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Arsenic uptake and toxicity threshold for lettuce plants (*Lactuca sativa* L.) grown in poultry litter amended soils

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Poultry litter (PL) is used as a soil amendment agriculture, and can contain toxic As, which can accumulate in soils and absorb by plants. A greenhouse study was conducted to evaluate the uptake of As in Kirkham and Sunset soils which received PL for over 20 years. The soils received 0, 5, 10, 15, 20, and 25 mg kg⁻¹ arsenic and black seeded 'Simpson' lettuce grown. At maturity, plants were divided into roots and shoots, and analyzed for total As. Shoot dry weight declined regardless of soil series or whether poultry litter was applied. Harvest index decreased linearly with increasing As concentrations and was lowest at the 25 mg kg⁻¹ level. As in shoots and roots was greater on Sunset compared to Kirkham soil and greater in PL than in non-PL soils. Root and shoot As was greater in Kirkham NPL than PL soils, 5 times greater in roots than shoots of plants in Kirkham soils, and 11 times higher in roots than shoots of plants in PL-amended Kirkham soils. There were 109 µg/plant As in Sunset soils and 78.7 µg/plant in the Kirkham soil. Toxicity threshold in Kirkham and Sunset soils was 20 and 10 mg kg⁻¹ decreasing leaf area, plant height and yield 46 and 83% in Kirkham and Sunset soils, respectively. As in the edible parts was low and acute exposure was not problematic. Transfer factor in PL amended soils was higher in roots than shoots in Kirkham than Sunset soil, and decreased with increasing soil As.

Key words: Poultry litter, transfer factor, arsenic, animal manure.

INTRODUCTION

Animal manures such as poultry litter are inexpensive compared to commercial fertilizers, and readily available. Manures are excellent source of plant nutrients and organic matter (Nookabkaew et al., 2016; Toor et al., 2007) and can improve soil physical and chemical properties as well as enhance growth of food crops (Wang et al., 2013; Russo, 2010). However, the long-term

practice of applying animal manures to farmland as fertilizer is a short-term solution with potential unforeseen long-term consequences.

Studies show that animal wastes contain trace and non-trace elements such as As, Pb, and Cd. Farmers sometimes add Cu, Zn, and As to animal feeds to control parasites and to ensure weight gain (Foust et al., 2018;

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Moore et al., 1995), of which approximately 95% pass through the animal's digestive track unchanged. Animal wastes can also contain Cd, Mn, Pb and Mn; Foust et al. (2018) and Sims and Wolf (1994) reported that As, Cd, Ca, Mn, Pb, and Zn tend to accumulate in chicken litter, and are about six times more concentrated in the litter than in the carcasses. Once generated, chicken litter is processed (mixed with feathers, peanut shells) and surface applied to soils where crop plants are cultivated for animal and human consumption.

A major concern with application of fertilizer containing trace elements such as As is that over time, they may accumulate in the root zone (Netthisinghe et al., 2016; Toor et al., 2007; Ayari et al., 2010), and may be taken up by plants.

Arsenic is an extremely toxic naturally occurring metalloid (Naujokas et al., 2013; Zhai et al., 2017; Beni et al., 2011). Its uptake by plants is species dependent (Huang et al., 2013; Matschullat, 2000), and is influenced by As oxidation state (Campbell and Nordstrom, 2014), the concentration of soil As and the soil's physical property (Jiang and Singh, 1994), and the presence of other ions (Zhou et al., 2005). Vegetable crops grown in contaminated soils can accumulate As in tissues (Upadhyay et al., 2019; Geng et al., 2017; Zhou et al., 2005), and a hyperaccumulating plant such as the Chinese Brake fern (*Pteris vittata* L.) can accumulate unusually high levels of arsenic. In a greenhouse pot experiment in which soils were spiked with high levels of As, Han et al. (2016) and Martinez (2017) reported that As concentration in fronds of Chinese brake fern reached levels as high as 6017 and 4841 mg kg⁻¹ with no reduction in plant yield, presumably by either compartmentalizing the As in vacuoles where it does not interfere with metabolic activities, or by transforming As to less phytotoxic species.

Other plants, including most leafy vegetables (which are moderately tolerant to As) can accumulate much lower levels, but at soil concentrations higher than about 200 mg kg⁻¹, most of these plants exhibit signs of stress. Plant species that are not resistant to As tend to take up higher levels in the above ground biomass and exhibit noticeable symptoms of stress including inhibition of root development (Latowski et al., 2018).

Abbas et al. (2018) and Metiva (2002) reported an As toxicity threshold of tomatoes (*Lycopersicon esculentum* L.) grown on high As soils is mediated by the addition of phosphorous. Liu et al. (2012) reported that soil concentration of 80 to 100 mg kg⁻¹ reduced yields in wheat (*Triticum aestivum* L.) but not for rape (*Brassica napus*).

Plants grown on sites where animal manures are applied repeatedly over a long period tend to absorb trace elements which may accumulate near the root zone (Tangahu et al., 2011; Sorboni et al., 2013). However, farmers do not see the potential long-term impact of this practice. The cultivation of vegetables in soils amended with poultry litter can serve as a pathway for introduction

of toxic metals/metalloids in the food chain, potentially increasing risks to humans. The objective of this study was to determine the toxicity threshold, and to compare yield, physiological growth responses and As concentration and content in lettuce plants grown on soils which received poultry litter for over 20 years.

