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SPECIAL ISSUE: IMPROVING LIVABILITY IN URBAN AREAS: EXAMINING URBAN AND PERI-URBAN SOIL AND PLANT MANAGEMENT

Mycorrhizal fungi and soil factors influence toxic element uptake in urban grown produce

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Abstract

Despite the need for more urban-grown produce, toxic elements contaminated soils continue to be a major barrier to food production and food sovereignty in urban areas and a continued health and environmental justice issue. Although the US EPA provides recommendations regarding levels of soil lead that are safe for gardening, soil abiotic and biotic factors as well as plant identity play a major role in determining the actual crop uptake of toxic elements. This study evaluated the role of crop identity, harvested tissue, and soil factors, including arbuscular mycorrhizal (AM) fungi on crop uptake of lead (Pb) and arsenic (As) in an urban community farm. Crop species varied in their Pb and As accumulations, both by crop identity and also by plant tissue. Crop uptake of lead increased with lower soil pH (range 5.3–6.9) and lower soil P (range 365–1771 mg kg^{-1} total P). For mycorrhizal crops, greater intensity of AM fungal colonization and the prevalence of arbuscules were associated with greater lead uptake, but the presence of more storage vesicles was related to less As uptake into leaves. These findings can help inform crop selection and soil management to improve soil stabilization of toxic elements in moderately contaminated soils while serving as a platform for community conversations about the importance of soil management in healthy urban food production.

1 | INTRODUCTION

In recent years, urban farming and gardening initiatives have increased in many of the metropolitan areas around the United States (Palmer, 2018). Urban-grown food sources have the potential to increase food security, decrease reliance on industrial agriculture, and empower communities to grow their food through a grassroots framework. Because two thirds of the world's population is expected to live in cities by 2050, and peri-urban cropland is at risk of loss to development, broad-based governmental and nongovernmental support for urban agriculture is growing, for example, with the United Nations Food and Agriculture Organization Urban Food Agenda (FAO, 2019). However, a major limiting factor to urban food sovereignty is contaminated soils from years of industrial pollution and development. Further exacerbating the problem, contaminated soils occur more frequently in low-income communities and communities of color due to industrial history, urban renewal, and redlining (Aelion et al., 2013;

Abbreviations: AMF, arbuscular mycorrhizal fungi; UNFAO, United Nations Food and Agriculture Organization.

McClintock, 2015). Costly remediation strategies involving heavy equipment, imported soil, and infrastructure are not always feasible, and thus, wider knowledge of soil management practices can be an important tool to limit exposure and risk.

Some of the most problematic soil contaminants in urban soils are toxic elements (e.g., lead, zinc, arsenic, and cadmium) (Wortman & Lovell, 2013). Although toxic elements are naturally present in trace amounts in many soils, anthropogenic activity has caused increased concentrations in many urban soils over time (e.g., Clark & Knudsen, 2013; McClintock, 2012). For example, agricultural soils in Europe have an average lead (Pb) concentration of 16 mg kg^{-1} (Reimann et al., 2012), whereas urban soils were found to have average concentrations of 106 mg kg^{-1} (Aelion et al., 2013). A recent review by Paltseva et al. (2022) reported median soil Pb concentrations range from 27 to 594 mg kg^{-1} in major US cities. In this study, we focus on arsenic (As) and Pb, which are common contaminants in urban soils due to past use as pesticides and have been widely emitted from industrial and municipal activities (Kabata-Pendias & Mukherjee, 2007). Both As and Pb pose significant health hazards when consumed chronically and are toxic at low concentrations (ATSDR, 2020).

Arsenic is an element that is prevalent throughout the earth's crust. Soil concentrations average about 5 mg kg^{-1} total As worldwide but vary greatly depending on factors, including parent material and volcanic activity (Kabata-Pendias & Mukherjee, 2007). Major sources of elevated As in anthropogenic soils are lead–arsenate insecticides that were extensively used in the first half of the 20th century for fruit tree crops (Ayuso et al., 2004) and chromated copper arsenate, which was used to treat wood used in most construction from the 1930s to 2004 (Jones et al., 2019; Selene et al., 2003). Although leaching from treated wood is perhaps a more common source of As in urban soils, insecticides were also used on orchards that existed in peri-urban settings now claimed by growing cities. Similar to As, Pb naturally occurs in soil at concentrations averaging, for example, 14 mg kg^{-1} in the Eastern United States (Wedepohl, 1995). One main source of elevated Pb in urban soils comes from leaded-gasoline combustion, as it was used as an antiknock agent in gasoline from the 1950s into the 1990s (Clark & Knudsen, 2013). Soils located near urban areas with heavy traffic often became contaminated with Pb from exhaust and fuel spills over time (Mielke & Reagan, 1998). The other main source of anthropogenic Pb comes from Pb-based paints made prior to 1978 (Clark & Knudsen, 2013; Mielke & Reagan, 1998), making areas near older buildings hotspots for Pb contamination due to chipping paint and construction.

