Environmental Toxicology

Effects of Lead and Arsenic in Soils from Former Orchards on Growth of Three Plant Species

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Abstract: Historical use of lead arsenate as a pesticide in former orchards of eastern Washington State (USA) has resulted in legacy lead (Pb) and arsenic (As) soil contamination. However, the impacts on plant growth in soils with residual Pb and As contamination have not yet been quantified. To this end, a comparative study of plant growth impacts was performed for native bluegrass (Poa secunda), invasive cheatgrass (Bromus tectorum), and buttercrunch lettuce (Lactuca sativa). Using standard plant growth protocols, germination frequency and biomass growth were measured over a wide range of Pb and arsenate concentrations, with maximum concentrations of 3400 and 790 mg kg⁻¹ for Pb and As, respectively. Results indicated that only the biomass growth for all species decreased in soils with the highest concentrations of Pb and As in the soil, with no impacts on soils with lower residual Pb and arsenate concentrations. No impact on percentage of germination was observed at any soil concentration. These results can be used to determine site-specific soil screening levels for use in ecological risk assessments for Pb and arsenate in soils. Environ Toxicol Chem 2022;41:1459–1465. © 2022 Battelle Memorial Institute. Environmental Toxicology and Chemistry published by Wiley Periodicals LLC on behalf of SETAC.

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INTRODUCTION

Before the development of synthetic organic pesticides, which became available after World War II, lead arsenate (PbHAsO₄) was a primary insecticide used to control codling moths (Cydia pomonella) in orchards (Johnson et al., 1927; Peryea, 1998). Many former orchards treated with PbHAsO₄ still have elevated lead (Pb) and arsenic (As) from historical applications (Peryea, 1998). Both Pb and As are recalcitrant metals that have limited mobility in soils (Delistraty & Yokel, 2011; Hood, 2006; Peryea & Creger, 1994; Schooley et al., 2008; Veneman et al., 1983). However, aged residues of Pb and As from PbHAsO₄ remain phytoavailable, and thus potentially toxic to plants (Gaw et al., 2008), driving the need to evaluate the ecotoxicological impacts of Pb and As on non-target organisms. Previous research has also indicated 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 & Peryea, 1992; Kenyon et al., 1979; MacLean & Langille, 1981).

At the Hanford Site, located in southeastern Washington State (USA), historical use of PbHAsO₄ as a pesticide in orchards occurred until 1954, resulting in Pb and As contamination in surface soils. The potential risk from PbHAsO₄ to ecological receptors at Hanford is currently being evaluated in terms of the need for remedial actions. Our current knowledge of As and Pb toxicity to terrestrial plants at Hanford remains limited (Delistraty & Yokel, 2011). Studies of Pb and As uptake in the literature (see Codling, 2009, 2014; Codling et al., 2015, 2016; McBride, 2013; Walsh et al., 1977) do not reflect the range of concentrations observed at former orchards on the Hanford Site, nor are they representative of species encountered at Hanford. Hence, there is a need to evaluate the effects...
of Pb- and As-contaminated soils at Hanford on terrestrial plant growth to provide a technical basis for soil screening levels (SSLs). The SSLs can then be used to support an evaluation of ecological risk and provide technical input into potential remedy decisions.

The purpose of the present study was to evaluate the effects of Pb- and As-contaminated soils from a former orchard on three terrestrial plant species, buttercrunch lettuce (*Lactuca sativa*), Sandburg’s bluegrass (*Poa secunda*), and cheatgrass (*Bromus tectorum*). Although buttercrunch lettuce is a standard test species used in plant bioassays (Delistraty & Yokel, 2011; US Environmental Protection Agency [USEPA], 2012), it is not native to or present in the arid environment of eastern Washington State. Therefore, two additional species were tested: a native bluegrass (*P. secunda*) and an invasive cheatgrass (*B. tectorum*), both of which are common across eastern Washington State.

Our study provides data that can be used to quantify phytotoxicity and assess the impact on plant growth of Pb and As in soil resulting from historical PbHAsO4 application. The present study complements an earlier study by Delistraty and Yokel (2011) but evaluates higher Pb and As soil concentrations, with concentrations up to 3400 and 790 mg kg\(^{-1}\) for Pb and As, respectively. This is in comparison with naturally occurring concentrations on the Hanford Site, which have been reported to average 20 and 5.7 mg kg\(^{-1}\) (90% upper confidence limit) for Pb and As, respectively. Our study represents, to the best of the authors’ knowledge, the only plant growth study for Pb/As-contaminated soils that extends to soil concentrations of Pb greater than 1400 mg kg\(^{-1}\) and As greater than 500 mg kg\(^{-1}\), providing information to support the determination of site-specific phytotoxicity for use in ecological risk assessments.

