Accumulation of Lead and Arsenic by Lettuce Grown on Lead-arsenate Contaminated Orchard Soils

E. E. Codling[*]

USDA-ARS, Environmental Management and Byproduct Utilization Laboratory, 10300 Baltimore Avenue Beltsville, MD. 20705

Abstracts: Lead-arsenate was used to control codling moth (*Cydia pomonella)* in apple (*Malus sylvestris* Mill) orchards from the 1900's to the1960's. Lead and arsenic are generally immobile and remain in the surface soil. Some orchard lands are being used for vegetable crop production. There are concerns of lead and arsenic accumulation in vegetables crops grown in these soils. Objectives were to 1) determine lead and arsenic uptake by lettuce and 2) determine the translocation into lettuce leaves. Lettuce (*Lactuca sativa* L) was grown for 60 days in four orchard soils (Bagstown, Hudson, Spike and Cashmont) with total lead and arsenic ranging from 350-961 and 93-291 mg kg^{-1} respectively. Plants were harvested and separated in younger and older leaves, rinsed and dried. Yield reduced to 18, 11, 38 and 31 percent for plants grown on Bagstown, Hudson, Spike and Cashmont soils respectively. Tissue lead and arsenic concentrations were higher in the plants grown on the lead-arsenate soils compared to control. Lead and arsenic concentrations ranged from 0.44 to 3.91 and 4.65 to 24.1 mg kg^{-1} respectively. Unlike arsenic levels in water, there are no established standard for arsenic in food; therefore, it is difficult to determine if arsenic levels observed in this study should be of concern to the consumer. Until there are established standard limits for arsenic in food, care should be taken when these soils are used for lettuce production without soil remediation. Further studies are needed to determine what fraction of lead and arsenic in lettuce is bioaccessible when consumed.

INTRODUCTION

Lead arsenate was used as a foliar spray from the 1900s to the1960's to control codling moth (*Cydia pomonella)* in apple (*Malus sylvestris* Mill) orchards [1, 2]. Lead (Pb) and arsenic (As) are generally immobile in soil and remain in the surface soil due to adsorption by fine silt and clay particles, amorphous oxides, and organic matter [3-6]. Concentrations of Pb and As in some orchard soils exceeded 900 and 200 mg kg^{-1} respectively, [7, 8]. These concentrations are much higher than the 400 and 40 mg kg^{-1} considered safe by EPA for Pb and As in contaminated soils [9].

Lead

Lead is a toxic element that occurs naturally in rocks, soil, plants, water, and the atmosphere. Lead has been used in many products such as in anti-knock compound, gasoline, pesticides, paints, and storage batteries [2]. It can be harmful to humans when inhaled, ingested directly through the consumption of contaminated water, or ingested indirectly by consuming crops grown on contaminated soils or irrigated with contaminated water [10, 11]. Diseases such as neurobehavioral impairment, hypertension, and cardiovascular

disease in humans are attributed to excessive Pb exposure, especially in growing children [11].

Lead in Plants

Plants generally do not accumulate substantial amounts of Pb in tops or edible tissues [12]. Lead is mostly available for plant uptake in soils with low organic matter, low pH, and low phosphate [13]. When phosphate is present in the rhizosphere, an insoluble Pb compound (chloropyromorphite) is formed near or in the plant roots [14]. Brown *et al.* (2004) [15] reported a reduction in soluble Pb with increased concentration of phosphate. Nriagu (1978) [16] suggested that Pb in soils with high pH and phosphate will precipitate as Pb phosphate, Pb carbonate, or Pb hydroxides that are not readily available for plant uptake.

Studies have shown that some vegetable crops remove Pb from Pb contaminated soils. Chisholm (1972) [17] reported that Pb concentration in carrots grown on two lead-arsenate contaminated soils exceeded the Canadian residue tolerance levels for human safety of 2 mg kg^{-1}. Boon and Soltanpour (1992) [18] found that Pb concentration of leafy vegetables such as spinach ranged from < 5 to 45 mg kg^{-1} when grown on soils with a total Pb concentration above 2000 mg kg^{-1}. Creger and Peryea (1994)[19] concluded that the increased Pb concentration of leafy vegetable crops grown on a Pb contaminated soil may have resulted from soil particles containing Pb adhering to the plant surface.