Core Ideas

- Lower soil phosphorus and greater mycorrhizal colonization were associated with significantly increased lead uptake.
- Lower soil pH and fewer mycorrhizal storage structures in roots were associated with significantly greater arsenic uptake.
- Leafy greens, but not root crops, grown in moderately contaminated urban soils were considered safe for consumption.

For the urban farmer or gardener, there can be some confusion over what levels of Pb and As are considered within safe ranges for growing food. For instance, recommendations for maximum safe levels of total soil As vary from state to state, for example, from 0.07 to 24 mg kg^{-1} in California and Texas, respectively (Teaf et al., 2010). For Pb, the EPA defines a bare soil hazard limit of 400 mg kg^{-1} (USEPA, 2020). The University of Connecticut Soil Nutrient Analysis Laboratory advises caution in crop selection when growing in soils with Pb levels between 100 and 400 mg kg^{-1} and to bring in clean soil if existing soil is >400 mg kg^{-1} (Pettinelli, 2007). For many, these recommendations seem considerably vague, and there is still uncertainty as to what concentrations of soil As and Pb are actually safe for farming (Cooper et al., 2020; Finster et al., 2004).

Furthermore, soil toxic element concentration is not always a good predictor of plant concentration because the mobility and bioavailability of toxic elements are context dependent (e.g., McBride et al., 2014). Various soil factors, including pH, phosphorus (P) levels, presence of arbuscular mycorrhizal fungi (AMF), percent organic matter, as well as crop type, all have been shown to affect plant uptake (Fendorf et al., 2010; Laperche et al., 1997; Leyval et al., 2002; Soares & Siqueira, 2008). Although some trends are emerging, we still lack a cohesive understanding of how soil factors and crop types interact to influence the safety of produce grown in urban soils that may be considered "borderline" contaminated according to the EPA.

There is evidence that toxic element accumulation is highest in root tissues, followed by shoot tissue, and the lowest in fruits (Nouri et al., 2009; Singh et al., 2012). Findings such as these have led to a common notion among urban farmers and gardeners that root vegetables should be avoided in urban soils and that only fruiting crops (e.g., tomatoes) are safe for consumption (USEPA, 2020). However, there is evidence that the distribution of toxic elements in plant tissue

can vary by site and crop type in ways that do not always follow this pattern, for example, when root tissues are sometimes found to have lower Pb levels than shoots tissues (Spittler & Feder, 1979).

Soil physicochemical factors can also be a strong determinant of crop plant uptake of toxic elements. For example, studies have found the availability of soil phosphorus (P) has a significant effect on Pb uptake into plants because, at a neutral pH, phosphate (HPO_4^{2-}) bonds with water-soluble Pb^{2+} ions to yield an insoluble pyromorphite ($Pb_5(PO_4)_3$) (Laperche et al., 1997; Soares & Siqueira, 2008). Because arsenate is chemically analogous to phosphate (Fendorf et al., 2010), As availability for plant uptake should be greatest at a neutral pH, similar to that of P. Dissolved Pb and As have the ability to bind with organic matter serving to immobilize them (Zeng et al., 2011) and compete for binding sites with essential nutrients (Hazelton & Murphy, 2016). Thus, the application of compost to gardens increases organic matter levels, and this may serve to immobilize toxic elements and limit uptake via these mechanisms in addition to diluting soil concentrations of toxic elements.

Soil biota can also play an easily overlooked role in plant accumulation of toxic elements. Approximately 90% of land plants form symbiotic, typically mutualistic, relationships with AMF; however, not all common agricultural crops have this relationship, with crops in the Brassicaceae and Amaranthaceae as notable examples of non-mycorrhizal crops (Smith & Read, 2010). AMFs have been found to affect toxic element uptake (Leyval et al., 2002; Javaid, 2011). Given that AMF are obligate biotrophs, it would make sense in contaminated soil for selection to favor symbiotic fungi that most effectively relieve toxic element stress for their host plants (Chen et al., 2006; Huang et al., 2018; Li et al., 2014), but how this plays out for urban soils and garden crop safety is not well known. As fungi, in general, are bioaccumulators of toxic elements (Kokkoris et al., 2019; Leyval et al., 2002) and popular interest in these fungal symbionts is high, more could be known and shared regarding this potential factor in urban crop safety.