**MATERIALS AND METHODS**

This section describes the methods used to prepare, characterize, execute, and monitor plant growth in the presence of Pb and As, using both a control soil and soil from a former orchard located at the Hanford Site (Bunn et al., 2014, 2019).

**Soil sampling and preparation**

Surface soils were collected from four spots at one general location within former orchard lands. This former orchard was in operation from the early 1900s through the mid-1940s. Once the government acquired the property in the 1940s as part of the war effort, trees were either cut down or irrigated and maintained as an orchard. The former orchard lands are located in a desert shrub–steppe, with vegetation consisting of native and invasive grasses and shrubs. The area where the samples were collected is classified as having an Ephrata sandy loam surface soil type (Hajek, 1966). This location was chosen because previous characterization research had indicated the presence of a wide range of soil concentrations. Being able to collect samples with a wide range of concentrations close to each other reduced the likelihood of other soil characteristics impacting the results (i.e., texture and pH). All four collection points were within 30 m of one another. At each collection point, 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 the soil was collected and transferred to a plastic storage bag using a scoop. Soil was collected up to 12 inches deep, as necessary to gather the necessary volume.

For the control, as recommended, a synthetic soil was prepared according to Washington State Department of Ecology guidelines (Norton, 1996). The synthetic soil included 70% silica sand, 20% kaolin clay, 10% peat moss, and a pH adjustment to approximately 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 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 min 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.

In the laboratory, each bag of soil collected at the former orchards was emptied into separate aluminum trays and labeled with the collection location. Soil was then cleared of detritus by removing plant and other nonsoil material. Sieving involved passing soil through sieves with meshes with 4.00- and 1.68-mm openings (Nos. 5 and 12 stainless-steel sieves; W.S. Tyler). The trays were then left uncovered at ambient temperature for 4 days to dry.

Trays of soil were mixed to homogenize the soil. Two trays were combined at a time and mixed by hand. When all trays for a particular location had been combined, soil was transferred to large plastic jars and placed on bench-top rollers. Mechanical mixing with the motorized rollers occurred for at least 12 h. During the soil homogenization process, any additional nonsoil material identified was removed. Soil sterilization was considered, but was not done. There was concern that thermal sterilization could impact the fractionation and bioavailability of Pb species (Islam & Park, 2017).

When soil homogenization was complete, a hand-held X-ray fluorescence (XRF) analyzer (Niton XL3t 950; ThermoFisher Scientific) was used to measure the concentrations of Pb and As. A quality check of the XRF was conducted prior to use, evaluating precision and accuracy with blank and reference samples using the methodology described in Bunn et al. (2014). The XRF analyzer is generally considered to be within 10% accuracy (Bisping et al., 2017; Bunn et al., 2014).

Repeat measurements resulted in a relative standard deviation of less than 13% for all samples and analytes (Table 1). Combining the analytical error and the variability as the sum of...
Soil and seed preparation

Three plant species were chosen for evaluation: a native grass (P. secunda, Sandberg's bluegrass, a non-native grass found on the Hanford Site (B. tectorum, cheatgrass), and a plant used in similar studies (L. sativa, buttercrunch lettuce; Delistraty & Yokel, 2011). Small plastic pots typically used by nurseries (nominally 10- x 10-cm square) were used for each of six replicates. Each pot had four drain holes in the bottom that 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.

Nine seeds were planted in each pot. A groove was created along one edge of the pot where the first three seeds were planted, and the soil was folded back over the seeds to avoid their disturbance during subsequent watering. The process was repeated twice more, for a total of three rows, each with three seeds, equally spaced within and between rows. Each combination of soil type and plant species had six replicates, resulting in 54 seeds for each plant species in each soil. Pots were labeled with soil concentration classification, seed type, and replicate number and placed randomly in trays in groups of nine, distributed on eight separate trays. Once arranged, the trays were moved to an environmental growth chamber (Conviron GR48; Controlled Environments).

Environmental conditions 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 μmol m⁻² s⁻¹. The light intensity was increased to 365 μmol m⁻² s⁻¹ on day 4. Trays were briefly moved from the environmental growth chamber to the adjoining laboratory each day to apply water and measure germination. Irrigation, or hydration, followed the method described by Norton (1996). Field capacity was determined in early studies; this volume of water was added to all replicates daily for the duration of each study.

A standard plant-growth study occurs over a 14-day period (Norton, 1996), but a 20-day growing period was used to compensate for the lower temperatures and slower growth of the bluegrass. Daily measurements of temperature, relative humidity, and seedling emergence were recorded. In addition, any observed sublethal effects such as wilting or discoloration were recorded. Light intensity, temperature, and relative humidity were measured to ensure that conditions were within the acceptable range proposed in Norton (1996). Observations of seed germination occurred daily, and final measurements were recorded on day 20.

At the end of the 20-day period, the number of surviving seedlings was recorded and used as the final survival measurement. Following this measurement, individual seedlings were harvested for biomass measurements by cutting the shoots at the soil interface.

