laboratory results, such as the one in Figure 9. Then use that equation to correct the XRF results to get a more representative estimate of the concentration expected from traditional laboratory methods.

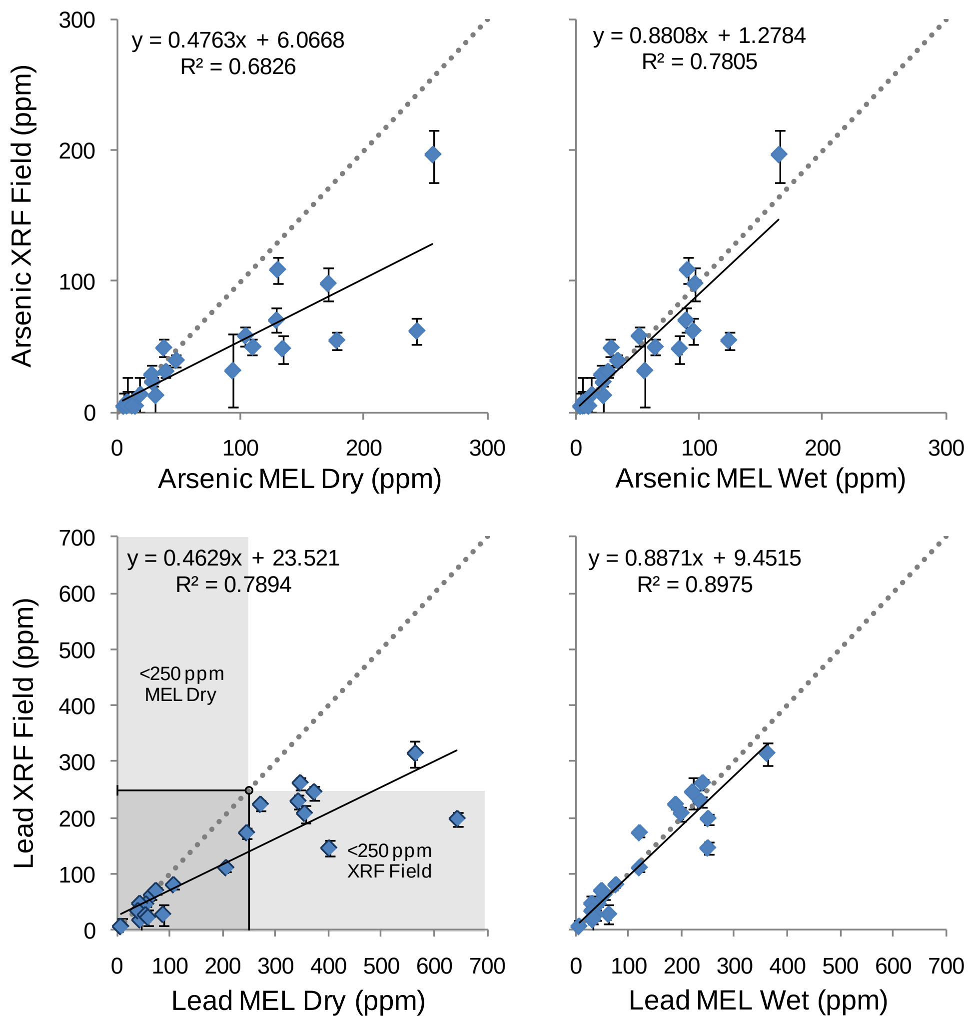

Figure 9. Tacoma Smelter Plume footprint laboratory results compared to XRF field results for arsenic and lead.

Error bars represent the 1 sigma error on the counting statistics of the XRF measurement.
The light gray boxes for the Lead XRF field vs. MEL dry graph indicate values below the MTCA human health screening level of 250 ppm.

XRF Dry and Wet Measurements vs. Laboratory Analyses

To investigate how moisture can affect XRF measurements, aliquots from the soil samples analyzed by MEL were dried in an oven at 60°F. XRF readings of the dried soils were taken and then the soils were wetted and re-measured by XRF.

The XRF dry and MEL dry results and XRF wet and MEL wet results were highly correlated, with each relationship having an R^2 greater than 0.90 (Figure 10). However, unlike the XRF field to laboratory relationships, the slopes were greater than 1. This means the XRF tended to give a higher result than the laboratory. The relationships for the MEL wet and XRF dry results are weaker and the slopes of the trends are greater than 2, indicating increased bias.

The XRF wet vs. MEL dry relationship mirrors the XRF field vs. MEL dry situation because field soils are likely to be moist rather than completely dry. The trends for XRF wet vs. MEL dry relationship are very similar to the XRF field vs. MEL dry with slopes less than 1. However, the relationship is little bit stronger. This is likely due to similar moisture content between the XRF wet soils and the heterogeneous moisture content of the XRF field soils. The agreement between these two datasets provides further evidence that the presence of moisture in soils produces lower XRF results than observed by laboratory methods.

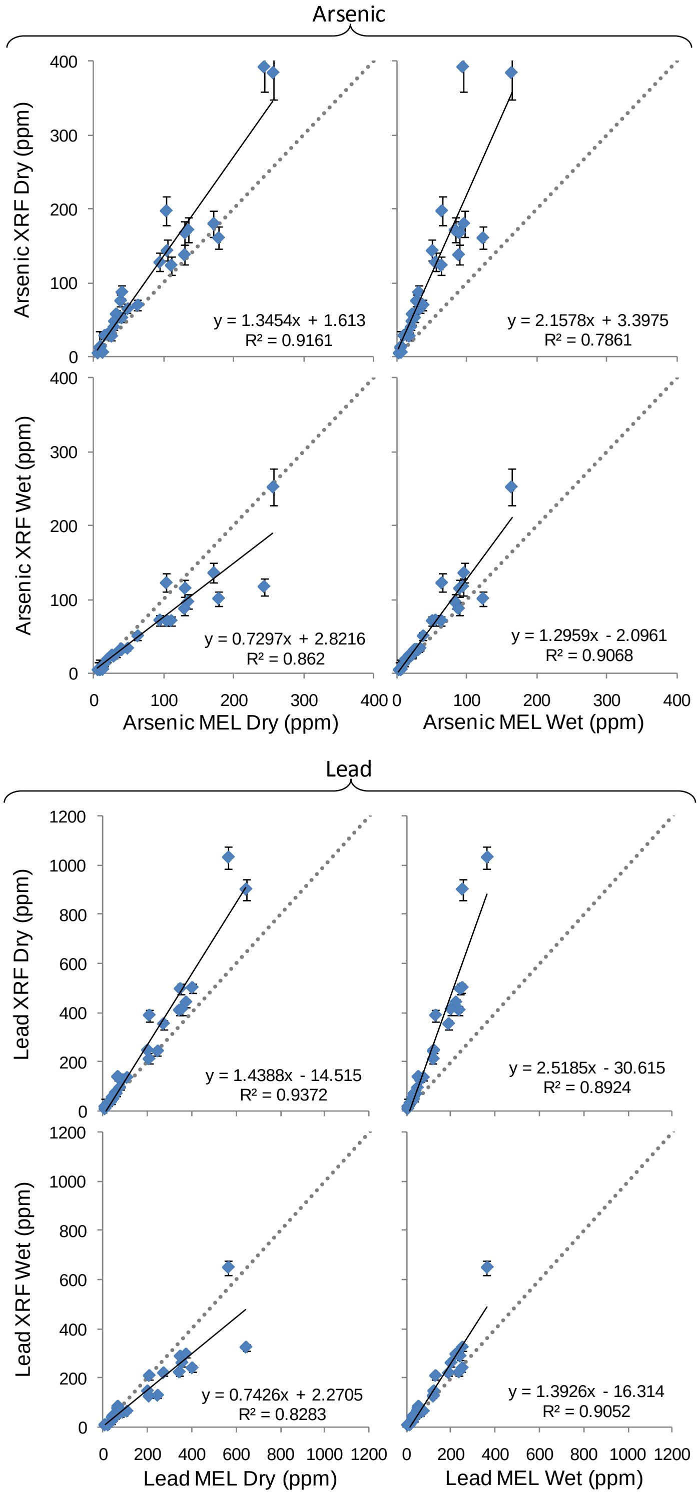

Figure 10. Tacoma Smelter Plume footprint laboratory results compared to dry and wet soil sample XRF results for arsenic and lead.

Error bars represent the 1 sigma error on the counting statistics of the XRF measurement.

Bioassay Results

Earthworm and lettuce bioassays were conducted at 21 of 36 locations sampled. Due to pH quality control issues, five locations were re-run for the earthworm or lettuce test. Toxicity was evident if the sample results were significantly more impacted than the control soil. Sample results were also compared to pre-selected reference locations representing low arsenic and lead concentrations within the Hanford Old Orchards area and each soil type within the Tacoma Smelter Plume footprint. If the soil type was unknown for the Tacoma Smelter Plume footprint locations, then results were only compared to the control. Table 13 shows the results of the bioassays; detailed results are in Appendix D.

Increased earthworm mortality compared to the control was seen for MORFORD-Har and MIMP-Kit-UNK. MORFORD-Har also showed toxicity when compared to the reference location. Toxicity was also observed for KCO-Evt and MIMP-Ald-UNK when compared to the control for the sublethal endpoint. Sublethal effects such as observing responsiveness, lesions, and general health of the earthworms at the end of the test are less severe than mortality effects.

No location exhibited poor lettuce survival. BURTON-Evt exhibited toxicity for the lettuce biomass endpoint, compared to the control. However, when BURTON-Evt was re-run due to pH issues, no lettuce toxicity was observed even though the pH had stayed the same. This indicates dissipating toxicity over time. NEWTAC-Spn, COLGATE-Ald, FTSTEILGC-Spn, and KCO-Evt all showed lettuce biomass toxicity, compared to the reference. Figure 11 shows the lettuce bioassay test at day 13 of 14.

Figure 11. Lettuce bioassay on day 13.

Table 13. Arsenic and lead concentrations with summary of bioassay results.

Location	Arsenic in Soil (mg/Kg dw)	Earthworm Survival	Earthworm Sublethal	Lettuce Survival	Lettuce Biomass
TSP Area					
PTROB-Kit	7.84				
IDLEWILD-Spn *	7.88				
KOPA-Har *	8.76				
KOPA-Har *^	8.76				
WING-Kit *	12.1				
WING-Ald *	14.7				
TACNAR-Har-UNK	27.8				
CORMOR-Evt *	31.2	Con[1]			
CORMOR-Evt *^	31.2	Con[1]			
TSP Lettuce ↓LOAEL					
FTSTEILGC-Spn	37.65				Con & Ref
TSP Earthworm ↓LOAEL					
MORFORD-Har	62.2	Con & Ref			
MORFORD-Har ^	62.2				
MIMP-Kit-UNK	93.6	Con	Con		
NEWTAC-Spn	103				Ref
BURTON-Evt	104				Con & Ref
BURTON-Evt ^	104				
COLGATE-Ald	131.5				Ref
MIMP-Ald-UNK	171		Con		
KCO-Evt	256		Con		Ref
KCO-Evt ^	256				
HOO Area					
HOO-06	6.35				
HOO-05*	12				
HOO-04	32.8				
HOO-01	68.6				Ref
HOO-03	71.6				
HOO-02	128				
HOO Earthworm and Lettuce ↑NOAEL					

Location	Lead in Soil (mg/Kg dw)	Earthworm Survival	Earthworm Sublethal	Lettuce Survival	Lettuce Biomass
TSP Area					
PTROB-Kit	8.06				
KOPA-Har *	22.9				
KOPA-Har *^	22.9				
TACNAR-Har-UNK	41				
IDLEWILD-Spn *	44.7				
WING-Ald *	55.2				
WING-Kit *	59.8				
TSP Lettuce ↓LOAEL					
FTSTEILGC-Spn	66.45				Con & Ref
CORMOR-Evt *	88	Con[1]			
CORMOR-Evt *^	88	Con[1]			
TSP Earthworm ↓LOAEL					
MORFORD-Har	200	Con & Ref			
MORFORD-Har ^	200				
NEWTAC-Spn	208				Ref
BURTON-Evt	246				Con & Ref
BURTON-Evt ^	246				
MIMP-Ald-UNK	355		Con		
COLGATE-Ald	372				Ref
MIMP-Kit-UNK	373	Con	Con		
KCO-Evt	565		Con		Ref
KCO-Evt ^	565				
HOO Area					
HOO-06	35.2				
HOO-05*	128.5				
HOO-04	261				
HOO-02	364				
HOO-03	368				
HOO-01	390				Ref
HOO Earthworm and Lettuce ↑NOAEL					

Results were compared to both the control and reference within each soil type.
If the soil type could not be verified (identified by the UNK) the location was only compared to the control.
Con: significantly different when compared to the control.
Ref: significantly different when compared to the reference for that soil type.
*: Reference location.
^ : Soil re-tested due to failure of pH criteria.
[1]Results omitted from further bioassay analyses. See data quality section text for discussion.
LOAEL: Lowest observed adverse effect level (for comparisons to the control).
NOAEL: No observed adverse effect level (for comparisons to the control).
The gray bars for the arsenic and lead soil concentrations are a visual aid to show increasing concentrations.

Bioassays Compared to Soil Concentrations

Traditionally, SSLs for plants and soil biota are determined by conducting bioassays with a soil spiked with the contaminant of interest, here arsenic or lead. This allows the researcher to be sure that the introduced contaminant is the cause of toxicity. In addition, the concentrations in the soil are controlled so that an effect level can be calculated or the lowest observed adverse effect level (LOAEL) determined (i.e. the lowest concentration tested where a negative effect was observed). The calculated effects concentration or LOAELs are then used as the SSLs for plants and soil biota for the element examined. However, laboratory spiked soils do not represent the effects of natural conditions such as metals speciation, pH, weathering, and particle size.

Bioassays on contaminated field soils do take into account the effects of natural conditions. However, they combine the effects of all potential toxicants in that sample, known or unknown to the researcher. Therefore when toxicity is exhibited, it is difficult to say with certainty what caused it. The LOAEL is appropriate to use as an estimate of the concentration at which a contaminant may have caused adverse effects in field soils, compared to the control. This study compares LOAELs derived from lettuce and earthworm bioassays on field soils to current MTCA and EPA plant and soil biota SSL values for the Tacoma Smelter Plume footprint and Hanford Old Orchards.

To determine the LOAEL in mg/Kg dw for each endpoint, bioassay results were compared to arsenic, lead, and copper concentrations in the soil. Note that since Hanford Old Orchards locations showed no adverse effects when compared to the control, no observed adverse effect level (NOAEL) values were used. Table 14 shows the LOAEL, NOAEL MTCA SSL, and EPA SSL values. Table 13 displays the determination of the LOAEL and NOAEL values graphically for arsenic and lead. Copper is shown in Appendix F.

Table 14. Lettuce and earthworm LOAEL values compared to current SSL values in mg/Kg dw.

Metal	Receptor	Bioassay Test	SSL		LOAEL	NOAEL
			MTCA	EPA[1]	TSP	HOO
Arsenic	Plant	Lettuce	10	18	38	128
	Soil Biota	Earthworm	60	NA	62	128
Lead	Plant	Lettuce	50	120	67	390
	Soil Biota	Earthworm	500	1,700	200	390
Copper	Plant	Lettuce	100	70	40	58
	Soil Biota	Earthworm	50	80	129	58

[1] EPA, 2005b and EPA, 2005c.
TSP: Tacoma Smelter Plume footprint.
HOO: Hanford Old Orchards.

Arsenic Bioassay Comparison

The arsenic LOAEL and NOAEL values for both the lettuce and earthworm bioassays are higher than the current SSL values. In addition, at least one location had no observed toxicity between the SSL values and the LOAEL or NOAEL for plants. This also holds true for the Hanford Old

Orchards location that did exhibit plant toxicity when compared to the reference at 69 mg/Kg dw, despite being lower than the NOAEL. This indicates that the effects of natural soil conditions may attenuate the impact of arsenic to plants in the Tacoma Smelter Plume footprint and Hanford Old Orchards areas. This trend also holds true for the earthworm bioassays for the Hanford Old Orchards area. Given this evidence there may be justification for using higher SSLs for Tacoma Smelter Plume footprint plants (38 mg/Kg dw) and Hanford Old Orchards plants and soil biota (128 mg/Kg dw).

The MTCA SSL and the LOAEL were virtually identical for the Tacoma Smelter Plume footprint earthworm bioassay. This indicates that 60 mg/Kg dw arsenic is appropriate for the protection of soil biota in the Tacoma Smelter Plume footprint. The earthworm LOAEL from the Tacoma Smelter Plume footprint is from the MORFORD-Har location which also had the highest arsenic (III) to total arsenic ratio of 12:100 vs. the ~1:100 ratio of the other locations measured. It is unknown if this contributed to the toxicity.

Lead Bioassay Comparison

The lead MTCA SSLs are significantly lower than the EPA SSLs for both plants and soil biota. No earthworm or lettuce toxicity was observed for any of the Hanford Old Orchards locations, compared to the control. Because the MTCA and EPA SSLs for soil biota are above the highest measured lead concentration, it is difficult to say whether the MTCA or EPA SSLs are appropriate. The only observed toxicity in the Hanford Old Orchards area was for lettuce, compared to the reference location. The toxicity occurred at the highest lead concentration of 390 mg/Kg, which is higher than both the MTCA and EPA SSL levels. This indicates that the Hanford soils may attenuate the toxicity of lead to plants and a higher SSL may be warranted.

In the Tacoma Smelter Plume footprint, there were two locations above the MTCA SSL but below the LOAEL with no lettuce toxicity. Therefore a higher lead SSL for plant toxicity may also be appropriate for the Tacoma Smelter Plume footprint. However, given that there was toxicity observed below the EPA SSL it does not seem appropriate to use the EPA SSL for the Tacoma Smelter Plume footprint. The Tacoma Smelter Plume footprint earthworm LOAEL was lower than both the MTCA and EPA lead SSLs. This indicates that there may be increased toxicity to soil biota in the soils of the Tacoma Smelter Plume footprint and an SSL closer to the LOAEL value of 200 mg/Kg may be more appropriate.

Copper Bioassay Comparison

Copper was analyzed in soils being tested with bioassays due to concerns about copper toxicity interference with interpreting the bioassay results. It is noted that other contaminants not measured here may also be present at the study locations and may cause toxicity in the bioassay tests. However, copper was of particular concern in the Tacoma Smelter Plume footprint because it was also emitted from the smelter along with arsenic and lead. Therefore it may be elevated above background concentrations. Copper was analyzed in the Hanford Old Orchards area to provide a consistent data set for comparison.

The lettuce LOAEL for copper was 40 mg/Kg and the earthworm LOAEL was 129 mg/Kg for the Tacoma Smelter Plume footprint. Interestingly for the Hanford Old Orchards, the lowest location for copper was the only one to show any toxicity for lettuce at 13 mg/Kg, compared to the reference. No toxicity was exhibited when compared to the control for earthworms or lettuce in the Hanford Old Orchards so the NOAEL for both is 58 mg/Kg copper. Copper results for this study exceeded those values ranging from 13-235 mg/Kg with multiple tests passing above the EPA SSL values.

Although copper may have contributed to the toxicity exhibited in these bioassays, it is not conclusive that copper was the sole cause. As stated above, bioassays combine the effects of all potential toxicants in a sample and it is difficult to tease out the exact cause of toxicity. If the results had shown that above a certain copper level there is always an adverse effect observed, this would have provided evidence that copper was the cause of toxicity. However, since this was not the case, it cannot be determined if copper was responsible for the toxicity.

Wildlife Exposure Model

In the MTCA TEE process, a wildlife exposure model can be used to set site specific SSLs for ecological risk, with parameter values from MTCA, the literature, or site- specific empirical data. This study used the wildlife exposure model to recommend area-wide SSLs for the Tacoma Smelter Plume footprint and Hanford Old Orchards areas. This addresses the study goal to compare current MTCA SSL values to updated SSLs. Figure 12 graphically shows the wildlife exposure model components and how they interact.

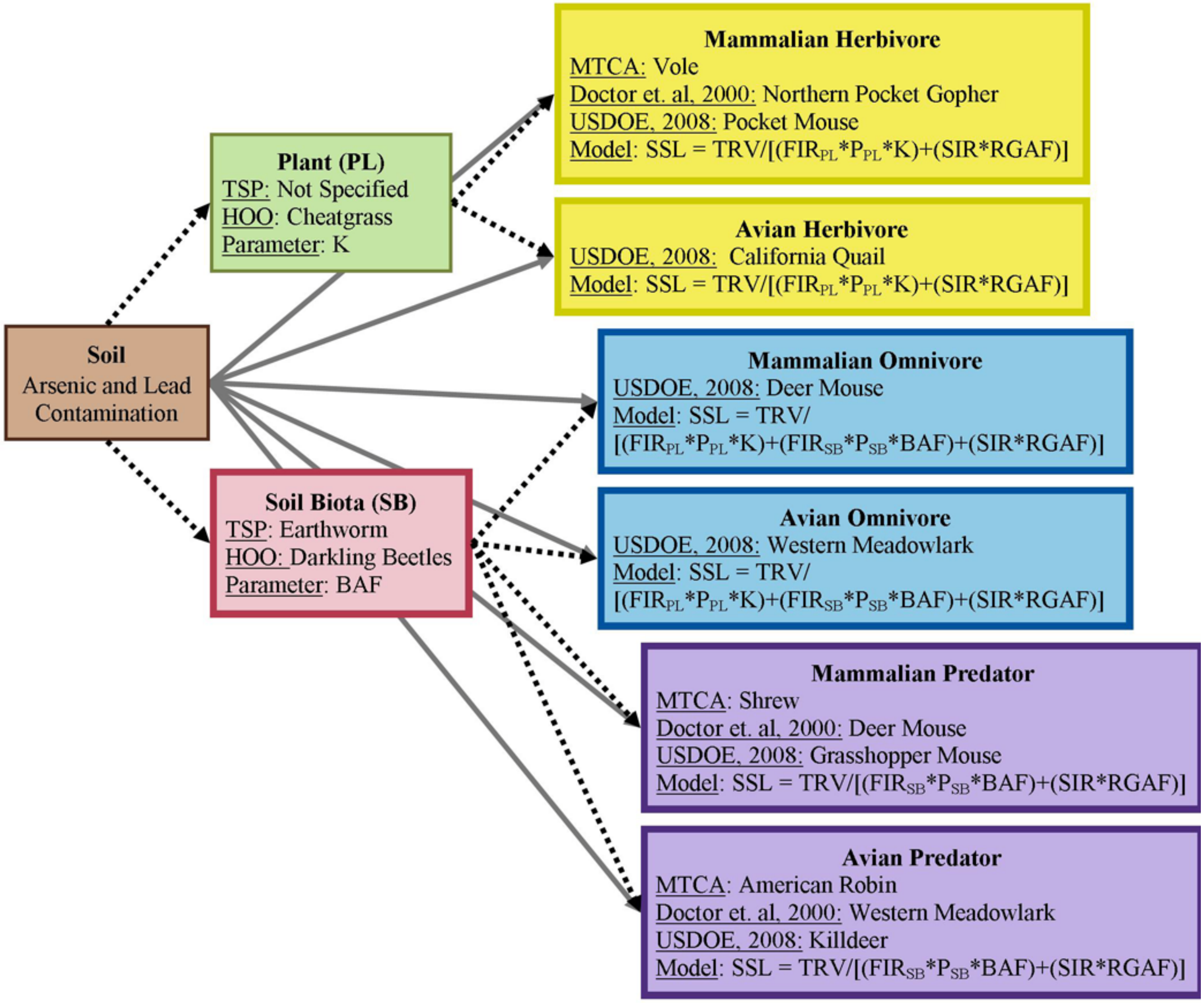

Figure 12. Diagram of the wildlife model used in a terrestrial ecological evaluation (TEE).

Outline of receptor species group parameters for the Tacoma Smelter Plume footprint (TSP) and Hanford Old Orchards (HOO) areas used in the TEE wildlife exposure model. Dotted lines represent the food pathway while solid grey lines represent the direct ingestion of soil pathway for arsenic and lead to enter organism. Surrogate receptor species in each receptor group are preceded by their parameter source.

Measured Parameters
BAF: Bioaccumulation factor
K: Plant Uptake Coefficient

Parameters for MTCA or Hanford Old Orchards receptor species groups
P: Diet contamination
SIR: Soil ingestion rate
FIR: Food ingestion rate
RGAF: Gut adsorption factor

Parameter from EPA or MTCA
TRV: Toxicity reference value

Plant Uptake Coefficient (K) and Soil Biota Bioaccumulation Factor (BAF)

Site-specific empirical data from this study was used in the wildlife exposure model for plant uptake coefficients (K) and soil biota bioaccumulation factors (BAF). K and BAF are calculated by dividing the contaminant concentration found in plants and soil biota respectively by the contaminant concentration in the soil. Before determining area-specific K and BAF values, the effects of soil type and plant species needed to be determined.

The influence of soil type on the bioaccumulation of arsenic and lead in plants and earthworms

It was hypothesized that soil type does not influence the body burden of arsenic and lead in plants and earthworms. To evaluate this, only Tacoma Smelter Plume footprint locations having confirmed soil types were assessed. K and BAF values were used as dependent variables in statistical models to test this hypothesis. Soil type as a factor on its own did not show statistically significant differences. Therefore, soil type was combined with other factors that may influence the uptake of metals to determine if soil type contributes to the bioaccumulation of arsenic and lead.

Boxplots in Figure 13 display the variability of the K and BAF values by area and soil type. Table 15 summarizes the best model runs. The only significant model was for predicting arsenic K using soil type, arsenic level, and plant type as factors (Model $p = 0.008$, soil type $p = 0.014$, $R^2 = 0.783$, Table 15). Tukey honestly significant difference (HSD) multiple comparisons found that the Alderwood, Everett, and Spanaway series had significantly lower arsenic K values than those of the Kitsap series (Table 16). Since the Kitsap series is the only silt loam soil while the remaining series are sandy loams, it makes sense that this series may exhibit different accumulation of arsenic in plants.

Given that only the arsenic uptake in plants for the Kitsap soil series is statistically different and only in combination with other factors, it seems reasonable to use area-wide K and BAF values instead of soil type-specific values. Additionally, the Kitsap series represents a very small proportion - less than 5%, of the Tacoma Smelter Plume footprint. Therefore it would not be advantageous to set a separate SSL value for this particular soil type, unless future studies find differences in soil texture in the Tacoma Smelter Plume footprint to be important in the bioaccumulation of arsenic and lead. While soil type does not appear to be important for determining uptake of metals, it is noted that a combination of soil properties (e.g., Eh, pH, Kd, and TOC) and biological properties do affect the transfer of metals from the soil to organisms (Sheppard et al. 1998).

Plant species influence on the accumulation of arsenic and lead

It was expected that arsenic and lead accumulation would not vary based on plant species. Statistical models were run using the same subset of data as the soil type statistical modeling. Individual plant species showed no significant relationships. Due to a lack of significance at the species level and the fact that some plant species were only collected at one location, plants were grouped into a woody or herbaceous plant type for analysis.

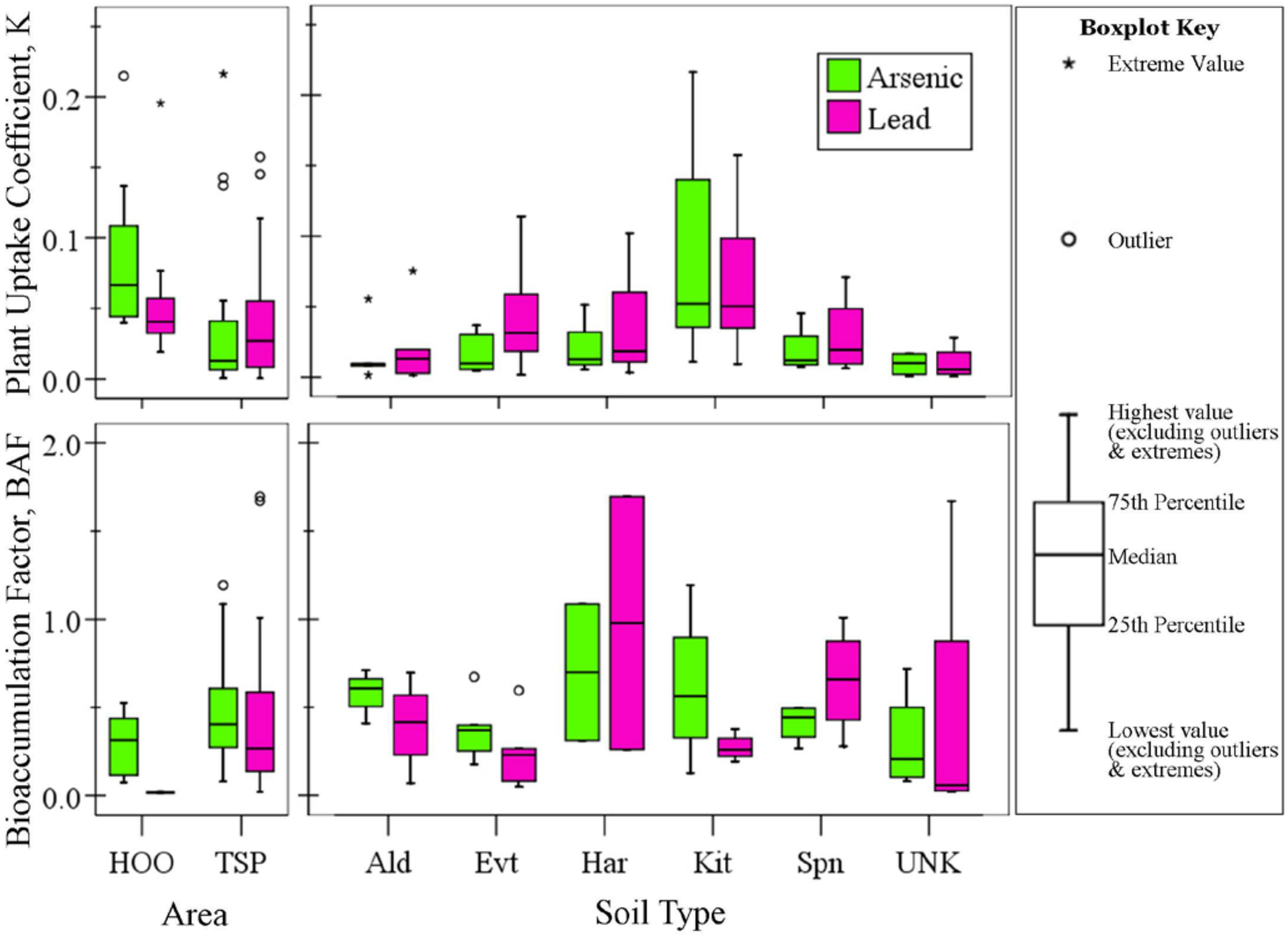

Figure 13. Box plots of K and BAF values by area and soil type.