*Address correspondence to this author at the USDA-ARS, Environmental Management and Byproduct Utilization Laboratory, 10300 Baltimore Avenue Beltsville, MD. 20705; Tel: 301-504-5708; Fax: 301-504-5456; E-mail: eton.codling@ars.usda.gov

Arsenic

Arsenic is a ubiquitous element that is found naturally in the environment [20]. Inorganic arsenite (As $^{3+}$) and arsenate (As $^{5+}$) are the dominant toxic As species found in food and drinking water [21-23]. High exposure to As has been shown to cause skin, lung and bladder cancer in humans [24-26]. Humans can be exposed to As directly through the consumption of water and indirectly by consuming crops grown on As contaminated soils or irrigated with contaminated water [22, 23, 27-29]. It has been estimated that 35 to 77 million people in Bangladesh are at risk of As poisoning from drinking As contaminated well water [28]. The major pathway for human As contamination is the consumption of drinking water [28, 30]. The recommended guideline set by Environmental Protection Agency (EPA) for As in drinking water is 10 μgL^{-1} [9], but it currently has no recommended guidelines for As in food crops.

Arsenic in Plants

Arsenic is not essential for plant growth and inorganic As species are generally regarded as potentially phytotoxic when they accumulate in soils [23, 31]. Uptake, accumulation, and translocation of As by plants are influenced by factors such as 1) soil properties, 2) soil As concentration, 3) presence of other ions which compete with As for sorption on soil surfaces, and 4) plant species and age [23, 32, 33]. Geng *et al.* (2006) [34] stated that soil-plant transfer of As is one pathway for human exposure to As. It is believed that most food crops are injured by soil As before the crops can accumulate enough As to become a health risk to humans and animals [35, 36]. For example, Gusman *et al.*, (2013) [21] observed yield reduction in lettuce grown hydroponically. There are concerns that Pb and As will enter the food chain through the consumption of vegetables crops grown on lead-arsenate contaminated soils. Therefore, the objectives of this study were to 1) determine Pb and As uptake by lettuce 2) determine movement of Pb and As in lettuce leaf tissue.

MATERIALS AND METHODS

Soil Collection

Bagstown loam (*Oxyaquic Hapludults*), Spike sandy loam (*Psammentic Haplaquolls*), Hudson silty clay loam (*Glossaquic Hapludalf*), and Cashmont silt loam (*Aridic Haploxerolls*) were collected from the surface 15 cm depth from four orchards in Maryland, Michigan, New York and Washington State with histories of lead-arsenate use. A non-orchard soil, Christiana (control) fine sandy loam *(Typic Paleudult)*, was collected from Maryland for use as a control soil.

Soil Analysis before pH Adjustment

Soil pH was measured in a 1:1 soil to water slurry using a combined electrode; electrical conductivity (EC) measured in a 1:2 soil to water slurry using a Orion conductivity meter and organic carbon was determined with a LECO-CN analyzer (St Joseph, MI). Soil texture was determined by the pipette method [37]. Total soil Pb and As were determined using the aqua regia digestion method [38]. Soil Pb concentrations in the digested solution were determined using a

Perkin Elmer inductively coupled plasma-optical emission spectrometer (ICP-OES) with scandium as an internal standard. Soil As concentrations in solution were determined by an ICP-OES hydride method outlined by Anderson and Isaacs (1995) [39]. The procedure was as follows: 4 ml of soil extract solution was placed into 15 mL test tubes and 1.5 mL concentrated HCl (trace element grade), 2 mL potassium iodide solution, and 2.5 mL 1.73 M sulfamic acid were mixed and allowed to stand for 30 minutes for reaction to occur. A 0.5 % sodium borohydride and 0.05% sodium hydroxide solution was used to generate arsine gas as it entered the nebulizer of the ICP-OES.

Soil Analysis after pH Adjustment

In order to attain a pH of 6.5 calcium and magnesium carbonate were mixed with the soil and allowed to incubate for two weeks. After two weeks, pH and EC were determined as outlined above. The Mehlich-3 (M-3) method [40] was used to determine extractable soil Pb and As. Lead and As concentrations in the extracting solution were analyzed using ICP-OES as outlined above.

Experiment

This growth chamber pot study was conducted at the United States Department of Agriculture; Agricultural Research Service in Beltsville Maryland, USA. The pH adjusted soils were fertilized with 300 kg ha^{-1} of P as calcium phosphate $Ca(H_2PO_4)_2$ and potassium phosphate (KH_2PO_4), 100 kg ha^{-1} nitrogen as ammonium nitrate (NH_4NO_3), 60 kg ha^{-1} Mg as magnesium sulfate ($MgSO_4$) and magnesium carbonate ($MgCO_3$) and 232 kg ha^{-1} K as (KH_2PO_4). Soil and fertilizers were mixed and incubated moist (near field capacity) for four weeks. Fertilizer rates used were based on the fertilizer recommendation for lettuce production.