There remains uncertainty about the practical application of these concepts when growing produce in marginally contaminated soils common to urban environments. We conducted a field trial at an urban community farm with varying levels of soil toxic element contamination to answer three main questions: (1) Do commonly grown crops differentially take up toxic elements into roots and shoots?; (2) Which soil factors, including AMF colonization, are the most important predictors of trace element uptake?; and (3) Are some of these mycorrhizal or non-mycorrhizal crops safe to grow on a moderately contaminated soil? This information is needed for marginally contaminated soils that are common in formerly industrial cities globally.

TABLE 1 Mean (+SE) soil physicochemical factors for terrace and basement sites at depths of 0–15 cm, $n = 25$ sets of pooled soil cores from each of the five-replicate plot at each site.

	Site	
Soil factors	**Basement**	**Terrace**
% Soil organic matter	15.1 + 0.7	8.8 + 0.4
pH	6.7 + 0.03	5.9 + 0.5
% Sand	70	65
% Silt	22	24
% Clay	8	11
P (pseudototal[a]) mg kg^{-1}	868 + 44	1319 + 69
P (exchangeable) mg kg^{-1}	9.6 + 1.0	12.5 + 0.8
Pb (pseudototal) mg kg^{-1}	872 + 58	280 + 38
Pb (exchangeable) mg kg^{-1}	0.91 + 0.11	0.46 + 0.03
As (pseudototal) mg kg^{-1}	6.1 + 0.3	12.0 + 0.7

[a]Pseudototal concentrations include all of a given element not in the primary silicate mineral lattice (see Section 2.8).

2 | METHODS

2.1 | Site description

We conducted a field trial at an urban community farm located in New London, CT, USA. Past industry in the Hempstead Historic District, where the farm sits, has included a tannery, painting shops, clothing and frame manufacturing, and an auto showroom (Churchill & Herzan, 1986). Currently, a local food justice nonprofit operates a small urban community farm in this centrally located area. The farm sits on an east-facing slope broken up into three large terraces separated by two ~3 m stone retaining walls built before the 1860s. For this study, we utilized two potential growing spaces at the farm: (1) a section that runs along the top terrace with moderately elevated toxic elements ("terrace"); and (2) a section on street level where an old house stood previously and a higher concentration of toxic elements are found ("basement"). The terrace site is currently used to grow mostly fruiting crops in-ground using best practices (i.e., compost-amended soil, mulch to minimize soil interaction, and splashing), whereas the basement site is not used for farming aside from several raised beds on wood chip mulch adjacent to the study area. Soil at the site is a fine sandy loam (Carlton–Chatfield complex) with glacial till parent material (Natural Resources Conservation Service,2018). Soil properties for both terrace and basement sites are given in Table 1.

2.2 | Experimental design

Crops were selected based on three factors: (1) relevance to community members who farm and eat food grown here; (2) crops grown for roots, leaves, and/or both roots and leaves;

FIGURE 1 Experimental planting layout at terrace (left) and basement (right) sites. Each crop was planted in plots (diamond planting for lettuce and collards, single row for beans, three rows for beets and carrots), which were randomized within each of five blocks along a single bed on the terrace (left) or along two adjacent beds in the basement (right).

and (3) mycorrhizal and non-mycorrhizal crop families (Brundrett, 2008). The two crops that were planted for leafy greens were *Lactuca sativa* L.—a lettuce variety brought from China and shared by a community gardener (mycorrhizal) and *Brassica oleracea* var. *viridis* L.—"Champion" collard greens (non-mycorrhizal). The two crops harvested for roots, but also with edible leaves, were *Daucus carota* L. subsp. *sativus*—"Scarlet Nantes" carrots (mycorrhizal) and *Beta vulgaris* L.—"Detroit Dark Red" beets (non-mycorrhizal). *Phaseolus vulgaris* L.—"Provider" bush beans (mycorrhizal) were planted for representation of a crop grown for fruits.

To prepare beds prior to planting, each was shaped using hoes and rakes to create slightly mounded 1-m-wide vegetable beds. To mimic growing practices at the site, we added 2.5–5 cm of commercial compost (Fleming's Feed, Stonington, CT, USA) and incorporated it into the top 10–15 cm of soil. At each site (terrace and basement), we established plots in a complete randomized block design with five replicates on five blocks and at least five plants plus border plants in each block (Figure 1).