TSP: Tacoma Smelter Plume footprint; HOO: Hanford Old Orchards.

Table 15. Arsenic and lead K and BAF predictive model results.

Test	Dependent	Model	Sig.	R^2
GLM	Arsenic K	Intercept + SOIL_TYPE + AS_LEVEL + PLANT_TYPE + SOIL_TYPE * PLANT_TYPE + AS_LEVEL * PLANT_TYPE	0.008 **	0.783
	Arsenic BAF	Intercept + SOIL_TYPE + TOC_SOIL	0.053	0.532
Kruskal-Wallis	Lead K	SOIL_TYPE	0.397	
	Lead BAF	SOIL_TYPE	0.180	
	Lead K	PLANT_TYPE	0.114	

AS_LEVEL: Expected arsenic level that was used to select each location; represents the project design.
PLANT_TYPE: Herbaceous or woody.
* $p \leq 0.05$ ** $p \leq 0.01$

Table 16. Arsenic K multiple comparisons of soil type.

	Ald	Evt	Har	Spn
Evt	1.00			
Har	0.999	0.999		
Spn	1.00	1.00	1.00	
Kit	0.013*	0.004**	0.062	0.024*

* $p \leq 0.05$
** $p \leq 0.01$

Plant type was not significant when used as the sole factor in the statistical models. However, arsenic K was predicted by a model that included plant type along with soil type and arsenic level (Table 15). Other combinations of factors did not produce significant statistical models for arsenic in plants. Lead in plants showed no significant combination of factors. Given these results it is reasonable to conclude that plant type alone does not have a significant effect on K values. Therefore, K values were pooled together for calculating SSLs without considering plant type.

Calculating K and BAF values for the Tacoma Smelter Plume footprint and Hanford Old Orchards areas

Area-wide K and BAF values are preferable for use in large-scale cleanup efforts because they simplify the cleanup process. Given that soil and plant type are not major factors in determining the uptake of metals by plants and soil biota, it is reasonable to establish a single K and BAF for each of the study areas.

A regression model type approach for calculating K and BAF values is often more accurate for site specific uses, however site specific soil concentrations are needed to determine SSL values. Given that this studies goal is to provide area wide rather than site specific SSL values and the soil concentrations in these areas have a large range, regression modeling was not an appropriate approach. Instead the median K and BAF values for each of the Tacoma Smelter Plume footprint and Hanford Old Orchards datasets were used. This approach follows previous establishment of statewide K and BAF values by Ecology and the method used by the EPA when an acceptable regression model is not available (EPA, 2005a).

Table 17 shows the median K and BAF values by area. These are the values that were used in the wildlife exposure model to derive new SSL values for the Tacoma Smelter Plume footprint and Hanford Old Orchards areas.

Table 17. Median K and BAF values by area.

Grouping Parameter	Median Plant Uptake Coefficient, K (mg/Kg dw plant/mg/Kg dw soil)			Median Bioaccumulation Factor, BAF (mg/Kg dw biota/mg/Kg dw soil)		
	N	Arsenic	Lead	N	Arsenic	Lead
TSP	32	0.0125	0.0271	24	0.403	0.268
HOO	11	0.0668	0.0403	9	0.314	0.0180
TSP and HOO	43	0.0301	0.0298	33	0.381	0.219
MTCA		0.06	0.0047		1.16	0.69

TSP: Tacoma Smelter Plume footprint.
HOO: Hanford Old Orchards.

Surrogate Species Receptors

Surrogate species are used to represent the receptor species groups: mammalian herbivores, avian herbivores, mammalian omnivores, avian omnivores, mammalian predators, and avian predators (Figure 12). Each surrogate species has a number of parameters specific to that species that determine the uptake of contaminants. These parameters are: the proportion of contaminated food in the diet (P), food ingestion rate (FIR), soil ingestion rate (SIR), and gut absorption factor (RGAF). The wildlife exposure model produces an SSL for each surrogate receptor species based on species-specific parameters. The lowest soil screening level produced by the wildlife exposure model becomes the overall soil screening level for the protection of wildlife.

The default surrogate species used in MTCA are the vole, shrew, and American robin. These are appropriate for the Tacoma Smelter Plume footprint where forest habitats dominate and these species have the potential of being present. However, Doctor et al. (2000) recommended that these default surrogate species be replaced with shrub-steppe habitat species for the Hanford site. They recommended Hanford surrogates of the northern pocket gopher, deer mouse, and Western meadowlark. More recently USDOE (USDOE, 2008, in draft) recommended Hanford site surrogate receptors of the pocket mouse, California quail, deer mouse, Western meadowlark, grasshopper mouse, and killdeer.

This study evaluates SSLs using the MTCA surrogate species for the Tacoma Smelter Plume footprint locations and the MTCA, Doctor et al. (2000) and USDOE (2008) surrogate species for the Hanford Old Orchards.

Toxicity Reference Value

The toxicity reference value (TRV) is calculated for mammals and birds, representing the dose above which adverse effects are expected. MTCA TRVs are from literature searches conducted during rule development. Although currently in rule for the state of Washington, they do not reflect the best available science. In 2005 EPA published new TRVs, based on a new literature search, that result in more stringent SSLs. SSLs using both the MTCA and EPA TRVs have been calculated for this study to aid in comparisons. Table 18 summarizes the TRVs used in the wildlife exposure model.

Table 18. Toxicity reference values used in the wildlife exposure model in mg/Kg/day.

Receptor Group	MTCA		EPA, 2005b & c	
	Arsenic	Lead	Arsenic	Lead
Mammalian Herbivore, Omnivore, or Predator	As(V) = 35	20	1.04	4.7
Avian Herbivore, Omnivore, or Predator	As(V) = 22	11.3	2.24	1.63

As(V) value is used in this study because in dry soils As(III) is rarely present in sufficient quantities.

Soil Screening Levels for the Protection of Wildlife

SSLs were calculated using the wildlife exposure model with empirical values from this study and parameter values from MTCA, EPA (2005b,c), Doctor et al. (2000), and USDOE (2008). SSLs were calculated for each surrogate receptor for a combination of parameter values. The lowest SSL value of any receptor group is reported in Table 19 for different combinations of parameter value sources. Full details for each receptor group and parameter values are in Appendix E.

SSL values based on the median K, median BAF, and MTCA parameters for both the Tacoma Smelter Plume footprint and Hanford Old Orchards areas are considerably higher than the current MTCA SSLs of 7 and 118 mg/Kg for arsenic and lead, respectively. This indicates that the median field-derived K and BAF values for the receptor group with the lowest SSL are lower than the values in MTCA. The avian predator receptor group produced the lowest SSL values of the three receptor groups. The median field-derived BAF was used to calculate these avian predator SSL values and is indeed below the MTCA BAF values (Table 17). The newly calculated SSLs continue to be higher even when the wildlife exposure model parameter values are changed for arsenic. However, this does not hold true for lead if the parameters are varied.

Table 19. SSL values using different wildlife exposure model parameters in mg/Kg dw.

Surrogate Species Source	Arsenic		Lead	
Toxicity Reference Value ->	EPA	MTCA	EPA	MTCA
Tacoma Smelter Plume footprint				
MTCA	11	339	32	224
Hanford Old Orchards area				
MTCA	14	398	70	482
Doctor et al., 2000	36	583	73	504
USDOE, 2008	21	254	34	239

Values from field data used in model: median BAF and K.
Receptor species parameters source: MTCA (MTCA default), Doctor et al., 2000 (Hanford Old Orchards specific), and USDOE, 2008(Hanford Old Orchards specific).
Parameters include: Diet contamination (P), Soil ingestion rate (SIR), Food ingestion rate (FIR), and Gut adsorption factor (RGAF).
Toxicity reference value (TRV) source: MTCA or EPA, 2005bc.

The EPA TRV SSL values in the Tacoma Smelter Plume footprint are 11 and 32 mg/Kg dw for arsenic and lead, respectively. The EPA TRV Hanford Old Orchards SSL values are higher than the Tacoma Smelter Plume footprint values especially when using the Hanford Old Orchards specific receptors at 21 or 36 mg/Kg dw for arsenic and 34 or 73 mg/Kg dw for lead, respectively. Given that the EPA TRV values are more current than the MTCA TRVs, it is recommended that SSLs calculated using these values be used in cleanups.

Using Hanford Old Orchards specific surrogate receptor species increased the lowest EPA TRV Hanford Old Orchards SSL values for arsenic from 14 mg/Kg dw to 21 and 36 mg/Kg dw. This increase illustrates that the MTCA default receptor species provide a more conservative estimate of risk than the Hanford Old Orchards specific surrogate receptor species. However, for lead this increase in SSL value only holds true for the MTCA to Doctor et al. (2000) comparison. The USDOE (2008) surrogate species are more sensitive to lead than the MTCA surrogate species. Since the Hanford Old Orchards specific receptor species better represent the food web that exists in a shrub-steppe environment, these species should be used to develop SSLs in the Hanford Old Orchards area rather than MTCA default receptors.

In general, the SSL values changed the most dramatically when comparing MTCA and EPA TRV values, used in the wildlife exposure models (e.g., reading Table 19 horizontally). However the SSLs were fairly consistent when the surrogate receptor values were altered (e.g., reading Table 19 vertically).

Summary of Soil Screening Levels from Bioassays and Wildlife Exposure Models

Table 20 summarizes the various SSL values identified in this report. For all sets of SSLs, the lowest SSL is the value that would be used as a screening level at a cleanup site. The Tacoma Smelter Plume footprint and Hanford Old Orchards values are derived from this study. Since these SSL values most closely represent the conditions in the Tacoma Smelter Plume footprint and Hanford Old Orchards areas, it is recommended that these be considered for use with cleanup sites in these areas. The plant and soil biota SSL values are derived from the bioassay LOAEL and NOAEL values from this study, while the wildlife SSLs are from wildlife exposure modeling.

Interestingly, the lowest MTCA, EPA, and Tacoma Smelter Plume footprint arsenic SSLs are fairly similar, at 10, 18, and 11 mg/Kg respectively. The Hanford Old Orchards arsenic SSL is higher when using either the Doctor et al. (2000) or USDOE (2008) receptors at 36 and 21 mg/Kg respectively. The higher Hanford Old Orchards SSLs may represent a lower exposure risk by species in the Hanford Old Orchards environment. Plants were the driving receptor for the MTCA and EPA arsenic SSLs, while wildlife receptors had the lowest values for the Tacoma Smelter Plume footprint and Hanford Old Orchards arsenic SSLs.

The lowest lead SSL values span a much larger range than the arsenic SSLs. The MTCA lead SSL is driven by plant receptors while the EPA, Tacoma Smelter Plume footprint, and Hanford Old Orchards lead SSLs are lowest for the wildlife receptors. The Tacoma Smelter Plume footprint lowest SSL and Hanford Old Orchards with USDOE (2008) receptors fall between the

MTCA and EPA values at 32 and 34 mg/Kg respectively. The Hanford Old Orchards with Doctor et al. (2000) receptors SSL is the highest of the four values, implying reduced risk to species in the steppe-shrub habitat similar to arsenic.

Table 20. Summary of recommended SSL values in mg/Kg dw.

Source->	Arsenic					Lead				
	MTCA As(V)	EPA, 2005b	TSP^	HOO[1] Doctor	HOO[1] USDOE	MTCA	EPA, 2005c	TSP[1]	HOO Doctor[1]	HOO USDOE[1]
Plants	10	18	38*	128^		50	120	67*	390^	
Soil Biota	60	-	62*	128^		500	1700	200*	390^	
Wildlife	132	43	11	36	21	118	11	32	73	34
Lowest SSL	10	18	11	36	21	50	11	32	73	34

*Values from bioassay LOAEL values, not true SSL values.

^Values from bioassay NOAEL values, not true SSL values, MTCA specifies the use of the LOAEL for SSLs; however, since an LOAEL cannot be calculated from this dataset the NOAEL has been reported.

[1]Wildlife SSLs from wildlife exposure model using empirical values from this study, EPA, 2005bc TRVs, MTCA surrogate species for Tacoma Smelter Plume footprint, and Doctor et al. (2000) or USDOE (2008) surrogate species for the Hanford Old Orchards area.

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Conclusions

Twenty-five locations in the Tacoma Smelter Plume footprint and 11 locations in Hanford Old Orchards were sampled for soil, plants, and soil biota in 2010. Samples were analyzed to evaluate the effects of arsenic and lead on ecological receptors in these two areas. Lettuce and earthworm bioassays were conducted to explore the effects of arsenic and lead on plants and soil biota. From these data, lowest observed adverse effect levels (LOAELs) were determined to establish a guideline for the protection of plants and soil biota. Before evaluating appropriate soil screening levels (SSLs) for the protection of wildlife, several questions were answered.

Using Total vs. Speciated Arsenic to Establish SSLs

Arsenic (III) was found in very small quantities, with arsenic (V) making up the majority of the total arsenic present. Given the lack of arsenic (III), total arsenic is appropriate to use for establishing SSL values for dry soils; arsenic species should continue to be considered for wetted soils.

Influence of Soil Type on Plants and Soil Biota Bioaccumulation of Arsenic and Lead

Arsenic uptake by plants differed significantly in statistical models that included soil type and other factors. While the influences of soil type on arsenic uptake in plants may logically be attributed to differences in soil texture (silt loam vs. sandy loam), more evidence is needed to conclusively demonstrate this effect. No significant difference was found for soil biota or lead based on soil type. Based on this outcome, plant uptake (K) and bioaccumulation factor (BAF) values were not distinguished based on soil type. This reflects the practicality of using one value for an entire area, simplifying the use of SSLs for cleanup actions.

Influence of Plant Type on Bioaccumulation of Arsenic and Lead

Arsenic uptake was moderately different between woody and herbaceous plant types in combination with other factors. No significant differences were found for lead uptake. Therefore, since arsenic uptake was only moderately different and no difference was found for lead, K values for woody and herbaceous plants were pooled together for use in establishing SSLs.

Parameters Used in the Wildlife Exposure Model

Since accumulation differences by soil type and plant type did not justify separate SSL values, one K and one BAF value were established for each of the two study areas. This decision is both practical and warranted until there is stronger evidence that soil texture or plant type contributes to increased accumulation of arsenic or lead.

Two sources of toxicity reference values (TRVs) were considered in this study. The EPA (2005b and c) TRVs were selected for calculating the recommended SSL values for this study because they reflect the best available science. It is noted that these updated TRV values dramatically lower the SSL values compared to the Model Toxics Control Act (MTCA) TRVs when all other variables in the wildlife exposure model are held constant.

Default surrogate receptor species were used for the Tacoma Smelter Plume footprint, and surrogate receptor species more representative of a steppe-shrub habitat were used for the Hanford Old Orchards SSLs. Use of the steppe-shrub species did not dramatically influence the calculated SSL values, but it is still important to use representative species in the Wildlife Exposure Model.

Recommendations

As a result of this study, the following recommendations are made:

- Soil screening levels (SSLs) derived from EPA toxicity reference values (TRVs) should be used for determining risks to wildlife.
- Hanford-specific surrogate receptor species should be used to determine SSLs for the Hanford Old Orchards area instead of the default Model Toxics Control Act (MTCA) species.
- Total arsenic is appropriate to use for establishing SSL values for dry soils, instead of arsenic species.
- The influence of a silt loam soil vs. a sandy loam soil on plant uptake (K) and bioaccumulation factor (BAF) values for arsenic and lead should be further investigated.
- Additional information on the differences in uptake of arsenic and lead in different plant species should be collected.
- When using an Innov-X Systems X-ray Fluorescence Instrument (XRF) for screening purposes, it is recommended that the relationship between the XRF results and traditional laboratory results be evaluated for bias. If a bias by the XRF is found, an equation to correct the XRF results should be developed to provide more representative comparison to the concentration expected from traditional laboratory methods.
- Numeric criteria for terrestrial bioassays should be developed in addition to the statistical comparison used in this study (i.e., bioassay tests should be analyzed consistently in MTCA for both the sediment management standards and terrestrial ecological evaluations).
- Future studies should use a wet sieving grain size method for soils containing silts or clays.
- Freeze drying is not recommended for earthworms due to difficulties homogenizing the sample. Earthworm tissues should be ground and homogenized wet.

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References

APHA, AWWA, and WEF, 1998. Standard Methods for the Examination of Water and Wastewater 20th Edition. Method SM2540G. American Public Health Association, Washington, D.C.

Button M., M.J. Watts, M.R Cave., C.F. Harrington, and G.T Jenkin., 2009. Earthworms and in vitro physiologically-based extraction tests: complementary tools for a holistic approach towards understanding risk at arsenic-contaminated sites. Environmental Geochemistry and Health 31(2):273-82.

Doctor, P.G., K.A. Gano, and N.K. Lane, 2000. Evaluation of a Terrestrial Foodweb Model to Set Soil Cleanup Levels. Environmental Toxicology and Risk Assessment: Recent Achievements in Environmental Fate and Transport: ninth volume. ASTM, West Conshohocken, PA.

Ecology, 2007. Tacoma Smelter Plume Management Plan - Objectives, Priorities, Goals, and Implementation Steps**.** Washington State Department of Ecology, Olympia, WA. www.ecy.wa.gov/programs/tcp/sites/tacoma_smelter/tsp_mgmt_plan.html.

Efroymson, R.A., M.E. Will, and G.W. Suter II, 1997a. Toxicological benchmarks for contaminants of potential concern for effects on soil and litter invertebrates and heterotrophic process: 1997 revision. Prepared for the U.S. Department of Energy at by the Oak Ridge National Laboratory, ES/ER/TM-126/R2.

Efroymson, R.A., M.E. Will, G.W. Suter II, and A.C. Wooten, 1997b. Toxicological benchmarks for contaminants of potential concern for effects on soil terrestrial plants: 1997 revision. Prepared for the U.S. Department of Energy at by the Oak Ridge National Laboratory, ES/ER/TM-85/R3.

Eisler, R., 1988a. Arsenic Hazards to Fish, Wildlife, and Invertebrates: a Synoptic Review. U.S. Fish and Wildlife Service. Biological Report 85(1.12).

Eisler, R., 1988b. Lead Hazards to Fish, Wildlife, and Invertebrates: a Synoptic Review. U.S. Fish and Wildlife Service. Biological Report 85(1.14).

EPA, 1991. Methods for the Determination of Metals in Environmental Samples, Method 200.8. Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio. EPA-600/4-91-010.

EPA, 2004. Test Methods for Evaluating Solid Waste, Method 9045D Revision 4. U.S. Environmental Protection Agency, Office of Solid Waste. SW-846, Physical/Chemical Methods. www.epa.gov/wastes/hazard/testmethods/sw846/online/index.htm.

EPA, 2005a. Attachment 4-1: Guidance for developing ecological soil screening levels (Eco-SSLs) – Exposure factors and bioaccumulation models for derivation of wildlife Eco-SSLs. OSWER Directive 9285.7-55. U.S. Environmental Protection Agency, Washington D.C.

EPA, 2005b. Ecological soil screening levels for arsenic, interim final. OSWER Directive 9285.7-62. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington D.C.

EPA, 2005c. Ecological soil screening levels for lead, interim final. OSWER Directive 9285.7-70. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington D.C.

EPA, 2007. Test Methods for Evaluating Solid Waste, Method 6200 Update IVA. U.S. Environmental Protection Agency, Office of Solid Waste. SW-846, Physical/Chemical Methods. www.epa.gov/wastes/hazard/testmethods/sw846/online/index.htm

EPA, 2010. Site Description and Cleanup Progress for Commencement Bay, Nearshore/ Tideflats - Washington. U.S. Environmental Protection Agency. EPA ID# WAD980726368 EPA Region 10 - Pierce County - Tacoma and Ruston. http://yosemite.epa.gov/r10/nplpad.nsf/88d393e4946e3c478825631200672c95/06e1c0cda0d11fc285256594007559fd?OpenDocument.

Langdon, C.J., C. Winters, S.R. Sturzenbaum, A.J. Morgan, J.M. Charnock, A.A. Meharg, T.G. Piearce, P.H. Lee, and K.T. Semple, 2005. Ligand arsenic complexation and immunoperoxidase detection of metallothionein in the earthworm Lumbricus rubellus inhabiting arsenic-rich soil. Environmental Science and Technology 39:2042-2048.

Ma, W.K., B.A. Smith, G.L. Stephenson, and S.D. Siciliano, 2009. Development of a Simulated Earthworm Gut for Determining Bioaccessible Arsenic, Copper, and Zinc from Soil. Environmental Toxicology and Chemistry 28(7):1439-46.

Norton, D., 1996a. Early Seedling Growth Protocol for Soil Toxicity Screening. Washington State Department of Ecology, Olympia, WA. Publication No. 96-324. www.ecy.wa.gov/biblio/96324.html.

Norton, D., 1996b. Earthworm Bioassay Protocol for Soil Toxicity Screening. Washington State Department of Ecology, Olympia, WA. Publication No. 96-327. www.ecy.wa.gov/biblio/96327.html.

Pacific Groundwater Group and TeraStat Inc, 2005. Tacoma Smelter Plume Project Extended Footprint Study. Prepared for the Washington State Department of Ecology, Olympia, WA. www.ecy.wa.gov/programs/tcp/sites/tacoma_smelter/extended_footprint_study_kc/e_f_s.html.

PSEP (Puget Sound Estuary Program), 1997. Recommended Guidelines for Measuring Organic Compounds in Puget Sound Water, Sediment and Tissue Samples. U.S. Environmental Protection Agency, Region 10, Office of Puget Sound, Seattle, Washington.

Sheppard, S., J. Bembridge, M. Holmstrup, and L. Posthuma, 1998. Advances in earthworm ecotoxicology. Proceedings from the Second International Workshop on Earthworm Ecotoxicology. 2-5 April 1997. Amsterdam, The Netherlands. Pensacola, FL: Society of Environmental Toxicology and Chemistry (SETAC) 472pp.

Sloan, J., 2010. Quality Assurance Project Plan: Evaluating the toxicity of arsenic and lead in the soils of the Tacoma Smelter Plume footprint and Hanford Old Orchards areas. Washington State Department of Ecology, Olympia, WA. Publication No. 10-03-107. www.ecy.wa.gov/biblio/1003107.html.

Soil Survey Staff, 2008. Official Soil Series Descriptions: Natural Resources Conservation Service, United States Department of Agriculture (USDA-NRCS), Lincoln, NE. http://soils.usda.gov/technical/classification/osd/index.html.

USDOE, 2008. River Corridor Baseline Risk Assessment (RCBRA): Source and groundwater component. Vol. 1: Ecological risk assessment. U.S. Department of Energy. DOE/RL-2007-21, Draft B Regulator Review.

Walsh, L.M., M.E. Sumner, and D.R. Keeney, 1977. Occurrence and Distribution of Arsenic in Soils and Plants. Environmental Health Perspectives 19: 67-71.

Yokel, J. and D. Delistraty, 2003. Arsenic, Lead, and Other Trace Elements in Soils Contaminated with Pesticide Residues at the Hanford Site (USA). Environmental Toxicology 18(2): 104-114.

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Appendices

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Appendix A. Site Characteristics

Table A- 1. Sampling location collection date and coordinates.

User_Location_ID	Date Sampled	Time	NAD 83 Latitude (N)	NAD 83 Longitude (W)	County
Tacoma Smelter Plume footprint					
BONN-Kit	5/10/2010	13:52	47.39657	122.43419	King
BURTON-Evt	5/11/2010	15:00	47.39053	122.45082	King
COLGATE-Ald	5/17/2010	9:37	47.22586	122.54850	Pierce
CORMOR-Evt	5/17/2010	17:29	47.15944	122.61184	Pierce
DOCKTON-Ald	5/14/2010	12:44	47.36803	122.45336	King
FTSTEILGC-Spn	5/19/2010	10:02	47.18542	122.56323	Pierce
FTSTEILP-Evt	5/12/2010	14:34	47.16774	122.56283	Pierce
FTSTEILP-Spn	5/12/2010	11:42	47.16909	122.55455	Pierce
ICF-Evt	5/10/2010	10:26	47.44170	122.47703	King
IDLEWILD-Spn	5/17/2010	14:04	47.15889	122.54675	Pierce
KCO-Evt	5/18/2010	14:19	47.37102	122.43804	King
KOPA-Har	5/20/2010	14:06	47.30727	122.68131	Pierce
KOPA-Kit	6/8/2010	12:40	47.30902	122.68609	Pierce
LOWJOHN-Har	5/12/2010	9:19	47.16876	122.68980	Pierce
MIMP-Ald-UNK	5/21/2010	11:48	47.37942	122.41401	King
MIMP-Kit-UNK	5/21/2010	16:32	47.38393	122.40138	King
MORFORD-Har	5/20/2010	10:51	47.29301	122.55722	Pierce
MORN-Evt	5/18/2010	10:39	47.34209	122.50013	King
NEILLPT-Kit-UNK	6/9/2010	10:32	47.33223	122.49384	King
NEWTAC-Spn	5/19/2010	15:00	47.20330	122.56090	Pierce
PTROB-Kit	5/14/2010	10:01	47.38903	122.37677	King
TACNAR-Har-UNK	5/20/2010	8:42	47.27299	122.56462	Pierce
THEMGIL-Ald	6/8/2010	9:08	47.21952	122.52047	Pierce
WING-Ald	5/11/2010	9:57	47.49690	122.46355	King
WING-Kit	5/11/2010	12:50	47.49748	122.45988	King
Hanford Old Orchards					
HOO-01	5/25/2010	10:12	46.69017	119.47470	Benton
HOO-02	5/25/2010	10:33	46.69016	119.47494	Benton
HOO-03	5/25/2010	11:42	46.69002	119.47473	Benton
HOO-04	5/26/2010	10:00	46.68525	119.47555	Benton
HOO-05	5/26/2010	12:37	46.68418	119.47070	Benton
HOO-06	5/26/2010	10:30	46.68710	119.47499	Benton
HOO-07	5/25/2010	12:03	46.68984	119.47466	Benton
HOO-08	5/26/2010	12:31	46.68471	119.46996	Benton
HOO-09	5/26/2010	14:28	46.68783	119.47479	Benton
HOO-10	5/26/2010	13:41	46.68422	119.47051	Benton
HOO-11	5/26/2010	11:10	46.68525	119.47535	Benton

Table A- 2. Sampling location soil texture, land use, habitat, and vegetation.

User_Location_ID	Soil Texture	Land Use	Habitat	Dominant Non-tree vegetation
Tacoma Smelter Plume footprint				
BONN-Kit	Silt Loam	Natural and Business	Deciduous Forest	Shrub
BURTON-Evt	Sandy Loam	Natural and Recreation	Mature Mixed Forest toward Deciduous	Forbs
COLGATE-Ald	Loamy Sand	Recreation and Natural	Mixed Forest	Shrub
CORMOR-Evt	Sandy Loam	Recreation and Natural	Second Timber Deciduous Forest	Shrub
DOCKTON-Ald	Loam	Natural and Recreation	Mixed Forest	Forbs
FTSTEILGC-Spn	Sandy Loam	Recreation	Open meadow with Deciduous	Grass
FTSTEILP-Evt	Loamy Sand	Recreation and Natural	Mature Conifer Forest	Forbs
FTSTEILP-Spn	Loamy Sand	Recreation and Natural	Open meadow with Deciduous	Grass
ICF-Evt	Loamy Sand	Natural and Recreation	Second Timber Mixed Forest	Forbs
IDLEWILD-Spn	Sandy Loam	School and Natural	Deciduous Forest	Forbs
KCO-Evt	Sandy Loam	Natural and Recreation	Mature Conifer Forest	Shrub
KOPA-Har	Loamy Sand	Natural and Recreation	Mixed Forest	Forbs
KOPA-Kit	Silt Loam	Recreation and Natural	Mature Deciduous Forest	Forbs
LOWJOHN-Har	Loamy Sand	Recreation and Natural	Mixed Forest	Shrub
MIMP-Ald-UNK	Sandy Clay Loam	Natural and Recreation	Mature Deciduous Forest	Shrub
MIMP-Kit-UNK	Sandy Loam	Natural and Recreation	Mature Deciduous Forest	Forbs
MORFORD-Har	Loamy Sand	Natural and Recreation	Mature Conifer Forest	Forbs
MORN-Evt	Sandy Loam	Recreation and Natural	Mixed Size Conifer Forest	Bare Soil
NEILLPT-Kit-UNK	Sandy Clay	Natural	Mature Deciduous Forest	Forbs
NEWTAC-Spn	Loamy Sand	Natural and Business	Sparse Conifer Forest	Grass
PTROB-Kit	Silt Loam	Recreation and Natural	Second Timber Deciduous Forest	Forbs
TACNAR-Har-UNK	Sandy Clay	Natural and Recreation	Mature Mixed Forest toward Conifer	Forbs
THEMGIL-Ald	Sandy Loam	Recreation and Natural	Mature Conifer Forest	Shrub
WING-Ald	Sandy Clay Loam	Natural and Recreation	Deciduous Forest	Forbs
WING-Kit	Silty Clay Loam	Recreation and Natural	Mixed Forest	Forbs
Hanford Old Orchards				
HOO-01	Loamy Sand	Natural	Shrub-Steppe with cheatgrass	Forbs
HOO-02	Loamy Sand	Natural	Shrub-Steppe with cheatgrass	Forbs
HOO-03	Loamy Sand	Natural	Shrub-Steppe with cheatgrass	Forbs
HOO-04	Loamy Sand	Natural	Shrub-Steppe with cheatgrass	Forbs
HOO-05	Loamy Sand	Natural	Shrub-Steppe with cheatgrass	Forbs
HOO-06	Loamy Sand	Natural	Shrub-Steppe with cheatgrass	Forbs
HOO-07	Loamy Sand	Natural	Shrub-Steppe with cheatgrass	Forbs
HOO-08	Sandy Loam	Natural	Shrub-Steppe with cheatgrass	Forbs
HOO-09	Loamy Sand	Natural	Shrub-Steppe with cheatgrass	Forbs
HOO-10	Sandy Loam	Natural	Shrub-Steppe with cheatgrass	Forbs
HOO-11	Loamy Sand	Natural	Shrub-Steppe with cheatgrass	Forbs

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Appendix B. Chemistry Data Quality Assurance

Earthworm tissues, plants, and soils were received by MEL frozen and in good condition. Earthworm tissue and plant samples were freeze dried within one month of arriving at the lab, ground, and homogenized. Metals analyses were performed on the freeze dried tissues and soil samples using EPA method 200.8 or 1632 modified within established holding times. Data quality was assessed by the measurement quality objectives (MQO) outlined in the quality assurance project plan (Sloan, 2010). Tables A- 1 through A- 6 show the project objectives and quality assurance results.