MATERIALS AND METHODS

Collection and preparation of soils

Kirkham and Sunset soils were obtained from two separate farms located in Utah county, UT, (40.2130° N, 111.8025° W) where poultry litter has been repeatedly applied annually at a rate of 18.0 mg ha⁻¹ for at least 20 years. Application occurred once each year during the fall after harvest or in the spring before planting followed by discing to a depth of 10 to 15 cm. These soils have been used to cultivate corn, barley and wheat (on a corn-corn-barley-wheat rotation) and watered using the border irrigation method. The water used for irrigation is obtained from Strawberry Reservoir located in Wasatch County, Utah. Soil samples were collected during the summer months of 2010 at depths ranging from 0 to 15 cm. Additional counterpart non-amended soils were also collected within 0.95 km south from the amended Sunset soil, and an approximate distance of 2.7 km from the Kirkham soil to be used as controls. In these soils, corn/alfalfa and hay/wheat, respectively are cultivated. Climatic conditions are similar throughout the entire area where the samples are taken (both amended and non-amended soils) in Utah county and can be characterized as having low humidity, with an annual precipitation of 25.4 to 37.5 cm and an average temperature of 21.1°C during the summer months. A subsample was obtained to determine selected physical and chemical properties of each soil (Table 1). The subsamples were air dried at room temperature and ground to pass a 2 mm sieve and physical and chemical properties as follows: soil particle size distribution using the standard pipette technique (Day and Funk, 1965), pH (soil: water or CaCl₂ 1:2.5 ratio) by glass electrode and cation exchange capacity by the ammonium acetate method (Thomas, 1982). Soils were classified as silty clay loam (fine-silty, mixed, mesic, aquic fluventic) for the Kirkham soil series, and loam (Coarse-Loamy, mixed, mesic, Aquic Fluventic Haplustolls) for Sunset soil series. The remainder of the soils used in the greenhouse study were passed through a 5 cm screen to remove large particles and debris and sterilized at 140°F for 30 min. After sterilization soils were separated into 6 kg portions and spiked above background concentration with 0, 5, 10, 15, 20, and 25 mg kg⁻¹ As, respectively, as sodium arsenate, thoroughly mixed and placed into 20 cm pots for one week before transplanting.

Starting transplants and planting

Black seeded lettuce (*Lactuca sativa* L.) cv. Simpson (IFA Payson, Utah), is heat tolerant, slow to bolt and highly productive. It is an excellent heirloom variety in the Western states, it ships well, and is great for the growing organic vegetable market. Two or three seeds were sown in a tray containing 72 Jiffy peat pellets (Jiffy Eugene, OR) and covered with approximately 2 cm of medium and watered to keep the pellets moist. At fourteen days after planting, one seedling was transplanted to each pot and plants were watered twice each week. Initially, the plants were watered with approximately 250 mL of water but not to saturation, to reduce leaching, or with 250 mL nutrient solution (Rahman and Naidu, 2009). Greenhouse conditions included 12 h/12 h photoperiod, 25.2°C, and photosynthetic photon flux (PPF) at canopy level of

Table 1. Selected chemical and physical properties[†] of the soils (0-15 cm) used in the greenhouse experiment

Soil	Amendment	pH	Sand Silt Clay			OM	Texture	CEC	EC	N	P	K	Fe	Al	Ca	As
			mg kg ⁻¹					cmol kg ⁻¹	dS/M							
Kirkham	NPL	7.2	120	382	498	32.0	Clay	16.4	0.98	50.0	115	432	99440	19520	311	6.40
Kirkham	PL	7.1	140	312	548	38.0	Clay	23.0	1.85	117	24.0	146	117300	20150	342	12.6
Sunset	NPL	6.8	280	422	298	19.0	Loam	15.6	1.70	7.70	5.90	259	94960	13130	292	5.20
Sunset	PL	7.5	280	402	318	24.0	Loam	18.7	2.20	75.2	60.1	234	101500	13710	319	13.0

[†]NPL= Non-poultry litter amended soil, PL= poultry litter amended soil; OM=organic matter; CEC= cation exchange capacity; EC=electrical conductivity.

Table 2. Statistical significance from analysis of variance of soil, As concentration, amendments, soil x amendment and As x amendment of lettuce height, leaf area, shoot and root yield, uptake, and transfer factor for Black Seeded Simpson lettuce grown in Sunset and Kirkham soils as affected by added As.[‡]

Effects	df	Yield		HI	Plant height	As tissue conc.		Uptake		Transfer factor	
		Shoot	Root			Shoot	Root	Shoot	Root	Shoot	Root
		mg kg ⁻¹				µg plant ⁻¹		µg plant ⁻¹		µg plant ⁻¹	
Soil	1	***	NS	NS	***	***	***	***	*	*	***
As Conc.	5	***	*	*	***	***	***	**	***	***	**
Amendments	1	***	***	***	***	**	***	***	NS	**	***
Soil x Concentration	5	**	NS	NS	NS	NS	***	***	***	**	***
Soil x Amendment	1	***	*	*	***	NS	NS	***	***	***	***
As x Amendment	5	***	NS	NS	***	NS	*	***	*	***	***

[‡]Dry weight (DW), harvest index (HI), non-amended poultry litter (NPL), poultry litter amended (PL), and significant at P= 0.05 (*), 0.01 (**), 0.001 (***), 0.001 (****), or nonsignificant (NS). Conc. = concentration.

