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Plant Growth Study

Growth of Three Plant Species in 100-OL-1 Operable Unit Soils

April 2021

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U.S. DEPARTMENT OF ENERGY

Prepared for the U.S. Department of Energy
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Abstract

Soil contamination from historical application of lead arsenate pesticides persists in the 100-OL-1 Operable Unit (OU) of the U.S. Department of Energy's Hanford Site in Benton County, Washington. A remedial investigation used a portable X-ray fluorescence analyzer to estimate and map the concentrations of metals in surface soil in the 133 decision units comprising the OU. There is variability in lead and arsenic concentrations among the non-contiguous decision units, and an incomplete understanding of the ecological and human risks from soil contamination. While general screening criteria for lead are reported in literature, limited information is available on the impacts of heavy metals on site-specific plant and animal life. To address this information gap, a study on plant growth in Hanford soils was conducted using native bluegrass (*Poa secunda*), invasive cheatgrass (*Bromus tectorum*), and buttercrunch lettuce (*Lactuca sativa*). The latter is included because it is a common species used in plant growth studies.

Soil samples were collected from a single general location within the 100-OL-1 OU, prepared, and categorized as having low, medium, or high concentrations of lead and arsenic, with the high-concentration soil having concentrations of up to 3,400 and 790 mg/kg lead and arsenic, respectively. Additionally, a synthetic soil was prepared and used as a control, in accordance with standard plant growth protocols. Fifty-four seeds of each plant species were planted in each of the four soil types following the procedure outlined by the Washington State Department of Ecology.[1] Germination rates and biomass measurements were recorded for 20 days. Both bluegrass and lettuce germination rates appeared to be reduced at the highest lead and arsenic concentrations (Pb: 3400 mg/kg, As: 790 mg/kg), while cheatgrass germination rates were unaffected. Total biomass for all species appeared to be related to the relative concentrations of lead and arsenic in the soil.

Results of this growth study agree reasonably well with previous Hanford Site plant growth studies[2] and provide additional data for assessing ecological risk at the Hanford Site.

[1] Norton D. 1996. *Early Seedling Growth Protocol for Soil Toxicity Screening*. Publication No. 96-324, Washington State Department of Ecology, Olympia, WA.
[2] Delistraty D and J Yokel. 2011. "Ecotoxicological Study of Arsenic and Lead Contaminated Soils in Former Orchards at the Hanford Site, USA." *Environmental Toxicology*. doi:10.1002/tox.20768

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Acronyms and Abbreviations

ACME	*Achillia millefolium*
BRTE	*Bromus tectorum*
ELEL	*Elymus elymoides*
LASA	*Lactuca sativa*
NQAP	Nuclear Quality Assurance Program
OU	operable unit
POSE	*Poa secunda*
PUTR	*Purshia tridentate*
XRF	X-ray fluorescence

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1.0 Introduction

Prior to the acquisition of the Hanford Site by the U.S. federal government in 1943, the area was used for agricultural purposes, including fruit tree orchards. As was typical of the era, farmers in the region applied lead arsenate pesticide ($PbHAsO_4$) to their fruit trees to control codling moths (*Cydia pomonella*) (Johnson et al. 1927; Peryea 1998). Investigations have demonstrated that residual lead arsenate persists in the soils in the former Hanford orchard sites. This is expected, as lead (Pb) and arsenic (As) are generally immobile or slow moving in soil (Delistraty and Yokel 2011; Hood 2006; Peryea and Creger 1994; Schooley et al. 2008; Veneman et al. 1983).

Aged residues of Pb and As remain phytoavailable to plants (Gaw et al. 2008), which has motivated studies of ecotoxicity impacts of Pb and As on non-target organisms. Studies show that early symptoms of metal toxicity on seedlings include root growth inhibition and reduction in photosynthetic pigments (Ali et al. 2000; Fargašová 1999; Gadallah 1995; Vassilev et al. 1998). Studies also indicate that the uptake of Pb and As is generally low in fruit crops but higher in leafy vegetables and root crops (Aten et al. 1980; Creger and Peryea 1992; Kenyon et al. 1979; MacLean and Langille 1981). McBride et al. (2013) found that leafy green vegetables grown in As and Pb soil concentrations of 200 and 1,300 mg/kg, respectively, exceeded World Health Organization health-based standards for As, but not Pb.

The purpose of this study is to evaluate the effects of Pb- and As-contaminated soils from the Hanford Site's 100-OL-1 Operable Unit (OU) on terrestrial plants, specifically, buttercrunch lettuce *(Lactuca sativa)*, Sandburg's bluegrass *(Poa secunda)*, and cheatgrass (*Bromus tectorum*). While buttercrunch lettuce is a standard species used in plant bioassays (Delistraty and Yokel 2011; EPA 2012), it is not present on the Hanford Site. Therefore, two additional species found on the Hanford Site were tested: a native bluegrass *(P. secunda)* and invasive cheatgrass *(B. tectorum).*

This study provides data that can be used to establish toxicity levels and assess the impact of Pb and As soil concentrations found in Hanford Site soils on plant growth. This work complements an earlier study by Delistraty and Yokel (2011) but increases the maximum Pb and As soil concentrations tested from 390 and 128 mg/kg to 3,400 mg/kg and 790 mg/kg, respectively.

2.0 Methods

To determine the impact of Pb and As on soil growth, both Hanford sediments and a control soil were used, with X-ray fluorescence (XRF) used to measure Pb and As concentrations in the Hanford soil. This section describes the methods used to execute, prepare, characterize, and monitor plant growth in the presence of Pb and As.

2.1 Soil Collection

Soil for this study was collected from a former orchard within the 100-OL-1 OU on the Hanford Site (Bunn et al. 2014, 2019). The area where the samples were collected is classified as having an Ephrata sandy loam surface soil type (Hajek 1966). Four locations for soil sample collection were selected from the general area (Table 1). All four locations were within 30 meters of one another. The proximity of soil collection locations was targeted to minimize soil type and nutrient differences between the various soil samples with various Pb and As concentrations. A portable XRF analyzer was used to survey the region in situ and determine the nominal concentrations of Pb and As at the soil sample collection locations. In each sampling area, the vegetation and other detritus were removed by scraping the surface with a spatula. When this was completed, the spatula was used to loosen the soil in the sample area and then a scoop was used to transfer soil to a Ziploc bag. Per the collection permit requirements, soil was removed no deeper than 10 cm from the surface of each sample area. After soil collection was complete, the XRF instrument was used to analyze the newly exposed soil at the bottom of the hole. This second XRF reading was used with the initial XRF reading to determine the nominal concentrations of Pb and As in the bulk soil collected.

Table 1. Soil sample collection location and in situ XRF scoping measurements.