Carrots, beets, and beans were planted by direct seeding. Lettuce and collards were started in a greenhouse 5 weeks prior to planting. Starts were grown in 128-cell plastic flats in Pro-Mix + Mycorrhizae potting soil (Premier Tech Horticulture, Quebec, Canada). For lettuce, beans, and collards, eight plants were planted in a 1 m by 1 m section for each block (0.6 m of plants with 0.15 m of space on either side). For beets and carrots, 0.45 m by 1 m space was used for each block with four rows of carrots and three rows of beets planted at 2.5–5 cm spacing. Beets and carrots were thinned once they had at least two-to-four true leaves to 10 and 12.5 cm spacings, respectively.

2.3 | Plot management

Beds were irrigated with two lines of drip tape with emitters spaced 20.3 cm apart, delivering water at a rate of 1.9 L per 30 cm of tape per hour. The experimental plots were connected to the same mainline as the rest of the urban farm and were therefore irrigated based on the timed schedule for the farm, which was 1.5 h daily via the drip system. This is equivalent to an irrigation depth of ~20 mm applied at each irrigation event. Crops were planted at the terrace site on June 2, 2021, and the basement site was planted on June 8, 2021. Beds were hand-weeded every 2 days. Collard greens were treated once with *Bacillus thuringiensis* Berliner (*Bt*) (Bonide Products, LLC, Oriskany, NY, USA) to control for imported cabbageworm. Plot management was consistent over the 10-week period. Top site crops of beans, lettuce, and collards were harvested 57 days after planting. Beets and carrots were harvested at 66 days. Bottom site crops of beans, lettuce, and collards were harvested 59 days after planting. Beets and collars were harvested after 65 days.

2.4 | Sample collection

Interior (non-border) plants within replicate plots were harvested to avoid edge effects between replicates. For each replicate, two mature, healthy, non-exterior leaves from three interior plants were harvested (a total of six leaves) and stored in Ziploc bags in a cooler, taking care to avoid soil contact. For beans, in addition to six trifoliate leaves, two edible-mature fruits (pods) were sampled from each of three bean plants, totaling six fruits per replicate plot. After sampling all the aboveground tissues, the remaining shoots were cut at the base and discarded to make room for soil coring.

Five pooled cores from each of two depths (0–15 cm and 15–30 cm) were taken per replicate plot. Cores were taken from points distributed throughout the same sample area where plant tissues were grown. Cores were then pooled together in plastic bags and mixed thoroughly. Soil probes were cleaned with paper towels between replicate plots.

For root harvesting, the three root systems from above-ground tissue-sampled plants were harvested. Root systems were dug up, and rhizosphere soils (soil adhering to the root system) were shaken into a plastic bag for collection. Entire root systems were then placed into a separate plastic bag. For beets and carrots, additional two edible roots were harvested (five total, to ensure enough biomass was collected for analysis). The remaining root systems were dug up and discarded. Each specific tissue (i.e., shoots, fruits, and roots) from each replicate plot was directly placed into labeled plastic bags and then directly placed into a cooler after the initial harvest. Tissues were stored at 4˚C for 2 days until processing.

2.5 | Sample processing

Soil samples were stored at −20˚C for 2 days. The soils were then dried in an oven at 60–65˚C for 48 h and then sieved to 2 mm to remove any debris and larger rocks.

Root samples for each species were washed thoroughly with tap water until all remaining soil was gone. For each crop, approximately 150 g of randomly sampled absorptive root tissue was removed from the system for drying. Another random sample of roots was used for AMF staining and analysis. Those roots were stored at 4˚C in 30% ethanol until being used within 1 week. In addition, the six shoots collected from each replicate and six fruits collected from beans were rinsed with tap water and oven-dried at 60–65˚C.

For beets and carrots, edible root tissues were subsampled for toxic element analysis. Enlarged edible roots were sliced into quarters lengthwise. One-quarter section from each of the five harvested carrots was used for each replicate. Root, shoot, and fruit samples were dried in an oven at 60–65˚C for 24 h. After drying, tissues were transferred to labeled ziplock bags and sent to the UMass Amherst Trace Metal Biogeochemistry Laboratory for toxic element concentration analysis.

2.6 | AMF sample preparation and analysis

From the random samples of absorptive roots collected, approximately 20–25, 1–2 cm root segments were placed in biopsy cassettes. Three cassettes were filled for each replicate of mycorrhizal (AM) crops (i.e., carrots, lettuce, and beans) and one cassette for non-AM crops (i.e., beets and collards).