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1191 µmol m⁻² s⁻¹. The CO₂ ranged from 292 to 502 µmol mol⁻¹, and relative humidity was approximately 32%.

Harvest

Plants were harvested at 45-days after planting (DAP) and total fresh weight and leaf area determined. Each plant was separated into roots and shoots, and final plant height recorded. Roots were dipped in tap water followed by three successive rinses to remove soil adhering to root surfaces. Root and shoot samples were oven dried at 65°C for 72 h, and dry weights determined. Dried plant tissues were ground to pass a 2 mm screen and analyzed for total As concentration at the Soils and Plant Laboratory at Brigham

Young University, Provo, Utah.

Statistical analysis

Experiments were conducted using a randomized complete block design with a 2x2x6 (two soil types, two amendments, at six As concentrations) factorial treatment arrangement and three replications. Data were analyzed using the general linear model (SAS Institute Inc., 2009). For each tissue source, a full model ANOVA was performed. This included the main effects of soil series, use of poultry litter, and arsenic dosage along with their two and three way interactions and the three-way interaction. From this model the insignificant interaction

terms were dropped. All main effects that were included in retained interactions were included in the reduced model regardless of their significance. Post hoc analyses of means were done using Tukey's adjusted t-tests (P<0.05) (data not shown).

RESULTS AND DISCUSSION

There were significant interactions between soil series and As concentrations, soil series and amendments, and As concentrations and amendments (Table 2). Where significant interactions were not observed, the main effects

are presented. The interactions indicate that effect of arsenic concentration on variables were interdependent, that is, soil series effects were different for different amendments and amendment effects were different for different soil series.

Effects of PL applications on plant-available As

Selected chemical and physical properties of the non-amended and amended Kirkham and Sunset soils (prior to being spiked with As) are shown in Table 1. Mineral concentrations as well as CEC, clay, and organic matter (OM) increased with long-term poultry litter application. These increases were greater in Kirkham compared to Sunset soils; total As. The retention of total As by Kirkham and Sunset soils may be attributed to their high clay, OM and ion (Fe, Al and Ca) contents. A number of studies have shown that adding PL increased trace metal concentration in soils (Cadet et al., 2012; Ayari et al., 2010). Han et al. (2004) reported that oxides of Fe, Al and Mn are strongly sorbed to As, and that As was strongly correlated with clay minerals.

The rates of As uptake depend on the metalloid's mobility and bioavailability. Once PL is added to soils, some trace elements, including As is initially quite mobile because As is in a soluble form (Rutherford et al., 2003). Under alkaline conditions, As is more strongly sorbed to Ca^{++} and becomes less bioavailable. Onken and Adriano (1997) reported that As becomes rapidly recalcitrant in soil following arsenate and arsenite treatment under saturated and subsaturated conditions within only 68 days. Han et al. (2004) also reported that residual As is a major solid phase fraction in soils that have been amended over long periods, where the As in the residual solid phase account for 72% of the total As.

In addition, OM in animal manures rapidly and easily decomposed following PL addition. but a small fraction persisted. McGrath et al. (2000) reported that approximately 15% of this OM remained in the soil, and may persist for over 20 years before reverting to background levels. When this residual OM in soil decomposes soluble As is slowly released and can be taken up by plants. In this study, Kirkham soil retained more As than Sunset soil while Clay and Ca^{++} contents were higher in Sunset soil. However, As in Kirkham soil was immobilized being sorbed strongly to OM, ions, and soil particles. Thus, As accumulated at a more rapid rate in lettuce roots and shoots in Sunset but slower in Kirkham. However, when the persistent OM decomposes, As is slowly adsorbed by lettuce roots, allowing for higher As content in the plant tissues. Therefore, PL added to soils in past years contained residual quantities of As that slowly decomposed contributing to plant toxicity observed in lettuce plants years later, particularly in Kirkham soil.

Shoot and root yield

Shoot dry weight declined regardless of soil series

(Figure 1A) or whether poultry litter was applied (Figure 1B). However, the magnitude of decrease was greater in Sunset soil or where poultry litter was applied. Shoot dry weight declined 46% in Kirkham soils and 84% in Sunset soils. A similar trend was observed for shoot yield for plants in PL and NPL soils. Thus the threshold of As phytotoxicity may have approached critical levels in plant tissues, and was achieved at a lower concentration in Sunset than in Kirkham soil which had a higher clay content, retaining As at surfaces, making it less available for uptake.

Tu and Ma (2002) reported a 64% reduction in shoot biomass of *P. vittata* L when grown on soils contaminated with arsenic. Furthermore, McBride (1995) reported that As formed strong bonds with Fe and Al oxides, clay, and OM in soils. Beni et al. (2011) and Abedin et al. (2002) observed that in sandy soils As is five times more phytotoxic than in clay soils. In the present study, Fe, Al, Ca^{++} , clay and OM contents were relatively higher in Kirkham than in Sunset soils (Table 1). With the exception of stunted growth, very few if any symptoms of phytotoxicity were evident with the 0 to 25 mg kg^{-1} range. Similarly, Évio et al. (2012) observed no phytotoxic symptoms in sunflower (*Helianthus annuus* L.) and castor bean (*Ricinus communis* L.) in spite of the reduction in yield.