Coordinates		XRF Reading	Pb	As	
Latitude	Longitude	#	(mg/kg)	(mg/kg)	Comments
46.606541	-119.423302	15	28.4	5.54	Surface
46.606525	-119.423332	27	1484	253	Bottom
46.606266	-119.423294	26	403	27.8	Surface
46.606289	-119.423248	37	614	54.9	Bottom
46.606552	-119.423241	17	5703	1252	Surface
46.606564	-119.423225	49	1493	460	Bottom
46.606297	-119.422943	41	55.2	7.86	Surface
46.606323	-119.422958	50	15.7	33.4	Bottom

2.2 Soil Preparation

This study used two types of soils: (1) the primary test soils, which were collected from the field and used to compare the impact of different concentrations of Pb and As; and (2) a synthetic soil that was prepared to serve as a control (Norton 1996).

2.2.1 Field Soil

In the laboratory, each Ziploc bag of soil was emptied into separate aluminum trays and labeled with the nominal concentration of Pb and As (low, medium, high) and ID number. Working from low concentration to high, soil was then cleared of detritus using a sieve, tweezers, and hand picking to

remove plant and other non-soil material. The trays were then left uncovered at ambient temperature for 4 days to dry.

Trays of soil for each of the relative concentrations of Pb and As were mixed together to homogenize the soil. Two trays were combined at a time and mixed by hand. When all trays had been combined, soil was transferred to large plastic jars and placed on bench-top rollers (Figure 1). Mechanical mixing with the motorized rollers occurred overnight, or for at least 12 hours. Soil samples of the same concentration class were then combined and mixed by hand again. During the soil homogenization process, any additional non-soil material identified was removed. When soil homogenization was complete, the XRF analyzer was used to measure the concentrations of Pb and As.

Figure 1. Mixing of soil samples using bench-top rollers.

2.2.1.1 X-ray Florescence

After soil homogenization and mixing, the soil concentrations of Pb and As were measured using the XRF analyzer and lead to a general classification of soil samples as low, medium, or high concentration (Table 2).

The soil concentrations of Pb and As were measured using a handheld XRF analyzer (Niton XL3t 950, Thermo-Fisher Sci., Waltham, MA). This same instrument was used in field evaluations of Pb and As concentrations in orchards on the Hanford Site (Bisping et al. 2017; Bunn et al. 2014, 2019). The XRF analyzer has been demonstrated to be capable of providing data with acceptable detection limit, accuracy, and precision for characterization and decision-making (Bisping et al. 2017; Bunn et al. 2014). A quality check of the XRF was conducted prior to use; precision and accuracy were evaluated by measuring blank and reference samples according the methodology described by Bunn et al. (2014).

The XRF analyzer accuracy is generally considered to be within 10% (Bisping et al. 2017; Bunn et al. 2014). Given the research team's experience with the instrument, and that it was demonstrated to be functioning properly on the day of use, the individual measurements of Pb and As in soil should be considered accurate to within 10%. Additionally, the standard deviation of repeat measurements made for this study was less than 13%; this included repeat measurements of the test soil (see Table 2) and repeat measurements of reference samples done as part of the daily quality assurance check (Appendix B). Combining the analytical error and the variability as the sum of squares, the total combined uncertainty in individual measurements of Pb and As concentrations is considered to be on the order of 15%.

Table 2. Homogenized soil metal concentrations. Note that values are rounded to two significant figures.

	Control	Low	Medium	High
Lead				
Number of measurements	1	4	3	2
Average (mg/kg)	5.7	200	660	3400
Standard deviation (mg/kg)	N/A	5.1	32	2.8
Relative standard deviation	N/A	3.0%	5.0%	0.0%
Arsenic				
Number of measurements	1	4	3	2
Average (mg/kg)	4.0	34	58	790
Standard deviation (mg/kg)	N/A	4.0	6.5	73
Relative standard deviation	N/A	12%	11%	9.0%

2.2.2 Control Soil

For the control, a synthetic soil was prepared according to Washington State Department of Ecology guidelines (Norton 1996). This recipe specifies 70% silica sand, 20% kaolin clay, 10% peat moss, and a pH adjustment to ~7 using calcium carbonate ($CaCO_3$). Soil was prepared in small batches (~2 kg at a time) and thoroughly mixed in large plastic jars using the bench-top rollers. Each jar was weighed and $CaCO_3$ equal to 0.40% of total weight was added. After thorough individual mixing, jar contents were emptied into two aluminum pans and thoroughly mixed. For each tray, the pH of the control soil was measured by first performing a slurry test and then measuring the supernatant after 30 minutes as described in Norton 1996. Additional $CaCO_3$ was added in small increments (~25 g at a time) to increase the pH to 7.0 ± 0.5.

2.3 Seeding

Based on the results of a seed viability study (Appendix A), three plant species were chosen for the test: a native grass [*P. secunda* (POSE), Sandberg's bluegrass], a non-native grass found on the Hanford Site [*B. tectorum* (BRTE), cheatgrass], and a plant used in similar studies [*L. sativa* (LASA), buttercrunch lettuce]. Small plastic pots typically used by nurseries (nominally 10 cm x 10 cm square) were used for each of the six replicates. Each pot had four drain holes in the bottom; these were covered with glass-fiber filter paper to prevent soil loss. For the site-specific soils, 600 ± 10 g of soil was added to each pot. Due to the lower bulk density, only 400 ± 10 g of synthetic soil was added to each pot.

Each pot was planted with nine seeds. Seeds were planted by creating a groove along one edge of the pot; three seeds were added to the groove and the soil was folded back over the seeds, with effort made not to disturb the seeds. The process was repeated twice more, for a total of three rows, each with three seeds. Every effort was made to ensure the rows and seeds were equally distanced. Each combination of soil type and plant species had six replicates, resulting in 54 seeds for each plant species in each soil type. Pots were labeled with soil type, seed type, and replicate number and placed randomly in trays in groups of nine. Once arranged, the trays were moved to the environmental growth chamber to initiate the test.

In the environmental growth chamber, pots were arranged randomly in groups of nine pots on each tray (Figure 3). Trays were numbered 1 through 8 and placed on a table; even-numbered trays were on the right-hand side of the environmental growth chamber and odd-numbered trays were on the left side.

2.4 Test Conditions

Plants for this study were grown inside an environmental growth chamber (Conviron GR48, Controlled Environments, Ltd., Manitoba, Canada). Environmental conditions for the test were based on guidelines from the Washington State Department of Ecology (Norton 1996), with modifications to accommodate cool season lettuce and winter annual cheatgrass. Test conditions included a constant temperature of 18°C, with an initial light intensity of 300 $\mu molm^{-2}s^{-1}$. The light intensity was increased to 365 $\mu molm^{-2}s^{-1}$ on day 4. Daily condition measurements are reported in Appendix B.

Trays were moved from the environmental growth chamber to the adjoining lab briefly each day to apply water and measure germination.

Figure 2. Plants growing in environmental growth chamber.

2.5 Measurements

The standard plant-growth study uses a 14-day period (Norton 1996); for this study, a 20-day growing period was used to compensate for the lower temperatures and slower growth of the bluegrass (POSE).