Table B- 1. Laboratory blanks.

Analyte	Matrix	QC Result	Units
Arsenic (MEL)	Soil	0.008 U	mg/Kg dw
	Tissue	0.03 U	
		0.10 U	
Arsenic (BRL)	Soil	0.003 UJ	
Arsenic (III)	Soil	0.05 UJ	
Copper	Soil	0.006 U	
Lead	Soil	0.003 U	
	Tissue	0.02 U	
		0.10 U	
Solids (MEL)	Soil	0	%
Solids (BRL)	Soil	0.21 UJ 0.35 J	
Total Organic Carbon	Soil	0.03 U	

QC: Quality control.
MEL: Manchester Environmental Laboratory.
BRL: Brooks Rand Laboratory.
U: Analyte was not detected at or above the reported result.
UJ: Analyte was not detected at or above the reported estimate.
J: Analyte was positively identified, but the reported value is an estimate.

Table B- 2. Laboratory duplicates.

Analyte	Matrix	Sample ID	Sample Site Name	Sample Result	QC Result	RPD	Units
Arsenic (MEL)	Soil	1005024-01	MIMP-Ald-UNK	171	166	2	mg/Kg dw
		1005024-39	HOO-11	19.6	18	8	
	Tissue	1005024-67	PTROB-Kit	1.12	1.11	0.6	
		1005024-90	NEILLPT-Kit-UNK	4.11	2.85	**36**	
		1005024-AF	MIMP-Kit-UNK	26.1	19.6	**28**	
		1005024-BD	NEILLPT-Kit-UNK	31.6	31.2	1	
		1005024-BQ	WING-Ald	0.141	0.108	**27**	
Arsenic (BRL)	Soil	1024022-01	BURTON-Evt	88.5	96.86	9	
		1024022-12	HOO-01	59.7	64.47	8	
Arsenic (III)	Soil	1024022-01	BURTON-Evt	0.503	0.515	2	
		1024022-12	HOO-01	0.559 J	0.451	**21**	
Copper	Soil	1005024-01	MIMP-Ald-UNK	220	224	2	
		1005024-39	HOO-11	14.5	14.7	2	
Lead	Soil	1005024-01	MIMP-Ald-UNK	355	365	3	
		1005024-39	HOO-11	215	216	0.5	
	Tissue	1005024-67	PTROB-Kit	1	1	4	
		1005024-90	NEILLPT-Kit-UNK	5	3	**38**	
		1005024-AF	MIMP-Kit-UNK	30	35	16	
		1005024-BD	NEILLPT-Kit-UNK	13	14	2	
		1005024-BQ	WING-Ald	0.7	0.4	**53**	
Solids (MEL)	Soil	1005024-03	COLGATE-Ald	68.9	69	0.2	%
		1005024-15	KOPA-Har	69.1	68.7	0.6	
		1005024-16	WING-Kit	55.2	53.8	3	
		1005024-39	HOO-11	92.5	92.5	0.09	
Solids (BRL)	Soil	1024022-01	BURTON-Evt	55.79	59.42	6	
		1024022-12	HOO-01	97.47	97.57	0	
Total Organic Carbon	Soil	1005024-01	MIMP-Ald-UNK	10.2	8.63	16	
					9.83	3	
		1005024-21	ICF-Evt	3.74	4.4	16	
					4.65	**22**	

QC: Quality control.
RPD: Relative Percent Difference.
MEL: Manchester Environmental Laboratory.
BRL: Brooks Rand Laboratory.
J: Analyte was positively identified, but the reported value is an estimate.

Table B- 3. Field replicates.

Analyte	Matrix	Tissue Type	Sample ID	QC Sample ID	Sample Site Name	Sample Result	QC Result	RPD (%)	Units
Arsenic (MEL)	Soil		1005024-02	1005024-03	COLGATE-Ald	134	129	4	mg/Kg dw
			1005024-11	1005024-12	FTSTEILGC-Spn	35.8	39.5	10	
			1005024-32	1005024-33	HOO-05	11.4	12.6	10	
	Tissue	Salal	1005024-60	1005024-61	COLGATE-Ald	0.26	0.12	**74**	
		Grass	1005024-69	1005024-70	FTSTEILGC-Spn	2.67	0.74	**113**	
		Cheat Grass	1005024-75	1005024-76	HOO-05	0.79	0.81	3	
		Earthworm	1005024-99	1005024-AA	COLGATE-Ald	104	82.8	**23**	
		Earthworm	1005024-AI	No Sample	Fort S GC	18.7	-	**-**	
Arsenic (BRL)	Soil		1024022-02	1024022-03	COLGATE-Ald	155	122	24	
			1024022-16	1024022-18	HOO-05	14.6	12.2	18	
Arsenic (III)	Soil		1024022-02	1024022-03	COLGATE-Ald	1.22	1.1	10	
			1024022-16	1024022-18	HOO-05	0.135J	0.119 J	13	
Copper	Soil		1005024-02	1005024-03	COLGATE-Ald	206	198	4	
			1005024-11	1005024-12	FTSTEILGC-Spn	41.1	38.7	6	
			1005024-32	1005024-33	HOO-05	52.2	62.9	19	
Lead	Soil		1005024-02	1005024-03	Colgate Park	401	343	16	
			1005024-11	1005024-12	FTSTEILGC-Spn	67.7	65.2	4	
			1005024-32	1005024-33	HOO-05	125	132	5	
	Tissue	Salal	1005024-60	1005024-61	COLGATE-Ald	0.81	0.02 U	**-**	
		Grass	1005024-69	1005024-70	FTSTEILGC-Spn	7.48	1.93	**118**	
		Cheat Grass	1005024-75	1005024-76	HOO-05	2.49	2.43	2	
		Earthworm	1005024-99	1005024-AA	COLGATE-Ald	286	231	**21**	
		Earthworm	1005024-AI	No Sample	Fort S GC	67.1	-	**-**	
Solids (MEL)	Soil		1005024-02	1005024-03	COLGATE-Ald	62.4	68.9	10	%
			1005024-11	1005024-12	FTSTEILGC-Spn	79	79.3	0	
			1005024-32	1005024-33	HOO-05	93.4	93.2	0	
Solids (BRL)	Soil		1024022-02	1024022-03	COLGATE-Ald	63.42	74.65	16	
			1024022-16	1024022-18	HOO-05	95.51	94.58	1	
Total Organic Carbon	Soil		1005024-02	1005024-03	COLGATE-Ald	10	7.88	**24**	
			1005024-11	1005024-12	FTSTEILGC-Spn	5.97	5.68	5	
			1005024-32	1005024-33	HOO-05	1.23	1.14	8	

QC: Quality control.
RPD: Relative Percent Difference.
MEL: Manchester Environmental Laboratory.
BRL: Brooks Rand Laboratory.
U: Analyte was not detected at or above the reported result.
J: Analyte was positively identified, but the reported value is an estimate.

Table B- 4. Matrix spike recoveries and duplicates in mg/Kg dw.

Analyte	Matrix	Tissue Type	Sample ID	Sample Site Name	Sample Result	Spike Level	Dup Flag	QC Result	Recovery (%)	RPD (%)
Arsenic (MEL)	Soil		1005024-19	THEMGIL-Ald	39.4	20		51.8	**62**	
							Dup	51.7	**61**	0.2
			1005024-31	HOO-04	32.8	20		51.7	94	
							Dup	53.7	105	4
	Tissue	Salal	1005024-80	KOPA-Har	0.45	40		42	104	
							Dup	42	104	0.02
		Cheat Grass	1005024-97	HOO-11	1.09	40		42.7	104	
							Dup	43	105	0.5
		Earthworm	1005024-AD	TACNAR-Har-UNK	20	40		61	102	
							Dup	61.9	105	2
		Earthworm	1005024-BE	KOPA-Kit	6.18	39.92		45	97	
						39.84	Dup	45	97	0.1
		Raspberry	1005024-BL	BONN-Kit	2.68	40		43.8	103	
							Dup	44.5	105	2
		Darkling Beetle	1008066-14	HOO-10-13	8.23	75.8		88.2	106	
						76.3	Dup	86.8	103	2
Copper	Soil		1005024-19	THEMGIL-Ald	43	20		59.2	81	
							Dup	62	95	5
			1005024-31	HOO-04	16.5	20		34.7	91	
							Dup	35.2	93	1
Lead	Soil		1005024-19	THEMGIL-Ald	74.7	20		87.1	**62**	
							Dup	89.4	**74**	3
			1005024-31	HOO-04	261	20		275	**71**	
							Dup	278	82	0.8
	Tissue	Salal	1005024-80	KOPA-Har	2	40		41	98	
							Dup	42	100	2
		Cheat Grass	1005024-97	HOO-11	8	40		49	103	
							Dup	48	100	2
		Earthworm	1005024-AD	TACNAR-Har-UNK	68	40		127	**146**	
							Dup	143	**185**	12
		Earthworm	1005024-BE	KOPA-Kit	2	39.92		39	93	
						39.84	Dup	39	93	0.6
		Raspberry	1005024-BL	BONN-Kit	6	40		45	98	
							Dup	46	99	1
		Darkling Beetle	1008066-14	HOO-10-13	3	75.8		78	99	
						76.3	Dup	79	99	0.3

QC: Quality control.
Dup: Duplicate.
RPD: Relative Percent Difference.
MEL: Manchester Environmental Laboratory.
BRL: Brooks Rand Laboratory.

Table B- 5. Laboratory control standards in mg/Kg dw.

Analyte	Matrix	Laboratory Control Standard Value	QC Result	Recovery (%)
Arsenic (MEL)	Soil	20	20.7	104
			21.2	106
	Tissue	40	41.6	104
			41.8	104
			42.6	107
			43.6	109
			37.8	94
			41.9	105
Arsenic (BRL)	Soil	0.05000	0.053	106
			0.054	108
			0.056	112
Arsenic (III)	Soil	0.5000	0.519	104
			0.52	104
			0.536	107
			0.56	112
Copper	Soil	20	20.1	100
			20.5	102
Lead	Soil	20	20.1	101
			20.7	103
	Tissue	40	41	102
			42	105
			38	94
			40	99

QC: Quality control.
MEL: Manchester Environmental Laboratory.
BRL: Brooks Rand Laboratory.

Table B- 6. Standard reference material.

Analyte	Matrix	Standard Reference Material Value	QC Result	Recovery (%)	Units
Arsenic (MEL)	Soil	158	153	97	mg/ Kg dw
			159	100	
	Tissue	0.112	0.154	**138**	
			0.16	**143**	
			0.141	**126**	
Arsenic (BRL)	Soil	18.62	18.77	101	
			19.56	105	
Arsenic (III)	Soil	4.752	3.473	**73**	
			4.685	99	
Copper	Soil	129	122	95	
			130	101	
Lead	Soil	172	165	96	
			172	100	
Total Organic Carbon	Soil	2.99	2.71	91	%
			2.76	92	

QC: Quality control.
MEL: Manchester Environmental Laboratory.
BRL: Brooks Rand Laboratory.

Appendix C. Chemistry Data

Tacoma Smelter Plume Footprint Chemistry

Table C- 1. pH, metals, total organic carbon, and percent solids soils data for the Tacoma Smelter Plume footprint locations.

Soil	Field	Analyses by Brooks Rand Laboratory				Analyses by Manchester Environmental Lab				
User_Location_ID	pH	Arsenic (mg/Kg)	Arsenic III (mg/Kg)	Arsenic V (mg/Kg)	Total Solids (%)	Arsenic (mg/Kg)	Copper (mg/Kg)	Lead (mg/Kg)	Total Solids (%)	Total Organic Carbon (%)
BONN-Kit	4.5					110		207	58.2	12
BURTON-Evt	4.7	88.5	0.503	88	55.79	104	102	246	49	18.4
COLGATE-Ald	5.31	155	1.22	154	63.42	134	206	401	62.4	10
COLGATE-Ald*	5.67	122	1.1	121	74.65	129	198	343	68.9	7.88
CORMOR-Evt	6.06					31.2	87	88	70.8	9.55
DOCKTON-Ald	5.52					130		347	69.3	9.53
FTSTEILGC-Spn	6.61	45.4	0.432	45	78.74	35.8	41.1	67.7	79	5.97
FTSTEILGC-Spn*	6.28					39.5	38.7	65.2	79.3	5.68
FTSTEILP-Evt	6.6					28.7		56.1	75.6	5.66
FTSTEILP-Spn	6.62					47.8		108	70.1	14.3
ICF-Evt	5.61					24.9		34.8	72.5	3.74 J
IDLEWILD-Spn	6.16					7.88	20	44.7	71.4	8.45
KCO-Evt	4.98	284	1.93	282	62.2	256	235	564	64.3	10
KOPA-Har	5.5					8.76	13.4	22.9	69.1	6.52
KOPA-Kit	6.79					5.18		8.74	65.6	3.74
LOWJOHN-Har	6.36					18.7		44.3	67.3	6.55
MIMP-Ald-UNK	4.53	166	1.62	164	56.35	171	220	355	56.3	10.2
MIMP-Kit-UNK	4.89	122	0.743	121	59.05	93.6	195	373	59.3	8.58
MORFORD-Har	5.83	8.14	1	7.14	61.79	62.2	129	200	61	16.3
MORN-Evt	5.29					178		272	69.5	10.4
NEILLPT-Kit-UNK	4.81					243		643	39	19.5
NEWTAC-Spn	6.4	113	1.23	112	65.08	103	101	208	62.7	7.84
PTROB-Kit	7.12	110	0.258	110	68.82	7.84	25.5	8.06	69.7	2.81
TACNAR-Har-UNK	7.11	30	0.267	29.7	73.33	27.8	42.8	41	73.4	4.01
THEMGIL-Ald	5.72					39.4 J		74.7	65	13
WING-Ald	6.58					14.7	22.3	55.2	68.4	9.14
WING-Kit	6.47					12.1	32.8	59.8	55.2	6.87

* Field Replicate.
J: Analyte was positively identified, but the reported value is an estimate.

Table C- 2. Metals in soil biota data for the Tacoma Smelter Plume footprint locations in mg/Kg dw.

User_Location_ID	Generic Name	Scientific Name	Arsenic	Lead
BONN-Kit	Earthworm	*Oligochaeta*	14	40
BURTON-Evt	Mixed Invertebrates	*Insecta*	18.4	19.6
COLGATE-Ald	Earthworm	*Oligochaeta*	104	286
COLGATE-Ald*	Earthworm	*Oligochaeta*	82.8	231
CORMOR-Evt	Earthworm	*Oligochaeta*	11.9	21.4
DOCKTON-Ald	Earthworm	*Oligochaeta*	53.1	23.8
FTSTEILGC-Spn	Earthworm	*Oligochaeta*	18.7	67.1
FTSTEILP-Evt	Earthworm	*Oligochaeta*	19.3	33.4
FTSTEILP-Spn	Earthworm	*Oligochaeta*	12.7	62.5
ICF-Evt	Earthworm	*Oligochaeta*	6.28	9.21
IDLEWILD-Spn	Earthworm	*Oligochaeta*	3.12	12.4
KCO-Evt	Earthworm	*Oligochaeta*	102	27.2
KOPA-Kit	Earthworm	*Oligochaeta*	6.18	2.19
LOWJOHN-Har	Earthworm	*Oligochaeta*	20.3	75.1
MIMP-Ald-UNK	Mixed Invertebrates	*Insecta*	13.3	11.6
MIMP-Kit-UNK	Earthworm	*Oligochaeta*	26.1 J	29.7
MORFORD-Har	Earthworm	*Oligochaeta*	19.4	52.1
MORN-Evt	Earthworm	*Oligochaeta*	63.3	59.5
NEILLPT-Kit-UNK	Earthworm	*Oligochaeta*	31.6	13.3
NEWTAC-Spn	Earthworm	*Oligochaeta*	50.6	154
PTROB-Kit	Earthworm	*Oligochaeta*	4.69	3.04
TACNAR-Har-UNK	Earthworm	*Oligochaeta*	20	68.5 J
THEMGIL-Ald	Earthworm	*Oligochaeta*	24.2	29.2
WING-Ald	Earthworm	*Oligochaeta*	8.83	24.4
WING-Kit	Earthworm	*Oligochaeta*	6.37	16.2

* Field replicate.
Analyses by Ecology's Manchester Environmental Laboratory.
J: Analyte was positively identified, but the reported value is an estimate.

Table C- 3. Metals in plants data for the Tacoma Smelter Plume footprint locations in mg/Kg dw.

User_Location_ID	Common Name	Scientific Name	Arsenic	Lead
BONN-Kit	Grass	Poaceae	23.8	32.6
BONN-Kit	Raspberry	*Rubus sp.*	2.68	5.96
BURTON-Evt	Sweet Cicely	*Osmorhiza berteroi*	3.81	7.32
COLGATE-Ald	Salal	*Gaultheria shallon*	0.26	0.81
COLGATE-Ald*	Salal	*Gaultheria shallon*	0.12	0.1 U
CORMOR-Evt	Salal	*Gaultheria shallon*	0.17	0.14
DOCKTON-Ald	Grass	*Poaceae*	7.21	26.1
FTSTEILGC-Spn	Grass	*Poaceae*	2.67	7.48
FTSTEILGC-Spn*	Grass	*Poaceae*	0.74	1.93
FTSTEILP-Evt	English Ivy	*Hedera helix*	0.16	1.03
FTSTEILP-Spn	Grass	*Poaceae*	0.5	1.35
ICF-Evt	Salal	*Gaultheria shallon*	0.75	2.05
ICF-Evt	Sweet Cicely	*Osmorhiza berteroi*	0.55	2.45
IDLEWILD-Spn	English Ivy	*Hedera helix*	0.1	1.17
KCO-Evt	Oregon Grape	*Mahonia sp.*	2.47	17.6
KCO-Evt	Salal	*Gaultheria shallon*	1.17	17.6
KOPA-Har	Salal	*Gaultheria shallon*	0.45	2.34
KOPA-Kit	Nettles	*Urtica dioica*	0.27	0.45
KOPA-Kit	Raspberry	*Rubus sp.*	0.24	0.44
LOWJOHN-Har	Salal	*Gaultheria shallon*	0.1 U	0.14
MIMP-Ald-UNK	Salal	*Gaultheria shallon*	0.1 U	0.18
MIMP-Kit-UNK	Salal	*Gaultheria shallon*	0.32	1.38
MORFORD-Har	Raspberry	*Rubus sp.*	0.77	3.67
MORN-Evt	Evergreen Huckleberry	*Vaccinium ovatum*	0.91	3.28
MORN-Evt	Salal	*Gaultheria shallon*	6.43	31
NEILLPT-Kit-UNK	Nettles	*Urtica dioica*	4.11 J	4.72 J
NEWTAC-Spn	Grass	*Poaceae*	0.74	1.29
PTROB-Kit	Nettles	*Urtica dioica*	1.12	1.17
TACNAR-Har-UNK	Raspberry	*Rubus sp.*	0.45	1.15
THEMGIL-Ald	Salal	*Gaultheria shallon*	0.32	0.2
WING-Ald	English Ivy	*Hedera helix*	0.14	0.72
WING-Ald	Nettles	*Urtica dioica*	0.14	1.08
WING-Kit	Grass	*Poaceae*	0.13	0.56
WING-Kit	Vascular Plant	*Tracheobionta*	1.66	2.44

* Field replicate.
Analyses by Ecology's Manchester Environmental Laboratory.
J: Analyte was positively identified, but the reported value is an estimate.
U: Analyte was not detected at or above the reported result.

Hanford Old Orchards Chemistry

Table C- 4. pH, metals, total organic carbon, and percent solids soils data for the Hanford Old Orchards locations.

Soil	Field	Brooks Rand Laboratory				Manchester Environmental Laboratory				
User_Location _ID	pH	Arsenic (mg/Kg)	Arsenic III (mg/Kg)	Arsenic V (mg/Kg)	Total Solids (%)	Arsenic (mg/Kg)	Copper (mg/Kg)	Lead (mg/Kg)	Solids (%)	Total Organic Carbon (%)
HOO-01	7.68	59.7	0.559 J	59.1	97.47	68.6	13	390	95.8	1.32
HOO-02	7.4	118	0.77	117	98.45	128	14.8	364	95.6	0.87
HOO-03	6.7	78.5	0.493	78	96.97	71.6	13	368	96.4	1.03
HOO-04	6.85	50.5	0.416	50.1	94.89	32.8	16.5	261	94.7	1.64
HOO-05	7.25	14.6	0.135 J	14.5	95.51	11.4	52.2	125	93.4	1.23
HOO-05*	7.19	12.2	0.119 J	12.1	94.58	12.6	62.9	132	93.2	1.14
HOO-06	7.15	83	0.086 J	82.9	95.51	6.35	14.3	35.2	94.7	1.21
HOO-07	6.65					75.2	13.1	332	93.6	0.82
HOO-08						6.54	25.7	50.3	93.5	1.39
HOO-09						6.08	14.7	38.9	92.4	1.29
HOO-10						7.35	19.4	103	89.4	1.5
HOO-11	6.76					19.6	14.5	215	92.5	2.17

* Field replicate.
J: Analyte was positively identified, but the reported value is an estimate.

Table C- 5. Metals in soil biota data for the Hanford Old Orchards locations in mg/Kg dw.

User_Location_ID	Generic Name	Scientific Name	Arsenic	Lead
HOO-01	Darking Beetle	*Tenebrionidae*	8.95	5.58
HOO-03-07	Darking Beetle	*Tenebrionidae*	5.42	8.59
HOO-04-11	Darking Beetle	*Tenebrionidae*	8.23	3.39
HOO-05	Darking Beetle	*Tenebrionidae*	4.48	1.76
HOO-06	Darking Beetle	*Tenebrionidae*	3.19	0.76
HOO-07	Darking Beetle	*Tenebrionidae*	7.63	6.85
HOO-09	Darking Beetle	*Tenebrionidae*	3.2	0.7 U
HOO 01,02,03,06,&09	Darking Beetle	*Tenebrionidae*	1.85	3.67
HOO 01,02,03,06,&09	Mixed Invertebrates	*Insecta*	38.6	47.8
HOO-General	Darking Beetle	*Tenebrionidae*	6.15	17.8
HOO-General	Mixed Invertebrates	*Insecta*	12.7	28.3

Analyses by Ecology's Manchester Environmental Laboratory.
U: Analyte was not detected at or above the reported result.

Table C- 6. Metals in plant data for the Hanford Old Orchards locations in mg/Kg dw.

User_Location_ID	Common Name	Scientific Name	Arsenic	Lead
HOO-01	Cheat Grass	*Bromus tectorum*	3.11	7.72
HOO-02	Cheat Grass	*Bromus tectorum*	27.5	71.2
HOO-03	Cheat Grass	*Bromus tectorum*	3.09	15.2
HOO-04	Cheat Grass	*Bromus tectorum*	1.39	6.95
HOO-05	Cheat Grass	*Bromus tectorum*	0.79	2.49
HOO-05*	Cheat Grass	*Bromus tectorum*	0.81	2.43
HOO-06	Cheat Grass	*Bromus tectorum*	0.87	2.7
HOO-07	Cheat Grass	*Bromus tectorum*	2.99	13.2
HOO-08	Cheat Grass	*Bromus tectorum*	0.68	2.41
HOO-09	Cheat Grass	*Bromus tectorum*	0.64	2.57
HOO-10	Cheat Grass	*Bromus tectorum*	0.82	4.15
HOO-11	Cheat Grass	*Bromus tectorum*	1.09	8.21

* Field replicate.
Analyses by Ecology’s Manchester Environmental Laboratory.

XRF Results

Table C- 7. Average XRF field results for the Tacoma Smelter Plume footprint locations in ppm.

Site Name	XRF Field					
	Arsenic			Lead		
	Mean	St. Dev.	+/-	Mean	St. Dev.	+/-
BONN-Kit	50	14	6	111	24	7
BURTON-Evt	58	15	7	173	112	9
COLGATE-Ald	49	11	11	146	60	13
COLGATE-Ald-Rep	70	20	9	229	118	12
CORMOR-EVT	13	5	16	28	14	19
DOCKTON-Ald	108	79	9	261	64	11
FTSTEILGC-Spn	49	16	7	62	30	8
FTSTEILGC-Spn-Rep						
FTSTEILP-Evt	23	3	4	46	19	5
FTSTEILP-Spn	40	7	4	81	15	6
ICF-Evt						
IDLEWILD-Spn	8	4	18	18	8	26
KCO-Evt	196	32	20	313	13	22
KOPA-Har						
KOPA-Kit	5	0	10	7	0	14
LOWJOHN-Har	14	8	13	48	48	6
MIMP-Ald	98	25	12	207	83	15
MIMP-Kit	32	12	28	245	93	12
MORFORD-Har						
MORN-Evt	55	12	7	223	137	9
NEILLPT-Kit-UNK	62	38	10	198	194	13
NEWTAC-Spn						
PTROB-Kit	5	0	10	7	0	14
TACNAR-Har-UNK	29		8	35		10
THEMGIL-Ald	31	14	4	70	39	5
WING-Ald	5	0	10	27	10	5
WING-Kit	5	0	10	23	11	15

LOD: Limit of Detection.
St. Dev.: Standard Deviation.

Table C- 8. XRF dried soil and re-wetted soil results for the Tacoma Smelter Plume footprint locations in ppm.

Name	Soil Dried at 60°C (XRF Dry)				Re-Wetted Soil (XRF Wet)			
	As	As +/-	Pb	Pb +/-	As	As +/-	Pb	Pb +/-
BONN-Kit	124	12.36	210	14.55	71	5.855	124	7.055
BURTON-Evt	144	14.07	242	16.36	71	6	128	7
COLGATE-Ald	172	16.23	500	20.12	97	10	240	12
COLGATE-Ald-Rep	138	14	409	17	88	8.65	223	10.635
CORMOR-EVT	58	5.785	126	7.205	26	3	58	4
DOCKTON-Ald	168	16	496	20	115	11	288	14
FTSTEILGC-Spn	76	7	138	9	33	4	84	5
FTSTEILGC-Spn-Rep	87	9	138	10	32	3	75	4
FTSTEILP-Evt	48	5	67	6	24	3	40	4
FTSTEILP-Spn	65	6	134	8	34	3	63	4
ICF-Evt	28	5.01	35	6.445	25	3	17	4
IDLEWILD-Spn	13	4	46	5	<LOD	10	30	5
KCO-Evt	385	36.12	1031	44.865	252	24.27	649	29.59
KOPA-Har	14	4	31	5	<LOD	10	<LOD	14
KOPA-Kit	<LOD	10	<LOD	14	<LOD	10	<LOD	14
LOWJOHN-Har	30	4	54	5	19	3	44	4
MIMP-Ald	180	18	413	21	136	13	261	15
MIMP-Kit	128	13	441	16	72	7	296	9
MORFORD-Har	70	7	246	9	51	5	146	6
MORN-Evt	161	15.545	353	18.55	101	10	219	12
NEILLPT-Kit-UNK	392	34	900	41	118	12	324	14
NEWTAC-Spn	198	19	388	22	123	12	208	14
PTROB-Kit	<LOD	24	<LOD	33	<LOD	12	<LOD	16
TACNAR-Har-UNK	40	4	55	5	23	3	38	5
THEMGIL-Ald	53	5	93	6	33	3	66	4
WING-Ald	29	4	73	6	13	3	42	4
WING-Kit	<LOD	12	69	5	<LOD	11	44	5

LOD: Limit of Detection.

Table C- 9. Average XRF field results for the Hanford Old Orchards locations in ppm.

Name	XRF Field					
	Arsenic			Lead		
	Mean	St. Dev.	+/-	Mean	St. Dev.	+/-
HOO-01	87	68	40	538	306	20
HOO-02	103	101	22	520	371	30
HOO-03	69	67	15	470	304	19
HOO-04	68	120	24	570	552	34
HOO-05	15	13	19	136	103	9
HOO-06	8	0	15	25	3	7
HOO-07	35	16	8	378	184	11
HOO-08	7	0	15	44	11	7
HOO-09	8	0	15	32	20	21
HOO-10	9	4	29	80	37	13
HOO-11	14	15	15	158	43	8

LOD: Limit of Detection.
St. Dev.: Standard Deviation.

Table C- 10. XRF dried soil and re-wetted soil results for the Hanford Old Orchards locations in ppm.