There were five soils x four replications for each soil. Four kilograms of each soil (based on air dried weight) were placed in 20 ×18 cm plastic pots and planted with Parris Island romaine lettuce (*Lactuca sativa* L.). Soil surfaces of each pot were covered with 2 cm of plastic beads to reduce splashing during watering. Pots were placed into a plastic saucer, and then placed in a growth chamber in a randomized complete block design with 16 hr light at 25°C and 8 hr dark at 19°C. Plants were watered every two days or as needed to maintain field capacity. Any leachate collected was returned to the pots. Eight days, after germination, plants were thinned to 2 plants per pot and grown for 60 days. Lettuce was harvested by cutting the stem above the fourth lowest leaves. The harvested leaves were separated into newer and older leaves, washed with sodium lauryl sulfate and rinsed with deionized water and freeze-dried for 3 days for yield determination. After yield was determined, plant tissue was ground using a Whaley mill, mixed-well, and stored until analysis.

At end of the experiment, soil in each pot was mixed and a sample was collected for pH, EC and M-3 extractable Pb and As determination.

Plant Analysis

A microwave digestion method as outlined by Codling and Ritchie (2005) [7] was used for plant sample digestion.

Table 1. Texture, pH, organic carbon (OC), electrical conductivity (EC), total and Mehlich-3 (M-3) extractable Pb and As in soil used in experiment before and after pH adjustment.

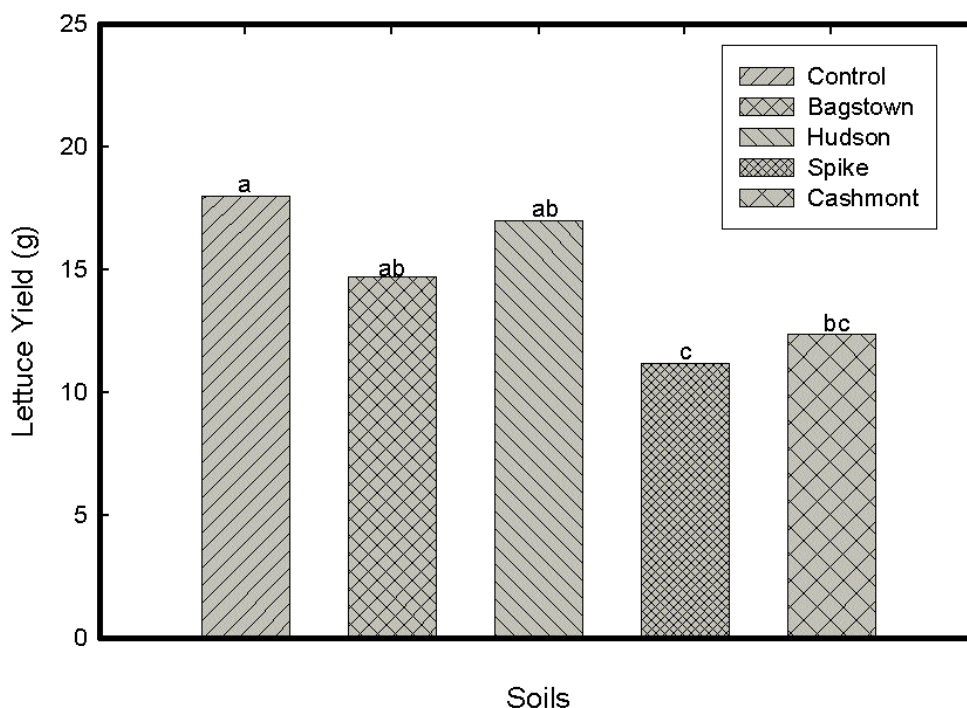| Soils	Texture	OC	pH	pH	EC	EC	Total Pb	M-3 Pb	Total As	M-3 As
	Before	**Before**	**Before**	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**
		mg kg^{-1}	--------- ms cm^{-1}---------				----------------------mg kg^{-1}-----------------------			
Control	Sandy loam	18.0	5.32	6.29	0.48	1.00	20	<0.01	4.0	0.45
Bagstown	Sandy loam	100	5.16	6.69	0.80	1.19	676	263	133	20.29
Hudson	Silt loam	43.9	4.49	6.12	1.05	1.24	435	164	153	13.15
Spike	Sandy loam	14.4	7.08	6.07	0.35	0.62	350	252	93	37.75
Cashmont	Silt loam	12.4	5.26	6.32	0.20	0.55	961	524	291	110

Fig. (1). Yield of lettuce grown on a control soil and four lead-arsenate contaminated orchard soils. Columns with similar letters are not significant at p <0.05.