2.5.1 Daily Observations

Daily measurements of temperature, relative humidity, and seedling emergence were recorded. Additionally, any observed sublethal effects such as wilting or discoloration were recorded. The light intensity, temperature, and relative humidity measurements are only provided to demonstrate that the test was conducted within the acceptable range established by Norton (1996). The absolute accuracy of the pH, temperature, relative humidity, and light intensity is not considered relevant to interpretation of these results. Observations of seed germination occurred daily, and final measurements were recorded on day 20.

2.5.2 Seedling Survival

Seedling survival is defined as the number of emerged seedlings that are still alive at the end of the test period. At the end of the 20-day test duration, the final number of seedlings that emerged and survived was recorded and used as the final survival measurement. Following this measurement, individual seedlings were harvested for biomass measurements by cutting at the soil interface. For some of the replicates, non-test species were observed to sprout. These sprouts were not harvested for the biomass measurements.

2.5.3 Biomass Growth

All combined shoots harvested from each replicate were placed in pre-tared aluminum foil pans and weighed (wet weight). Shoots were then placed in a drying oven at 65°C for 24 hours, followed by cooling in a desiccator for 30 minutes. The plants were then weighed to obtain an initial dry-weight using a calibrated 4-place balance. Plants were then placed in the drying oven for an additional 2 hours, the desiccator for 30 minutes, and then reweighed to obtain a second dry-weight measurement. If the difference between the two weight measurements was less than 0.5%, the mass was considered stable and the material fully dry. In all instances, the weights were within 0.5% and no additional drying was necessary. The biomass dry weight was determined as the average mass of the two measurements minus the tare weight of the aluminum pan.

The accuracy of the biomass measurements can be determined as the sum of squares of the accuracy of the dry-weight measurement and the tare weight of the pan. It is assumed that the accuracy of the measurement is two times larger than the resolution, or 0.0002 g. Therefore, the combined accuracy of the biomass measurements is assumed to be 0.0003 g; the lowest calculated dry-mass result was 0.0041 g, making the maximum uncertainty in the biomass measurements 7%.

2.5.4 pH

Soil pH was measured in accordance with guidelines from the Washington State Department of Ecology (Norton 1996). A slurry of water and soil was mixed at a 1:1 mass ratio; 25 g of soil and 25 mL of deionized water were mixed in a 100-mL beaker. The slurry was mixed with a magnetic stir bar on a stir plate for 5 minutes. The pH was then measured using a calibrated benchtop pH meter (Mettler Toledo, Spectrum Technologies, Inc., Aurora, IL) and recorded. Then, the slurry was allowed to settle for 30 minutes and the pH of the supernatant was measured again. This second measurement was considered the pH reported here.

The pH results are only provided here to demonstrate that the soil pH was within the correct range, and that all the soil types had a similar pH. The absolute accuracy of the pH is not considered relevant to interpretation of these results.

The pH of the soil was measured before and after the test. Prior to the test, only the bulk soil was tested (before packing into pots). After the test, the pH of each soil type and species was tested (Table 3).

Table 3. Soil pH measured before and after test.

	Cheatgrass	Lettuce	Bluegrass
Post-experiment			
Control	7.6	7.7	7.6
Low	(a)	7.6	(a)
Medium	6.4	6.5	6.3
High	6.4	6.6	6.5
Pre-experiment			
Control		6.4	
Low		6.8	
Medium		6.1	
High		6.1	

(a) Data not necessary. Extra seeds sprouted, making counts unreliable.

3.0 Results and Discussion

While seeds germinated in all cases, lettuce and bluegrass germination rates appeared to be reduced in soils with high Pb and As concentrations (Figure 3). No relationship between cheatgrass germination rates and Pb and As concentrations was readily observed. The Washington State Department of Ecology classifies results as invalid if the germination rate in control soils is less than 90%; both the lettuce and the bluegrass failed to meet this threshold (Table 4) (Norton 1996). It was also noted that extra seedlings sprouted in the low-concentration soil. While it was easy to differentiate between the lettuce and the extra sprouts, these additional sprouts were visually similar to the cheatgrass and bluegrass sprouts. This called into question the accuracy of the cheatgrass and bluegrass results in the low-concentration soil; therefore, all measurements for cheatgrass and bluegrass in the low-concentration soil were excluded.

Table 4. Germination success as measured on day 20 of the experiment.

	Total Number of Seedlings on Day 20	% Germination
Cheatgrass (BRTE)		
Control	51	94%
Low	(a)	(a)
Medium	49	91%
High	47	87%
Lettuce (LASA)		
Control	48	89%
Low	32	59%
Medium	45	83%
High	39	72%
Sandberg's Bluegrass (POSE)		
Control	41	76%
Low	(a)	(a)
Medium	35	65%
High	42	78%

(a) Data not used. Extra seeds sprouted, making counts unreliable.