Name	Soil Dried at 60°C (XRF Dry)				Re-Wetted Soil (XRF Wet)			
	As	As +/-	Pb	Pb +/-	As	As +/-	Pb	Pb +/-
HOO-01	80	8	458	10	80.36	8.03	363.78	10.43
HOO-02	156	15	432	19	81.89	8.15	298.2	10.41
HOO-03	90	9	500	12	64.46	6.44	359.91	8.51
HOO-04	56	6	326	8	51.22	5.31	247.1	6.94
HOO-05	16	5	141	7	<LOD	11.5	96.19	5.3
HOO-05-Rep	<LOD	14	135	6	<LOD	11.93	101.77	5.44
HOO-06	<LOD	26	45	12	<LOD	10.31	36.74	4.77
HOO-07	110	11	530	14	83.61	7.35	340.48	9.45
HOO-08	<LOD	12	66	6	<LOD	10.08	39.67	4.66
HOO-09	<LOD	11	55	5	<LOD	10.02	35.65	4.64
HOO-10	<LOD	13	118	6	<LOD	11.26	76.97	5.12
HOO-11	30	6	246	8	15.62	4.41	172.11	6.01

LOD: Limit of Detection.

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Appendix D. Bioassay Data and Quality Assurance

The bioassay laboratory report appendices (after Table D-9) are not included in this report but are available upon request.

Table D- 1. *Eisenia foetida* 14-day survival and growth test summary.

Scientific Name of Organism	*Eisenia foetida*		
Test Type/Duration	14-day Earthworm Survival		
Method Used	Norton, D. 1996. Earthworm bioassay protocol for soil toxicity screening. WA Department of Ecology. Publication No. 96-327 www.ecy.wa.gov/biblio/96327.html		
Chain of Custody Followed?	Yes		
Storage Temperature	4°C	Remarks:	None
Batch #	1		
Sample Details	Sample ID	Date Collected	Date Received
	Burton Acres	5/11/2010	5/24/2010
	Colgate Park	5/17/2010	5/24/2010
	Cormorant Park	5/17/2010	5/24/2010
	Wing-Ald	5/14/2010	5/24/2010
	Eagle Ridge	5/20/2010	5/24/2010
	Fort Steilacoom GC	5/19/2010	5/24/2010
	Idlewild	5/17/2010	5/24/2010
	Kopachuck-HAR	5/20/2010	5/24/2010
	King Co. MP-Ald	5/21/2010	5/24/2010
	King Co. MP-Kit	5/21/2010	5/24/2010
	King Co. Owned	5/18/2010	5/24/2010
	Morford's	5/20/2010	5/24/2010
	Pt. Robinson	5/14/2010	5/24/2010
	Tacoma Cem	5/19/2010	5/24/2010
	Winghaven-Kit	5/11/2010	5/24/2010
	HOO-01	5/25/2010	5/27/2010
	HOO-02	5/25/2010	5/27/2010
	HOO-03	5/25/2010	5/27/2010
	HOO-04	5/26/2010	5/27/2010
	HOO-05	5/26/2010	5/27/2010
	HOO-06	5/26/2010	5/27/2010
Medium Used in Test	Soil		
Bioassay Start Date	5/28/2010	Bioassay Start Time:	15:15
Bioassay End Date	6/11/2010	Bioassay End Time:	13:30
Endpoints Measured	Survival and sublethal effects (behavioral/morphological)		
Treatments	None		
Positive Control Toxicant	2-chloroacetamide		
Positive Control Chemical Name	2-chloroacetamide		
Positive Control Chemical Formula	C2-H4-Cl-N-O		
Positive Control 100% Concentration	80 mg/Kg		
Positive Control LC50 or EC50	55.3 mg/Kg	Acceptable Range:	0.60 - 81.5 mg/Kg
Deviations from Protocols	<u>Sample Holding Time Deviation:</u> Burton Acres and Winghaven Kit not expected to have influenced results <u>pH Deviation:</u> Wing-Ald, King Co. MP-Ald, Colgate Park, Cormorant Park, King Co. Owned, Burton Acres, Kopachuck-HAR, Eagle Ridge, Morford's, Fort Steilacoom GC, and Idlewild. Not expected to have influenced results <u>Moisture Content:</u> HOO-01, HOO-02, HOO-03, and HOO-06 were hydrated to between 20 and 25 percent moisture content. Not expected to have influenced results.		
Data Acceptability	Deviation from protocols occurred but are not expected to influence results. Data are considered good and usable.		

Table D- 2. *Eisenia foetida* 14-day survival data.

Test Initiated May 28, 2010			Survival					Sublethal Effects				
Soil Series	Sample ID	Rep	# Alive	% Survival	Mean % Survival	St. Dev.	% of Control	# Sublethal Effects	% Normal	Mean % Normal	St. Dev.	% of Control
	Laboratory Control	1	10	100				1	90.0			
		2	10	100	100	0.0		1	90.0	93.3	5.77	
		3	10	100				0	100			
Alderwood	S10-447 Wing-Ald Reference	1	10	100				0	100			
		2	10	100	100	0.0	100	0	100	100	0.00	107
		3	10	100				0	100			
	S10-452 King Co. MP-Ald	1	10	100				2	80.0			
		2	10	100	100	0.0	100	2	80.0	76.7	5.77	82.1
		3	10	100				3	70.0			
	S10-445 Colgate Park	1	10	100				0	100			
		2	10	100	100	0.0	100	0	100	100	0.00	107
		3	10	100				0	100			
Everett	S10-446 Cormorant Park Reference	1	0	0.0				NA*	NA*			
		2	0	0.0	0.0	0.0	0.0	NA*	NA*	NA*	NA*	NA*
		3	0	0.0				NA*	NA*			
	S10-454 King Co. Owned	1	10	100				2	80.0			
		2	10	100	96.7	5.8	96.7	2	80.0	75.6	7.70	81.0
		3	9	90.0				3	66.7			
	S10-444 Burton Acres	1	10	100				2	80.0			
		2	10	100	100	0.0	100	10	0.0	43.3	40.4	46.4
		3	10	100				5	50.0			
Harstine	S10-451 Kopachuck-HAR Reference	1	10	100				2	80.0			
		2	10	100	100	0.0	100	3	70.0	83.3	15.3	89.3
		3	10	100				0	100			
	S10-448 Eagle Ridge	1	10	100				0	100			
		2	10	100	100	0.0	0.0	6	40.0	80.0	34.6	85.7
		3	10	100				0	100.0			
	S10-455 Morford's	1	8	80.0				1	87.5			
		2	9	90.0	86.7	5.8	86.7	0	100	92.1	6.85	98.7
		3	9	90.0				1	88.9			

NA*: Not available, no survivors so endpoint could not be measured.

Table D- 2 continued. *Eisenia foetida* 14-day survival data.

	Sample ID	Rep	Survival					Sublethal Effects				
			# Alive	% Survival	Mean % Survival	St. Dev.	% of Control	# Sublethal Effects	% Normal	Mean % Normal	St. Dev.	% of Control
Kitsap	S10-458 Winghaven-Kit Reference	1	10	100	100	0.0	100	0	100	96.7	5.77	104
		2	10	100				0	100			
		3	10	100				1	90.0			
	S10-453 King Co. MP-Kit	1	7	70.0	66.7	15.3	67	4	42.9	44.3	5.15	47
		2	5	50.0				3	40.0			
		3	8	80.0				4	50.0			
	S10-456 Pt. Robinson	1	10	100	96.7	5.8	97	2	80.0	93.3	11.5	100
		2	9	90.0				0	100			
		3	10	100				0	100			
Spanaway	S10-450 Idlewild Reference	1	10	100	100	0.0	100	0	100	90.0	17.3	96.4
		2	10	100				0	100			
		3	10	100				3	70.0			
	S10-457 Tacoma Cem	1	10	100	100	0.0	100	0	100	100	0.00	107
		2	10	100				0	100			
		3	10	100				0	100			
	S10-449 Fort Steilacoom GC	1	8	80.0	86.7	11.5	86.7	1	87.5	84.2	8.04	90.2
		2	10	100				1	90.0			
		3	8	80.0				2	75.0			

NA*: Not available, no survivors so endpoint could not be measured.

Table D- 3. *Eisenia foetida* 14-day Survival Data.

Soil Series	Sample ID	Rep	Survival					Sublethal Effects				
			# Alive	% Survival	Mean % Survival	St. Dev.	% of Control	# Sublethal Effects	% Normal	Mean % Normal	St. Dev.	% of Control
Hanford Old Orchards	S10-466 HOO-05 Reference	1	10	100				0	100			
		2	10	100	100	0.0	100	0	100	100	0.00	107
		3	10	100				0	100			
	S10-462 HOO-01	1	10	100				0	100			
		2	10	100	100	0.0	100	0	100	100	0.00	107
		3	10	100				0	100			
	S10-463 HOO-02	1	10	100				0	100			
		2	10	100	100	0.0	100	2	80.0	93.3	11.5	100
		3	10	100				0	100			
	S10-464 HOO-03	1	10	100				0	100			
		2	10	100	100	0.0	100	0	100	100	0.00	107
		3	10	100				0	100			
	S10-465 HOO-04	1	10	100				0	100			
		2	10	100	100	0.0	100	0	100	100	0.00	107
		3	10	100				0	100			
	S10-467 HOO-06	1	10	100				0	100			
		2	10	100	100	0.0	100	0	100	100	0.00	107
		3	10	100				0	100			

Table D- 4. *Lactuca sativa* 14-day survival and growth test summary.

Scientific Name of Organism	*Lactuca sativa*			
Test Type/Duration	14-day Lettuce Seedling Survival and Growth			
Method Used	Norton, D. 1996. Early Seedling Growth Protocol for Soil Toxicity Screening. WA Department of Ecology. Publication No. 96-324, www.ecy.wa.gov/biblio/96324.html			
Chain of Custody Followed?	Yes			
Storage Temperature	4°C		Remarks:	None
Batch #	1			
Sample Details	Sample ID		Date Collected	Date Received
	Burton Acres		5/11/2010	5/24/2010
	Colgate Park		5/17/2010	5/24/2010
	Cormorant Park		5/17/2010	5/24/2010
	Wing-Ald		5/14/2010	5/24/2010
	Eagle Ridge		5/20/2010	5/24/2010
	Fort Steilacoom GC		5/19/2010	5/24/2010
	Idlewild		5/17/2010	5/24/2010
	Kopachuck-HAR		5/20/2010	5/24/2010
	King Co. MP-Ald		5/21/2010	5/24/2010
	King Co. MP-Kit		5/21/2010	5/24/2010
	King Co. Owned		5/18/2010	5/24/2010
	Morford's		5/20/2010	5/24/2010
	Pt. Robinson		5/14/2010	5/24/2010
	Tacoma Cem		5/19/2010	5/24/2010
	Winghaven-Kit		5/11/2010	5/24/2010
	HOO-01		5/25/2010	5/27/2010
	HOO-02		5/25/2010	5/27/2010
	HOO-03		5/25/2010	5/27/2010
	HOO-04		5/26/2010	5/27/2010
	HOO-05		5/26/2010	5/27/2010
	HOO-06		5/26/2010	5/27/2010
Medium Used in Test	Soil			
Bioassay Start Date	5/27/2010		Bioassay Start Time:	14:15
Bioassay End Date	6/10/2010		Bioassay End Time:	13:50
Endpoints Measured	Survival and biomass			
Treatments	None			
Positive Control Chemical Name	Boric Acid		Toxicant:	Boric Acid
Positive Control Chemical Formula	BH3O3		100% Concentration:	460 mg/Kg
Positive Control LC50 or EC50	Survival:	183 mg/Kg	Acceptable Range:	121 - 398 mg/Kg
	Biomass:	135 mg/Kg	Acceptable Range:	97.0 - 202 mg/Kg
Deviations from Protocols	<u>Sample Holding Time Deviation:</u> Burton Acres and Winghaven Kit not expected to have influenced results. <u>pH Deviation:</u> Wing-Ald, Colgate Park, King Co. Owned, Burton Acres, Kopachuck-HAR, Morford's, Pt. Robinson, and Fort Steilacoom GC. Not expected to have influenced results. <u>Oven Temperature Deviation:</u> All TSP samples, HOO-02, HOO-03, and HOO-06. Not expected to have influenced results. <u>Number of organisms per replicate deviation:</u> Fort Steilacoom GC, Morford's, Winghaven-Kit. Not expected to have influenced results, with the exception of Burton Acres.			
Data Acceptability	Deviations from protocol occurred but are not expected to influence results. Data are considered good and usable.			

Table D- 5. *Lactuca sativa* 14-day survival and growth data.

			Survival					Growth						
Soil Series	Sample ID	Rep	# Alive	% Survival	Mean % Survival	St. Dev.	% of Control	Tare Weight (mg)	Total Weight (mg)	Total Seedling Weight (mg)	Growth per Seedling (mg)	Mean Growth per Org (mg)	St. Dev.	% of Control
	Laboratory Control	1	11	91.7				1237.05	1269.82	32.77	2.98			
		2	11	91.7				1249.44	1260.88	11.44	1.04			
		3	12	100	93.3	3.7		1244.60	1263.53	18.93	1.58	1.93	0.76	
		4	11	91.7				1264.85	1282.83	17.98	1.63			
		5	11	91.7				1245.79	1272.19	26.40	2.40			
Aderwood	S10-447 Wing-Ald	1	10	83				1232.05	1248.76	16.71	1.67			
		2	12	100				1248.47	1282.11	33.64	2.80			
		3	9	75.0	88.3	11.2	95	1242.52	1264.37	21.85	2.43	2.50	0.60	130
		4	10	83.3				1278.29	1311.21	32.92	3.29			
		5	12	100				1244.10	1271.68	27.58	2.30			
	S10-452 King Co. MP-Ald	1	12	100				1236.22	1258.40	22.18	1.85			
		2	12	100				1306.44	1331.43	24.99	2.08			
		3	12	100	96.7	7.5	104	1249.65	1273.47	23.82	1.98	1.94	0.43	101
		4	10	83.3				1267.73	1292.69	24.96	2.50			
		5	12	100				1286.20	1301.89	15.69	1.31			
	S10-445 Colgate Park	1	12	100				1249.75	1264.03	14.28	1.19			
		2	12	100				1228.85	1245.15	16.30	1.36			
		3	12	100	95.0	11.2	102	1227.75	1248.19	20.44	1.70	1.34	0.21	69.5
		4	9	75.0				1253.87	1264.66	10.79	1.20			
		5	13	100				1240.54	1256.65	16.11	1.24			
Everett	S10-446 Cormorant Park	1	11	91.7				1223.03	1258.18	35.15	3.20			
		2	9	75.0				1273.35	1297.30	23.95	2.66			
		3	12	100	93.3	10.9	100.0	1275.95	1306.51	30.56	2.55	2.80	0.25	145
		4	12	100				1271.53	1304.40	32.87	2.74			
		5	12	100				1234.14	1268.53	34.39	2.87			

Table D- 5 continued. *Lactuca sativa* 14-day survival and growth data.

			Survival					Growth						
Soil Series	Sample ID	Rep	# Alive	% Survival	Mean % Survival	St. Dev.	% of Control	Tare Weight (mg)	Total Weight (mg)	Total Seedling Weight (mg)	Growth per Seedling (mg)	Mean Growth per Org (mg)	St. Dev.	% of Control
Everett	S10-454 King Co. Owned	1	12	100	91.7	8.3	98	1292.02	1308.20	16.18	1.35	1.58	0.25	82.2
		2	10	83.3				1261.09	1279.57	18.48	1.85			
		3	12	100				1251.52	1273.69	22.17	1.85			
		4	11	91.7				1227.12	1243.71	16.59	1.51			
		5	10	83.3				1252.74	1266.43	13.69	1.37			
	S10-444 Burton Acres	1	12	100	96.7	7.5	103.6	1309.76	1321.95	12.19	1.02	1.17	0.13	60.7
		2	10	83.3				1269.48	1282.56	13.08	1.31			
		3	12	100				1283.33	1296.12	12.79	1.07			
		4	12	100				1291.84	1305.87	14.03	1.17			
		5	12	100				1233.84	1249.32	15.48	1.29			
Harstine	S10-451 Kopachuck-HAR	1	11	91.7	96.7	4.6	103.6	1252.40	1273.57	21.17	1.92	1.86	0.35	96.4
		2	12	100				1220.80	1241.10	20.30	1.69			
		3	11	91.7				1252.93	1278.65	25.72	2.34			
		4	12	100				1258.74	1282.08	23.34	1.94			
		5	12	100				1229.90	1246.51	16.61	1.38			
	S10-448 Eagle Ridge	1	12	100	96.7	4.6	104	1236.35	1266.91	30.56	2.55	2.15	0.28	112
		2	11	91.7				1243.98	1265.27	21.29	1.94			
		3	11	91.7				1275.17	1295.65	20.48	1.86			
		4	12	100				1268.53	1295.91	27.38	2.28			
		5	12	100				1253.31	1278.75	25.44	2.12			
	S10-455 Morford's	1	12	100	96.7	7.5	104	1217.85	1244.51	26.66	2.22	1.98	0.32	103
		2	10	83.3				1271.50	1294.50	23.00	2.30			
		3	13	100				1277.74	1305.15	27.41	2.11			
		4	13	100				1232.35	1254.51	22.16	1.70			
		5	12	100				1234.69	1253.55	18.86	1.57			

Table D- 5 continued. *Lactuca sativa* 14-day survival and growth data.

Soil Series	Sample ID	Rep	Survival: # Alive	% Survival	Mean % Survival	St. Dev.	% of Control	Growth: Tare Weight (mg)	Total Weight (mg)	Total Seedling Weight (mg)	Growth per Seedling (mg)	Mean Growth per Org (mg)	St. Dev.	% of Control
Kitsap	S10-458 Winghaven-Kit	1	13	100	88.3	12.6	94.6	1297.96	1330.13	32.17	2.47	2.74	0.58	142
		2	11	91.7				1296.56	1338.10	41.54	3.78			
		3	8	66.7				1258.88	1279.00	20.12	2.51			
		4	11	91.7				1247.40	1274.03	26.63	2.42			
		5	11	91.7				1248.44	1275.99	27.55	2.50			
	S10-453 King Co. MP-Kit	1	11	91.7	93.3	7.0	100.0	1255.20	1285.45	30.25	2.75	2.23	0.30	116
		2	10	83.3				1283.20	1305.10	21.90	2.19			
		3	11	91.7				1278.60	1302.07	23.47	2.13			
		4	12	100				1297.16	1321.27	24.11	2.01			
		5	12	100				1286.41	1311.17	24.76	2.06			
	S10-456 Pt. Robinson	1	11	91.7	91.7	10.2	98.2	1252.86	1283.08	30.22	2.75	3.10	0.67	161
		2	12	100				1255.17	1290.79	35.62	2.97			
		3	11	91.7				1282.90	1326.27	43.37	3.94			
		4	9	75.0				1262.93	1295.20	32.27	3.59			
		5	12	100				1244.40	1271.33	26.93	2.24			
Spanaway	S10-450 Idlewild	1	8	66.7	88.3	13.9	94.6	1212.48	1230.87	18.39	2.30	2.57	0.46	133
		2	12	100				1255.70	1286.51	30.81	2.57			
		3	12	100				1268.76	1307.14	38.38	3.20			
		4	10	83.3				1266.18	1294.15	27.97	2.80			
		5	11	91.7				1245.87	1267.68	21.81	1.98			
	S10-457 Tacoma Cem	1	11	91.7	91.7	5.9	98.2	1308.51	1327.57	19.06	1.73	1.58	0.33	82.0
		2	12	100				1286.10	1301.42	15.32	1.28			
		3	10	83.3				1270.12	1286.71	16.59	1.66			
		4	11	91.7				1228.29	1250.37	22.08	2.01			
		5	11	91.7				1297.17	1310.56	13.39	1.22			
	S10-449 Fort Steilacoom GC	1	7	58	78.3	19.2	83.9	1236.82	1240.02	3.20	0.46	0.59	0.11	30.8
		2	11	91.7				1247.37	1254.59	7.22	0.66			
		3	10	83.3				1220.97	1225.99	5.02	0.50			
		4	15	100				1258.69	1268.72	10.03	0.67			
		5	7	58.3				1245.19	1249.98	4.79	0.68			

Table D- 5 continued. *Lactuca sativa* 14-day survival and growth data.

Soil Series	Sample ID	Rep	Survival: # Alive	Survival: % Survival	Survival: Mean % Survival	Survival: St. Dev.	Survival: % of Control	Growth: Tare Weight (mg)	Growth: Total Weight (mg)	Growth: Total Seedling Weight (mg)	Growth: Growth per Seedling (mg)	Growth: Mean Growth per Org (mg)	Growth: St. Dev.	Growth: % of Control
Hanford Old Orchards	S10-466 HOO-05	1	11	91.7	90.0	9.1	96.4	1281.56	1301.39	19.83	1.80	2.12	0.34	110.2
		2	9	75.0				1246.74	1268.57	21.83	2.43			
		3	12	100				1267.35	1297.81	30.46	2.54			
		4	11	91.7				1278.71	1298.79	20.08	1.83			
		5	11	91.7				1241.50	1263.73	22.23	2.02			
	S10-462 HOO-01	1	6	50.0	90.0	22.4	96.4	1259.73	1272.95	13.22	2.20	1.72	0.28	89.4
		2	12	100				1285.15	1305.41	20.26	1.69			
		3	12	100				1225.80	1244.29	18.49	1.54			
		4	12	100				1242.68	1262.66	19.98	1.67			
		5	12	100				1290.39	1308.58	18.19	1.52			
	S10-463 HOO-02	1	6	50.0	86.7	21.7	92.9	1282.15	1290.05	7.90	1.32	1.86	0.37	97
		2	10	83.3				1282.16	1303.93	21.77	2.18			
		3	13	100				1289.28	1317.90	28.62	2.20			
		4	12	100				1242.94	1263.26	20.32	1.69			
		5	13	100				1279.96	1304.77	24.81	1.91			
	S10-464 HOO-03	1	11	91.7	91.7	10.2	98.2	1299.25	1320.96	21.71	1.97	2.40	0.45	125
		2	13	100				1253.64	1278.81	25.17	1.94			
		3	11	91.7				1243.92	1269.95	26.03	2.37			
		4	12	100				1267.13	1300.87	33.74	2.81			
		5	9	75.0				1248.23	1274.38	26.15	2.91			
	S10-465 HOO-04	1	10	83.3	86.7	12.6	92.9	1226.63	1248.06	21.43	2.14	2.60	0.50	135
		2	12	100				1213.91	1240.25	26.34	2.19			
		3	11	91.7				1260.70	1297.53	36.83	3.35			
		4	8	66.7				1267.35	1289.85	22.50	2.81			
		5	11	91.7				1281.59	1309.36	27.77	2.52			
	S10-467 HOO-06	1	9	75.0	93.3	10.9	100	1237.34	1258.67	21.33	2.37	2.53	0.63	131
		2	13	100				1230.59	1268.59	38.00	2.92			
		3	13	100				1279.61	1301.40	21.79	1.68			
		4	11	91.7				1233.95	1270.46	36.51	3.32			
		5	12	100				1235.08	1263.27	28.19	2.35			

Table D- 6. *Eisenia foetida* 14-day survival and growth repeated test summary.

Scientific Name of Organism:	*Eisenia foetida*		
Test Type/Duration:	14-day Earthworm Survival		
Method Used:	*Norton, D. 1996. Earthworm bioassay protocol for soil Toxicity screening.* *WA Department of Ecology. Publication No. 96-327* *www.ecy.wa.gov/biblio/96327.html*		
Chain of Custody Followed?	Yes		
Storage Temperature	4°C	Remarks:	None
Batch #	3		
Sample Details:	Sample ID	Date Collected	Date Received
	Cormorant Park	5/17/2010	5/24/2010
	Kopachuck-HAR	5/20/2010	5/24/2010
	King Co. Owned	5/18/2010	5/24/2010
	Morford's	5/20/2010	5/24/2010
Medium Used in Test:	Soil		
Bioassay Start Date:	8/9/2010	Bioassay Start Time:	16:13
Bioassay End Date:	8/23/2010	Bioassay End Time:	17:00
Endpoints Measured:	Survival and sublethal effects (behavioral/morphological)		
Treatments:	None		
Positive Control Toxicant:	2-chloroacetamide		
Positive Control Chemical Name:	2-chloroacetamide		
Positive Control Chemical Formula:	C2-H4-Cl-N-O		
Positve Control 100% Concentration	80 mg/Kg		
Positve Control LC50 or EC50	54.3 mg/Kg	Acceptable Range:	4.04-81.6 mg/Kg
Deviations from Protocols:	Sample Holding Time Deviation:		
	All samples past holding time		
	pH Deviation:		
	Kopachuck-HAR, King Co. Owned, and Morford's. Not expected to have influenced results.		
Data Acceptability	Deviation from protocols occurred but are not expected to influence results. Data are considered good and usable.		

Table D- 7. *Lactuca sativa* 14-day survival and growth repeated test summary.

Scientific Name of Organism:	*Lactuca sativa*			
Test Type/Duration:	14-day Lettuce Seedling Survival and Growth			
Method Used:	*Norton, D. 1996. Early Seedling Growth Protocol for Soil Toxicity Screening. WA Department of Ecology. Publication No. 96-324 www.ecy.wa.gov/biblio/96324.html*			
Chain of Custody Followed?	Yes			
Storage Temperature	4°C	Remarks:	None	
Batch #	3			
Sample Details:	Sample ID	Date Collected	Date Received	
	Cormorant Park	5/17/2010	5/24/2010	
	Burton Acres	5/11/2010	5/24/2010	
Medium Used in Test:	Soil			
Bioassay Start Date:	8/9/2010	Bioassay Start Time:	15:10	
Bioassay End Date:	8/23/2010	Bioassay End Time:	16:10	
Endpoints Measured:	Survival and biomass			
Treatments:	None			
Positive Control Toxicant:	Boric Acid			
Positive Control Chemical Name:	Boric Acid			
Positive Control Chemical Formula:	BH3O3			
Positve Control 100% Concentration	640 mg/Kg			
Positve Control LC50 or EC50	Survival:	179 mg/Kg	Acceptable Range:	113-385 mg/Kg
	Biomass:	126 mg/Kg	Acceptable Range:	98.0-198 mg/Kg
Deviations from Protocols:	Sample Holding Time Deviation:			
	All samples past holding time			
	pH Deviation:			
	All samples, not expected to have influenced results.			
Data Acceptability	Deviation from protocols occurred but are not expected to influence results. Data are considered good and usable.			

Table D- 8. Repeated *Eisenia foetida* 14-day survival.

Soil Series	Sample ID	Rep	Survival: # Alive	Survival: % Survival	Survival: Mean % Survival	Survival: St. Dev.	Survival: % of Control	Sublethal Effects: # Sublethal Effects	Sublethal Effects: % Normal	Sublethal Effects: Mean % Normal	Sublethal Effects: St. Dev.	Sublethal Effects: % of Control
	Laboratory Control	1	10	100.0	96.7	5.8		0	100	100.0	0.00	
		2	9	90.0				0	100			
		3	10	100.0				0	100			
Everett	S10-446 Cormorant Park Reference	1	5	50.0	50.0	10.0	51.7	1	80.0	87.8	10.72	87.8
		2	6	60.0				1	83.3			
		3	4	40.0				0	100			
	S10-454 King Co. Owned	1	11	100.0	93.3	5.8	96.6	0	100	100	0.00	100
		2	9	90.0				0	100			
		3	9	90.0				0	100			
Harstine	S10-451 Kopachuck-HAR Reference	1	10	100.0	100	0.0	103.4	0	100	100.0	0.0	100.0
		2	10	100.0				0	100			
		3	10	100.0				0	100			
	S10-455 Morford's	1	9	90.0	96.7	5.8	100.0	0	100	100.0	0.00	100.0
		2	10	100.0				0	100			
		3	10	100.0				0	100			

Table D- 9. Repeated *Lactuca sativa* 14-day survival and growth.

Soil Series	Sample ID	Rep	Survival: # Alive	% Survival	Mean % Survival	St. Dev.	% of Control	Growth: Tare Weight (mg)	Total Weight (mg)	Total Seedling Weight (mg)	Growth per Seedling (mg)	Mean Growth per Org (mg)	St. Dev.	% of Control
	Laboratory Control	1	11	91.7				1300.02	1313.80	13.78	1.25			
		2	11	91.7				1249.12	1266.87	17.75	1.61			
		3	11	91.7	91.7	5.9		1247.85	1263.02	15.17	1.38	1.55	0.28	
		4	12	100.0				1288.43	1306.78	18.35	1.53			
		5	10	83.3				1280.39	1300.18	19.79	1.98			
Everett	S10-446 Cormorant Park	1	11	91.7				1296.35	1311.25	14.90	1.35			
		2	11	91.7				1285.71	1299.06	13.35	1.21			
		3	10	83.3	90.0	3.7	98.2	1289.65	1303.58	13.93	1.39	1.33	0.11	85.5
		4	11	91.7				1265.53	1281.60	16.07	1.46			
		5	11	91.7				1257.50	1270.75	13.25	1.20			
	S10-444 Burton Acres	1	11	91.7				1286.84	1311.82	24.98	2.27			
		2	12	100.0				1252.62	1279.32	26.70	2.23			
		3	12	100.0	95.0	4.6	103.6	1280.21	1305.12	24.91	2.08	2.33	0.22	150.4
		4	11	91.7				1294.98	1324.11	29.13	2.65			
		5	11	91.7				1299.65	1326.48	26.83	2.44			

Nautilus Environmental, LLC

Earthworm Soil Toxicity Evaluation

Tacoma Smelter Plume and Hanford Old Orchards Study, WA

Final Report

Date: July 28, 2010

Submitted to:

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SIGNATURE PAGE

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Washington Laboratory Manager

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Project Manager

This report has been prepared based on data and/or samples provided by our client and the results of this study are for their sole benefit. Any reliance on the data by a third party is at the sole and exclusive risk of that party.