All combined shoots harvested from each replicate were placed in pre-tared aluminum foil pans and weighed (wet wt). Shoots were then dried at 65 °C for 24 h, followed by cooling in a desiccator for 30 min. The plants were then weighed to obtain an initial dry weight using a calibrated four-place balance (AX105DR; Mettler Toledo). Plants were then placed in an oven to dry for an additional 2 h and back in the desiccator for 30 min, 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 greater than the resolution, or 0.0002 g. Therefore, the combined accuracy of the biomass measurements is assumed to be 0.0003 g, with the lowest calculated dry-mass of 0.0041 g, making the maximum uncertainty in the biomass measurements estimated at 7%.

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, with 25 g of soil and 25 ml of deionized water mixed in a 100-ml beaker using a magnetic stir bar on a stir plate for 5 min. The pH was then measured using a calibrated bench-top pH meter (Mettler Toledo; Spectrum Technologies). The pH of each soil classification was measured prior to the seed planting. After the 20-day period, the pH of the soil in three replicate containers of each exposure and vegetation type was measured (Table 2). The standard deviation of the replicates ranged from 1% to 3% of the measurement, or less than the accuracy of the instrument.

| TABLE 1: Homogenized soil concentrations |
|------------------------------------------|
| Lead | Control | Low | Medium | High |
|------|---------|-----|--------|------|
| No. of measurements | 1 | 4 | 3 | 2 |
| Average (mg kg⁻¹) | 5.7 | 200 | 660 | 3400 |
| Standard deviation (mg kg⁻¹) | N/A | 5.1 | 32 | 2.8 |
| Relative standard deviation | N/A | 3.0% | 5.0% | 0.082% |

N/A = not applicable.
TABLE 2: Soil pH measured before and after test

| Soil     | Before experiment | Cheatgrass | Lettuce | Bluegrass |
|----------|-------------------|------------|---------|-----------|
| Control  | 6.4               | 7.6        | 7.7     | 7.6       |
| Low      | 6.8               | —a         | 7.6     | —a        |
| Medium   | 6.1               | 6.4        | 6.5     | 6.3       |
| High     | 6.1               | 6.4        | 6.6     | 6.5       |

aData not reported. Sprouts exceeded numbers planted.

RESULTS AND DISCUSSION

Although the germination of seeds within the control soil (87%) was below the 90% standard, temperatures were lower than the conditions specified in Norton (1996). Hence, results are conditionally acceptable given that the temperature is outside the standard, consistent with guidelines described in Norton (1996).

It was also noted that the number of seedlings sprouted in the low-concentration soil exceeded the number of seeds added to a replicate. Although it was easy to differentiate between the lettuce and the extra sprouts, these additional sprouts were visually similar to the cheatgrass and bluegrass sprouts. Therefore, all measurements for cheatgrass and bluegrass in the low-concentration soil were excluded. As the medium- and high-concentration soils were collected from areas with little to no surface vegetation, we assumed that all sprouted plants were from seeds planted as part of our study. In addition, the sprouts in the medium- and high-concentration soil all came at expected locations (per the gridded planting pattern). None of the medium- and high-concentration pots had more sprouts than the number of seeds planted, and the appearance of the sprouts was more consistent than for the low-concentration soil. We assumed that the variation in depth of native seeds in the low-concentration soil resulted in this timing difference. Finally, there were no grass sprouts identified in the medium- and high-concentration samples for lettuce. All these factors provide evidence that the extra seeds were only present in the low-concentration sample, and that the results for the medium- and high-concentration cheatgrass and bluegrass are valid.

Although the percentage of germination varied across the various species and concentration combinations, there were no obvious trends in the germination data (Table 3). The low-concentration soil had the lowest germination occurrence for lettuce, and the high-concentration soil had the highest percentage of germination occurrence for bluegrass. This would indicate that the concentrations were likely not high enough to significantly impact germination and that differences in percentage of germination may be a function of other conditions. For example, a previous local study (McCarthy, 2012) showed that the percentage of germination of Sandberg's bluegrass increased as soil nitrogen, pH, percentage of silt/clay increased and decreased as the percentage of sand increased.

The biomass measurements indicated a difference between the treatments (Table 4). Specifically, the high-concentration soil appeared to induce a reduction in seedling growth (Figure 1). A series of independent two-sample Student’s t-tests was conducted ($\alpha = 0.01$) to compare the biomass growth of the plants in different soils (Table 5). Differences in plant mass/seedling among the control and low-, and medium-concentration soils might be explained by other factors or statistical variation ($p > \alpha$), but the observed reduction in the high-concentration soil relative to the control and low- and medium-concentration soils was statistically significant ($p < \alpha$).