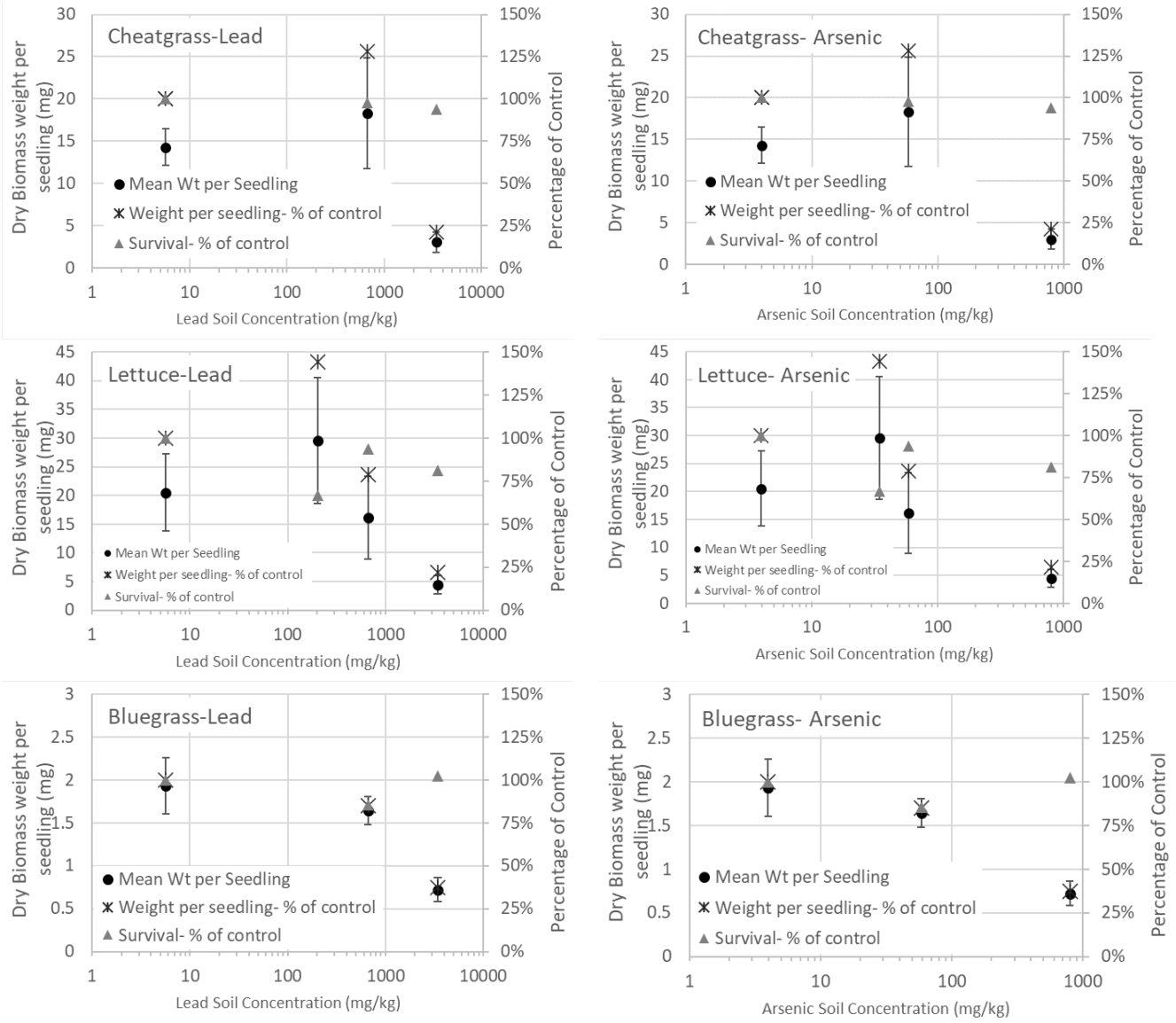

Figure 3. Plots of biomass dry weight normalized to the control soil for cheatgrass (BRTE), lettuce (LASA), and bluegrass (POSE).

The results of this test agree with the findings of Delistraty and Yokel (2011) but evaluated higher Pb and As soil concentrations (Figure 4). The present study considers the combined effects of Pb and As on plant growth without considering the individual impacts of Pb and As, which is consistent with the approach in Delistraty and Yokel (2011). Both studies evaluated growth of lettuce in soils collected from the former Hanford Site orchard site, with elevated Pb and As concentrations from residual lead arsenate pesticide application. Relative to the control soil, both studies indicated an initial increase in lettuce (LASA) plant growth with increasing Pb and As concentrations, which decreased once concentrations reached ~400 and ~50 mg/kg for lead and arsenic, respectively. This pattern was not observable in the cheatgrass or bluegrass data because the results for the low-concentration soil were excluded from the study because additional sprouts whose species could not be differentiated were observed.

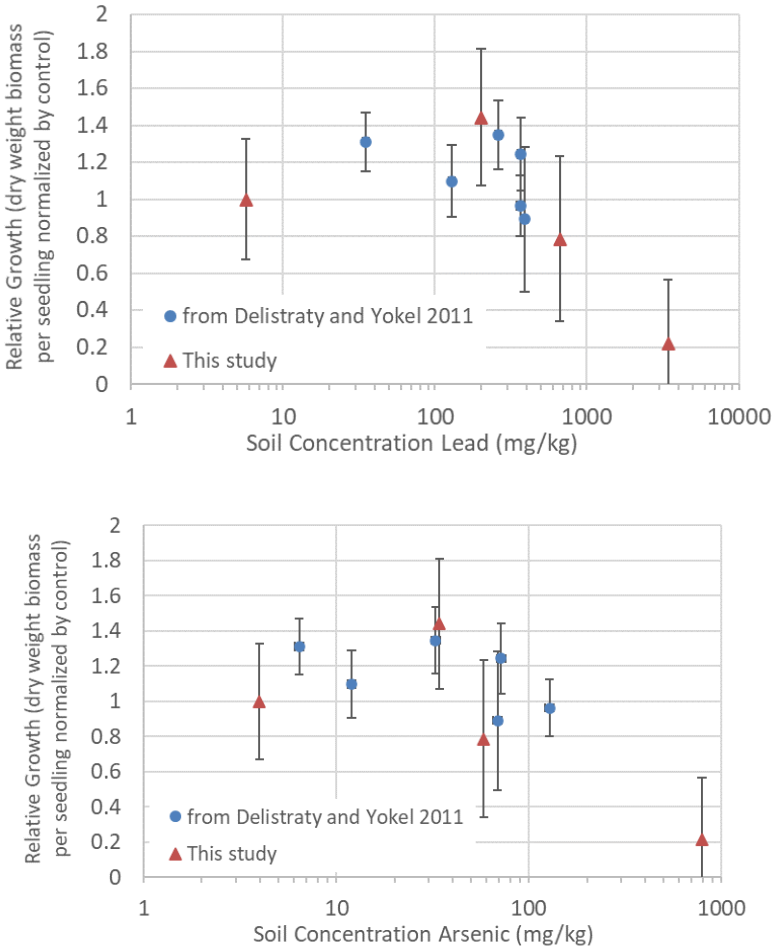

Figure 4. Comparison of lettuce (LASA) dry biomass per seedling, relative to the control, for this study and Delistraty and Yokel (2011). Error bars represent the relative error of the replicate measurements (standard deviation of the measurements divided by the mean). Note that the control sample from Delistraty and Yokel (2011) is not shown; it was reported to have a lead concentration equal to zero, which does not appear on this semi-log plot.

Table 5. Summary of biomass measurements (total dry weight and normalized). Note that values are rounded to two significant figures.

	Control	Low	Medium	High
Soil Concentration (mg/kg)				
Lead	5.7	200	660	3400
Arsenic	4.0	34	58	790
Biomass Growth Total Dry Weight (mg)				
Cheatgrass (BRTE)	730	[a]	900	140
Lettuce (LASA)	990	880	700	170
Bluegrass (POSE)	78	[a]	57	30
Biomass Growth Average Dry Weight per Seedling (mg)				
Cheatgrass (BRTE)	14	[a]	18	3.1
Lettuce (LASA)	21	30	16	4.5
Bluegrass (POSE)	1.9	[a]	1.6	0.72
Relative Growth (percentage of control)				
Cheatgrass (BRTE)	1	[a]	130%	21%
Lettuce (LASA)	1	144%	79%	22%
Bluegrass (POSE)	1	[a]	85%	37%

(a) Data not used. Extra seeds sprouted, making counts unreliable.

4.0 Conclusion

Soil contamination by Pb and As is a common problem in former agricultural areas that may pose human and ecological risks. While some studies have been conducted to determine general screening criteria for heavy metals, the ecological response is expected to be site-specific, requiring more detailed analysis.

The plant growth study consisted of analytical chemistry and 20-day bioassay results for cheatgrass, lettuce, and Sandberg's bluegrass. Bioassays for all three test species were conducted on 100-OL-1 OU soils with medium and high arsenic and lead concentrations, plus a laboratory control. Tests with lettuce were also performed on 100-OL-1 OU soils with low arsenic and lead concentrations.