Executive Summary

This report contains a biological evaluation of the condition of soils at different sites affected by the Tacoma Smelter plume in Pierce and King Counties, as well as sites affected by arsenic and lead containing pesticides in the Old Orchards area at the Department of Energy Hanford site. The evaluation was conducted using the 14-day lettuce survival and biomass, and 14-day earthworm survival bioassays. Twenty-one soil samples were analyzed. This report contains the results for the earthworm bioassay, which was conducted on May 28, 2010 using the species *Eisenia foetida.* Performance in the test samples was compared to a negative control. In addition, performance of soil samples within a soil series, samples having the same soil characteristics, was compared to a reference sample within that series. Testing was initiated within 14 days of soil collection for all but three sites, meeting holding time requirements for 18 of the 21 sites.

The earthworm survival bioassay met all performance criteria for the negative and positive controls. Cormorant Park, King Co. MP-Kit, and Morford's were significantly different from the negative control for the survival endpoint.

1.0 INTRODUCTION

Laboratory toxicity testing was conducted on soil samples collected from 15 sites within the Tacoma Smelter Plume footprint, and six sites in the Hanford Old Orchards area within the Department of Energy Hanford site, Washington, following site-specific terrestrial ecological evaluation (TEE) methodology under the Model Toxics Control Act (MTCA). The specific contaminants of concern for the bioassay component of the TEE were arsenic and lead. Soil samples were collected from areas within the Tacoma Smelter Plume footprint, and Hanford Old Orchards, representing different soil types and a range of concentrations of arsenic and lead. A toxicity test was conducted using the earthworm *Eisenia foetida*, of the family lumbricidae. Testing was initiated on May 28, 2010 at the Washington Laboratory of Nautilus Environmental, located in Tacoma, Washington. Test procedures followed methods published by Washington State Department of Ecology for the Toxics Cleanup Program.

2.0 METHODS

2.1 Sample Receipt and Manipulation

Twenty-one soil samples were collected by Department of Ecology personnel between May 11th and 26th, 2010 into 3.5 L HPDE containers. Samples were received by Nautilus Environmental on May 24th and May 27th, 2010. All samples were transported in coolers containing ice. Individual samples were in labeled plastic jars. Upon receipt in the laboratory, the coolers were opened and the contents inspected and compared with documentation provided on the chain-of-custody forms (COC). Sample temperatures were measured upon receipt and recorded on both the COC and in a bound logbook maintained in the laboratory. Samples were held in the dark at 4 ± 2°C until testing.

Large pieces of wood, debris and rocks were removed from soils prior to testing. No sieving was performed on the samples. Analysis of soil pH, conductivity, and moisture content were performed upon sample receipt.

Sample ID's with corresponding dates of collection, receipt, holding time expiration (sampling date plus fourteen days), and test initiation are provided in Table 1.

Table 1. Sample collection, receipt, expiration, and test initiation dates.

Client ID	Nautilus Log-In Number	Date Collected	Date Received	Date Holding Time Expired	Test Initiation Date
Burton Acres	S10-444				
Winghaven-Kit	S10-458	May 11, 2010	May 24, 2010	May 25, 2010 [1]	May 27, 2010
Wing-Ald	S10-447				
Pt. Robinson	S10-456	May 14, 2010	May 24, 2010	May 28, 2010	May 27, 2010
Colgate Park	S10-445				
Cormorant Park	S10-446	May 17, 2010	May 24, 2010	May 31, 2010	May 27, 2010
Idlewild	S10-450				
King Co. Owned	S10-454	May 18, 2010	May 24, 2010	June 1, 2010	May 27, 2010
Fort Steilacoom GC	S10-449	May 19, 2010	May 24, 2010	June 2, 2010	May 27, 2010
Tacoma Cem	S10-457				
Eagle Ridge	S10-448				
Kopachuck-HAR	S10-451	May 20, 2010	May 24, 2010	June 3, 2010	May 27, 2010
Morford's	S10-455				
King Co. MP-Ald	S10-452	May 21, 2010	May 24, 2010	June 4, 2010	May 27, 2010
King Co. MP-Kit	S10-453				
HOO-01	S10-462				
HOO-02	S10-463	May 25, 2010	May 27, 2010	June 8, 2010	May 27, 2010
HOO-03	S10-464				
HOO-04	S10-465				
HOO-05	S10-466	May 26, 2010	May 27, 2010	June 9, 2010	May 27, 2010
HOO-06	S10-467				

[1]Deviation from holding time requirements in protocol. Not expected to influence results of the test (see QA/QC section 4.0)

2.2 Earthworm survival test methods

An earthworm survival test was conducted on samples received May 24th and 27th, 2010 using the red wiggler worm, *Eisenia foetida*. The organisms were obtained from Aquatic Research Organisms, NH. Nautilus Environmental received the organisms at the laboratory on May 26, 2010 in good condition. Tests were initiated on May 28th, 2010 according to procedures presented by WADOE (1996) and ASTM (1994). Detailed test methods are documented in the Nautilus Environmental Standard Operating Procedure T-1560-14 (Appendix F). Test procedures are summarized in Table 2.

Table 2. Summary of testing conditions for the earthworm survival test.

Test start date	May 28, 2010
Test end date	June 11, 2010
Test organism	*Eisenia foetida*
Test organism source	Aquatic Research Organisms, Hampton, NH
Test organism age	>90 days
Test duration	14 days
Test chamber	1-L glass jar
Test soil/replicate	200 g dry weight
Water source for hydration	De-ionized water
Control soil	70% sand, 20% kaolin clay, 10% peat moss, 0.45% $CaCO_3$
Number of organisms/replicate	10
Number of replicates/sample	3
Test temperature	22± 2°C
Illumination	Continuous lighting
Test acceptance criterion	≥90% mean survival of control organisms
Positive control reference toxicant	2-chloroacetamide

Twenty-four hours prior to testing, 25 g of soil was removed from each sample, the initial weight of soil and vessel was obtained, and samples were then placed in a Thelco 28 oven set to between 103 and 105°C to dry for 24 hours. After 24 hours, samples were removed from the oven, allowed to cool, and final weights were obtained to determine the moisture content of each sample. Samples with a moisture content of less than 40 percent were then hydrated to match control levels or control friability, as required. Moisture content upon receipt of the samples, as well as hydration requirements and amount of water added to samples is contained in Table 3.

On test initiation, pH and conductivity measurements were conducted on a slurry of de-ionized (DI) water and soil in a 1:1 ratio (i.e., 25 mL DI water:25 g soil). Soil slurry pH was measured after allowing soil/water mixture to stir for 5 minutes. Once the measurement was taken, the slurry was allowed to settle for 30 minutes, after which the pH of the supernatant liquid was measured. Conductivity and pH measurements were conducted utilizing an Orion 130A and Orion 320 meter, respectively.

Sample soils were hydrated with DI water where necessary, and distributed into 1-L labeled glass jars prior to test initiation. Three replicates and a surrogate were used for each sample, each containing 200 g of soil. Moisture content was also determined at test initiation. Sample distribution took place according to a randomization sheet created in Excel. Organisms, greater than 90 days old, were added following sample distribution, once samples were confirmed to be within acceptable temperature range. Jars were placed in an environmental chamber at 22 ± 2°C under continuous light conditions.

Test temperatures were measured daily from surrogate test chambers. Test chambers were misted daily with DI water in order to maintain proper moisture levels.

Table 3. Pre-test hydration used for visual match of control friability.

Client ID	Nautilus Log-In	Initial Moisture Content (%)	Hydration Needed (%)	Amount of Water Added to Sample (ml)
Burton Acres	S10-444	107	0	0
Colgate Park	S10-445	58.5	0	0
Cormorant Park	S10-446	37.4	0	0
Wing-Ald	S10-447	42.8	0	0
Eagle Ridge	S10-448	36.2	0	0
Fort Steilacoom GC	S10-449	40.4	0	0
Idlewild	S10-450	39.1	0	0
Kopachuck-HAR	S10-451	47.2	0	0
King Co. MP-Ald	S10-452	74.7	0	0
King Co. MP-Kit	S10-453	69.0	0	0
King Co. Owned	S10-454	56.2	0	0
Morford’s	S10-455	62.9	0	0
Pt. Robinson	S10-456	40.6	0	0
Tacoma CEM	S10-457	53.9	0	0
Winghaven-Kit	S10-458	82.0	0	0
HOO-01	S10-462	3.01	22.0[1]	176[1]
HOO-02	S10-463	1.75	18.3[1]	146[1]
HOO-03	S10-464	9.46	15.5[1]	124[1]
HOO-04	S10-465	5.44	30.0	236
HOO-05	S10-466	8.27	26.7	214
HOO-06	S10-467	5.98	14.0[1]	112[1]

[1] Samples pre-hydrated to below 35% moisture (see section 4.0)

The tests were terminated on day 14, June 11, 2010. At test termination, prior to counting, observations were made of each test chamber, including dead organisms on the surface or any behavior abnormalities. To count test organisms, sample replicates were transferred to a flat surface lined with moistened paper towels, and animals were counted, and any behavior (e.g., lack of burrowing, coiling, “balling” together), or morphological changes (e.g., contraction, rigidity, ulceration of the integument, segmental constriction, segmental loss) were noted.

Upon client request, animals from each site were placed in glass jars and saved at 4°C for further studies. The surrogate chamber was used to determine final moisture content, and final pH and conductivity measurements.

The endpoints calculated were the earthworm survival, and occurrence of abnormal behavior and morphological changes. Abnormal behavior and morphological changes were categorized as sublethal effects. For statistical comparisons the sublethal effects were normalized compared to survival. The test acceptance criterion for the negative control was earthworm survival of ≥ 90 percent. Statistics were run using Biostat software on all sites where survival or sublethal effects (normality) were less than control or their respective reference site, using a level of significance of 0.05.

A reference toxicant test (positive control) was conducted in conjunction with the earthworm survival tests using 2-chloroacetamide. Test organisms were exposed to control, 10, 20, 40, and 80 mg/kg 2-chloroacetamide for the same duration as the concurrent soil tests, and the results of this test were compared with historical data for the species to determine whether the sensitivity of the organisms was appropriate.

3.0 RESULTS

Results of toxicity tests conducted using *E. foetida* starting May 28, 2010 are summarized in Tables 4 and 5. Detailed results of the soil toxicity tests and statistical analyses are provided in Appendix A. Copies of the laboratory bench sheets, QA/QC summary, reference toxicant test results, SOP, and chain-of-custody forms are in Appendices B, C, D, E and F.

3.1 Toxicity results

Mean survival was 100 percent for the artificial soil control as well as for Wing-Ald, Kopachuck-HAR, Winghaven-Kit, Idlewild, and HOO-05 reference sites. The reference site Cormorant Park, had 100 percent mortality. The mean survival in the test soils ranged from 66.7 to 100 percent. Cormorant Park, King Co. MP-Kit, and Morford's exhibited significant toxic effects when compared to the negative control.

Sublethal effects occurred in 6.67 percent of the surviving organisms for the artificial soil control, and 0, 16.7, 3.3, 10 and 0 percent for those reference sites with surviving organisms (Wing-Ald, Kopachuck-HAR, Winghaven-Kit, Idlewild, and HOO-05, respectively). Burton

Acres, King Co. MP-Ald, King Co. MP-Kit, and King Co. Owned were significantly different from the negative control. King Co. MP- Ald, and King Co. MP-Kit were significantly different than their respective reference sites. There are no sublethal criteria defined in the test protocol. However, these comparisons provide additional information about effects on the test organisms.

Table 4. Results (means ± standard deviations) for *E. foetida* survival

Soil Series	Site ID/Nautilus Log-In Number	Survival (%)	Mean Survival (%)	% of Control	Significant Decrease from Control? ($p<0.05$)	Significant Decrease from Reference? ($p<0.05$)
	Negative Control	100 100 100	100 ± 0.0	--	--	--
Alderwood	Wing-Ald S10-447 (Reference)	100 100 100	100 ± 0.0	100	No	--
Alderwood	King Co. MP-Ald S10-452	100 100 100	100 ± 0.0	100	No	No
Alderwood	Colgate Park S10-445	100 100 100	100 ± 0.0	100	No	No
Everett	Cormorant Park S10-446 (Reference)	0.0 0.0 0.0	0.0 ± 0.0	0.0	**Yes**	--
Everett	King Co. Owned S10-454	100 100 90.0	96.7 ± 5.8	96.7	No	No
Everett	Burton Acres S10-444	100 100 100	100 ± 0.0	100	No	No
Harstine	Kopachuck-HAR S10-451 (Reference)	100 100 100	100 ± 0.0	100	No	--
Harstine	Eagle Ridge S10-448	100 100 100	100 ± 0.0	100	No	No
Harstine	Morford's S10-455	80.0 90.0 90.0	86.7 ± 5.8	86.7	**Yes**	**Yes**
Kitsap	Winghaven-Kit S10-458 (Reference)	100 100 100	100 ± 0.0	100	No	--
Kitsap	King Co. MP-Kit S10-453	70.0 50.0 80.0	66.7 ± 15.3	66.7	**Yes**	**Yes**
Kitsap	Pt. Robinson S10-456	100 90.0 100	96.7 ± 5.8	96.7	No	No

Table 4 cont. Results (means ± standard deviations) for *E. foetida* survival

Soil Series	Site ID/Nautilus Log-In Number	Survival (%)	Mean Survival (%)	% of Control	Significant Decrease from Control? (p<0.05)	Significant Decrease from Reference? (p<0.05)
Spanaway	Idlewild S10-450 (Reference)	100 100 100	100 ± 0.0	100	No	--
	Tacoma Cem S10-457	100 100 100	100 ± 0.0	100	No	No
	Fort Steilacoom GC S10-449	80.0 100 80.0	86.7 ± 11.5	86.7	No	No
Hanford Old Orchards	HOO-05 S10-466 (Reference)	100 100 100	100 ± 0.0	100	No	--
	HOO-01 S10-462	100 100 100	100 ± 0.0	100	No	No
	HOO-02 S10-463	100 100 100	100 ± 0.0	100	No	No
	HOO-03 S10-464	100 100 100	100 ± 0.0	100	No	No
	HOO-04 S10-465	100 100 100	100 ± 0.0	100	No	No
	HOO-06 S10-467	100 100 100	100 ± 0.0	100	No	No

Table 5. Results (means ± standard deviations) for *E. foetida* sublethal effects

Soil Series	Site ID/Nautilus Log-In Number	Normal[2] (%)	Mean Normal (%)	% of Control	Significant Decrease from Control? ($p<0.05$)	Significant Decrease from Reference? ($p<0.05$)
	Negative Control	90.0 90.0 100	93.3± 5.8	--	--	--
Alderwood	Wing-Ald S10-447 (Reference)	100 100 100	100 ± 0.0	107	No	--
	King Co. MP-Ald S10-452	80.0 80.0 70.0	76.7 ± 5.8	82.2	**Yes**	**Yes**
	Colgate Park S10-445	100 100 100	100 ± 0.0	107	No	No
Everett	Cormorant Park S10-446 (Reference)	NA[1] NA[1] NA[1]	NA[1]	NA[1]	NA[1]	NA[1]
	King Co. Owned S10-454	80.0 80.0 66.7	75.6 ± 7.7	81.0	**Yes**	NA[1]
	Burton Acres S10-444	80.0 0.0 50.0	43.3 ± 40.41	46.4	No	NA[1]
Harstine	Kopachuck-HAR S10-451 (Reference)	80.0 70.0 100	83.3 ± 15.3	89.2	No	--
	Eagle Ridge S10-448	100 40.0 100	80.0 ± 34.6	85.7	No	No
	Morford's S10-455	87.5 100 88.9	92.1 ± 6.9	98.7	No	No
Kitsap	Winghaven-Kit S10-458 (Reference)	100 100 90.0	96.7 ± 5.8	103	No	--
	King Co. MP-Kit S10-453	42.9 40.0 50.0	44.3 ± 5.2	47.5	**Yes**	**Yes**
	Pt. Robinson S10-456	80.0 100 100	93.3 ± 11.5	100	No	No

[1]Not Applicable—all organisms died in reference site, [2]Percent of surviving organisms without sublethal effects

Table 5, cont. Results (means ± standard deviations) for *E. foetida* sublethal effects

Soil Series	Site ID/Nautilus Log-In Number	Normal[2] (%)	Mean Normal (%)	% of Control	Significant Decrease from Control? (p<0.05)	Significant Decrease from Reference? (p<0.05)
Spanaway	Idlewild S10-450 (Reference)	100 100 70.0	90.0 ± 17.3	96.4	No	--
	Tacoma Cem S10-457	100 100 100	100 ± 0.0	107	No	No
	Fort Steilacoom GC S10-449	87.8 90.0 75.0	84.2 ± 8.0	90.2	No	No
Hanford Old Orchards	HOO-05 S10-466 (Reference)	100 100 100	100 ± 0.0	107	No	--
	HOO-01 S10-462	100 100 100	100 ± 0.0	107	No	No
	HOO-02 S10-463	100 80.0 100	93.3 ± 11.5	100	No	No
	HOO-03 S10-464	100 100 100	100 ± 0.0	107	No	No
	HOO-04 S10-465	100 100 100	100 ± 0.0	107	No	No
	HOO-06 S10-467	100 100 100	100 ± 0.0	107	No	No

[2]Percent of surviving organisms without sublethal effects

3.2 Soil Chemistries

Soil chemistry data are provided in Appendices A and B. Sample pH and conductivity data from test initiation and termination are provided in Tables 6 and 7. A summary of physical and chemical characteristics measured during testing is provided in Table 8. Temperatures ranged between 20.0 and 21.0ºC for the duration of the test. Values for conductivity in soil slurry ranged from 21 to 856 µS/cm, and in soil supernatant from 21 to 669 µS/cm. Values for pH in soil slurry ranged from 3.65 to 7.56, and in soil supernatant from 3.70 to 7.83.

Wing-Ald, King Co. MP-Ald, Colgate Park, Cormorant Park, King Co. Owned, Burton Acres, Kopachuck-HAR, Eagle Ridge, Morford's, Idlewild, and Fort Steilacoom GC had initial soil slurry pH values which were below the acceptable range for testing (pH < 5.0), and according to protocol requirements, should not have been tested. Cormorant Park and Morford's were the only samples with low pH (pH of 4.78 and 4.26, respectively) that exhibited toxicity of the survival endpoint, while King Co. Owned and King Co. Ald (pH of 4.07 and 4.30, respectively) exhibited a higher incidence of sublethal effects than the control. While pH cannot be ruled out as a factor in the toxicity or increased sublethal effects, Wing-Ald (pH 3.73) and Kopachuck-HAR (pH 3.83) had lower pH values and complete survival suggesting pH may not have been the only factor in the toxicity observed.

Past experiences with soil toxicity testing indicate that addition of water to sample soils during pre-test hydration can have an unpredictable effect on pH, as it may raise or lower a soil's pH depending on its chemical composition. In this case, however, sample soils with initial pH readings outside the acceptable range were not hydrated prior to test initiation, ruling out pre-test hydration as a cause for the samples' low pH readings. The method for obtaining pH measurements requires addition of DI water (pH ~4.5), which may cause variation in a sample's pH reading as different metals and/or chemicals go into solution. As the complete sample composition is unknown, the cause of pH variation cannot be definitively identified.

Cormorant Park had an increase of 1 pH unit during the course of testing. This rather significant pH change could have been caused by leaching out of calcium and/or magnesium from the soil due to daily watering. It is, however, not possible to say with certainty that watering was the cause for the change, as soil pH can be affected by other factors (e.g., organic matter decomposition, activities of soil biota, nutrient leaching, etc.). Therefore, the high mortality seen in this sample cannot be definitively assigned to the pH change, as it could have been caused by a number of different biotic and abiotic factors in the sample.

Table 6. Initial and final pH values for *E. foetida* test on May 28, 2010

Soil Series	Sample ID	Nautilus Log-In Number	Soil Slurry pH		Soil Supernatant pH	
			Initial	Final	Initial	Final
	Negative Control		7.56	7.83	7.83	8.08
Alderwood	Wing-Ald	S10-447	3.75[1]	3.96	4.73[1]	4.43
	King Co. MP-Ald	S10-452	4.30[1]	4.50	5.20	5.00
	Colgate Park	S10-445	4.53[1]	4.59	5.29	5.03
Everett	Cormorant Park	S10-446	4.78[1]	5.89	5.63	6.37
	King Co. Owned	S10-454	4.07[1]	4.82	4.76[1]	5.18
	Burton Acres	S10-444	3.44[1]	3.34	4.06[1]	3.63
Harstine	Kopachuck-HAR	S10451	3.83[1]	4.48	4.86[1]	4.99
	Eagle Ridge	S10-448	4.59[1]	4.72	5.63	5.27
	Morford's	S10-455	4.26[1]	4.89	4.84[1]	5.13
Kitsap	Winghaven-Kit	S10-458	5.95	5.52	6.19	5.51
	King Co. MP-Kit	S10-453	5.15	5.29	6.04	5.67
	Pt. Robinson	S10-456	5.55	5.21	6.00	5.31
Spanaway	Idlewild	S10-450	4.73[1]	4.94	5.87	5.43
	Tacoma Cem	S10-457	5.08	5.97	5.39	5.92
	Fort Steilacoom GC	S10-449	3.79[1]	4.29	4.89[1]	4.88
Hanford Old Orchards	HOO-05	S10-466	7.03	7.62	7.05	7.71
	HOO-01	S10-462	6.57	6.31	6.70	6.27
	HOO-02	S10-463	7.26	6.67	7.29	6.53
	HOO-03	S10-464	6.20	6.74	6.29	6.50
	HOO-04	S10-465	6.51	7.03	6.55	7.11
	HOO-06	S10-467	7.39	7.19	7.31	7.12

[1] Deviation from allowable pH range in protocol (see section 3.2)

Table 7. Initial and final conductivity values for *E. foetida* test on May 28, 2010

Soil Series	Sample ID	Nautilus Log-In Number	Soil Slurry Conductivity (µS/cm)		Soil Supernatant Conductivity (µS/cm)	
			Initial	Final	Initial	Final
	Negative Control		264	310	335	324
Alderwood	Wing-Ald	S10-447	80	409	83	409
	King Co. MP-Ald	S10-452	81	326	86	328
	Colgate Park	S10-445	27	151	27	160
Everett	Cormorant Park	S10-446	48	484	51	503
	King Co. Owned	S10-454	39	158	80	162
	Burton Acres	S10-444	99	336	101	378
Harstine	Kopachuck-HAR	S10451	50	175	51	236
	Eagle Ridge	S10-448	21	158	23	170
	Morford's	S10-455	33	146	34	149
Kitsap	Winghaven-Kit	S10-458	122	497	115	486
	King Co. MP-Kit	S10-453	44	423	46	418
	Pt. Robinson	S10-456	48	378	53	381
Spanaway	Idlewild	S10-450	53	576	56	566
	Tacoma Cem	S10-457	26	122	29	119
	Fort Steilacoom GC	S10-449	21	121	22	137
Hanford Old Orchards	HOO-05	S10-466	66	148	72	159
	HOO-01	S10-462	323	483	343	469
	HOO-02	S10-463	650	856	677	869
	HOO-03	S10-464	291	362	304	373
	HOO-04	S10-465	351	412	363	467
	HOO-06	S10-467	137	364	154	392

Table 8. Summary of Chemical/Physical Characteristics measured during *E. foetida* testing

Parameter	Criteria	Count	Minimum	Maximum	Average	Acceptable? Samples affected
Initial Moisture Fraction (%)	35-45	21	20.2	103	47.2	No[1] Burton Acres, Colgate Park, King Co. MP-Ald, King Co. MP-Kit, King Co. Owned, Morford's, Tacoma Cem, Winghaven-Kit, HOO-01, HOO-02, HOO-03, HOO-06
Initial Slurry pH	>5.0	21	3.44	7.39	5.18	No[1] Burton Acres, Colgate Park, Cormorant Park, Wing-Ald, Eagle Ridge, Fort Steilacoom GC, Idlewild, Kopachuck-HAR, King Co. MP-Ald, King Co. Owned, Morford's
Initial Supernatant pH	>5.0	21	4.06	7.31	5.74	No[1] Burton Acres, Wing-Ald, Fort Steilacoom GC, Kopachuck-HAR, King Co. Owned, Morford's, Pt. Robinson
Initial Slurry Conductivity (µS/cm)		21	21	650	124	Yes
Initial Supernatant Conductivity (µS/cm)		21	22	677	132	Yes
Temperature (°C)	22±2	15	20.1	21.0	20.6	Yes

[1]Deviation from protocol not expected to influence results of the test

4.0 QA/QC

Testing was initiated within 14 days of soil collection in 18 of the 21 samples, meeting holding time requirements for these samples. Three samples, Burton Acres, Wing-Ald, and Winghaven-Kit fell outside of holding time by two days. However, due to the nature of sampling, and upon the client's request tests were still run on these samples. The extra holding time is not thought to have affected the levels of the chemicals of concern, as they do not exhibit high volatility.

Due to the nature of the Hanford Old Orchards soils, which consisted mostly of very fine sand and contained very little to no organic matter that could hold moisture, several of the samples (HOO-01, HOO-02, HOO-03, and HOO-06) were hydrated to between 20 and 25 percent moisture content. Hydration to 35 percent moisture content (recommended lower level in the protocol) would have created an inhospitable environment to earthworms, as they would have been submerged in water. With the lower moisture content, samples took on the same appearance and friability as the control soil. In addition, some samples contained more than the 45 percent moisture content recommendation. No manipulation was done on these samples and the extra moisture should not have affected the results.

A summary of all test deviations is provided in Appendix C.

The test met the acceptability criterion for negative control performance. Temperature readings remained within parameters for the duration of the test.

Results of reference toxicant test (positive control) conducted with the test organism are provided in Table 9. EC50 values fell within the acceptable range of mean ± two standard deviations for historical data. Indicating that the test organisms appeared to have been of an appropriate degree of sensitivity.

Table 9. Reference toxicant test results.

Species	Endpoint	Date Initiated	EC50 (mg/kg 2-Chloroacetamide)	Historical range (mean ± 2 SD)	Coefficient of Variation (%)
Eisenia foetida	Survival	5/28/2010	55.3	0.60 – 81.5	49.3

5.0 REFERENCES

American Society of Testing and Materials (ASTM). 1999. Standard guide for conducting terrestrial plant toxicity tests. ASTM designation E1963-98.

American Society of Testing and Materials (ASTM). 1997. Standard guide for conducting laboratory soil toxicity or bioaccumulation tests with the lumbricid earthworm *Eisenia* fetida. ASTM designation E1676-97.

Biostat. DMMP/SMS Bioassay Statistics Program for Microsoft Windows. Developed by Corps of Engineers, Seattle District

Washington State Department of Ecology (WDOE). 1996. Earthworm bioassay protocol for soil toxicity screening. WDOE Environmental Investigations and Laboratory Services Program Publication No. 96-327.

APPENDIX A – Summary of Results and Statistics

APPENDIX B – Laboratory Datasheets

APPENDIX C – QA/QC Summary

APPENDIX D - Reference Toxicant Test Results

APPENDIX E – Chain-of-Custody Forms

APPENDIX F – 14-day Earthworm Survival SOP

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Nautilus Environmental, LLC

Lettuce Soil Toxicity Evaluation

Tacoma Smelter Plume and Hanford Old Orchards Study, WA

Final Report

Date: July 28, 2010

Submitted to:

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SIGNATURE PAGE

Washington Laboratory Manager

Project Manager

This report has been prepared based on data and/or samples provided by our client and the results of this study are for their sole benefit. Any reliance on the data by a third party is at the sole and exclusive risk of that party.

Executive Summary

This report contains a biological evaluation of the condition of soils at different sites affected by the Tacoma Smelter plume in Pierce and King Counties, as well as sites affected by arsenic and lead containing pesticides in the Old Orchards area at the Department of Energy Hanford site. The evaluation was conducted using the 14-day lettuce survival and biomass, and 14-day earthworm survival bioassays. Twenty-one soil samples were analyzed. This report contains the results for the lettuce seedling bioassay, which was conducted on May 27, 2010 using the species *Lactuca sativa.* Performance in the test samples was compared to a negative control. In addition, performance of soil samples within a soil series, samples having the same soil characteristics, was compared to a reference sample within that series. Testing was initiated within 14 days of soil collection for all but three sites, meeting holding time requirements for 18 of the 21 sites.

The lettuce survival and biomass bioassay met all performance criteria for the negative and positive controls. No effects on survival were found in any of the test sites. Burton Acres and Fort Steilacoom GC sites were significantly different than the negative control for the biomass endpoint.

1.0 INTRODUCTION

Laboratory toxicity testing was conducted on soil samples collected from 15 sites within the Tacoma Smelter Plume footprint, and six sites in the Hanford Old Orchards area within the Department of Energy Hanford site, Washington following site-specific terrestrial ecological evaluation (TEE) methodology under the Model Toxics Control Act (MTCA). The specific contaminants of concern for the bioassay component of the TEE were arsenic and lead. Soil samples were collected from areas within the Tacoma Smelter Plume footprint, and Hanford Old Orchards representing different soil types and a range of concentrations of arsenic and lead. A toxicity test was conducted using the butter crunch lettuce seed *Lactuca sativa*. Testing was initiated on May 27, 2010 at the Washington Laboratory of Nautilus Environmental located in Tacoma, Washington. Test procedures followed methods published by Washington State Department of Ecology for the Toxics Cleanup Program.

2.0 METHODS

2.1 Sample Receipt and Manipulation

Twenty-one soil samples were collected by the Department of Ecology between May 11th and 26th, 2010, and were received by Nautilus Environmental on May 24th and May 27th, 2010. All samples were held in 3.5 L HPDE containers, labeled and transported in coolers containing ice. Upon receipt at the laboratory, the coolers were opened and the contents inspected and compared with documentation provided on the chain-of-custody forms (COC). Sample temperatures were measured upon receipt and recorded on both the COC and in a bound logbook maintained in the laboratory. Samples were held in the dark at 4 ± 2°C until testing.

Large pieces of wood, debris and rocks were removed from soils prior to testing. No sieving was performed on the samples.

Sample IDs, log-in numbers, collections dates, receipt date, holding time expiration dates (sample date plus fourteen days) and test initiation dates for the samples undergoing testing are provided in Table 1.