Pb in the digested solution was determined using ICP-OES as outlined above. Plant As concentration was determined using the hydride procedure previously outlined above. To ensure accuracy and precision, all samples were analyzed in duplicate with one blank for every ten samples. Peach leaves from the National Institute of Standards (NIST), Gaithersburg MD, was included as standard after every 30 samples.

Statistical analysis of the data was carried out using SAS Proc Mixed to model the treatment effects. Means comparisons were done with Sidak adjusted p-values so that the experiment-wise error was 0.05 [41].

RESULTS AND DISCUSSION

Selected properties of soils used in the experiment are presented in (Table **1**). Adjusting the soil pH before the ex-

periment influenced the pH and EC values. M-3 extractable As concentrations in the contaminated orchard soils were much lower than those of the total concentrations. Total Pb concentration in three of the four orchard soils exceeded the 400 mg kg^{-1} level considered hazardous to exposed organisms [42], and could pose health risk if these soils are used for crop production without remediation. Total As concentration in these orchard soils exceeded the 40 mg kg^{-1} limit USEPA has set for As contaminated soil [9].

Lettuce Yield

Lettuce grown on lead-arsenate soils yielded lower than those grown on the control soil (Fig. **1**). The difference, however, was only significant for the plants grown on the Spike and Cashmont soils. For example, yields were 38 and 31 percent lower for the Spike and Cashmont soils respectively compared to the control soil. Plants grown on the

Table 2. Lead (Pb) and arsenic (As) concentrations in lettuce leaves grownon lead-arsenate contaminated orchard and control soils.

Soils	Plant	Pb	As
	Leaves	-------------mg kg^{-1}--------------	
Control	New	BDL†	0.05 f
Control	Old	BDL	0.48 f
Bagtown	New	0.27 c	3.15 ef
Bagtown	Old	0.61 c	19.05 c
Hudson	New	0.38 c	2.00 f
Hudson	Old	0.66 c	7.30 de
Spike	New	0.50 c	7.42 de
Spike	Old	1.97 b	24.04 b
Cashmont	New	2.11 b	9.67 d
Cashmont	Old	5.81 a	41.44 a

† BDL = below detection limit.

Hudson soil had the highest yield of plants grown on the lead-arsenate contaminated soils. This soil had the lowest M-3 extractable Pb and As of the four soils (Table **1**). Yield reduction in lettuce was also observed by Michalska and Asp (2001) [43] when grown 0.5µM Pb solution. Reduction in lettuce yield in plants grown in an As rich environment may result from interference of As in the photochemical and biochemical steps of photosynthesis [21].

Lettuce Tissue Pb and As Concentrations

Lead concentration in the control soil was below the instrument detection limits of 0.03 mg kg^{-1}. Lead and As concentration were highly influenced by soil, leaf age and interaction soil x leaf age (p<0.0001). In all cases, Pb concentrations were higher in plants grown on the lead-arsenate soils compared to the control soil (Table **2**). Lead concentrations were higher in the older leaves compared to the younger leaves. For example, Pb concentrations were 56, 42, 75 and 64 percent higher in the older leaves compared to the younger leaves for the plants grown on Bagstown, Hudson, and Spike and Cashmont soils, respectively.

Arsenic concentrations were higher in the plants grown on the lead-arsenate contaminated soil compared to the control (Table **2**). Arsenic concentrations in the older leaves were higher than in the younger leaves. For example, As concentrations were 84, 73, 69 and 77 percent higher in the older leaves compared to the younger leaves for plants grown on the Bagstown, Hudson, Spike and Cashmont soil respectively.

The higher Pb and As concentrations observed in the older leaves demonstrated that Pb and As are not readily translocated from older to younger leaves. The higher Pb and As concentrations observed in the older leaves is not a result of soil contamination from splashing during watering as concluded by Greger and Peryea (1994) [19], because in this study the soil surface of each pot was covered with plastic beads and the four lower leaves were not harvested.