Results shows that lettuce survival in the tests was significantly reduced in the low-concentration sample but not in the medium samples or the high-concentration samples. Growth of the different species was significantly reduced in the high-concentration sample but not in any other sample. Results are in relative agreement with previous reporting on Hanford Site plant growth studies (Delistraty and Yokel 2011).

Phytotoxicity data obtained in this study are intended to be integrated with literature data, analyzed, interpreted, and compiled into the ecological risk analyses to support decision-making related to the development of cleanup guidelines for remediation of the 100-OL-1 OU.

5.0 Quality Assurance

This work was performed in accordance with the Pacific Northwest National Laboratory Nuclear Quality Assurance Program (NQAP). The NQAP complies with DOE Order 414.1D, *Quality Assurance*. The NQAP uses NQA-1-2012, *Quality Assurance Requirements for Nuclear Facility Application*, as its consensus standard and NQA-1-2012, Subpart 4.2.1 as the basis for its graded approach to quality.

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Appendix A – Initial Seed Viability Study

Initially, eight different species of plants were tested to evaluate seed viability: *Achillia millefolium* (ACME, yarrow), *Elymus elymoides* (ELEL, bottlebrush squirreltail), *Bromus tectorum* (BRTE, cheatgrass), *Purshia tridentate* (PUTR, Antelope bluegrass), *Poa secunda* (POSE, Sandberg's bluegrass), and *Lactuca sativa* (LASA, buttercrunch lettuce). LASA seeds from two different sources were tested concurrently. Ten seeds from each species were placed on a filter paper moistened with deionized water in a petri dish. The petri dish was then sealed with laboratory tape and placed in the germination chamber. The germination chamber was maintained at 65-68°F. Seeds were removed from the germination chamber daily, the number of seeds germinated was counted, and the filter paper was remoistened if needed.

It was determined that the original sample of BRTE seeds did not have enough seeds left to be used in this study. A new set of BRTE seeds was collected from the Hanford Site. These seeds were started on the sixth day of the initial germination study following the procedure previously described. By the fifth day, none of the BRTE seeds had germinated and additional set of BRTE seeds was collected (from the Volpentest HAMMER Federal Training Center in Richland, WA, and the Hanford Site 300 Area). BRTE seeds from the Hanford Site that had been rinsed in a 10% bleach solution were also started this day.

A secondary study was conducted with two additional samples of POSE and ELEL. This germination study was slightly different in that dark blue filter paper was used on the top and bottom of the seeds, and both filter papers were moistened. The dark blue filter paper was used to account for the possibility of light inhibition in these two species.

Appendix B – Data

This appendix provides all data, both observations and results, related to the plant growth study (i.e., including daily observations of chamber conditions, germination results, etc.).

Table B.1. Daily observations of chamber conditions.

Measurements	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Temperature (°C)	17.9	17.9	17.9	17.9	17.9	18.1	18.1	17.9	18.1	18
Humidity (%)	72	72	71	72	70	72	70	70	70	68
Light (µmolm-2s-1)	297	297	290	292	355	355	342	340	347	342
Measurements	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20
Temperature (°C)	18.1	18.2	18	17.9	18.1	18.1	18.1	18.2	18.1	18.1
Humidity (%)	71	70	68	68	68	70	69	70	71	75
Light (µmolm-2s-1)	345	342	357	350	347	352	347	347	348	270

Table B.2. Germination results. Only counts from even numbered days provided; data from day 20 used for final count. Gaps in ID numbers indicate data from BRTE and POSE in low-concentration soil removed.

Pot ID #	Plant Species	Soil	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16	Day 18	Day 20
2	LASA	Control	0	7	9	8	8	8	8	8	8	8
3	BRTE	High	0	0	9	9	9	9	9	9	9	9
4	LASA	Medium	0	4	7	7	7	7	7	7	7	8
5	POSE	Control	0	0	1	6	6	7	7	7	7	7
6	LASA	High	0	1	3	3	3	3	4	4	4	4
7	BRTE	Medium	0	0	9	9	9	9	9	9	9	8
9	POSE	Control	0	0	3	5	6	6	6	6	6	8
10	BRTE	High	0	0	5	6	6	6	6	6	6	6
11	BRTE	Control	0	0	8	8	8	8	8	9	8	9
12	POSE	Medium	0	1	2	2	4	4	4	4	4	4
13	POSE	High	0	0	0	4	5	7	6	6	6	7
15	BRTE	Control	0	0	8	8	8	8	8	8	8	8
16	LASA	Low	0	3	7	7	7	5	7	7	5	5
17	BRTE	High	0	0	4	6	6	7	7	7	7	7
18	POSE	Control	0	0	2	5	6	6	6	7	6	7
19	BRTE	Control	0	0	10	10	10	10	10	10	10	10
20	BRTE	High	0	0	4	5	9	9	9	9	9	9
21	BRTE	Medium	0	0	7	8	8	8	8	8	8	8
22	LASA	Low	0	4	6	7	8	7	7	7	6	6
23	POSE	Medium	0	1	3	4	9	9	9	9	9	9
26	LASA	High	0	1	5	5	5	5	5	7	7	7
27	LASA	Medium	0	3	5	4	4	5	6	6	6	6

Pot ID #	Plant Species	Soil	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16	Day 18	Day 20
28	LASA	High	0	0	3	2	2	5	6	8	7	7
29	LASA	Medium	0	3	5	5	5	6	6	6	6	6
30	BRTE	Medium	0	0	8	8	8	8	8	8	8	9
31	BRTE	Control	0	0	8	8	8	8	8	8	8	8
32	BRTE	Control	0	0	9	9	9	9	9	9	9	9
33	LASA	Control	0	7	8	8	9	9	9	9	9	9
34	LASA	Control	0	5	6	6	6	6	6	6	6	6
35	LASA	Medium	0	7	8	8	8	8	8	8	8	8
36	LASA	High	0	4	4	5	7	7	7	7	7	7
40	POSE	High	0	0	0	4	5	6	7	7	7	7
41	BRTE	Medium	0	0	5	7	8	8	8	8	8	8
44	POSE	Medium	0	1	4	4	6	6	6	6	6	6
45	POSE	High	0	0	0	2	5	5	6	7	7	7
46	LASA	Medium	0	4	6	9	9	9	9	9	9	9
47	POSE	Control	0	0	1	4	4	5	5	5	5	5
49	BRTE	Control	0	0	7	7	7	7	7	7	7	7
50	LASA	Control	0	6	7	8	8	8	8	8	8	8
51	BRTE	High	0	0	2	4	7	7	7	7	7	7
52	BRTE	Medium	0	0	9	9	9	9	9	9	9	9
53	BRTE	High	0	0	1	2	8	9	9	9	9	9
54	POSE	Medium	0	0	1	2	3	3	4	3	3	3
55	POSE	High	0	0	1	4	6	7	7	7	7	7
56	LASA	Medium	0	4	8	9	9	7	10	10	8	8
57	LASA	Low	0	3	5	6	6	6	7	7	7	7
58	LASA	Low	0	4	6	6	6	5	6	6	5	5
59	LASA	High	0	0	4	5	6	6	6	7	7	7
60	LASA	Control	0	5	9	9	9	9	9	9	9	9
61	POSE	Medium	0	0	0	6	8	8	8	8	8	8
62	POSE	High	0	0	1	3	5	6	6	6	6	6
63	POSE	Control	0	0	4	6	6	7	7	7	7	7
64	POSE	Medium	0	0	1	5	5	5	5	5	5	5
65	POSE	Control	0	0	0	5	6	6	7	7	7	7
66	LASA	Low	0	2	4	4	4	3	4	4	3	3
67	BRTE	Medium	0	0	5	7	7	7	7	7	7	7
68	LASA	Control	0	7	8	8	8	8	8	8	8	8
69	POSE	High	0	0	1	2	2	4	5	6	7	8
70	LASA	Low	0	2	7	6	6	6	7	7	6	6
72	LASA	High	0	0	6	6	6	6	7	7	7	7