The assessment that seedling growth was impacted at the highest concentration soil is consistent with the results of two previous studies (Delistraty & Yokel, 2011; Fleming et al., 1943). Our study followed a protocol similar to that of Delistraty and Yokel (2011), whereas Fleming et al. (1943) used a protocol with some differences, including growing the plants outside.

TABLE 3: Germination success as measured on day 20 of the experiment

|          | Total no. of seedlings on day 20 | % Germination |
|----------|----------------------------------|--------------|
| Cheatgrass |                                  |              |
| Control   | 51                               | 94%          |
| Low       | —a                               | —a           |
| Medium    | 49                               | 91%          |
| High      | 47                               | 87%          |
| Lettuce   |                                  |              |
| Control   | 48                               | 89%          |
| Low       | 32                               | 59%          |
| Medium    | 45                               | 83%          |
| High      | 39                               | 72%          |
| Sandberg’s bluegrass |                          |              |
| Control   | 41                               | 76%          |
| Low       | —a                               | —a           |
| Medium    | 35                               | 65%          |
| High      | 42                               | 78%          |

aData not reported. Sprouts exceeded numbers planted, making counts unreliable.

TABLE 4: Summary of soil concentrations and biomass measurements

| Soil concentration (mg kg$^{-1}$) | Control Low Medium High |
|----------------------------------|-------------------------|
| Lead                             | 5.7 200 660 3400        |
| Arsenic                          | 4.0 34 58 790           |
| Biomass growth total dry weight (mg) | 730 —a 900 140 |
| Cheatgrass                       | 730 —a 900 140        |
| Lettuce                          | 990 880 700 170        |
| Bluegrass                        | 78 —a 57 30           |
| Biomass growth average dry weight/seedling (mg) | 14 (2.2) —a 18 (6.5) 3.1 (1.2) |
| Cheatgrass                       | 14 (2.2) —a 18 (6.5) 3.1 (1.2) |
| Lettuce                          | 21 (6.7) 30 (11) 16 (7.2) 4.5 (1.6) |
| Bluegrass                        | 1.9 (0.32) —a 1.6 (0.17) 0.72 (0.14) |
| Relative growth (% of control)   |                         |
| Cheatgrass                       | 100 —a 130 21          |
| Lettuce                          | 100 144 79 22          |
| Bluegrass                        | 100 —a 85 37%          |

aData not reported. Sprouts exceeded numbers planted.

bIndicates standard deviation of the average.
and measuring growth after 30 days. In addition, Fleming et al. (1943) only reported the range of growth decrease (as a percentage of the control) for groups of vegetables. In the present study, for consistency, we only considered the grouping of vegetables that included lettuce (beets, cabbage, sweet corn, lettuce, muskmelon, parsley, radish, turnip). In addition, the concentrations of Pb and As in the Fleming et al. (1943) study were calculated based on the mass application rate/acre, mixed to a depth of 3 inches, and assuming a bulk density of 1.4 g cm$^{-3}$.

Despite some of the methodology differences, all three studies indicated similar responses to increasing soil concentrations of Pb and As. It is important to note that all these studies considered the combined effects of Pb and As (Delistraty & Yokel, 2011; Fleming et al., 1943). Whereas there was little impact on germination, lettuce growth was reduced at higher Pb and As concentrations (Figure 2). The results of all three studies appear to be consistent and indicate that impact on growth begins when Pb and As concentrations are greater than (nominally) 500 and 200 mg kg$^{-1}$, respectively. These results extend the range of concentrations tested relative to previously reported tests, and show a statistically significant impact on seedling growth at the highest concentrations.

For instance, the results of these bioassays represent one line of evidence for realistic evaluation of the risks that historically applied PbHASO$_4$ presents to ecological receptors in the former orchard lands at the Hanford Site. In the present study, minimal impacts were observed on growth and germination in the medium-concentration soil (660 and 58 mg/kg for Pb and As, respectively). For comparison, ecological indicator soil concentrations for plants from Table 749–3 of the State of Washington Model Toxics Control Act (Washington State Legislature, 2007) are 50 and 10 mg/kg for Pb and As, respectively. The site- and PbHASO$_4$–specific bioassay results will serve to better focus development of remedial actions for the former orchards to minimize contaminant risks to the plant community in the former orchards while maximizing the maintenance of existing habitat.

**CONCLUSIONS**

Our results demonstrate the combined impacts on early plant growth of Pb and As in soil. These results will be critical for the development of dose–response models, because the range of concentrations evaluated extends to higher soil concentrations than previously reported in the literature. This extended range allows for the observation of statistically significant impacts on seedling growth for all three plant species tested (i.e., the median effect concentration).
Although individual impacts of each metal have not been evaluated, the combined impact is relevant for former orchard lands. These findings add to published data on the impacts of Pb and As on plant growth that can be used for determining site-specific SSLs for ecological risk assessments.

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