Harvest index

Harvest index (root mass/total plant mass) decreased linearly with increasing As (Figure 2), and was lowest at 25 mg kg^{-1} , suggesting high As negatively affected that harvest index. According to Velayudhan et al. (1995), typical harvest index for lettuce is 80%, while the present study highest harvest index was approximately 70%. Thus, assimilate partitioning to above ground biomass was reduced because of high As concentration.

Plant height and leaf area

Plant height (Figure 3) decreased significantly ($P < 0.001$) regardless of whether PL was applied (Figure 3A), at a greater magnitude in PL-amended soils. At background concentration, the difference in height between NPL and PL amended soil was negligible, but at 25 mg kg^{-1} , plants grown in PL soil were 4.79 cm taller than those grown in NPL soil.

In NPL soils, the leaf area declined by 44% at the highest soil As level while with PL addition, leaf area declined only 6.5%. The interaction between soil series and As concentration showed that leaf area was greater in Kirkham than in Sunset soil, but declined 17% in Kirkham and 46% in Sunset soils, respectively. The lower leaf area and shorter plants indicated that the plants were physiologically stressed. Arsenic in plants can induce oxidative stress which in turn inhibits normal plant

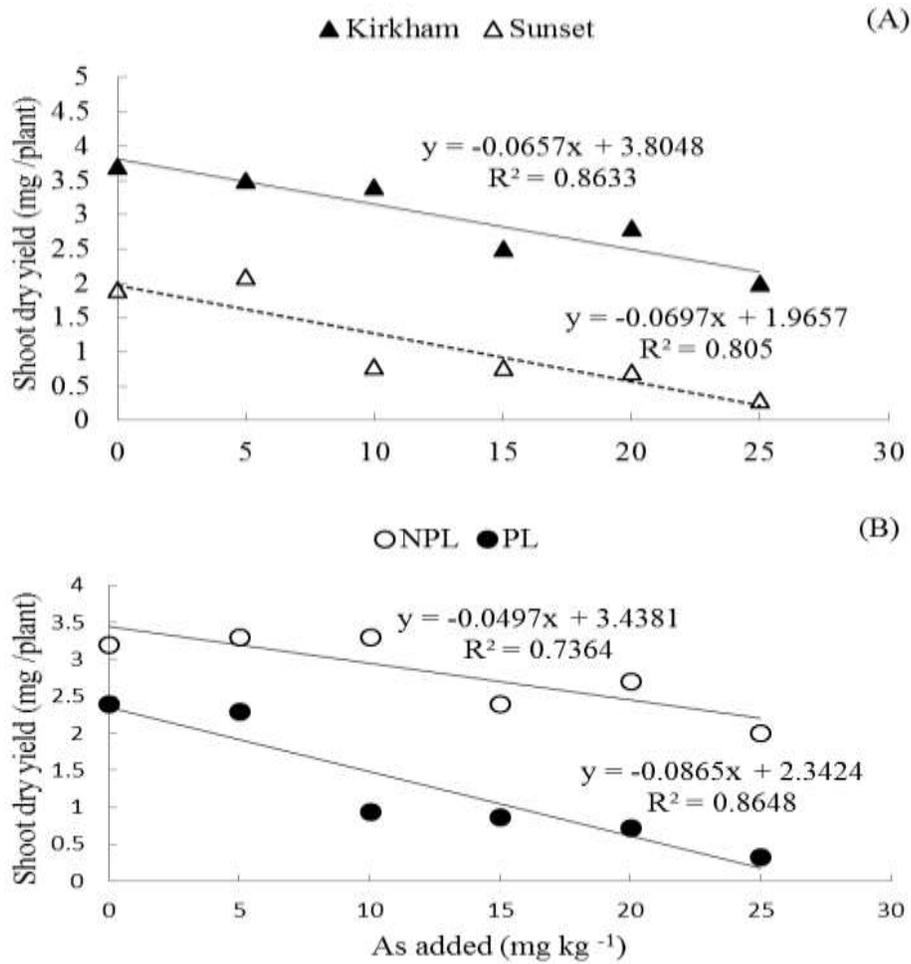


Figure 1. Relationship of arsenic x amendment (A) and arsenic x soil series (B) (x-axis) on dry shoot yield (y-axis) of lettuce grown in Kirkham and Sunset soils amended (PL) or not-amended (NPL) with poultry litter.