Table B.3. Data summary: plant germination and survival summary. Gaps in ID numbers indicate data from BRTE and POSE in low-concentration soil removed.

ID#	Plant Type	Soil Type	Pb (mg/kg)	As (mg/kg)	Rep-licate #	Count on Day 20	Percent Survival	Total Survival	Mean Survival	Std. Dev.	% of Control
11	BRTE	Control	5.68	3.98	4	9	100%	51	93%	0.09	1.00
15	BRTE	Control	5.68	3.98	3	8	89%				
19	BRTE	Control	5.68	3.98	2	10	100%				
31	BRTE	Control	5.68	3.98	1	8	89%				
32	BRTE	Control	5.68	3.98	5	9	100%				
49	BRTE	Control	5.68	3.98	6	7	78%				
3	BRTE	High	3421	791.5	2	9	100%	47	87%	0.15	0.94
10	BRTE	High	3421	791.5	1	6	67%				
17	BRTE	High	3421	791.5	3	7	78%				
20	BRTE	High	3421	791.5	4	9	100%				
51	BRTE	High	3421	791.5	5	7	78%				
53	BRTE	High	3421	791.5	6	9	100%				
7	BRTE	Medium	663.7	58.3	4	8	89%	49	91%	0.08	0.98
21	BRTE	Medium	663.7	58.3	3	8	89%				
30	BRTE	Medium	663.7	58.3	5	9	100%				
41	BRTE	Medium	663.7	58.3	6	8	89%				
52	BRTE	Medium	663.7	58.3	1	9	100%				
67	BRTE	Medium	663.7	58.3	2	7	78%				
2	LASA	Control	5.68	3.98	1	8	89%	48	89%	0.12	1.00
33	LASA	Control	5.68	3.98	4	9	100%				
34	LASA	Control	5.68	3.98	6	6	67%				
50	LASA	Control	5.68	3.98	2	8	89%				
60	LASA	Control	5.68	3.98	3	9	100%				
68	LASA	Control	5.68	3.98	5	8	89%				
6	LASA	High	3421	791.5	3	4	44%	39	72%	0.14	0.81
26	LASA	High	3421	791.5	2	7	78%				
28	LASA	High	3421	791.5	1	7	78%				
36	LASA	High	3421	791.5	5	7	78%				
59	LASA	High	3421	791.5	6	7	78%				
72	LASA	High	3421	791.5	4	7	78%				
16	LASA	Low	201.5	34.3	2	5	56%	32	59%	0.15	0.67
22	LASA	Low	201.5	34.3	5	6	67%				
57	LASA	Low	201.5	34.3	1	7	78%				
58	LASA	Low	201.5	34.3	4	5	56%				
66	LASA	Low	201.5	34.3	6	3	33%				
70	LASA	Low	201.5	34.3	3	6	67%				
4	LASA	Medium	663.7	58.3	1	8	89%	45	83%	0.14	0.94
27	LASA	Medium	663.7	58.3	4	6	67%				
29	LASA	Medium	663.7	58.3	3	6	67%				
35	LASA	Medium	663.7	58.3	5	8	89%				
46	LASA	Medium	663.7	58.3	2	9	100%				
56	LASA	Medium	663.7	58.3	6	8	89%				
5	POSE	Control	5.68	3.98	3	7	78%	41	76%	0.11	1.00
9	POSE	Control	5.68	3.98	1	8	89%				

ID#	Plant Type	Soil Type	Pb (mg/kg)	As (mg/kg)	Rep-licate #	Count on Day 20	Percent Survival	Total Survival	Mean Survival	Std. Dev.	% of Control
18	POSE	Control	5.68	3.98	4	7	78%				
47	POSE	Control	5.68	3.98	6	5	56%				
63	POSE	Control	5.68	3.98	2	7	78%				
65	POSE	Control	5.68	3.98	5	7	78%				
13	POSE	High	3421	791.5	6	7	78%	42	78%	0.07	1.02
40	POSE	High	3421	791.5	5	7	78%				
45	POSE	High	3421	791.5	4	7	78%				
55	POSE	High	3421	791.5	3	7	78%				
62	POSE	High	3421	791.5	2	6	67%				
69	POSE	High	3421	791.5	1	8	89%				
12	POSE	Medium	663.7	58.3	6	4	44%	35	65%	0.26	0.85
23	POSE	Medium	663.7	58.3	3	9	100%				
44	POSE	Medium	663.7	58.3	4	6	67%				
54	POSE	Medium	663.7	58.3	5	3	33%				
61	POSE	Medium	663.7	58.3	1	8	89%				
64	POSE	Medium	663.7	58.3	2	5	56%				

Table B.4. Data summary continued: plant biomass data. Gaps in ID numbers indicate data from BRTE and POSE in low-concentration soil removed.