The clearing and staining processes served to remove root pigments and stain only fungal tissues that colonized the roots, following a modified method of Vierheilig et al. (1998). Cassettes were fully submerged in 10% KOH for clearing of plant cell contents at room temperature for 4–7 days, depending on crop as different times in KOH are needed to clear roots due to variations in root thickness and pigmentation (Orchard et al., 2017; Vierheilig et al., 1998). Lettuce roots were soaked in 10% KOH at room temperature for 4 days, whereas collards, beets, carrots, and beans were soaked in 10% KOH at room temperature for 7 days. Once cleared, roots in cassettes were then rinsed in distilled water and stained in a 5% Sheaffer Ink (William Penn Pvt. Ltd., Bangalore, India) in 6% acetic acid solution at 70˚C (heated using hot water bath) for 5 min. After the staining process, the cassettes were then destained in water and acidified with several drops of acetic acid for 20 min.

Stained roots from each cassette were cut into 1-cm long segments before mounting onto slides. For mycorrhizal crops, five roots from each cassette (15 total) were mounted onto each slide. Two slides for each sample were made for AM crops, whereas a single slide was prepared for non-AM crops to confirm colonization had not occurred. For each slide, polyvinyl lacto-glycerol was applied, a cover slip was placed over roots, and nail polish was applied to seal the edges and prevent air bubbles. Slides then dried for 24 h before analysis. A total of 150 slides were made for AM crops (i.e., 50 lettuces, 50 carrots, and 50 beans). Additionally, 50 slides were made for non-AM crops (i.e., 25 collards and 25 beets).

For carrots, lettuce, and bean slides, AMF colonization intensity was estimated using a compound light microscope at 100× magnification. Total colonization, arbuscular colonization, and vesicular colonization were scored using the Trouvelot method (Trouvelot, 1986). Scores were recorded for each field of view along each of the 15 root segments on each slide and averaged for each replicate. For non-mycorrhizal beets and collards, slides were observed to confirm that AMF did not colonize any of the root tissue.

2.7 | Soil physicochemical analyses

Soil pH and percent soil organic matter were measured for each soil sample. For soil pH, a 5.0 g subsample was mixed with 20 g of 0.01 M $CaCl_2$ in a 50 mL centrifuge tube, shaken for 30 min, and settled for 2 h. Measurements were taken using a calibrated pH meter (8015 VWR). Loss-on-ignition was used to estimate percent soil organic matter, which is a

qualitative method for estimating the organic content of soil. For loss-on-ignition, a 4.00–6.00 g of air-dried subsample was combusted at 550°C for 6 h and reweighed to determine the mass loss.

2.8 | Soil and plant tissue elemental analysis

Dried and sieved soil samples were assessed for exchangeable and pseudototal concentrations of nutrients and toxic elements. First, 2 g of soil was weighed into a 50 mL centrifuge tube. Next, 15 mL of 0.1 M ammonium acetate was added to each tube and shaken for 24 h. The slurry was centrifuged at 2700 rpm, and the supernatant was collected, filtered with a Whatman 1 ashless filter, and acidified to pH 1 with ~0.5 mL of concentrated trace metal grade nitric acid. Second, we used strong acid digestion following USEPA method 3050B to quantify the pseudototal fraction of elements, which are elements not within primary silicate mineral lattice (Chen & Ma, 1998). The soil slurry following the exchangeable extraction was subsequently digested with 5 mL of 9:1 ratio of trace metal grade nitric acid to hydrochloric acid (15 M HNO_3 + 10 M HCl, Fisher Scientific) heated to 70°C for 45 min using a heating element. The digest was allowed to cool and diluted to 50 mL using 18.2 MΩ cm deionized water. For every 30 samples, a preparation blank, duplicate, and standard reference material (NIST 2709a San Joaquin Soil) were included. Solutions were diluted further and analyzed for macroelements (Al, Fe, Ca, K, Mg, Mn, Ba, Cu, Na, Zn, P, and S) using an Agilent 5110 Inductively Couple Plasma–Optical Emission Spectrometer (ICP–OES) (Agilent Technologies, Santa Clara, CA, USA) and trace elements (As, B, Cd, Cr, Cu, Ni, and Pb) with an Agilent 7700× Inductively Couple Plasma–Mass Spectrometer (ICP–MS). Preparation blanks for Al, Fe, Ca, K, Mg, Mn, Ba, Cu, Na, Zn, P, and S were <0.02 mg L^{-1} via ICP–OES analysis, and preparation blanks for As, B, Ba, Cd, Cu, Na, Ni, Pb, Sr, and Zn were <0.3 μg L^{-1} via ICP–MS analysis. Exchangeable fractions are not certified for NIST 2709a, but pseudototal digestions had strong acid digestion recovery rates of 87%–109% for Al, Fe, Ca, K, Mg, Mn, As, B, Ba, Cd, Cu, Na, Ni, Pb, Sr, and Zn of their certified values. Duplicates had <5% intrasample variation for most elements but 6%–11% intrasample variation for Fe, As, Cd, and Cr due to either their low concentration (As, Cd, and Cr) or variations in Fe digestion efficiency of oxides and Fe bearing minerals.