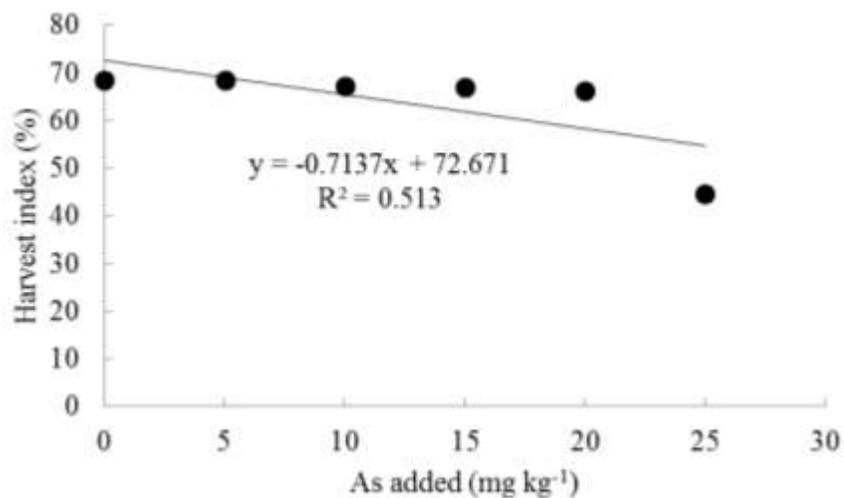


Figure 2. The relationship between added soil (x-axis) and harvest index (y-axis) of lettuce grown in Kirkham and Sunset soils amended (PL) or non-amended (NPL) with poultry litter.

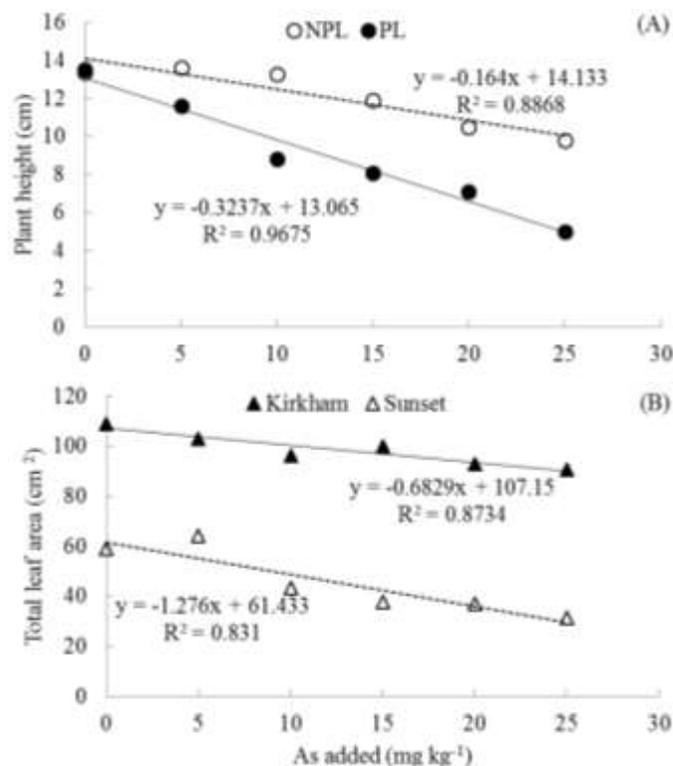


Figure 3. Relationship between As in soils (x-axis) amended (PL) or Not-amended (NPL) with poultry litter on plant height (A) and soil series on total leaf area (B) (y-axis) of lettuce grown in Kirkham and Sunset soils.

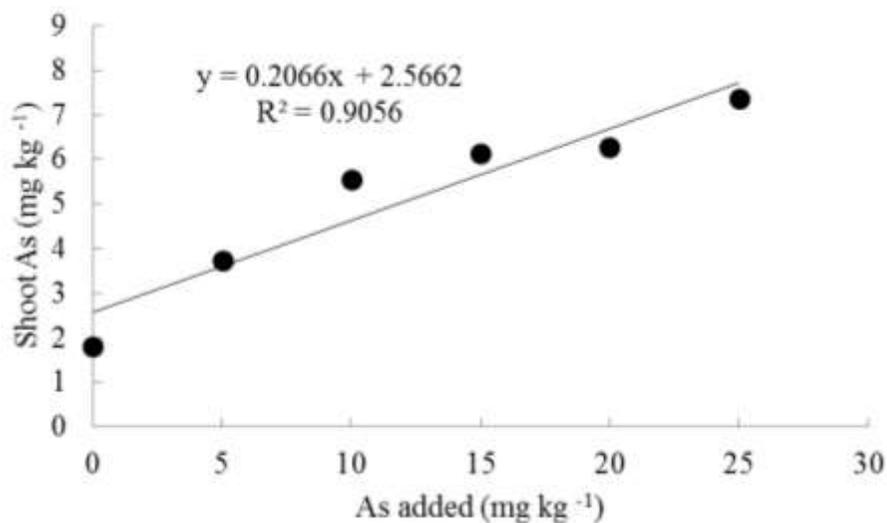


Figure 4. The relationship between added soil (x-axis) shoot As concentration (y-axis) of lettuce grown in Kirkham and Sunset soils amended (PL) or not-amended (NPL) with poultry litter.

development due to deleterious effects on photosynthetic and respiration rates (Llamas and Sanz, 2008). These

findings are consistent with those of Al-Qurainy (2009), who showed that heavy metals such as Ni and Al