Table 1. Sample collection, receipt, expiration and test initiation dates.

Sample ID	Nautilus Log-In Number	Date Collected	Date Received	Date Holding Time Expired	Test Initiation Date
Burton Acres	S10-444				
Winghaven-Kit	S10-458	May 11, 2010	May 24, 2010	May 25, 2010[1]	May 27, 2010
Wing-Ald	S10-447				
Pt. Robinson	S10-456	May 14, 2010	May 24, 2010	May 28, 2010	May 27, 2010
Colgate Park	S10-445				
Cormorant Park	S10-446	May 17, 2010	May 24, 2010	May 31, 2010	May 27, 2010
Idlewild	S10-450				
King Co. Owned	S10-454	May 18, 2010	May 24, 2010	June 1, 2010	May 27, 2010
Fort Steilacoom GC	S10-449	May 19, 2010	May 24, 2010	June 2, 2010	May 27, 2010
Tacoma Cem	S10-457				
Eagle Ridge	S10-448				
Kopachuck-HAR	S10-451	May 20, 2010	May 24, 2010	June 3, 2010	May 27, 2010
Morford's	S10-455				
King Co. MP-Ald	S10-452	May 21, 2010	May 24, 2010	June 4, 2010	May 27, 2010
King Co. MP-Kit	S10-453				
HOO-01	S10-462				
HOO-02	S10-463	May 25, 2010	May 27, 2010	June 8, 2010	May 27, 2010
HOO-03	S10-464				
HOO-04	S10-465				
HOO-05	S10-466	May 26, 2010	May 27, 2010	June 9, 2010	May 27, 2010
HOO-06	S10-467				

[1] Deviation from holding time requirements in protocol. Not expected to influence results of the test (see QA/QC section 4.0)

2.2 Lettuce seedling survival and biomass test methods

A lettuce seedling survival and biomass test was conducted on samples received May 24th and 27th, 2010 using butter crunch lettuce seeds, *Lactuca sativa*. The organisms were obtained from Territorial Seed Company, Oregon. Nautilus Environmental received the organisms at the laboratory on April 9, 2010. The tests were initiated on May 27, 2010. Tests were performed according to procedures presented by WADOE (1996) and ASTM (1994). Detailed test methods are documented in the Nautilus Environmental Standard Operating Procedure T-1550 (Appendix E). Test procedures are summarized in Table 2.

Prior to test initiation, 300 g subsamples were collected from the negative control and each site, for use in the test, as well as for initial pH measurements. Using an Orion 230 meter, pH measurements were taken by making a slurry of de-ionized (DI) water and soil in a 1:1 ratio (i.e., 25 mL DI water: 25 g soil). Soil slurry pH was measured after allowing soil/water mixture to stir for 5 minutes. Once the measurement was taken, the slurry was allowed to settle for 30 minutes, after which the pH of the supernatant liquid was measured. Sample soils were hydrated with DI to match control friability where necessary, and distributed into three poly flat 36-cell trays with humidity domes. Five replicates per sample, each containing 50 g of soil, were randomly distributed into trays. Sample distribution took place according to a randomization sheet and planting maps created in Excel. Trays were placed in an environmental chamber at 20°C under a 16:8 hour light:dark photoperiod.

Lighting for the test was provided by 2-bulb gro-lights placed over each planting tray. Light measurements were taken upon test initiation, day seven, and at termination using a Milwaukee SM 700 photometer. Test temperatures were measured daily from a surrogate test chamber.

Table 2. Summary of testing conditions for the lettuce survival and biomass test.

Test start date	May 27, 2010
Test end date	June 10, 2010
Test organism	*Lactuca sativa*
Test organism source	Territorial Seed Company, Cottage Grove, OR
Test duration	14 days
Test chamber	60-mL planting cell with 4 drainage holes in bottom
Test soil/replicate	50 g dry weight
Water source for hydration	De-ionized water
Control soil	70% sand, 20% kaolin clay, 10% peat moss, 0.45% $CaCO_3$
Number of organisms/replicate	12
Number of replicates/sample	5
Test temperature	20-30ºC
Illumination	16:8 hr light:dark photoperiod
Test acceptance criterion	≥90% mean germination in control organisms
Positive control reference toxicant	Boric acid

The tests were terminated on day 14, June 10, 2010. At test termination, the number of seedlings in each replicate were counted and observations on seedling condition (e.g., chlorosis, wilting) were recorded. The above-soil portion of each seedling was then cut at the soil using scissors; and placed in a pre-tared weigh boat corresponding to the replicate number. A 25 g subsamble of soil from each site was collected for final pH measurements from a randomly chosen replicate.

Weigh boats containing seedlings were weighed immediately after cutting using a Mettler AE 240 scale, in order to obtain wet weights, and were subsequently placed in a Thelco 28 oven to dry for 24 hours. However, due to problems with oven temperature, it was deemed necessary to leave seedlings in the oven for an additional 24 hours in order to ensure all moisture was removed (See Section 4.0). Seedlings were weighed at the end of the drying period in order to obtain dry weights. The endpoints calculated were the number of seedling surviving and their biomass (evaluated on the basis of dry weight divided by initial count). The test acceptance criterion for the negative control was seedling survival of ≥ 90 percent. Statistics were run using Biostat software on all sites where survival or growth were less than control or their respective reference site, using a level of significance of 0.05.

A reference toxicant test (positive control) was conducted in conjunction with the lettuce seedling survival and biomass tests using boric acid as the toxicant. Test organisms were exposed to control, 40, 80, 160, 320 and 640 mg/kg boron for the same duration as the concurrent soil test, and the results of this test were compared with historical data for the species to determine whether the sensitivity of the organisms was appropriate.

3.0 RESULTS

Results of toxicity tests conducted using *L. sativa* on May 27, 2010 are summarized in Tables 3 and 4. Detailed results of the soil toxicity tests and statistical analyses are provided in Appendix A. Copies of the laboratory bench sheets, QA/QC summary, reference toxicant test results, test method SOP, and chain-of-custody forms are in Appendices B, C, D, E, and F.

3.1 Toxicity results

Mean survival was 93.3 percent for the artificial soil control, and 88.3, 93.3, 96.7, 90.0, 88.3, and 90.0 percent for the reference sites (Wing-Ald, Cormorant Park, Kopachuck-HAR, Winghaven-Kit, Idlewild, and HOO-05, respectively). The mean survival in the test soils ranged from 83.3 to 100 percent. None of the sites exhibited significant toxic effects when compared to negative control survival results. There were no differences in survival between the sites and their respective references.

Mean biomass was 1.93 mg per seedling for the artificial soil control, and 2.50, 2.80, 1.86, 2.74, 2.57, and 2.12 mg per seedling for the reference sites (Wing-Ald, Cormorant Park, Kopachuck-HAR, Winghaven-Kit, Idlewild, and HOO-05, respectively). Burton Acres and Fort Steilacoom GC were significantly different than the negative control for growth. Colgate Park, King Co.

Owned, Burton Acres, King Co. MP-Kit, Tacoma Cem, Fort Steilacoom GC, and HOO-01 were significantly different than their respective reference sites.

Table 3. Results (means ± standard deviations) for *L. sativa* survival

Soil Series	Site ID/ Nautilus Log-In Number	% Survival	Mean Survival (%)	% of Control	Significant Decrease from Control? ($p<0.05$)	Significant Decrease from Reference? ($p<0.05$)
	Negative Control	91.7 91.7 100 91.7 91.7	93.3 ± 3.7	--	--	--
Alderwood	Wing-Ald S10-447 (Reference)	83.3 100 75 83.3 100	88.3 ± 11.2	94.6	No	--
	King Co. MP-Ald S10-452	100 100 100 83.3 100	96.7 ± 7.5	104	No	No
	Colgate Park S10-445	100 100 100 75.0 100	95.0 ± 11.2	102	No	No
Everett	Cormorant Park S10-446 (Reference)	91.7 75.0 100 100 100	93.3 ± 10.9	100	No	--
	King Co. Owned S10-454	100 83.3 100 91.7 83.3	91.7 ± 8.3	98.2	No	No
	Burton Acres S10-444	100 83.3 100 100 100	96.7 ± 7.5	104	No	No

Table 3 cont. Results (means ± standard deviations) for *L. sativa* survival

Soil Series	Site ID/ Nautilus Log-In Number	% Survival	Mean Survival (%)	% of Control	Significant Decrease from Control? (p<0.05)	Significant Decrease from Reference? (p<0.05)
Harstine	Kopachuck-HAR S10-451 (Reference)	91.7 100 91.7 100 100	96.7 ± 4.6	104	No	--
	Eagle Ridge S10-448	100 91.7 91.7 100 100	96.7 ± 4.6	104	No	No
	Morford's S10-455	100 83.3 100 100 100	96.7 ± 7.5	104	No	No
Kitsap	Winghaven-Kit S10-458 (Reference)	100 91.7 66.7 91.7 91.7	88.3 ± 12.6	94.6	No	--
	King Co. MP-Kit S10-453	91.7 83.3 91.7 100 100	93.3 ± 7.0	100	No	No
	Pt. Robinson S10-456	91.7 100 91.7 75.0 100	91.7 ± 10.2	98.2	No	No
Spanaway	Idlewild S10-450 (Reference)	66.7 100 100 83.3 91.7	83.3 ± 13.9	94.6	No	--
	Tacoma Cem S10-457	91.7 100 83.3 91.7 91.7	91.7 ± 5.9	98.2	No	No

Table 3 cont. Results (means ± standard deviations) for *L. sativa* survival

Soil Series	Site ID/ Nautilus Log-In Number	% Survival	Mean Survival (%)	% of Control	Significant Decrease from Control? ($p<0.05$)	Significant Decrease from Reference? ($p<0.05$)
Spanaway	Fort Steilacoom GC S10-449	58.3 91.7 83.3 100 58.3	78.3 ± 19.2	83.9	No	No
Hanford Old Orchards	HOO-05 S10-466 (Reference)	91.7 75.0 100 91.7 91.7	90.0 ± 9.1	96.4	No	--
	HOO-01 S10-462	50.0 100 100 100 100	90.0 ± 22.4	96.4	No	No
	HOO-02 S10-463	50.0 83.3 100 100 100	86.7 ± 21.7	92.9	No	No
	HOO-03 S10-464	91.7 100 91.7 100 75.0	91.7 ± 10.2	98.2	No	No
	HOO-04 S10-465	83.3 100 91.7 66.7 91.7	86.7 ± 12.6	92.9	No	No
	HOO-06 S10-467	75.0 100 100 91.7 100	93.3 ± 10.9	100	No	No

Table 4. Results (means ± standard deviations) for *L. sativa* growth

Soil Series	Site ID/Nautilus Log-In Number	Growth per Seedling (mg)	Mean Growth per Organism (mg)	% of Control	Significant Decrease from Control? (p<0.05)	Significant Decrease from Reference? (p<0.05)
	Negative Control	2.98 1.04 1.58 1.63 2.40	1.93 ± 0.76	--	--	--
Alderwood	Wing-Ald S10-447 (Reference)	1.67 2.80 2.43 3.29 2.30	2.50 ± 0.60	130	No	--
	King Co. MP-Ald S10-452	1.85 2.08 1.98 2.50 1.31	1.94 ± 0.43	101	No	No
	Colgate Park S10-445	1.19 1.36 1.70 1.20 1.24	1.34 ± 0.21	69.5	No	**Yes**
Everett	Cormorant Park S10-446 (Reference)	3.20 2.66 2.55 2.74 2.87	2.80 ± 0.25	145	No	--
	King Co. Owned S10-454	1.35 1.85 1.85 1.51 1.37	1.58 ± 0.25	82.2	No	**Yes**
	Burton Acres S10-444	1.02 1.31 1.07 1.17 1.29	1.17 ± 0.13	60.7	**Yes**	**Yes**
Harstine	Kopachuck-HAR S10-451 (Reference)	1.92 1.69 2.34 1.94 1.38	1.86 ± 0.35	96.4	No	--

Table 4 cont. Results (means ± standard deviations) for *L. sativa* growth

Soil Series	Site ID/Nautilus Log-In Number	Growth per Seedling (mg)	Mean Growth per Organism (mg)	% of Control	Significant Decrease from Control? (p<0.05)	Significant Decrease from Reference? (p<0.05)
Harstine	Eagle Ridge S10-448	2.55 1.94 1.86 2.28 2.12	2.15 ± 0.28	112	No	No
	Morford's S10-455	2.22 2.30 2.11 1.70 1.57	1.98 ± 0.32	103	No	No
Kitsap	Winghaven-Kit S10-458 (Reference)	2.47 3.78 2.51 2.42 2.50	2.74 ± 0.58	142	No	--
	King Co. MP-Kit S10-453	2.75 2.19 2.13 2.01 2.06	2.23 ± 0.30	116	No	**Yes**
	Pt. Robinson S10-456	2.75 2.97 3.94 3.59 2.24	3.10 ± 0.67	161	No	No
Spanaway	Idlewild S10-450 (Reference)	2.30 2.57 3.20 2.80 1.98	2.57 ± 0.46	133	No	--
	Tacoma Cem S10-457	1.73 1.28 1.66 2.01 1.22	1.58 ± 0.33	82.0	No	**Yes**
	Fort Steilacoom GC S10-449	0.46 0.66 0.50 0.67 0.68	0.59 ± 0.11	30.8	**Yes**	**Yes**

Table 4 cont. Results (means ± standard deviations) for *L. sativa* growth

Soil Series	Site ID/Nautilus Log-In Number	Growth per Seedling (mg)	Mean Growth per Organism (mg)	% of Control	Significant Decrease from Control? (p<0.05)	Significant Decrease from Reference? (p<0.05)
Hanford Old Orchards	HOO-05 S10-466 (Reference)	1.80 2.43 2.54 1.83 2.02	2.12 ± 0.34	110	No	--
	HOO-01 S10-462	2.20 1.69 1.54 1.67 1.52	1.72 ± 0.28	89.4	No	**Yes**
	HOO-02 S10-463	1.32 2.18 2.20 1.69 1.91	1.86 ± 0.37	96.5	No	No
	HOO-03 S10-464	1.97 1.94 2.37 2.81 2.91	2.40 ± 0.45	125	No	No
	HOO-04 S10-465	2.14 2.19 3.35 2.81 2.52	2.60 ± 0.50	135	No	No
	HOO-06 S10-467	2.37 2.92 1.68 3.32 2.35	2.53 ± 0.63	131	No	No

3.2 Soil Chemistries

Soil chemistry data are provided in Appendices A and B. Temperatures ranged between 22.0 and 26.0ºC for the duration of the test. Sample pH data from test initiation and termination are provided in Table 5. A summary of physical and chemical characteristics measured during testing is provided in Table 6. Values for pH in soil slurry ranged from 3.65 to 7.54, and in soil supernatant from 3.26 to 7.41. At their low range, some pH values were outside the acceptable range according to the protocol (pH < 5.0). Wing-Ald, Colgate Park, King Co. Owned, Burton Acres, Kopachuck-HAR, Morford's, Pt. Robinson, and Fort Steilacoom GC had initial soil slurry or soil supernatant pH values which were outside acceptable range. None of the samples exhibited toxicity for the survival endpoint, however Burton Acres, the sample with the lowest pH, had significantly lower growth compared to the control. This low pH cannot be ruled out as a confounding factor of toxicity. The pH values below 5.0 were not detrimental to seedling survival or growth, only when pH dropped below 4.0 may there have been a problem.

Table 5. Initial and final pH values for *L. sativa* test on May 27, 2010

Soil Series	Sample ID	Nautilus Log-In Number	Soil Slurry pH		Soil Supernatant pH	
			Initial	Final	Initial	Final
	Negative Control		6.61	7.29	7.07	7.38
Alderwood	Wing-Ald	S10-447	4.68[1]	4.16	4.58[1]	4.31
	King Co. MP-Ald	S10-452	5.17	5.18	5.10	4.99
	Colgate Park	S10-445	5.14	5.25	4.95[1]	5.17
Everett	Cormorant Park	S10-446	5.46	4.72	5.31	4.80
	King Co. Owned	S10-454	4.86[1]	4.33	4.67[1]	4.57
	Burton Acres	S10-444	3.65[1]	3.70	3.26[1]	3.42
Harstine	Kopachuck-HAR	S10-451	4.91[1]	4.21	4.77[1]	4.83
	Eagle Ridge	S10-448	5.48	4.93	5.27	5.08
	Morford's	S10-455	4.92[1]	4.28	4.79[1]	4.48
Kitsap	Winghaven-Kit	S10-458	6.38	5.54	6.26	5.71
	King Co. MP-Kit	S10-453	6.10	5.91	5.93	5.68
	Pt. Robinson	S10-456	5.86	5.37	4.74[1]	5.63
Spanaway	Idlewild	S10-450	5.78	4.84	5.60	5.00
	Tacoma Cem	S10-457	5.52	5.02	5.42	5.20
	Fort Steilacoom GC	S10-444	4.72[1]	3.88	4.67[1]	4.04
Hanford Old Orchards	HOO-05	S10-466	7.26	7.17	7.13	7.14
	HOO-01	S10-462	6.66	6.30	6.67	6.43
	HOO-02	S10-463	7.31	6.81	7.41	7.26
	HOO-03	S10-464	6.43	6.11	6.38	6.36
	HOO-04	S10-465	6.80	6.53	6.70	6.53
	HOO-06	S10-467	7.54	7.22	7.35	7.33

[1] Deviation less than allowable pH range in protocol (see text above)

Table 6. Summary of Chemical/Physical Characteristics measured during *L. sativa* testing

Parameter	Criteria	Count	Minimum	Maximum	Average	Acceptable? Samples affected
Initial pH (Slurry)	>5.0	21	3.65	7.54	5.74	No Wing-Ald[1], Colgate Park[1], King Co. Owned[1], Burton Acres[2], Kopachuck-HAR[1], Morford's[1], Pt. Robinson[1], and Fort Steilacoom GC[1]
Initial pH (Supernatant)	>5.0	21	3.26	7.41	5.57	No Wing-Ald[1], Colgate Park[1], King Co. Owned[1], Burton Acres[2], Kopachuck-HAR[1], Morford's[1], Pt. Robinson[1], and Fort Steilacoom GC[1]
Temperature (°C)	20-30	15	21.4	26.0	24.1	Yes
Light Reading (Lux)	>1000	3	3380	6860	4766	Yes

[1]Deviation from protocol not expected to influence results of the test [2]Deviation from protocol may have influenced the results, see text for discussion.

4.0 QA/QC

Testing was initiated within 14 days of soil collection in 18 of the 21 samples, meeting holding time requirements for these samples. Three samples, Burton Acres, Wing-Ald and Winghaven-Kit fell outside of holding time by two days. However, due to the nature of sampling, and upon the client's request, tests were still run on these samples. The extra holding time is not thought to have affected the levels of chemicals of concern, as they do not exhibit high volatility.

At the onset of seed germination, it became apparent that several replicates (replicate 4 of Fort Steilacoom GC, 3 and 4 of Morford's, 1 of Winghaven-Kit, 3 and 5 of HOO-02, 2 of HOO-03, and 2 and 3 of HOO-06) contained more than 12 seeds. Start counts for these replicates were changed to appropriate numbers, so as to reflect the true number of seeds present. Several of the soil samples tested (Cormorant Park, Wing-Ald, Fort Steilacoom GC, Idlewild, King Co. MP-Ald, Pt. Robinson, and Winghaven-Kit) contained native seeds (identified by knowledgeable staff), which germinated during the test and were removed as their presence became apparent. Confirmation of native plants was determined by differences in cotyledon/leaf size, shape, and color, presence/absence of pubescence (leaf/stalk hairs), and leaf/cotyledon venation.

Upon test termination, seedlings were placed in an oven to dry for 24 hours. Oven temperature was set to be between 70 and 80°C, however at drying initiation, oven temperature was 40°C, which was attributed to handling time loading weigh boats into the oven. During morning temperature checks it was discovered that the oven temperature had remained at 40°C overnight. The temperature was increased and re-checked within the hour confirming that the temperature was acceptable. Seedlings remained in the oven for an additional 24 hours in order to ensure complete moisture removal. Observations made after this second 24-hour period showed the seedlings to be properly dessicated, and they were then weighed. A summary of all test deviations is provided in Appendix C.

The test met the acceptability criterion for negative control performance. Temperature and light readings remained within parameters for the duration of the test.

Results of reference toxicant test (positive control) conducted with the test organisms are provided in Table 7. EC50 values fell within the acceptable range of mean ± two standard deviations for historical data, indicating that the test organisms appeared to have been of an appropriate degree of sensitivity.

Table 7. Reference toxicant test results.

Species	Endpoint	Date Initiated	EC50 (mg/kg Boric Acid)	Historical range (mean ± 2 SD)	Coefficient of Variation (%)
Lactuca sativa	Survival	5/27/2010	183	121 – 398	26.6
	Biomass	5/27/2010	135	97.0 – 202	17.6

5.0 REFERENCES

American Society of Testing and Materials (ASTM). 1999. Standard guide for conducting terrestrial plant toxicity tests. ASTM designation E1963-98.

American Society of Testing and Materials (ASTM). 1994. Standard practice for conducting early seedling growth tests. ASTM designation E1598-94.

Biostat. DMMP/SMS Bioassay Statistics Program for Microsoft Windows. Developed by Corps of Engineers, Seattle District

Washington State Department of Ecology (WDOE). 1996. Early seedling growth protocol for soil toxicity screening. WDOE Environmental Investigations and Laboratory Services Program Publication No. 96-324.

APPENDIX A – Summary of Results and Statistics

APPENDIX B – Laboratory Datasheets

APPENDIX C – QA/QC Summary

APPENDIX D - Reference Toxicant Test Results

APPENDIX E – Lettuce Seedling Survival and Growth SOP

APPENDIX F - Chain-of-Custody Forms

Client: Washington Department of Ecology

Sample IDs: Cormorant Park, Burton Acres, King Co. Owned, Kopachuck-HAR, Morford's

INTRODUCTION AND METHODS:

This report summarizes a biological evaluation of the condition of soils at different sites affected by the Tacoma Smelter plume in Pierce and King Counties. Testing originally conducted in late May 2010 had QA/QC issues, primarily associated with low pH measurements, that brought into question the validity of the data. In an attempt to address concerns raised by the Washington State Department of Ecology, and confirm or rule out those results, the tests on sites included in Table 1 were repeated, although the samples were outside of the holding time. The evaluation was conducted using the 14-day lettuce (*Lactuca sativa*) survival and biomass, and 14-day earthworm (*Eisenia foetida*) survival bioassays, which were conducted on August 9, 2010. Details of test procedures are summarized in Tables 2 and 3. Moisture content upon receipt of the samples, as well as hydration requirements and amount of water added to samples for the earthworm test is contained in Table 4. Performance in the test samples was compared to a negative control. In addition, the performance of soil samples within a soil series (i.e. samples having the same soil characteristics) was compared to a reference sample within that series.

Table 1. Sample collection, receipt, expiration and test initiation dates.

Sample ID	Nautilus Log-In Number	Date Collected	Date Received	Date Holding Time Expired	Test Initiation Date
Burton Acres	S10-444	May 11, 2010	May 24, 2010	May 25, 2010	Aug. 9, 2010
Cormorant Park	S10-446	May 17, 2010		May 31, 2010	
King Co. Owned	S10-454	May 18, 2010		June 3, 2010	
Kopachuck-HAR	S10-451	May 20, 2010		June 3, 2010	
Morford's	S10-455				

Table 2. Summary of testing conditions for the lettuce survival and biomass test

Test start date	August 9, 2010
Test end date	August 23, 2010
Test organism	*Lactuca sativa*
Test organism source	Territorial Seed Company, Cottage Grove, OR
Test duration	14 days
Test chamber	60-mL planting cell with 4 drainage holes in bottom
Test soil/replicate	50 g dry weight
Water source for hydration	De-ionized water
Control soil	70% sand, 20% kaolin clay, 10% peat moss, 0.45% $CaCO_3$
Number of organisms/replicate	12
Number of replicates/sample	5
Test temperature	20-30ºC
Illumination	16:8 hr light:dark photoperiod
Test acceptance criterion	≥90% mean germination in control organisms
Positive control reference toxicant	Boric acid

Table 3. Summary of testing conditions for the earthworm survival test

Test start date	August 9, 2010
Test end date	August 23, 2010
Test organism	*Eisenia foetida*
Test organism source	Aquatic Research Organisms, Hampton, NH
Test organism age	>90 days
Test duration	14 days
Test chamber	1-L glass jar
Test soil/replicate	200 g dry weight
Water source for hydration	De-ionized water
Control soil	70% sand, 20% kaolin clay, 10% peat moss, 0.45% $CaCO_3$
Number of organisms/replicate	10
Number of replicates/sample	3
Test temperature	22± 2ºC
Illumination	Continuous lighting
Test acceptance criterion	≥90% mean survival of control organisms
Positive control reference toxicant	2-chloroacetamide

Table 4. Pre-test hydration used for visual match of control friability for *E. foetida*.

Client ID	Nautilus Log-In	Initial Moisture Content (%)	Hydration Needed (%)	Amount of Water Added to Sample (ml)
Cormorant Park	S10-446	37.4	0	0
King Co. Owned	S10-454	56.2	0	0
Kopachuck-HAR	S10-451	47.2	0	0
Morford's	S10-455	62.9	0	0

TOXICITY RESULTS:

Both tests met negative control criteria. Survival results for both lettuce and earthworm are summarized in Table 5. Growth data for lettuce are summarized in Table 6, while sublethal data for earthworms are in Table 7.

Lettuce

In the lettuce test, mean survival was 91.7 percent in the artificial soil control, 90.0 percent in the reference site (i.e., Cormorant Park), and 95.0 percent in Burton Acres. Neither of the sites exhibited significant toxic effects when compared to negative control survival results, and Burton Acres had higher survival than its corresponding reference site. These results are consistent with initial testing.

For lettuce, mean growth was 1.55 mg per seedling in the artificial soil control and 1.33 mg per seedling in the reference site. Burton Acres exhibited a mean growth of 2.33 mg per seedling, which was higher than growth in both the control and its reference site. This result is different from the initial test, in which Burton Acres exhibited significantly less growth compared to the control and its reference site.

Earthworm

In the earthworm test, mean survival was 96.7 percent in the artificial soil control. The reference site, Cormorant Park, exhibited 50 percent mortality, while in the initial test, earthworms exhibited 100 percent mortality at this site. However, both testing events identified statistically significant reductions in survival compared with the controls for Cormorant Park. Initial testing also indicted Morford's had significantly less survival than the controls (86.7 percent), however the current test results showed 96.7 percent survival in Morford's, and no significant decrease from the controls. All other sites tested in the current testing event exhibited survival of 93.3 to 100 percent, which was consistent with the response observed in the initial round of testing.

Sublethal effects overall were less apparent in the more recent test, compared with the initial test series in which up to 17 percent effects were seen. In the current test series, only earthworms exposed to Cormorant Park showed signs of sublethal effects, with 12.2 percent of the surviving organisms affected. In addition, no sublethal effects found on worms exposed to King Co. Owned, which is different from initial testing, in which a statistically significant increase in sublethal effects was observed in worms exposed to this sample (25 percent).

Table 5. Results (means ± standard deviations) for *L. sativa* and *E. foetida* survival

Soil Series	Site ID/ Nautilus Log-In Number	% Survival	Mean Survival (%)	% of Control	Significant Decrease from Control? (p<0.05)	Significant Decrease from Reference? (p<0.05)
		(*L. sativa*) Lettuce				
	Negative Control	91.7 91.7 91.7 100 83.3	91.7 ± 5.9	--	--	--
Everett	Cormorant Park S10-446 (Reference)	91.7 91.7 83.3 91.7 91.7	90.0 ± 3.7	98.2	No	--
Everett	Burton Acres S10-444	91.7 100 100 91.7 91.7	95.0 ± 4.6	104	No	No
		(*E. foetida*) Earthworms				
	Negative Control	100 90.0 100	96.7 ± 5.8	--	--	--
Everett	Cormorant Park S10-446 (Reference)	50.0 60.0 40.0	50.0 ± 10.0	51.7	Yes	--
Everett	King Co. Owned S10-454	100 90.0 90.0	93.3 ± 5.8	96.6	No	187
Harstine	Kopachuck-HAR S10-451 (Reference)	100 100 100	100 ± 0.0	103	No	--
Harstine	Morford's S10-455	90.0 100 100	96.7 ± 5.8	100	No	96.7

Table 6. Results (means ± standard deviations) for *L. sativa* growth

Soil Series	Site ID/Nautilus Log-In Number	Growth per Seedling (mg)	Mean Growth per Organism (mg)	% of Control	Significant Decrease from Control? (p<0.05)	Significant Decrease from Reference? (p<0.05)
	Negative Control	1.25 1.61 1.38 1.53 1.98	1.55 ± 0.28	--	--	--
Everett	Cormorant Park S10-446 (Reference)	1.35 1.21 1.39 1.46 1.20	1.33 ± 0.11	85.5	No	--
Everett	Burton Acres S10-444	2.27 2.23 2.08 2.65 2.44	2.33 ± 0.22	150	No	No

Table 7. Results (means ± standard deviations) for *E. foetida* sublethal effects

Soil Series	Site ID/Nautilus Log-In Number	Normal[1] (%)	Mean Normal (%)	% of Control	Significant Decrease from Control? (p<0.05)	Significant Decrease from Reference? (p<0.05)
	Negative Control	100 100 100	100± 0.0	--	--	--
Everett	Cormorant Park S10-446 (Reference)	80.0 83.3 100	87.8 ± 10.72	87.8	No	--
Everett	King Co. Owned S10-454	100 100 100	100± 0.0	100	No	No
Harstine	Kopachuck-HAR S10-451 (Reference)	100 100 100	100± 0.0	100	No	--
Harstine	Morford's S10-455	100 100 100	100± 0.0	100	No	No

[1]Percent of surviving organisms without sublethal effects

SOIL CHEMISTRY RESULTS:

Temperatures ranged between 23.0 and 25.0ºC for the duration of the lettuce test, and between 20.4 and 21.0ºC during the earthworm test. The primary reason for re-testing these samples was due to low pH values (pH < 5.0) found in the initial testing; therefore, to better characterize the pH of the soil samples, pH was measured at the start and end of each test by both a Nautilus pH meter and a Washington Department of Ecology pH meter, as each meter uses a different set of calibration standards. Initial and final pH values are found in Table 8 for both species, and Table 9 contains initial and final conductivity values for earthworms. A summary of physical and chemical characteristics measured during testing is provided in Table 10 for the lettuce test and Table 11 for the earthworm test.