ID#	Tare Wt (g)	Dry Wt (g)	Dry Wt (g)	Dry Biomass weight (g)	Total Dry Wt (g)	Weight per Seedling (mg)	Mean Weight per Seedling (mg)	Std. Dev.	% of Control
11	2.098	2.223	2.223	0.12455	0.73	13.8	14.3	2.16	1.00
15	2.149	2.265	2.2656	0.1166		14.6			
19	2.099	2.253	2.254	0.15425		15.4			
31	2.095	2.235	2.2358	0.1406		17.6			
32	2.109	2.209	2.2099	0.1007		11.2			
49	2.138	2.229	2.2307	0.09215		13.2			
3	2.105	2.142	2.1414	0.03675	0.14	4.1	3.1	1.24	0.21
10	2.055	2.076	2.075	0.02065		3.4			
17	2.122	2.142	2.1419	0.0199		2.8			
20	2.098	2.14	2.1396	0.0414		4.6			
51	2.142	2.156	2.1555	0.0137		2.0			
53	2.105	2.118	2.1178	0.01245		1.4			
7	2.135	2.337	2.3393	0.2033	0.90	25.4	18.3	6.52	1.28
21	2.114	2.271	2.2715	0.1572		19.7			
30	2.068	2.256	2.2572	0.18885		21.0			
41	2.097	2.149	2.1486	0.0521		6.5			
52	2.141	2.285	2.2853	0.1445		16.1			
67	2.103	2.252	2.2534	0.14915		21.3			
2	2.133	2.349	2.3516	0.21785	0.99	27.2	20.5	6.74	1.00
33	2.103	2.223	2.2235	0.1205		13.4			
34	2.101	2.189	2.1895	0.08785		14.6			
50	2.134	2.262	2.2646	0.12965		16.2			
60	2.123	2.324	2.3276	0.2025		22.5			
68	2.095	2.326	2.3306	0.2329		29.1			
6	2.114	2.143	2.1433	0.0288	0.17	7.2	4.5	1.55	0.22
26	2.087	2.109	2.1095	0.02245		3.2			
28	2.107	2.126	2.1259	0.0194		2.8			
36	2.133	2.164	2.164	0.03085		4.4			
59	2.136	2.166	2.1662	0.03035		4.3			
72	2.117	2.15	2.1504	0.0338		4.8			
16	2.136	2.27	2.2711	0.1341	0.88	26.8	29.6	10.93	1.44
22	2.084	2.248	2.2493	0.16415		27.4			
57	2.135	2.211	2.2113	0.07665		11.0			
58	2.136	2.325	2.3274	0.19055		38.1			
66	2.084	2.21	2.2115	0.12665		42.2			
70	2.133	2.323	2.3251	0.19165		31.9			
4	2.134	2.3	2.3021	0.16645	0.70	20.8	16.1	7.20	0.79
27	2.107	2.22	2.2212	0.1139		19.0			
29	2.067	2.204	2.204	0.13665		22.8			
35	2.136	2.286	2.2871	0.1503		18.8			
46	2.119	2.227	2.228	0.10825		12.0			

ID#	Tare Wt (g)	Dry Wt (g)	Dry Wt (g)	Dry Biomass weight (g)	Total Dry Wt (g)	Weight per Seedling (mg)	Mean Weight per Seedling (mg)	Std. Dev.	% of Control
56	2.106	2.129	2.1388	0.0275		3.4			
5	2.117	2.129	2.1293	0.01195	0.078	1.7	1.9	0.32	1.00
9	2.091	2.103	2.1027	0.0115		1.4			
18	2.096	2.112	2.1119	0.016		2.3			
47	2.11	2.121	2.1208	0.0103		2.1			
63	2.146	2.162	2.1619	0.01555		2.2			
65	2.122	2.136	2.1355	0.01315		1.9			
13	2.105	2.111	2.1105	0.0059	0.030	0.8	0.72	0.14	0.37
40	2.131	2.137	2.1367	0.0056		0.8			
45	2.112	2.116	2.116	0.00425		0.6			
55	2.106	2.111	2.1114	0.0051		0.7			
62	2.134	2.139	2.1386	0.00505		0.8			
69	2.151	2.155	2.1552	0.0041		0.5			
12	2.149	2.156	2.1565	0.00725	0.057	1.8	1.6	0.17	0.85
23	2.06	2.073	2.0732	0.0136		1.5			
44	2.092	2.102	2.1019	0.01005		1.7			
54	2.116	2.12	2.1198	0.0043		1.4			
61	2.142	2.155	2.1544	0.0127		1.6			
64	2.143	2.153	2.1526	0.00925		1.8			

Table B.5. Test soil XRF measurements, including a) hand recorded results and b) summary table.

Note that control soil was measured on a different day – no hand-recorded data

a) Hand-recorded results

Sample Count	Coordinates		Sample ID	XRF Reading #	Pb*	As*	Comments
	Latitude	Longitude					
1			Low-A	31	200	32	Duplicate XRF #32
3			Low-B	33	205	30	
4			Low-C	34	206	36	
5			Low-D	35	195	39	
6			Med-A	36	638	65	
7			Med-B	37	~~658~~ 654	58	
8			Med-C	38	699	52	
			High-A	39	3419	~~740~~	
			High-B	40	3423	843	Duplicate XRF #41

b) Summary table

Sample	Lead (mg/kg)	Arsenic (mg/kg)	Sample	Lead (mg/kg)	Arsenic (mg/kg)
Low-A	200	32	Med-B	654	58
Low-B	205	30	Med-C	699	52
Low-C	206	36	High-A	3419	740
Low-D	195	39	High-B	3423	843
Med-A	638	65	Control Soil	5.68	3.98

Table B.6. Blank, standard reference material, and duplicate measurements. Conducted as part of X-ray fluorescence (XRF) analyzer daily quality check prior to conducting measurements on test soil.

100-OL-1 OU Remedial Investigation: Soil Confirmatory Page ____ of _____

Date 7/22/14

Field Team (first and last name)
Katie Wagner

~~Decision Units~~ Sampled 7/22/14 KAW
Plant Growth Study

Blank Checks [1 detect, <7 mg/kg]		
ID: Blank-2	DU: NA	
XRF #	Pb	As
25	ND	ND
26	ND	ND
27	ND	ND
Pass?	Yes	Yes
ID: Blank-2	DU: NA	
45	ND	ND
46	ND	ND
47	ND	ND
Pass?	Yes	Yes
ID:	DU:	
Pass?		
ID:	DU:	

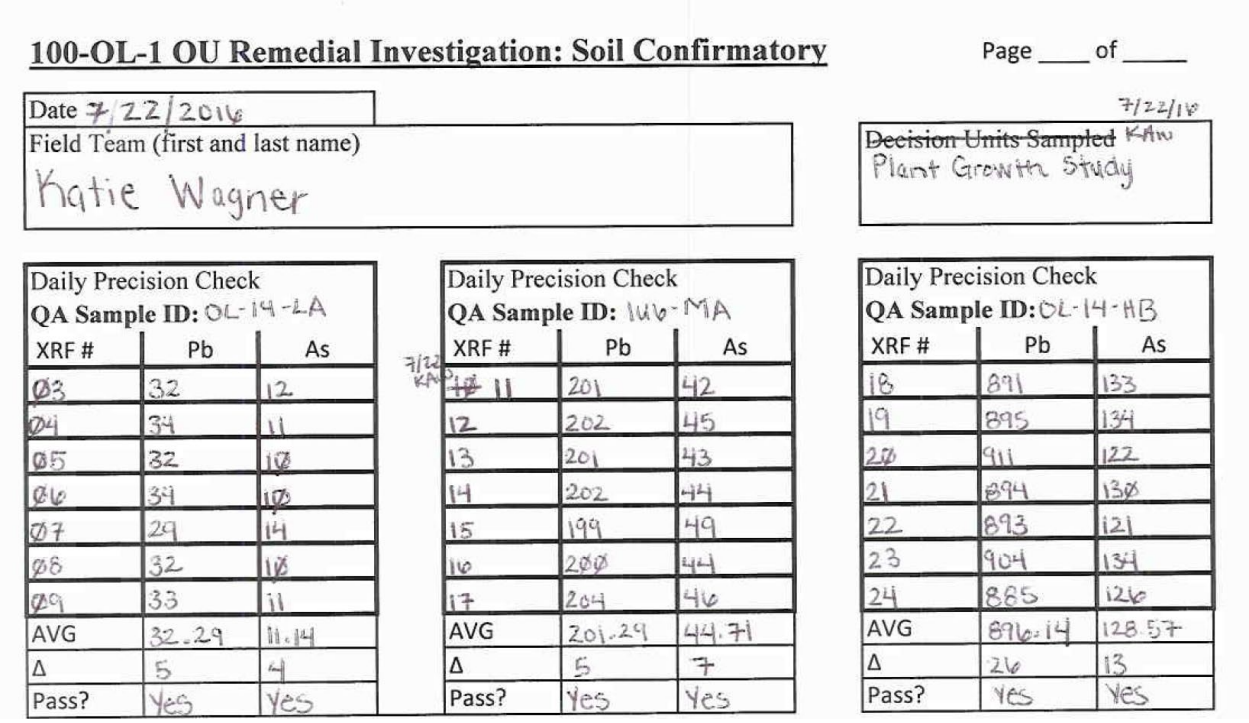
SRM Checks [PB 150-225] [As ~~24.2~~-36.4]		
ID: SRM-1	DU: NA	
XRF #	Pb	As
28	150	23
29	152	28
30	150	20
Pass?	Yes	Yes
ID: SRM-1	DU: ——— NA	
48	153	25
49	152	23
50	155	28
Pass?	Yes	Yes
ID:	DU:	
Pass?		
ID:	DU:	