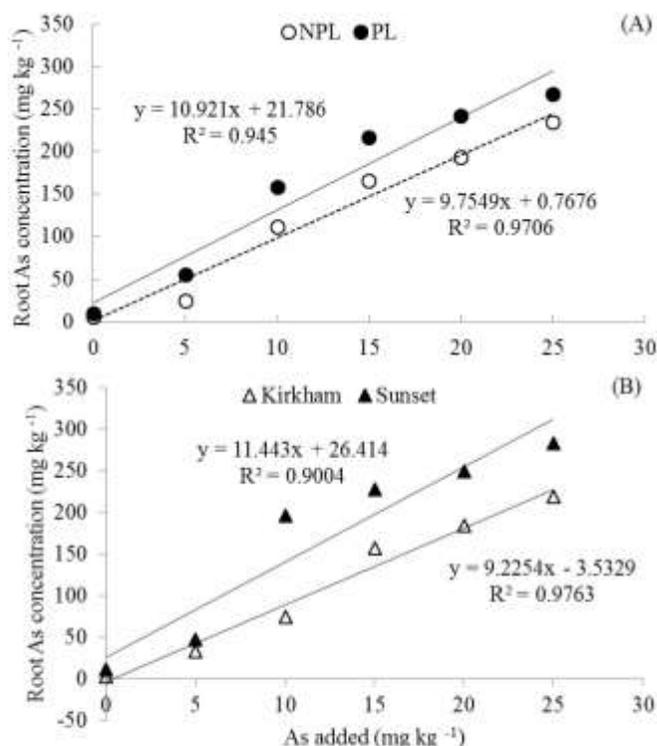


Figure 5. Relationship of interactions between As (x-axis) x amendments (A) and As x soil series (B) on root As concentrations (y-axis) of lettuce grown in Kirkham and Sunset soils amended (PL) or not-amended (NPL) with poultry litter.

reduced plant height and leaf area index.

Arsenic concentration in shoots and roots

As concentration in shoot increased linearly with soil As (Figure 4). There was a significant positive correlation between shoot As ($R^2=0.90$) and As added to soils, regardless of level and soil series. At 25 mg kg⁻¹ As, shoot As was 4 times greater in treated than in control plants, a 310% increase. The fact that there was no significant difference in As concentration in the plants grown in both soils suggested that plants were effective at restricting As in the roots.

Root As concentration increased linearly regardless of soil series, or whether PL was applied (Figure 5A and B). These observations confirm findings that As is not as mobile in clay soils (McBride, 1995; Hartley and Lepp 2008).

Sheppard (1992) reported that the threshold for As phytotoxicity varies with texture, thus, in sandy soils, 40 mg kg⁻¹ As is phytotoxic to plants, whereas, in clay soils it was to 200 mg kg⁻¹. This was attributed to the high retention capacity of As by clay soils, due to higher content of Fe and Al oxides (Évio et al., 2012; McBride, 1995). This means that As is highly mobile in coarse

textured soils (Beni et al., 2011), and is readily available for uptake. Burlo et al. (1999) showed that As concentrations in plant tissues increased with As levels in nutrient solution, or in soils (Évio et al., 2012; Gulz et al., 2005; Geng et al., 2006). The largest quantities of As residues taken up by plants are sequestered in roots, with a relatively smaller quantity in the shoot (Yuan-zhi et al., 2008). This is important because the accumulation of arsenic in the roots will affect the development of the leaves, therefore affecting productivity. Also, although As levels are low in the edible parts of lettuce, there is warrant for concern due to chronic exposure to the metalloid. At the background level (0 mg kg⁻¹ added As), shoot concentration was at 1.8 mg kg⁻¹ and increased to 7.8 mg kg⁻¹ (when soil concentration was 25 mg kg⁻¹). Although this falls below the maximum contaminant levels (MC) of 10.0 mg kg⁻¹ allowed by Environmental Protection Agency (EPA) in drinking water, life time exposure to low levels of arsenic can cause kidney damage and was reported to lower intelligence quotient (IQ) scores in children (ATSDR, 2007).

There is limited information in the literature on the As concentration in lettuce. Concentrations of As for a variety of vegetables, however, have been reported with large variations. Nevertheless, in most cases, root As levels were considerably higher than in shoots and levels