Values for pH in soil slurry ranged from 3.58 to 7.84, and in soil supernatant from 3.59 to 7.74, with similar values reported by both meters (Table 8). Of the samples tested, only Cormorant Park had pH values in the acceptable range according to the protocol (pH < 5.0); all other samples had initial soil slurry or soil supernatant pH values which were below the acceptable range. As Cormorant Park was the only sample to show toxicity in both testing events, it appears that the low pH consistently present in these samples in both events is not sufficient to cause toxicity on its own.

Table 8. Initial and final pH values for *L. sativa* and *E. foetida* test on August 9, 2010

Soil Series	Sample ID (Nautilus Log-In Number)	Soil Slurry pH				Soil Supernatant pH			
		Nautilus Meter		WDOE Meter		Nautilus Meter		WDOE Meter	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final
(*L. sativa*) Lettuce									
	Negative Control	6.98	7.04	7.10	7.75	6.85	7.05	7.04	7.70
Everett	Cormorant Park (S10-446)	5.10	4.46	5.14	5.11	5.05	4.52	5.24	5.06
Everett	Burton Acres (S10-444)	3.58	2.93	3.79	3.63	3.59	3.17	3.80	3.61
(*E. foetida*) Earthworm									
	Negative Control	6.98	7.16	7.10	7.84	6.85	7.24	7.04	7.74
Everett	Cormorant Park (S10-446)	5.10	4.59	5.14	5.25	5.05	4.75	5.24	5.26
Everett	King Co. Owned (S10-454)	4.51	4.07	4.89	4.70	4.57	4.13	4.54	4.70
Harstine	Kopachuck-HAR (S10-451)	4.76	3.98	4.75	4.52	4.63	4.00	4.74	4.56
Harstine	Morford's (S10-455)	4.80	4.03	4.92	4.73	4.76	4.17	4.89	4.74

Table 9. Initial and final conductivity values for *E. foetida* test on August 9, 2010

Soil Series	Sample ID	Nautilus Log-In Number	Soil Slurry Conductivity (µS/cm)		Soil Supernatant Conductivity (µS/cm)	
			Initial	Final	Initial	Final
	Negative Control		508	422	580	453
Everett	Cormorant Park	S10-446	251	498	244	522
Everett	King Co. Owned	S10-454	268	250	226	246
Harstine	Kopachuck-HAR	S10-451	152	300	159	315
Harstine	Morford's	S10-455	127	246	118	252

Table 10. Summary of Chemical/Physical Characteristics measured during *L. sativa* testing

Parameter	Criteria	Count	Minimum	Maximum	Average	Acceptable? Samples affected
Initial pH (Slurry)	>5.0	3	3.58	6.98	5.22	No Cormorant Park[1], Burton Acres[1]
Initial pH (Supernatant)	>5.0	3	3.59	6.85	5.16	No Cormorant Park[1], Burton Acres[1]
Temperature (°C)	20-30	15	23.0	25.0	24.0	Yes
Light Reading (Lux)	>1000	3	3032	6430	3187	Yes

[1]Deviation from protocol not expected to influence results of the test

Table 11. Summary of Chemical/Physical Characteristics measured during *E. foetida* testing

Parameter	Criteria	Count	Minimum	Maximum	Average	Acceptable? Samples affected
Initial Moisture Fraction (%)	35-45	5	38.9	62.3	48.4	No[1] King Co. Owned, Morford's,
Initial Slurry pH	>5.0	5	4.51	6.98	5.23	No[1] Kopachuck-HAR, King Co. Owned, Morford's
Initial Supernatant pH	>5.0	5	4.57	6.85	5.17	No[1] Kopachuck-HAR, King Co. Owned, Morford's
Initial Slurry Conductivity (μS/cm)		5	127	508	261	Yes
Initial Supernatant Conductivity (μS/cm)		5	118	580	265	Yes
Temperature (°C)	22±2	15	20.4	21.0	20.7	Yes

DISCUSSION

The extent and degree of toxicity observed in the initial round of tests were reduced in the current test series. All sites that exhibited effects in the initial testing, specifically Cormorant Park, King Co. Owned and Morford's (earthworm), and Burton Acres (lettuce), exhibited improved survival and sublethal responses in the current test series. However, because the pH of these samples did not change over the same time period, these results suggest that 1) low pH was not the cause of toxicity in the samples when originally tested; and 2) the cause of toxicity dissipated over the 10 week period the samples were in holding. In other words, the toxicity observed in the initial tests with these samples appears to be related to toxicity associated with the samples, and not the pH itself.

QA/QC

The second series of testing was initiated outside the two-week holding time by request, in order to follow-up on the initial responses observed with the samples of interest. Another deviation from the protocol was running the tests despite exhibiting low pH values at the time of initiation; this was also done by request to confirm the presence or absence of effects related to low pH. In addition, some samples contained more than the 45 percent moisture content recommendation for earthworms. No additional manipulation was done on these samples, and the extra moisture did not appear to have affected the results based on comparison to control performance. One replicate in the earthworm test for sample King Co. Owned was initiated with 11 organisms instead of the required 10. The starting number was corrected for in statistical analysis and this deviation is not expected to have influenced the results. Both tests met the acceptability criterion for negative control performance. Temperature and light readings remained within parameters for the duration of the test.

Results of reference toxicant test (positive control) conducted with the test organisms are provided in Table 12. EC50 values fell within the acceptable range of mean ± two standard deviations for historical data, indicating that the test organisms exhibited an appropriate degree of sensitivity.

Table 12. Reference toxicant test results

Species	Endpoint	Date Initiated	EC50	Historical range (mean ± 2 SD)	Coefficient of Variation (%)
Lactuca sativa	Survival	8/9/2010	179 mg/kg H_3BO_3	113 – 385	27.3
	Biomass	8/9/2010	126 mg/kg H_3BO_3	98.0 – 198	16.9
Eisenia foetida	Survival	8/9/2010	54.3 mg/kg 2-Chloroacetamide	4.04 – 81.6	45.3

REFERENCES

American Society of Testing and Materials (ASTM). 1999. Standard guide for conducting terrestrial plant toxicity tests. ASTM designation E1963-98.

American Society of Testing and Materials (ASTM). 1994. Standard practice for conducting early seedling growth tests. ASTM designation E1598-94.

Biostat. DMMP/SMS Bioassay Statistics Program for Microsoft Windows. Developed by Corps of Engineers, Seattle District

Washington State Department of Ecology (WDOE). 1996. Early seedling growth protocol for soil toxicity screening. WDOE Environmental Investigations and Laboratory Services Program Publication No. 96-324.

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Appendix E. Wildlife Exposure Model

MTCA Wildlife Exposure Model Parameters

Table E- 1. Wildlife exposure model parameter values.

Source	Parameter	Receptor Group	Surrogate Receptor	TRV mg dw/ Kg body/ day	FIR Kg food dw/ Kg body/ day	P	SIR Kg dw soil/ Kg body/ day	RGAF
MTCA	Arsenic	Mammalian Herbivore	vole	35	0.315	1	0.0079	1
MTCA	Arsenic	Mammalian Predator	shrew	35	0.45	0.5	0.0045	1
MTCA	Arsenic	Avian Predator	Robin	22	0.207	0.52	0.0215	1
MTCA	Lead	Mammalian Herbivore	vole	20	0.315	1	0.0079	1
MTCA	Lead	Mammalian Predator	shrew	20	0.45	0.5	0.0045	1
MTCA	Lead	Avian Predator	Robin	11.3	0.207	0.52	0.0215	1
EPA	Arsenic	Mammalian		1.04				
EPA	Arsenic	Avian		2.24				
EPA	Lead	Mammalian		4.7				
EPA	Lead	Avian		1.63				
Doctor, et al. 2000	Arsenic	Mammalian Herbivore	Northern Pocket Gopher		0.315	1	0.0079	1
Doctor, et al. 2000	Arsenic	Mammalian Predator	deer mouse		0.26	0.25	0.0045	1
Doctor, et al. 2000	Arsenic	Avian Predator	Western Meadowlark		0.207	0.25	0.0215	1
Doctor, et al. 2000	Lead	Mammalian Herbivore	Northern Pocket Gopher		0.315	1	0.0079	1
Doctor, et al. 2000	Lead	Mammalian Predator	deer mouse		0.26	0.25	0.0045	1
Doctor, et al. 2000	Lead	Avian Insectivore	Western Meadowlark		0.207	0.25	0.0215	1

Dw: Dry weight.
EPA: U.S. Environmental Protection Agency.
FIR: Food Ingestion Rate.
MTCA: Model Toxics Control Act.
P: Diet Contamination.
RGAF: Gut Adsorption Factor.
SIR: Soil Ingestion Rate.
TRV: Toxicity Reference Value.

Table E- 1 continued. Wildlife exposure model parameter values.

Source	Parameter	Receptor Group	Surrogate Receptor	TRV mg dw/ Kg body/ day	FIR Kg food dw/ Kg body/ day	P	SIR Kg dw soil/ Kg body/ day	RGAF
USDOE, 2008	Arsenic	Avian Herbivore	California quail		0.078	1	0.0053	1
USDOE, 2008	Arsenic	Mammalian Omnivore	deer mouse		0.18	0.5	0.002	1
USDOE, 2008	Arsenic	Mammalian Omnivore	deer mouse		0.18	0.5	0.002	1
USDOE, 2008	Arsenic	Mammalian Predator	Grasshopper Mouse		0.15	1	0.002	1
USDOE, 2008	Arsenic	Avian Predator	Killdeer		0.21	1	0.016	1
USDOE, 2008	Arsenic	Avian Omnivore	Meadowlark		0.2	0.37	0.042	1
USDOE, 2008	Arsenic	Avian Omnivore	Meadowlark		0.2	0.63	0.042	1
USDOE, 2008	Arsenic	Mammalian Herbivore	Pocket Mouse		0.18	1	0.0023	1
USDOE, 2008	Lead	Avian Herbivore	California quail		0.078	1	0.0053	1
USDOE, 2008	Lead	Mammalian Omnivore	deer mouse		0.18	0.5	0.002	1
USDOE, 2008	Lead	Mammalian Omnivore	deer mouse		0.18	0.5	0.002	1
USDOE, 2008	Lead	Mammalian Predator	Grasshopper Mouse		0.15	1	0.002	1
USDOE, 2008	Lead	Avian Predator	Killdeer		0.21	1	0.016	1
USDOE, 2008	Lead	Avian Omnivore	Meadowlark		0.2	0.37	0.042	1
USDOE, 2008	Lead	Avian Omnivore	Meadowlark		0.2	0.63	0.042	1
USDOE, 2008	Lead	Mammalian Herbivore	Pocket Mouse		0.18	1	0.0023	1

Dw: Dry weight.
FIR: Food Ingestion Rate.
P: Diet Contamination.
RGAF: Gut Adsorption Factor.
SIR: Soil Ingestion Rate.
TRV: Toxicity Reference Value.
USDOE: U.S. Department of Energy.

MTCA Wildlife Exposure Model Results

Soil screening levels (SSLs) were calculated using the model described in Figure 12. Plant uptake (K) and bioaccumulation factor (BAF) values are the median of an area or of the entire study. Both EPA and MTCA TRV values were used in the model as were a variety of sources for surrogate species parameter values. Tables E-2 to E-5 show the results of the model calculations.

Arsenic SSLs

Table E- 2. Arsenic SSLs using MTCA surrogate species values in mg/Kg dw.

Receptor Group	Surrogate Receptor	Area Represented					
		All Study Sites		Tacoma Smelter Plume Footprint		Hanford Old Orchards	
TRV Source->		EPA, 2005b	MTCA	EPA, 2005b	MTCA	EPA, 2005b	MTCA
Mammalian Herbivore	Vole	60	2014	88	2957	36	1209
Mammalian Predator	Shrew	12	388	11	368	14	466
Avian Predator	American Robin	36	352	35	339	41	398
Lowest		12	352	11	339	14	398

Table E- 3. Arsenic SSLs for Hanford Old Orchards in mg/Kg dw.

Receptor Group	Surrogate Receptor	Surrogate Receptor Parameter Source			
		Doctor et al., 2000		USDOE, 2008	
TRV Source->		EPA, 2005b	MTCA	EPA, 2005b	MTCA
Mammalian Herbivore	Northern Pocket Gopher	36	1209		
Mammalian Herbivore	Pocket Mouse			73	2443
Avian Herbivore	California Quail			213	2093
Mammalian Omnivore	Deer Mouse			29	254
Avian Omnivore	Western Meadowlark			26	965
Mammalian Predator	Deer Mouse	42	1405		
Mammalian Predator	Grasshopper Mouse			21	713
Avian Predator	Killdeer			27	268
Avian Predator	Western Meadowlark	59	583		
Lowest		36	583	21	254

Lead SSLs

Table E- 4. Lead SSLs using MTCA surrogate species values in mg/Kg dw.

Receptor Group	Surrogate Receptor	Area Represented					
		All Study Sites		Tacoma Smelter Plume Footprint		Hanford Old Orchards	
TRV Source->		EPA, 2005c	MTCA	EPA, 2005c	MTCA	EPA, 2005c	MTCA
Mammalian Herbivore	Vole	272	1217	286	1157	228	971
Mammalian Predator	Shrew	87	372	73	309	550	2339
Avian Predator	American Robin	36	251	32	224	70	482
Lowest		36	251	32	224	70	482

Table E- 5. Lead SSLs for Hanford Old Orchards in mg/Kg dw.

Receptor Group	Surrogate Receptor	Surrogate Receptor Parameter Source			
		Doctor et al., 2000		USDOE, 2008	
TRV Source->		EPA, 2005c	MTCA	EPA, 2005c	MTCA
Mammalian Herbivore	Northern Pocket Gopher	228	971		
Mammalian Herbivore	Pocket Mouse			492	2093
Avian Herbivore	California Quail			193	1338
Mammalian Omnivore	Deer Mouse			649	2760
Avian Omnivore	Western Meadowlark			34	239
Mammalian Predator	Deer Mouse	829	3527		
Mammalian Predator	Grasshopper Mouse			1000	4255
Avian Predator	Killdeer			82	571
Avian Predator	Western Meadowlark	73	504		
Lowest		73	504	34	239

EPA Exposure Model

The EPA exposure model is slightly different from the MTCA model. The equation is:

$$HQ=[FIR*(SSL*P_s+B_i)]/TRV$$

Where B_i is the K or BAF value, HQ = Hazard Quotient = 1, and
P_s= Soil ingestion as proportion of diet.

The EPA exposure model SSL values were not used in the report but are displayed in Tables E-7 and E-8.

Table E- 6. EPA wildlife exposure model parameter values.

Parameter	Receptor Group	Surrogate Receptor	TRV mg dw/ Kg body/day	FIR Kg food dw/ Kg body/day	P_s
Arsenic	Mammalian Herbivore	Vole	1.04	0.0875	0.032
Arsenic	Avian Herbivore	Dove	2.24	0.190	0.139
Arsenic	Mammalian Predator	Shrew	1.04	0.209	0.030
Arsenic	Avian Predator	Woodcock	2.24	0.214	0.164
Lead	Mammalian Herbivore	Vole	4.7	0.0875	0.032
Lead	Avian Herbivore	Dove	1.63	0.190	0.139
Lead	Mammalian Predator	Shrew	4.7	0.209	0.030
Lead	Avian Predator	Woodcock	1.63	0.214	0.164

Table E- 7. Arsenic SSLs using EPA exposure model and values in mg/Kg dw.

Receptor Group	Surrogate Receptor	Area Represented					
		All Study Sites		Tacoma Smelter Plume Footprint		Hanford Old Orchards	
TRV Source ->		EPA, 2005b	MTCA	EPA, 2005b	MTCA	EPA, 2005b	MTCA
Mammalian Herbivore	Vole	370	12499	371	12500	369	12498
Avian Herbivore	Dove	85	833	85	833	84	833
Mammalian Predator	Shrew	153	5569	152	5569	155	5572
Avian Predator	Woodcock	62	625	61	624	62	625
Lowest		62	625	61	624	62	625

Table E- 8. Lead SSLs using EPA exposure model and values in mg/Kg dw.

Receptor Group	Surrogate Receptor	Area Represented					
		All Study Sites		Tacoma Smelter Plume Footprint		Hanford Old Orchards	
TRV Source ->		EPA, 2005c	MTCA	EPA, 2005c	MTCA	EPA, 2005c	MTCA
Mammalian Herbivore	Vole	1678	7142	1678	7142	1677	7142
Avian Herbivore	Dove	62	428	62	428	61	428
Mammalian Predator	Shrew	742	3182	741	3181	749	3189
Avian Predator	Woodcock	45	321	45	320	46	322
Lowest		45	321	45	320	46	322

Appendix F. Additional Information

Copper LOAEL Calculation Table

Table F- 1. Copper concentrations with summary of bioassay results.

Location	Copper in Soil (mg/Kg dw)	Earthworm Survival	Earthworm Sublethal	Lettuce Survival	Lettuce Biomass
TSP Area					
KOPA-Har *	13.4				
KOPA-Har *^	13.4				
IDLEWILD-Spn *	20				
WING-Ald *	22.3				
PTROB-Kit	25.5				
WING-Kit *	32.8				
TSP Lettuce ↓LOAEL					
FTSTEILGC-Spn	39.9				Con & Ref
TACNAR-Har-UNK	42.8				
CORMOR-Evt *	87	Con[1]			
CORMOR-Evt *^	87	Con[1]			
NEWTAC-Spn	101				Ref
BURTON-Evt	102				Con & Ref
BURTON-Evt ^	102				
TSP Earthworm ↓LOAEL					
MORFORD-Har	129	Con & Ref			
MORFORD-Har ^	129				
MIMP-Kit-UNK	195	Con	Con		
COLGATE-Ald	202				Ref
MIMP-Ald-UNK	220		Con		
KCO-Evt	235		Con		Ref
KCO-Evt ^	235				
HOO Area					
HOO-01	13				Ref
HOO-03	13				
HOO-06	14.3				
HOO-02	14.8				
HOO-04	16.5				
HOO-05*	57.55				
HOO Earthworm and Lettuce ↑NOAEL					

Results were compared to both the control and reference within each soil type.
If the soil type could not be verified (identified by the UNK) the location was only compared to the control.
Con: Significantly different when compared to the control.
Ref: Significantly different when compared to the reference for that soil type.
*: Reference location.
^: Soil re-tested due to failure of pH criteria.
[1]Results omitted from further bioassay analyses. See data quality section text for discussion.
LOAEL: Lowest observed adverse effect level (for comparisons to the control).
NOAEL: No observed adverse effect level (for comparisons to the control).
The gray bars for the copper soil concentrations are a visual aid to show increasing concentrations.

XRF Hanford Graphs

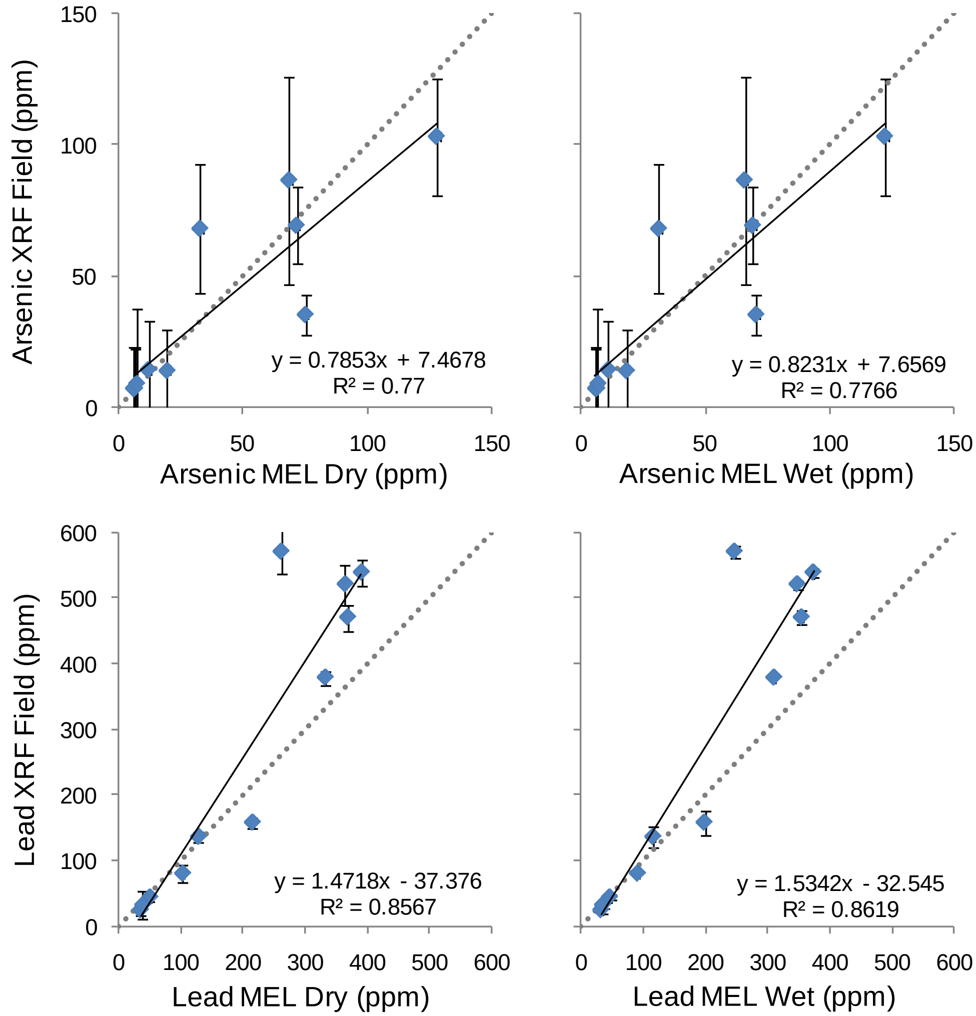

Figure F- 1. Hanford Old Orchards laboratory results compared to XRF field results for arsenic and lead.

Error bars represent the 1 sigma error on the counting statistics of the XRF measurement.

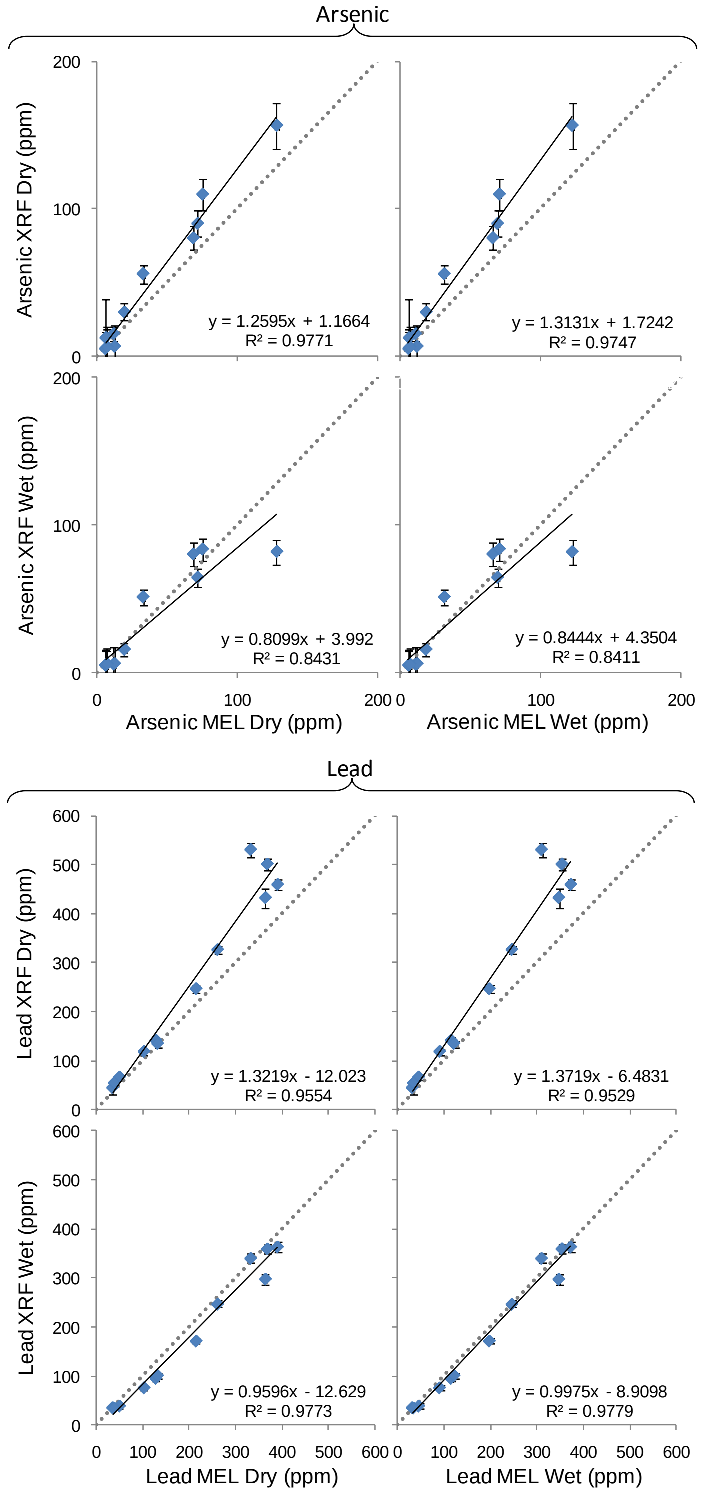

Figure F- 2. Hanford Old Orchards laboratory results compared to dry and wet soil sample XRF results for arsenic and lead.

Error bars represent the 1 sigma error on the counting statistics of the XRF measurement.

Total Arsenic Comparisons

Brooks Rand Laboratory (BRL) analyzed for total arsenic as part of the arsenic speciation method for a sub-set of locations. Ecology's Manchester Environmental Laboratory (MEL) analyzed all locations for arsenic. Figure F-3 shows the results for samples analyzed by both labs. Only three locations do not fall close to the 1:1 line, indicating different results between the labs. This difference is likely due to variability of concentrations in the soil rather than method.

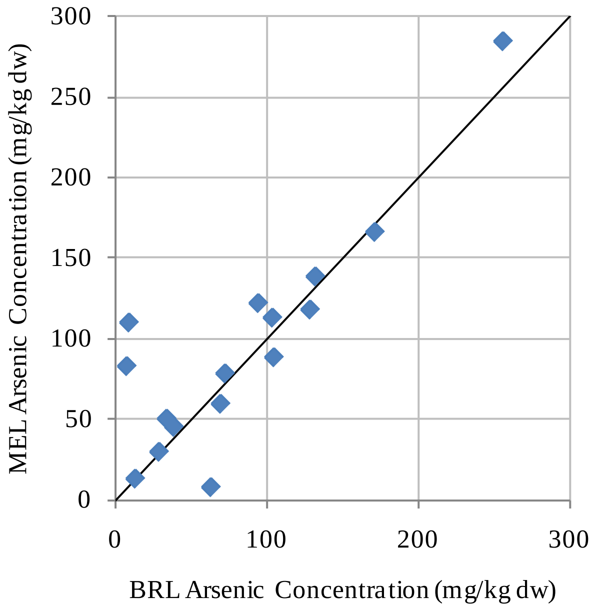

Figure F- 3. Comparison of BRL and MEL total arsenic values.

Tacoma Smelter Plume Footprint versus Hanford Old Orchards

Combining the two study areas for analysis was considered as part of this project to facilitate a more statewide approach to setting SSLs. However, it was decided to keep them separate due to several factors:

- Linear regressions of arsenic-versus-lead soil concentrations of the Tacoma Smelter Plume footprint and Hanford Old Orchards areas were compared. The slopes of the regressions were not statistically different, while the intercepts were statistically different. This indicates that the two data sets are distinct but parallel and therefore should be considered separately (Figure F-2).
- The wildlife exposure model receptors are unique to each area and resulted in different SSL values.
- These two locations are being considered individually for cleanup actions.

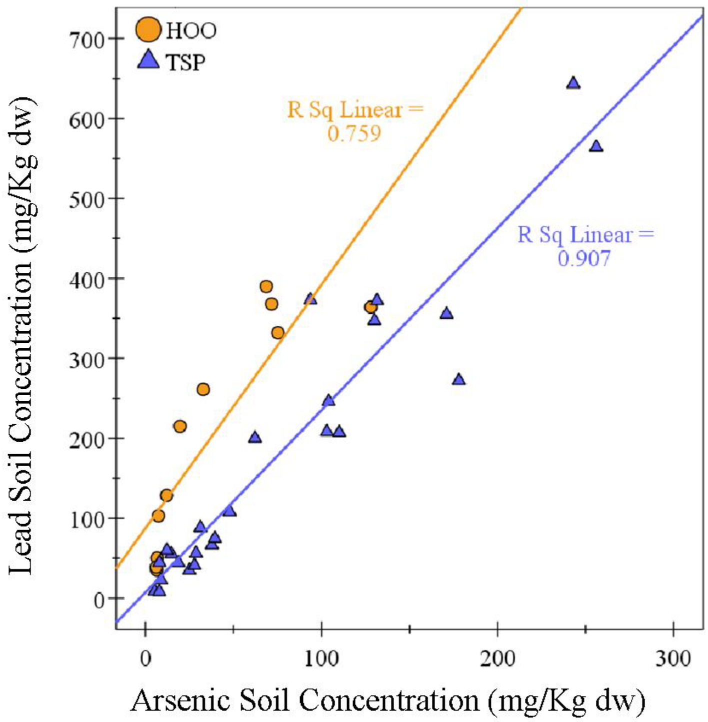

Figure F- 4. Arsenic vs. lead soil concentrations.