Total plant tissue digestions were carried out using a modified EPA 3050B method (Rechcigl & Payne, 1990), in which samples are ashed prior to strong acid, pseudototal digestion. Ground leaf and root samples were transferred to a ceramic vessel and combusted at 550°C for 8 h. The ashes were transferred to 50 mL centrifuge tubes and digested with 5 mL of reverse aqua regia (9:1 HNO_3:HCl trace metal grade) and lightly capped to degas overnight. After 12 h, the digest was heated to 80°C for 45 min using a hot plate and diluted to 50 g using deionized water. Every 20 samples included a preparation blank, duplicate, and SRM (NIST 1547 peach leaves). Total digestion recovery rates for peach leaves 1547 were 84%–104% of their certified values. Leaf and root digests were further diluted using 18.2 MΩ cm deionized water and analyzed with an Agilent 5110 ICP–OES and ICP–MS. As, Ca, Cu, Fe, K, Mg, Mn, P, Pb, and Zn concentrations in the preparation blanks were <0.1% of their respective measured concentrations, and intrasample variation was within 11% for all elements except Al, Fe, and S, which were between 11% and 16%. Recovery rates for Al, Fe, Ca, K, Mg, Mn, As, B, Ba, Cd, Cu, Na, Ni, Pb, Sr, and Zn were 93%–106%, except for S, which was only 86%.

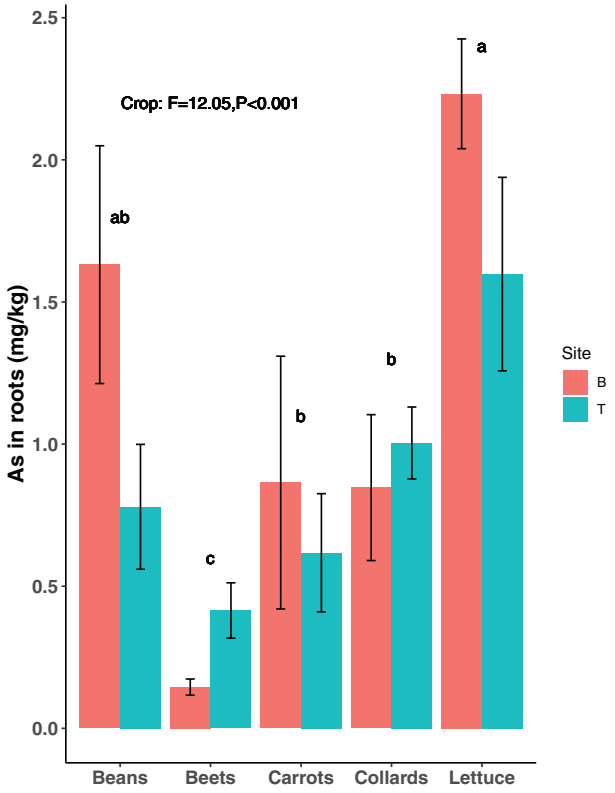

FIGURE 2 Dry weight As concentration (mg kg^{-1}) in root tissue compared across five crop types grown on basement (B) and terrace (T) sites. Different letters indicate significant overall differences between crops at both sites combined. Test statistics for crop, site, and any interaction are given as text on the plot area. Error bars indicate + SE, n = five samples of three root systems form each of the five-replicate plot.