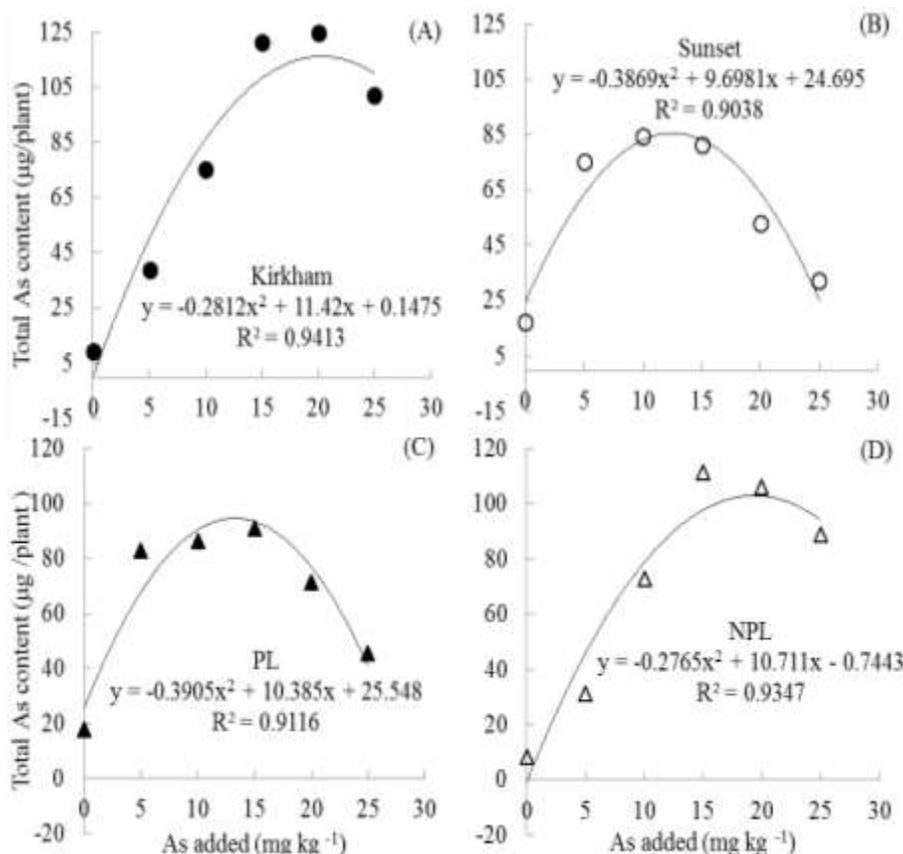


Figure 6. Relationship of interaction between As added (x-axis) x soils (A) and (B) and As added x amendments (C) and (D) on total As content (y-axis; $\mu\text{g plant}^{-1}$) of lettuce grown in Kirkham and Sunset soils, amended (PL) or not-amended (NPL) with poultry litter.

in this study fall within these reported ranges. Smith et al. (2009) reported As concentrations of up to 278 mg kg^{-1} in roots and approximately 3.18 mg kg^{-1} in the leaf of lettuce. In chard (*Beta vulgaris* L.) and radish (*Raphanus sativus* L.) 207 and 35.5 mg kg^{-1} , respectively, were detected in the roots, whereas 3.13 and 6.94 mg kg^{-1} , respectively, were observed in shoots. Others reported lower As levels in plant tissues. Cao et al. (2009) showed that root As for lettuce was less than 40 mg kg^{-1} when grown in a sandy soil. Above this level, root elongation was reduced showing that a toxicity threshold was reached. The results of the present study indicated higher levels of As in roots (250 mg kg^{-1}) and shoot (5.54 mg kg^{-1}) for Kirkham and Sunset soils, respectively. Additionally, shoot concentration in PL-amended soils (regardless of soil series) was 6.14 mg kg^{-1} compared to that in roots of plants grown in NPL soils (217 mg kg^{-1}).

Plant As content

Total plant As content varied with soil series (Figure 6A and B), or whether PL was applied (Figure 6 C and D).

Plants accumulated more As in Kirkham (Figure 6A) vs. Sunset soils (Figure 6B). Total plant As increased with soil As up to about 20 mg kg^{-1} , plateaued, and declined thereafter. In contrast, plants grown on Sunset soils had increased As up to approximately 13.0 mg kg^{-1} , plateaued and declined with increasing soil As (Figure 6B).

Similarly, when plants were grown in PL-amended soil, total As increased with soil As up to 13.0 mg kg^{-1} , but declined thereafter (Figure 6C). Total plant As increased with soil levels in NPL- amended soils up to 20.0 mg kg^{-1} but declined, thereafter, but at a smaller magnitude (Figure 6D). It appears that plants grown in the Sunset soil series and in PL-amended soils approach a toxicity threshold at a lower plant As content. The coefficients of determination (R^2) were 0.94 , 0.90 , 0.91 and 0.93 (Figure 6A-D), respectively, indicating that total plant As content can be predicted for a given level of added soil As. Therefore, on average, 90% or greater of the variation in total plant As can be explained by variation in soil As levels. This decrease in As uptake corresponded to a considerable reduction in shoot dry yield (from 2.13 to 0.79 mg kg^{-1}) and leaf area (from 64.2 to 43.3 cm^2) of lettuce. This constituted a toxicity threshold for lettuce in

Sunset soil since soil concentrations higher than 10.0 mg kg⁻¹ of As added resulted in decreased As uptake.

This finding was not unexpected in clay soils since As availability is generally low. Woolson (1972) observed that it was difficult to detect differences between treated and untreated soils at low soil As concentration because over 90% of the added As was fixed, thereby reducing availability. The toxicity threshold for lettuce grown in Kirkham soils occurred at 20 mg kg⁻¹ because at this concentration accumulation of As resulted in a corresponding reduction in shoot dry yield and leaf area, while that in Sunset soils occurred at 13.0 mg kg⁻¹. Sunset soils are coarse textured, with a sand content of approximately 280 mg kg⁻¹, more than twice that of Kirkham soils (averaging 130 mg kg⁻¹). Because of the relatively low clay content, As was mobile, and more readily available.

Thus As was taken up by lettuce plants at a greater rate (at lower soil As levels) and accumulated higher levels in roots than in the above ground biomass. In contrast, Kirkham soils which are higher in Fe, Al, Ca⁺⁺, clay and organic matter (OM) likely form strong complexes with As, reducing its mobility and availability.

Furthermore, previously added PL may not be available for plant uptake as As becomes more stable with time, thus, it was the recently added As in the PL that was taken up since it was in the soluble form. The rate of uptake is more rapid therefore accumulation of As was much quicker in Sunset soil amended with PL, and approached toxic levels earlier. Whereas, in Kirkham soil the rate of As uptake may be slower since much of it was complexed with soil constituents, especially OM. This allowed the plant to metabolize As to a less toxic form and translocate it to the shoots. This may explain why As content in lettuce is higher when grown in Kirkham compared to Sunset soil.