Averaged over leaf age, Pb concentrations were higher in the plants grown on the Cashmont and Spike soil compared to Bagstown and Hudson (Table **3**). There was no significant difference in lettuce Pb concentrations between the plants grown on Bagstown and Hudson soils. Even though, the total Pb concentrations in the Bagstown and Hudson were higher than in the Spike soil, tissue Pb concentrations in the plants grown in the Bagstown and Hudson were significantly lower than the plants grown on the Spike soil (Table **3**). The lower tissue Pb concentrations observed in the plants grown on the Bagstown and Hudson soils may have resulted from low extractable Pb in Bagstown and Hudson soils. Brown *et al*. (2001) [15] stated that change in soil Pb mineralogy through the application of soil amendment such as phosphate fertilizer can reduce the extractability and plant availability of Pb. The lettuce Pb concentrations observed in this study were much lower than the 37-87 mg kg^{-1} that was found by Bunzl *et al*. (2001) [44] when lettuce was grown in Black and Red slags with Pb concentrations ranging from 1580-5460 mg kg^{-1}.

Table 3. Lead (Pb) and arsenic (As) concentrations in lettuce grown on lead-arsenate contaminated orchard and control soils, averaged over leaf age.

Soils	Pb	As
	------------mg kg^{-1}-------------	
Control	<0.03 c†	0.26 e
Bagtown	0.44 c	11.57 c
Hudson	0.52 c	4.65 d
Spike	1.24 b	15.24 b
Cashmont	3.91 a	24.11 a

† Means within a column having letters in common is not significantly different at p < 0.05.

Table 4. Soil pH, electrical conductivity (EC), Mehlich-3 extractable Pb and As in lead-arsenate contaminated and control soils after lettuce crop.

Soils	pH	EC	Pb	As
		mSa/cm	----------mg kg^{-1}------------	
Control	6.08 b†	0.69 a	1.64 e	0.32 d
Bagstown	6.09 ab	0.36 b	271 b	30.90 b
Hudson	6.01 b	0.37 b	146 d	15.20 c
Spike	6.49 a	0.30 b	222 c	40.10 b
Cashmont	6.27 ab	0.46 b	461 a	117 a

† Means within a column having letters in common is not significantly different at $p < 0.05$.

Arsenic concentrations increased significantly in lettuce grown in the lead-arsenate soils (Table **3**). Among the lead-arsenate contaminated soils, As concentrations were highest in the plants grown Cashmont soil and lowest in those grown in the Hudson soil which reflects the M-3 extractable As (Tables **1** and **4**). Arsenic concentrations in the plants grown on the Cashmont soil were 52, 81 and 37 percent higher than plants grown on the Bagstown, Hudson and Spike soils, respectively.

Selective Properties of Soil after Experiment

Soil pH values between the control and the lead-arsenate contaminated soils were less than one pH unit. EC value was higher in the control soil compared to the lead-arsenate soils (Table **4**). Mehlich-3 extractable Pb concentrations were higher in the lead-arsenate contaminated soils compared to the control. Lead concentrations were highest in the Cashmont soil and lowest in the Hudson soil (Table **4**). M-3 extractable Pb concentrations in the Hudson, Spike and Cashmont soils were 18, 30 and 63 mg kg^{-1} lower than before the experiment (Tables **1** and **4**). The reduction in Pb may have resulted from the addition of P fertilizer to these soils. It has been demonstrated by [8, 15, 45] that phosphorus addition to Pb contaminated soils will reduce Pb solubility.

M-3 extractable As concentrations were higher in the lead-arsenate contaminated soils than the control. Arsenic concentrations were 10, 2, 2 and 7 mg kg^{-1} higher after the experiment for the Bagstown, Hudson, Spike and Cashmont soils, respectively. The higher extractable As after the experiment may have resulted from the addition P fertilizer. Codling (2007) [45] and Peryea (1998) [8] reported that adding P to lead-arsenate contaminated soil will increase As solubility because phosphate and arsenate exhibit similar physicochemical behaviors in soils and phosphate absorb more readily than arsenate on soil and organic matter surfaces.

CONCLUSION

Lettuce yield was reduced when grown on lead-arsenate contaminated soils. From this study lettuce plants did accumulate Pb and As, but mostly in the older leaves. Unlike As levels in water, there are no established standard for As in food; therefore, it is difficult to determine if the As levels observed in this study should be of concern to the consumer.

Until there are established standards limits for As in food, care should be taken when these soils are used for lettuce production without soil remediation. Further studies are also needed to determine what fraction of the Pb and As in the lettuce is bioaccessible when consumed by human and animals.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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