Soil Biota Percent Solids

During this project the goal was to collect 5 dry grams of soil biota to have enough mass for all analyses. Based on preliminary estimates of percent solids 50 wet grams of soil biota were targeted for collection. If using the minimum percent solids found of 13%, then 40 grams of soil biota would be needed. Therefore the approximation of 50 wet grams to result in 5 dry grams was a conservative goal.

Table F- 2. Soil biota wet weight, dry weight, and percent solids.

Location ID	Sample Type	Wet Weight (g)[1]	Dry Weight (g)	% Solids
HOO-1,2,3,6,&9	Beetle	Not measured	0.523	
BURTON-Evt	Insects	2	0.333	17
HOO-1,2,3,6,&9	Insects	Not measured	0.382	
MIMP-Ald-UNK	Insects	4.9	1.148	23
NEWTAC-Spn	Earthworm	Not measured	8.659	
FTSTEILP-Evt	Earthworm	Not measured	5.270	
WING-Kit	Earthworm	Not measured	9.301	
BONN-Kit	Earthworm	68.1	10.551	15
COLGATE-Ald	Earthworm	65	12.899	20
COLGATE-Ald Replicate	Earthworm	67.7	10.688	16
CORMOR-Evt	Earthworm	52	7.828	15
DOCKTON-Ald	Earthworm	30	4.725	16
FTSTEILGC-Spn	Earthworm	12	4.031	34
FTSTEILP-Spn	Earthworm	45	6.510	14
ICF-Evt	Earthworm	39.4	6.822	17
IDLEWILD-Spn	Earthworm	57	10.832	19
KCO-Evt	Earthworm	50	6.738	13
KOPA-Kit	Earthworm	54	9.011	17
LOWJOHN-Har	Earthworm	52	7.711	15
MIMP-Kit-UNK	Earthworm	51	8.046	16
MORFORD-Har	Earthworm	29	3.800	13
MORN-Evt	Earthworm	18.2	3.403	19
NEILLPT-Kit-UNK	Earthworm	54	7.630	14
PTROB-Kit	Earthworm	46	8.052	18
TACNAR-Har-UNK	Earthworm	62	10.452	17
THEMGIL-Ald	Earthworm	49	8.304	17
WING-Ald	Earthworm	47.6	7.518	16
			Median	16
			Mean	17
			Standard Deviation	4
			Minimum	13
			Maximum	34

[1]Wet weight is approximate. Worms were rinsed and then weighed; some additional water weight may be included here.

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Appendix G. Earthwork Biomarker Report

Tacoma Smelter Plume:
An opportunity to determine environmental risk using earthworms as bio indicators

By
Josh Sullivan
University Of Washington Tacoma
November 2010

Introduction

Earthworms have become increasingly studied as many correlations between high levels of metals in soil and worms have been found (Suthar and Singh, 2009). Earthworms with high metal content can pose a potential risk across trophic levels because they are the base of many food chains (Suthar et al., 2007). Worms are especially important to ecological processes, contributing greatly to the development and maintenance of soil structures, and are therefore excellent indicators of stresses on local ecosystems (Edwards and Bohlen, 1996).

Earthworms ingest soil to obtain food which leads to an increased exposure to metals in soils, some of which have 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 different metal concentrations in soils as having measurable physiological effects on MT and therefore qualifying the metal concentration levels at which earthworms begin to exhibit physiological effects.

The Tacoma Smelter Plume footprint is an example of an area where high concentrations of arsenic and lead may pose a health risk to earthworms. Under the Washington State Department of Ecology's Model Toxic Control Act (MTCA), human health cleanup levels for the Tacoma Smelter Plume footprint are 20 mg/kg for arsenic (III) and 250 mg/kg for lead, whereas ecological levels are set at 7 mg/kg for arsenic (III), 10 mg/kg arsenic (V) and 118 mg/kg for lead. These are the soil screening levels used to determine ecological risk at contaminated sites under the MTCA. Current wildlife cleanup levels have been established using tests conducted under controlled laboratory conditions and wildlife exposure models. However, laboratory tests used to set screening levels for earthworms may not be representative of actual effects of arsenic and lead on these organisms (Sloan, 2010).

This study focuses on earthworms collected in soils from the Tacoma Smelter Plume footprint to determine stresses on MT under different known arsenic and lead soil concentrations. Using an MT analysis method we hope to correlate these concentrations with measurable physiological changes in the organisms.

Methods

Study area

Sampling was completed March - May 2010 within the Tacoma Smelter Plume footprint (Figure G-1) in and around Tacoma and Vashon, Washington. Twenty-four sites within the area of the Tacoma Smelter Plume were chosen for known levels of arsenic and lead in the soil. Within each site, arsenic and lead concentrations were measured in the soil using an Innov-X Systems X-ray Fluorescence Instrument (XRF). Once the arsenic and lead levels were quantified, the perimeter of the sampling area was determined by using dice to randomize distance from the center in four cardinal directions. The randomized sample area was used to get a representative soils sample.

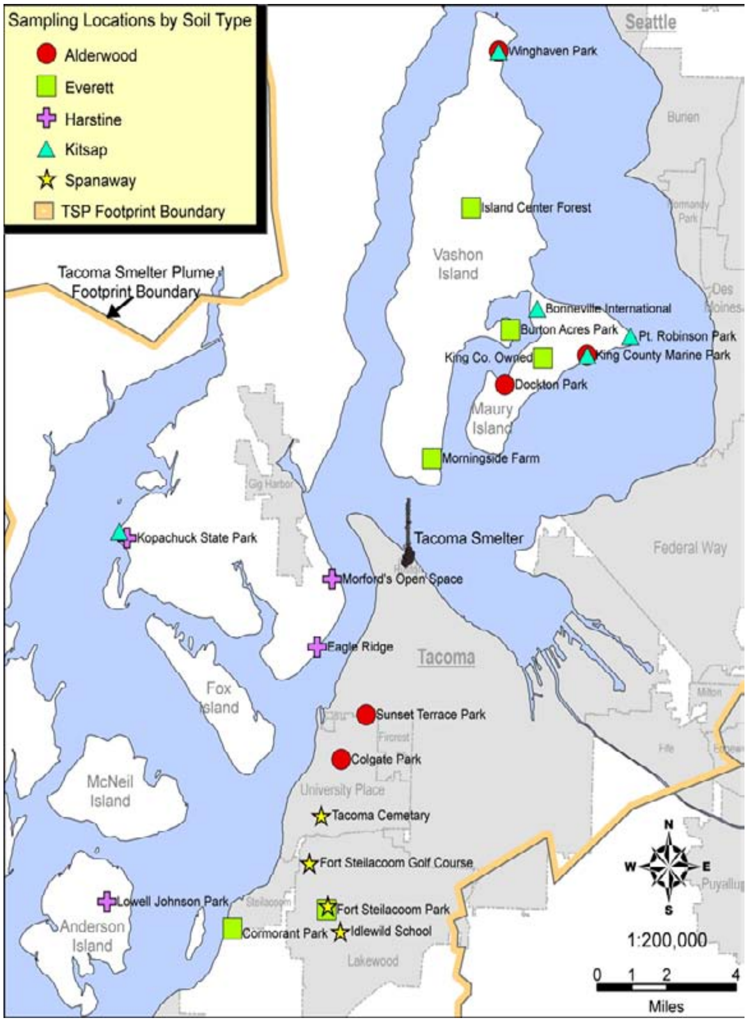

Figure G-1. Sampling area and soil types (Sloan, 2010).

Soil sampling

Soil was collected at four random points at each site using acid-washed utensils and bowls which were then covered with aluminum foil to protect the contents from contamination. Soil was sampled within 0 - 6 in. of the surface because it is the most biologically active zone (Suthar and 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 Ecology's Manchester Environmental Laboratory (MEL) for metal analysis by EPA method 200.8 (inductively coupled plasma/mass spectrometry).

Worm sampling

Worms were sampled within 10 feet of the center of the collection site. They were hand-collected, weighed, and then 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. This procedure was used to avoid contaminating the sample with soil in 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. Earthworms from 10-day bioassays conducted on the same soils by Sloan (2010) were handled similarly to field-collected earthworms after testing. The bioassays followed the protocol of Norton (1996).

Metallothionein (MT) analysis

Field-collected and bioassay earthworms were analyzed for MTs at University of Washington – Tacoma using the Viarengo et al. (1997) spectrophotometric method. 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.

Statistical analysis

Statistical analysis was done using Microsoft Excel. A Pearson correlation matrix was employed to investigate relationships between variables and a scatter plot made to examine relationship between variables. A Pearson probability matrix was also created to test the significance between the variables at the 95% level.

Results/Discussion

Metal content

Metal concentrations in the worms were significantly correlated with metal concentrations in the soils. There is a strong indication that soil arsenic has an influence on arsenic concentration in worms (Table G-1, Figure G-2). Arsenic was highly correlated ($p<0.01$) between soil and worms. This finding is consistent with a number of studies that have shown arsenic readily accumulates in the tissue of earthworms (Suthar and Sushma, 2009; Carpenèa et al., 2006; Meharg et al., 1998).

Lead soil concentrations were strongly correlated with arsenic soil concentrations, indicative of soils in the Tacoma Smelter Plume footprint. However, lead did not have a significant correlation in worm tissue (Table G-2, Figure G-3). This is to be expected as studies have shown that there is high variability between lead availability to earthworms (Smith et al., 2010). Even though lead in the soil did not correlate with higher concentrations in worm tissue, the overall results indicate that arsenic concentration in the soils do increase these concentrations in the worms. The results solidify worms as bio-indicators, as the metal concentrations in the tissue correspond with increased presence of metals in the soils.

Table G-1. Pearson correlations Mean Mole-Sulfhydryl/g.

	MEAN (mol-SH/g dry weight)	As Earthworms (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 weight)	1.000	0.125	0.270	-0.059	0.176
As Soil Biota (mg/kg dry weight)		1.000	0.703	0.677	0.689
As Soil (mg/kg dry weight)			1.000	0.184	0.949
Pb soil biota (mg/kg dry weight)				1.000	0.228
Pb soil (mg/kg dry weight)					1.000

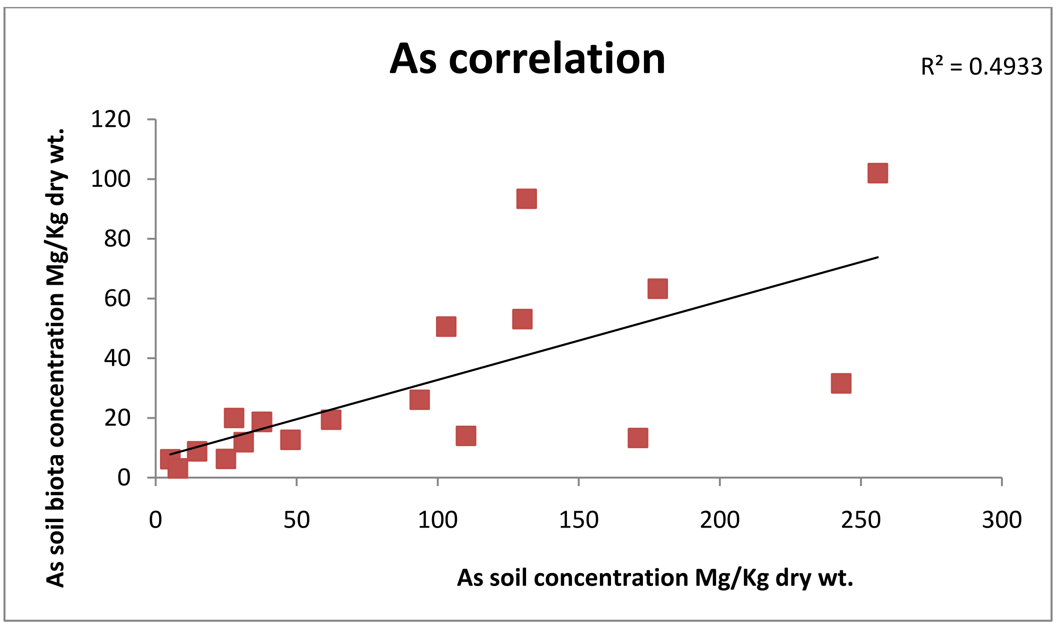

Figure G-2. Arsenic concentrations in soil vs. earthworms.

Table G-2. Pearson probabilities Mean mole-Sulfhydryl/g.

	MEAN (mol-SH/g dry weight)	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 weight)	-	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)					-

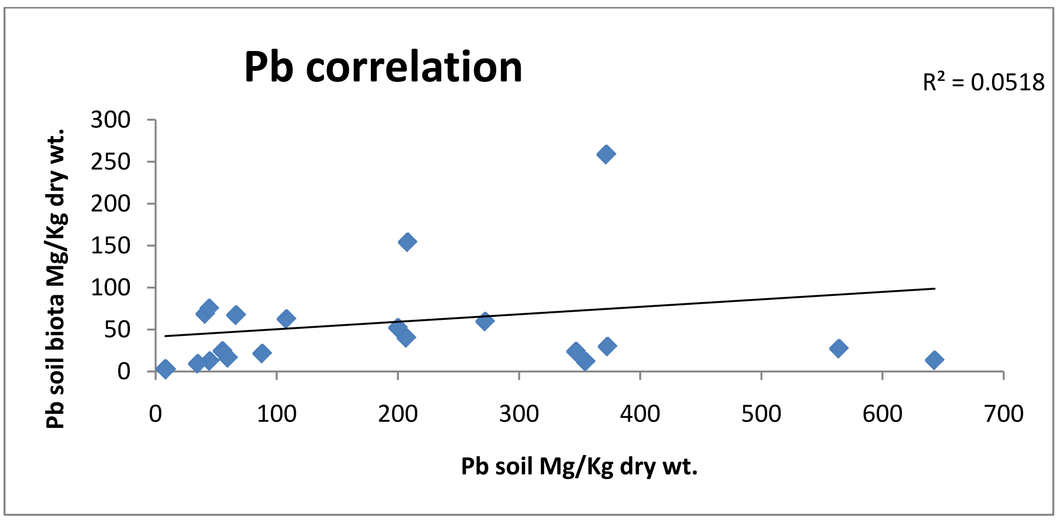

Figure G-3. Lead concentrations in soil vs. earthworms.

MT

MT in the worms did not have a statistically significant correlation to soil metal concentrations. As shown in Figures G-4 and G-5, there is no apparent trend that follows metal concentration in the soils, but the significance is far too poor (R2= .014 to .022) to infer any significant correlations.

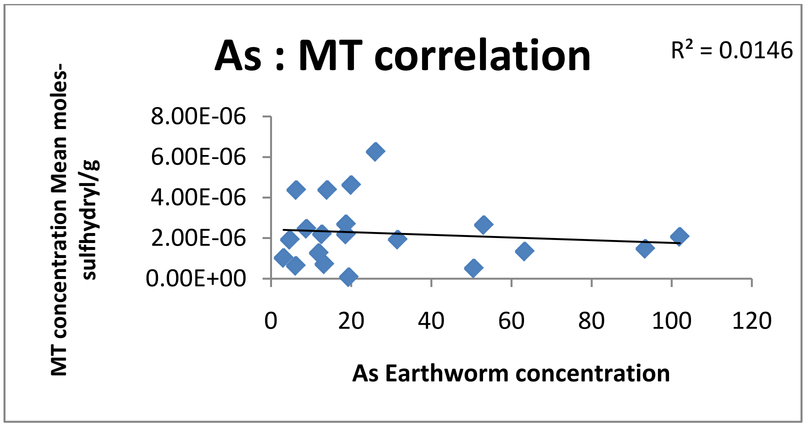

Figure G-4. Correlation between arsenic and MTs in earthworms.

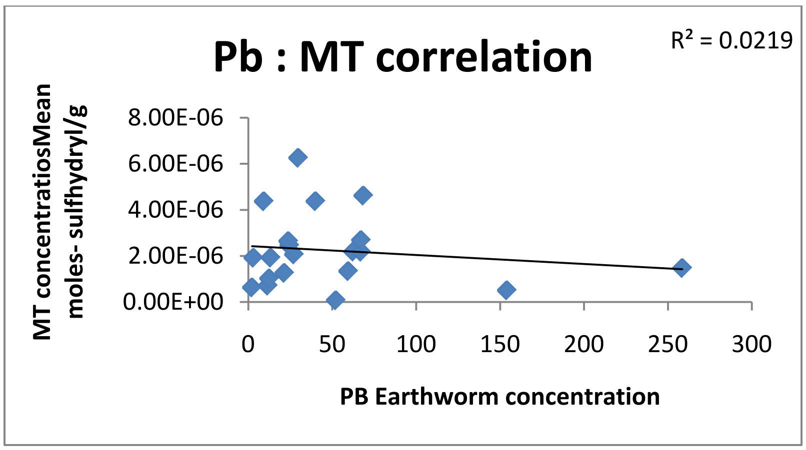

Figure G-5. Correlation between lead and MTs in earthworms.

There are studies that indicate that metal concentrations in soils and tissue do have a statistically significant probability of measurable increase in MT reactions. Stürzenbaum et al. (2001) found that MT has a significant role in the sequestering and detoxification of Cadmium in worms. The detoxification process in worms causes stresses in MT proteins which is a measurable reaction.

Our study did not prove any significant correlation between arsenic, lead, and MT and this may be due to the amount of samples processed. Each sample was homogenized from the total mass of worm collected at each site and then freeze-dried. Two samples per bag were run which accounts for the large variability in MT. The lack of samples may account for the weak correlation between soil and MT concentration. These results may also indicate that concentrations of metals in the Tacoma Smelter Plume may not be causing protein stresses in worms and therefore not causing quantifiable physiological effects. If this is the case then the current MTCA soil screening levels, if based on soil biota, may be inappropriate. If MTCA soil screening levels are to be indicative of actual in-situ conditions then more samples should be run in order to prove or disprove correlation between MT concentrations and increased physiological responses of organisms.

Bioassay Earthworms

The bioassay earthworms showed a difference in MT concentrations compared to the field-collected earthworms (Figure G-6). A paired two-tailed T-Test (P=0.00) illustrated that the in-situ worms had higher concentrations of MT than the bioassay earthworms. 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.

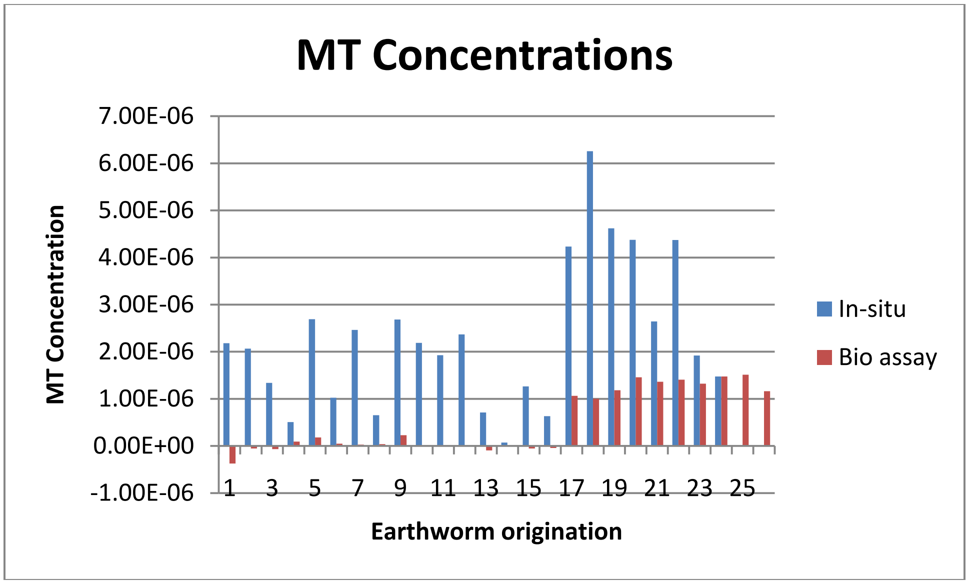

Figure G-6. MT concentrations in earthworms.

Conclusion

Because there is no significant correlation between MT concentrations and soil metals we can infer that the current MTCA ecological cleanup levels for arsenic and lead may be too high for earthworms. In-situ worms exhibit the ability to sequester toxic material and maintain a physiological balance due to soil variables which cannot be recreated in the lab (Meharg et al., 1998). 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 cleanup levels. More studies are needed to determine what levels of arsenic and lead cause earthworms to exhibit a quantifiable physiological response. MT concentrations in earthworms are an effective biomarker for soil metal concentration; however, more relevant studies for ecological responses of in-situ worms (i.e. mortality rates, reproduction etc.) should 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.
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References

Carpenèa, E., ANDREANI, g, MONARI, M., CASTELLANI, G. and 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.

MEHARG, A. A., SHORE, R. F. and BROADGATE, K., 1998. Edaphic factors affecting the toxicity and accumulation of arsenate in the earthworm *Lumbricus terrestris*. Environmental Toxicology and Chemistry, 17, 1124-1131.

Norton, D., 1996. Earthworm Bioassay Protocol for Soil Toxicity Screening. Washington State Department of Ecology, Olympia, WA. Publication No. 96-327. www.ecy.wa.gov/biblio/96327.html.

Sloan, J., 2010. Washington State. Quality Assurance Project Plan, 2010. Web. 14 Nov 2010.

SMITH, B. A., GREENBERG, B. and STEPHENSON, G. L., 2010. Comparison of biological and chemical measures of metal bioavailability in field soils: Test of a novel simulated earthworm gut extraction. Chemosphere, 81, 755-766.

Stürzenbaum, S. R., WINTERS, C., GALAY, M., MORGAN, A. J. and KILLE, P., 2001. Metal ion trafficking in earthworms - Identification of a cadmium-specific metallothionein. Journal of Biological Chemistry, 276, 34013-34018.

SUTHAR, S. and 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.

SUTHAR, S., SINGH, S., and DHAWAN, S., 2007. Earthworms as bioindicator of metals (Zn, Fe, Mn, Cu, Pb and Cd) in soils: Is metal bioaccumulation affected by their ecological category? Ecological Engineering, 32, 99-107.

VIARENGO, A., PONZANO, E., DONDERO, F. and 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.

Appendix for Appendix G

Table A-1 for Appendix G. Field-collected earthworm Metallothionein (MT) results.

Site Name	MT Result 1	MT Result 2	Average MT Result
Bonneville International	3.31E-04	4.43E-04	3.87E-04
Colgate Park	1.20E-04	2.78E-04	1.99E-04
Colgate Park Rep	1.22E-05	1.07E-04	5.94E-05
Cormorant Park	8.22E-05	2.74E-05	5.48E-05
Dockton Forest	4.11E-04	3.26E-04	3.69E-04
Fort Steilacoom Golf Course	1.04E-04	6.70E-05	8.52E-05
Fort Steilacoom Park (Evt)	6.09E-06	1.00E-04	5.33E-05
Fort Steilacoom Park (Spn)	2.49E-04	4.82E-04	3.65E-04
Idlewild School	6.09E-05	2.86E-04	1.74E-04
Island Center Forest	1.54E-04	2.90E-04	2.22E-04
King Co. Owned	1.67E-04	4.57E-05	1.07E-04
Kopachuck State Park (Kit)	3.04E-06	3.62E-04	1.83E-04
Lowell Johnson Park	1.54E-04	1.71E-04	1.63E-04
Maury Island Marine (Kit)	1.49E-04	1.73E-04	1.61E-04
Maury Island Marine Park (Ald)	6.09E-06		6.09E-06
Morford’s Open Space	7.00E-05	2.95E-04	1.83E-04
Morningside Farm	5.52E-04	4.96E-04	5.24E-04
Neill Point Natural Area	6.15E-05	6.54E-04	3.58E-04
Pt. Robinson Park	3.58E-05	4.99E-05	4.28E-05
Tacoma Cemetery	1.54E-04	2.97E-04	2.25E-04
Tacoma Narrows Park	1.44E-04	2.68E-04	2.06E-04
Thelma Gilmer	4.87E-05	1.98E-04	1.23E-04
Winghaven Park (Ald)	1.00E-04	1.25E-04	1.12E-04
Winghaven Park (Kit)	1.60E-04	2.92E-04	2.26E-04

Table A-2 for Appendix G. Bioassay earthworm Metallothionein (MT) results.

Site Name	MT Result 1	MT Result 2	Average MT Result
Negative Control	-6.24E-05	1.99E-05	-4.54E-08
Reference Toxicant 0 mg/Kg 2-chloroacetamide	6.09E-04	3.27E-04	1.06E-06
Reference Toxicant 10 mg/Kg 2-chloroacetamide	5.83E-05	1.89E-05	9.15E-08
Reference Toxicant 20 mg/Kg 2-chloroacetamide	1.34E-04	2.17E-05	1.81E-07
Reference Toxicant 40 mg/Kg 2-chloroacetamide	5.27E-05	-1.21E-05	4.95E-08
Burton Acres Park	-4.12E-06	6.17E-06	2.66E-09
Colgate Park	5.17E-04	5.57E-04	1.16E-06
FSGC Idle Mix	6.84E-04	5.17E-04	1.36E-06
Ft. Steilacoom Golf Course	7.21E-04	5.29E-04	1.41E-06
Idlewild School	4.58E-04	4.27E-04	1.00E-06
King County Owned	5.33E-04	4.63E-04	1.09E-06
Kopachuck State Park (Har)	-6.57E-05	1.89E-05	-5.47E-08
Maury Island Marine Park (Ald)	-6.93E-05	9.88E-05	3.89E-08
Maury Island Marine Park (Kit)	-6.58E-05	9.53E-05	2.78E-08
Morford's Open Space	6.39E-04	5.11E-04	1.32E-06
Pt. Robinson Park	5.08E-05	-5.21E-05	-2.98E-09
Tacoma Cemetery	-4.18E-05	3.70E-05	-2.73E-09
Tacoma Narrows Park	-5.90E-05	-3.16E-05	-9.51E-08
Winghaven Park (Ald)	-1.10E-05	-3.84E-05	-5.56E-08
Winghaven Park (Kit)	-3.16E-05	4.05E-05	8.39E-09
HOO 01	9.27E-04	4.51E-04	1.46E-06
HOO-02	2.67E-05	1.84E-04	2.27E-07
HOO-03	-4.59E-05	-1.21E-05	-6.88E-08
HOO-04	8.23E-04	5.02E-04	1.51E-06
HOO-05	-5.72E-05	-2.60E-04	-3.73E-07
HOO-06	6.25E-04	6.62E-04	1.47E-06

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Appendix H. Glossary, Acronyms, and Abbreviations

Glossary

Anthropogenic: Human-caused.

Bioassay: Usually a laboratory test which exposes organisms to the medium of interest (e.g., amphipod exposure to sediment). Results indicate the toxicity of the medium to that particular organism.

Biota: Flora (plants) and fauna (animals).

Depuration: Process of evacuating the guts of an organism by fasting.

Footprint: Area impacted by a pollution source, instead here to indicate the area impacted by emission from the Tacoma Smelter.

Parameter: Water quality constituent being measured (analyte). A physical, chemical, or biological property whose values determine environmental characteristics or behavior.

pH: A measure of the acidity or alkalinity of water. A low pH value (0 to 7) indicates that an acidic condition is present, while a high pH (7 to 14) indicates a basic or alkaline condition. A pH of 7 is considered to be neutral. Since the pH scale is logarithmic, a water sample with a pH of 8 is ten times more basic than one with a pH of 7.

Pollution: Such contamination, or other alteration of the physical, chemical, or biological properties, of any waters of the state. This includes change in temperature, taste, color, turbidity, or odor of the waters. It also includes discharge of any liquid, gaseous, solid, radioactive, or other substance into any waters of the state. This definition assumes that these changes will, or are likely to, create a nuisance or render such waters harmful, detrimental, or injurious to (1) public health, safety, or welfare, or (2) domestic, commercial, industrial, agricultural, recreational, or other legitimate beneficial uses, or (3) livestock, wild animals, birds, fish, or other aquatic life.

N^{th} Percentile: A statistical number obtained from a distribution of a data set, above which (100-N)% of the data exists and below which N% of the data exists.

Sublethal: Toxicity effects other than mortality. Effects may include lesions, behavioral changes such as lack of response, or overall health of the organism.

Toxicity: Negative effect on an organism caused by some stimulus. Mortality, decreased growth, and abnormal growth are examples of negative effects.

Acronyms and Abbreviations

Ald	Alderwood Series
As	Arsenic
BAF	Bioaccumulation Factor

BRL	Brooks Rand Laboratory
dw	Dry weight
Ecology	Washington State Department of Ecology
EIM	Environmental Information Management database
EPA	U.S. Environmental Protection Agency
Evt	Everett Series
FIR	Food Ingestion Rate
GIS	Geographic Information System software
Har	Harstine Series
HOO	Hanford Old Orchards
K	Plant Uptake Coefficient
Kit	Kitsap Series
LOAEL	Lowest Observed Adverse Effect Level
MEL	Manchester Environmental Laboratory
MTCA	Model Toxics Control Act
NOAEL	No observed adverse effect level
P	Diet Contamination
Pb	Lead
QAPP	Quality Assurance Project Plan
RGAF	Gut Adsorption Factor
RPD	Relative Percent Difference
RSD	Relative Standard Deviation
SIR	Soil Ingestion Rate
SOP	Standard Operating Procedure
Spn	Spanaway Series
SRM	Standard Reference Materials
SSL	Soil Screening Levels
StDev	Standard Deviation
TEE	Terrestrial Ecological Evaluation
TRV	Toxicity Reference Value
TSP	Tacoma Smelter Plume Footprint
UNK	Unknown
USDOE	U.S. Department of Energy
vs.	Versus
WAC	Washington Administrative Code
XRF	X-ray Fluorescence

Units of Measurement

°C	degrees centigrade
dw	dry weight
ft	feet
km	kilometer, a unit of length equal to 1,000 meters.
mg/Kg	milligrams per kilogram (parts per million)
ww	wet weight