Meharg and Harley-Whitaker (2002) reported that roots tend to accumulate high levels of As because of their association with sulfhydryl groups, suggesting that the translocation of As from roots to shoots is limited, resulting in a lower concentration in above ground biomass. Porter and Peterson (1975) showed that added As reacted with Fe and other soil constituents, reducing its toxicity. Walsh and Keeney (1975) observed that more As residues in plants are sequestered in roots than in above ground vegetative parts. Beni et al. (2011) reported that in sandy soils, As tended to leach faster due to inability of particles to form complexes with As contaminants. In contrast, Sheppard (1992) pointed out that As phytotoxicity is lower in sandy soils and relatively higher in clay soils; we found that in the Kirkham soil, which was high in clay, there was a greater total content in lettuce because As accumulation in clay soils occurred at lower soil As concentrations. High levels of As in plant tissues (Geng et al., 2006), disrupted the metabolic processes which may inhibit growth, but under more severe conditions (such as high As content in soils) can lead to death (Jiang and Singh, 1994).

Transfer factor (TF)

In PL-amended soils, the TF was higher in roots (ranging from 0.006 to 0.07) relative to the shoots (0.0003 to 0.02) regardless of soil series, and tended to be higher in roots of plants grown in Sunset soil (0.011 to 0.01) than in Kirkham soil (0.005 to 0.07), regardless of soil amendment. The TF, also known as bioconcentration factor (Huang et al., 2006), or transfer coefficient (Warren et al., 2003) is the ratio of metal concentration in plant tissues (dry weight basis) relative to that in soils in which the plants were grown (Cong et al., 2002). The TF is used to determine the ability of a metal to transfer from soil to the plant in a particular soil-plant system (Huang et al., 2006) and is used by the US EPA (1992) to determine the risk to humans who consume plants grown in As laden soils. The more elevated the TF, the more mobile or available the metal. According to Warren et al. (2003), the TF values of As for a number of vegetables ranged from 0.0007 to 0.032. Consequently, when the TF is above 1.0, the plant is said to be hyperaccumulating As (Évio et al., 2012), whereas a TF lower than 1.0 has been identified as a tolerant. Similar results were reported for sunflower and castor bean (Évio et al., 2012), in two soil types (Entisol and Oxisol) and tomato (Burlo et al., 1999). In the evaluation of the present study, the TF for lettuce was lower than that obtained by Cao and Ma (2004), but higher than those of Huang et al. (2005).

The results showed that transferability of As was greater in roots than in shoots of lettuce and was more easily transferred into roots of plants grown in Sunset than in Kirkham soil. The TF in both soils was <0.1, suggesting that 'Simpson' lettuce is tolerant mainly by accumulating higher As levels in the roots than in the shoots. A plant that is tolerant does not necessarily mean that it will not be impacted. Over time, plants develop mechanisms to survive high concentrations of metal toxicity in soil (Meharg and Harley-Whitaker, 2002). One such mechanism is exclusion, where the plants avoid the uptake of toxic metals altogether. Likewise, some plants utilize compartmentalization or chelation mechanisms to withstand high levels of metals in their tissues (Macnair and Cumbes, 1987). For lettuce grown in Kirkham and Sunset soils, As was mostly restricted to roots, with comparatively smaller quantities in shoots.

Hyperaccumulators such as the Chinese Brake fern (*P. vittata* L.), can uptake and sequester unusually high levels of As in shoots. Meharg and Harley-Whitaker (2002) reported that such plants have a TF greater than 1 while tolerant plants such as Tamarik (*Tamarix parviflora*) and Eucalyptus (*Eucalyptus camaldulensis*) (Tossell et al., 2000) can accumulate As ranging between 5 and 100 mg kg⁻¹ (Kabata-Pendias and Pendias, 1992) with a TF less than 1. Such was the case with the lettuce in this study. Non-tolerant plants, which are sensitive to metal toxicity, would suffer considerable stress at similar concentrations (MacNair and Cumbes, 1987). Although plants may be highly tolerant to excessive amounts of As,

they can still be impacted. For example, while *P. vittata* (a hyperaccumulator) can sequester up to 27,000 mg kg⁻¹ of As in the fronds, phytotoxicity symptoms begin to appear at approximately 10,000 mg kg⁻¹ (Wang et al., 2002). There is still an impact; however, as the toxicity threshold is much higher in hyperaccumulating plants and comparatively lower in tolerant or resistant plants, and even much more reduced in non-tolerant species.

Conclusion

Lettuce showed greater tolerance to As when grown in Kirkham compared to Sunset soil, primarily due to soil properties. Chronic impacts were observed in the lettuce grown in Kirkham soil because of the decomposition of residual As. Although this lettuce variety absorbed arsenic from both soil types, much of the metalloid was restricted to the roots. Because of the apparent tolerance of Black Simpson lettuce to As, it may not immediately be harmful if consumed by animals or humans. Although the concentration of As in the edible part of the plant was low, there is still reason for concern because increased PL application may increase As levels in soil that may negatively affect crop yield.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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