2.9 | Data analysis

Statistical analyses were run in R (R Core Team, 2013). All models were assessed at a 5% level of significance. First,

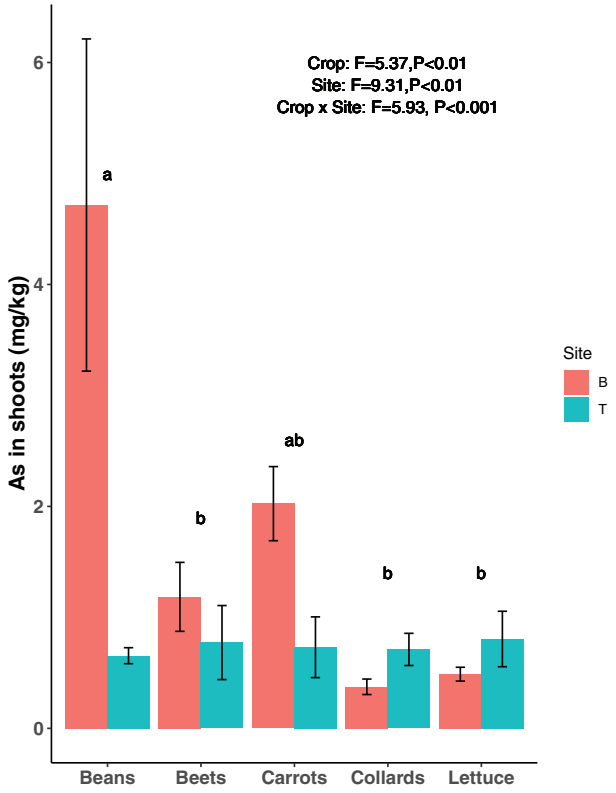

FIGURE 3 Dry weight As concentration (mg kg^{-1}) in shoot tissue compared across five crop types grown on basement (B) and terrace (T) plots. Different letters indicate significant overall differences between crops at both sites combined. Test statistics for crop, site, and any interaction are given as text on the plot area. Error bars indicate + SE, n = five samples of six pooled true leaves from each of the five replicate plots.

in order to address differences in Pb and As concentrations among crops, we used a two-factor analysis of variance with Pb or As as the response variable and crop, site, and replicate as factors. We included an interaction term to see if differences in crop concentrations depended on site (terrace or basement). All statistical analyses comparing plant tissue toxic element concentrations were performed and are reported on a dry weight basis.

Linear mixed-effects models were then used to assess the effect of soil factors, including soil phosphorous, pH, organic matter, mycorrhizal colonization and prevalence of fungal structures, and plant identity on shoot and root uptakes of Pb or As. Four separate models assessed the relationship between these factors on shoot uptake of Pb, shoot uptake of As, root uptake of Pb, and root uptake of As. Soil phosphorus, pH, and organic matter were included based on evidence in the literature that they may play an important role in toxic element uptake by plants (Fendorf et al., 2010; Laperche et al., 1997; Soares & Siqueira, 2008). We included fungal structures, vesicles and arbuscules, as factors because their functions (storage and nutrient exchange, respectively) may affect toxic element uptake in different ways. We created variables "Shoot uptake" and "Root uptake" to represent the transfer of either Pb or As from soils to plant tissue. These were made by dividing the specific toxic element in tissue (i.e., roots or shoots) concentration by the pseudototal toxic element concentrations in rhizosphere soil for each sample. In addition, we created variables representing the proportion of arbuscules and vesicles to total colonization as indicators of fungal functioning and to avoid colinearity between total colonization and total vesicular or arbuscular colonization. We also included Al concentration in shoots as a fixed factor in the models analyzing uptake into shoots to account for variation in data that may be due to remnants of soil dust on plant tissues after washing. Al has been used by others to account for surface contamination when analyzing plant uptake (McBride, 2013) because Al does not normally make it past the root cortex due largely to the Casparian strip (Ricachenevsky et al., 2018), and it occurs in relatively uniform and high concentrations in soils. Random effects included in the models were site and replicate to account for spatial heterogeneity in the field as well as differences in the soil–plant uptake relationship that may be due to unmeasured, inherent differences among sites. Data was scaled to allow for comparisons of the relative strength of each factor in the models. Models were constructed as linear mixed-effects models using the R package lme4 (Bates et al., 2015). Colinearity in models was checked for using the function "vif" in the R package "car" (Fox & Weisberg, 2018). *R*-squared statistics were estimated using the "r.squaredGLMM" function in the R package MuMIn (Bartón, 2000).