Duplicates			Related
XRF #	Pb	As	XRF #
32	191	39	31
41	3407	864	40
44	1132	175	43

Notes/Comments (units recorded as ppm (a.k.a. mg/kg) unless otherwise noted):

Table B.7. Replicate measurements (precision check) of reference soil. Conducted as part of XRF analyzer daily quality check prior to conducting measurements on test soil. Includes a) hand-recorded results and b) summary table.

a) Hand-recorded results



100-OL-1 OU Remedial Investigation: Soil Confirmatory Page ____ of _____

Date 7/22/2016

Field Team (first and last name)
Katie Wagner

7/22/16 KMW
~~Decision Units Sampled~~
Plant Growth Study

Daily Precision Check
QA Sample ID: OL-14-LA

XRF #	Pb	As
03	32	12
04	34	11
05	32	10
06	34	10
07	29	14
08	32	10
09	33	11
AVG	32.29	11.14
Δ	5	4
Pass?	Yes	Yes

Daily Precision Check
QA Sample ID: IU6-MA

XRF #	Pb	As
~~10~~ 11	201	42
12	202	45
13	201	43
14	202	44
15	199	49
16	200	44
17	204	46
AVG	201.29	44.71
Δ	5	7
Pass?	Yes	Yes

Daily Precision Check
QA Sample ID: OL-14-HB

XRF #	Pb	As
18	891	133
19	895	134
20	911	122
21	894	130
22	893	121
23	904	134
24	885	126
AVG	896.14	128.57
Δ	26	13
Pass?	Yes	Yes

b) Summary table

	OL-14-LA "Low"		IU6-MA "Medium"		OL-14-HB "High"	
Sample Count	Pb (mg/kg)	As (mg/kg)	Pb (mg/kg)	As (mg/kg)	Pb (mg/kg)	As (mg/kg)
1	32	12	201	42	891	133
2	34	11	202	45	895	134
3	32	10	201	43	911	122
4	34	10	202	44	894	130
5	29	14	199	49	893	121
6	32	10	200	44	904	134
7	33	11	204	46	885	126
Average	32.29	11.14	201.29	44.71	896.14	128.57
Standard Deviation	1.70	1.46	1.60	2.29	8.65	5.59
Relative Standard Deviation	5%	13%	1%	5%	1%	4%

Appendix C – Balance Calibration Certificate

Established 1974

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A2LA ACCREDITED
CERTIFICATE #1550.01

Battelle Pacific N.W. Natl. Lab
902 Battelle Blvd.
Richland, WA 99354

Report Number: BATN031125101490150918

A2LA ACCREDITED
CERTIFICATE OF CALIBRATION WITH DATA

INSTRUMENT INFORMATION

Item	Make	Model	Serial Number	Customer ID	Location
Balance	Mettler	AX105DR	1125101490	1125101490	BSF/2209
Units	**Readability**	**SOP**	**Cal Date**	**Last Cal Date**	**Cal Due Date**
g	0.00001/0.0001	QC012	9/18/15	9/5/13	9/2016

FUNCTIONAL CHECKS

ECCENTRICITY
Test Wt: 50 Tol: 0.0002
As-Found: Pass: ☑ Fail: ☐
As-Left: Pass: ☑ Fail: ☐

LINEARITY
Test Wt: 20x4 Tol: 0.0002
As-Found: Pass: ☑ Fail: ☐
As-Left: Pass: ☑ Fail: ☐

STANDARD DEVIATION
Test Wt: 10 Tol: 0.00005

1. 10.00000	5. 9.99998	9. 10.00003
2. 9.99999	6. 10.00000	10. 10.00004
3. 9.99998	7. 9.99999	**Result**
4. 10.00000	8. 10.00001	0.000019

ENVIRONMENTAL CONDITIONS
☐ Good ☑ Fair ☐ Poor
Temperature: 20.4°C

A2LA ACCREDITED SECTION OF REPORT

Standard	As-Found	As-Left	Expanded Uncertainty
100	100.0000	100.0000	0.00013
50	50.0000	50.0000	0.00012
30	30.00002	29.99999	0.000044
10	10.00002	10.00000	0.000042
5	5.00000	5.00000	0.000042
1	1.00000	0.99999	0.000041

CALIBRATION STANDARDS

Item	Make	Model	Serial Number	Cal Date	Cal Due Date	NIST ID
Weight Set	Rice Lake	30 kg-1mg	S751	12/2/14	12/2015	OR-13-314-C

Permanent Information Concerning this Equipment:

Comments/Info Concerning this Calibration:
9/15 PO #268748 As found / as left within tolerance.

Report prepared/reviewed by: D. Thompson **Date:** 1/13/21 (Reprint) **Technician:** R. Hintz

D. Thompson for R. Hintz
Signature:

The uncertainty is calculated according to the ISO Guide to the Expression of Uncertainty in Measurement and includes the uncertainty of standards used combined with the observed standard deviation and readability of the unit under test. The uncertainty is expanded with a k factor of 2 for an approximate 95% level of confidence. Instruments listed above were calibrated using standards traceable to the National Institute of Standards and Technology (NIST). Calibration data reflect results at the time and location of calibration. Calibration data should be reviewed to insure that the instrument is performing to its required accuracy. Calibrations comply with ISO/IEC 17025 and ANSI/Z540-1-1994 quality standards.

Member: National Conference of Standards Laboratories and Weights & Measures

PT ID: BATN03

Pacific Northwest National Laboratory

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