To compare Pb and As concentrations in our experimental crops in relation to perceived health risk, we compared them against UN FAO recommendations for fresh vegetables (The Codex, 2009) and also calculated target hazard quotients (THQ). THQ is calculated as the ratio of exposure to a reference dose, with the reference dose being the highest level of ingestion at which no adverse non-cancer effects are seen based on available studies. We used the following equation:

$$THQ = \frac{Ef \times Ed \times IR \times C}{RfD \times BW \times AT} \times 10^{-3}$$

where *Ef* is the exposure frequency (365 days $year^{-1}$), *Ed* is the exposure duration (70 years), *IR* is the ingestion rate (g $person^{-1}$ day^{-1}), *C* is the concentration of the toxic element (mg kg^{-1} on a fresh weight basis), *RfD* is the reference dose (mg kg body $weight^{-1}$ day^{-1}), *BW* is the average body weight for a US adult (83.6 kg) (Fryar et al., 2021), and *AT* is the average total exposure time (365 days × 70 years). The US EPA *RfD* for As is 3×10^{-4} mg kg body $weight^{-1}$ day (USEPA,1988). Although the US EPA no longer publishes an *RfD* for Pb (ATSDR, 2020), we used the *RfD* that has been used most frequently in the literature, 4×10^{-3} mg kg body

weight^{-1} day (Aendo et al., 2019; Nag & Cummins, 2022; Zhuang et al., 2009). Because we measured toxic element concentration on a dry weight basis and the equation assumes fresh weight concentrations, we adjusted our measurements for this metric using the reported percent water content for each vegetable given by the USDA Home and Garden Bulletin number 72 (Gebhardt & Thomas, 2002). Serving sizes from the USDA Nutrient Database (USDA ERS, 1997) were used for *IR* values. Serving sizes and moisture content for each crop were 67 g and 92% for collard greens, 60 g and 89% for beet greens and carrot greens, 56 g and 95% for lettuce, 62 g and 88% for beetroots, 62 g and 87% for carrots, and 63 g and 91% for green beans. These moisture contents were similarly used to adjust for fresh weight concentrations to compare against recommended UN FAO maximum safe levels. THQ values <1 indicate no risk of adverse non-cancer health effects given the assumptions of the equation, that is, body size, food intake rate, and so on. THQ calculations are not used for effects on children because the metric calculates an effect of chronic long-term exposure (over 70 years), and it is becoming clear that there are no safe levels of exposure for children (ATSDR, 2020).

3 | RESULTS

3.1 | Accumulation of As in different crops

In order to understand differences in crop accumulation of toxic elements, we compared above- and belowground tissue As concentrations across plant types. Crops varied in root As concentrations ($F = 12.05$, $p < 0.001$) with lettuce accumulating more As in roots (terrace = 1.60 ± 0.34 mg kg^{-1}, basement = 2.23 ± 0.19 mg kg^{-1}) than beets, carrots, and collards. Beans had higher concentrations of As in roots (terrace = 0.78 ± 0.22 mg kg^{-1}, basement = 1.63 ± 0.42 mg kg^{-1}) than beets (Figure 2). There was no difference in As root concentrations between sites.

There were also differences in As concentrations in shoot tissues between crop types ($F = 5.37$, $p < 0.01$) (Figure 3). Beans (terrace = 0.65 ± 0.07 mg kg^{-1}, basement = 4.72 ± 1.50 mg kg^{-1}) accumulated more As in shoots than beets (terrace = 0.77 ± 0.33 mg kg^{-1}, basement = 1.18 ± 0.31 mg kg^{-1}), collards (terrace = 0.71 ± 0.15 mg kg^{-1}, basement = 0.37 ± 0.07 mg kg^{-1}), and lettuce (terrace = 0.81 ± 0.25 mg kg^{-1}, basement = 0.49 ± 0.06 mg kg^{-1}). Plants grown at the basement site, where soil As was actually lower, accumulated more As in shoots overall ($F = 6.453$, $p < 0.001$), but this depended on crop identity ($F = 6.47$, $p < 0.001$) (Figure 3).

Bean fruit As concentration for the terrace was similar to shoot concentrations (0.68 ± 0.19 mg kg^{-1}), whereas bean fruit at the basement site accumulated less As than shoots or roots (0.29 ± 0.12 mg kg^{-1}).

3.2 | Influence of soil factors and AMF on As uptake

When looking at the relationship between soil factors and As uptake into roots, only higher soil pH (range: 5.3–6.88) was a significant predictor ($F = 7.2$, $p = 0.01$) (Table 2).

In the model looking at As uptake into shoots, there were several factors that affected As uptake across all crop types. Contrary to the effect of pH on root uptake, higher soil pH was associated with lower As uptake into the shoots ($F = 13.6258$, $p = <0.001$). Greater proportion of AMF vesicles was associated with lower uptake into the shoots ($F = 5.7752$, $p = <0.01$) (Table 2).

3.3 | Accumulation of Pb in different crops

There were significant differences in Pb concentrations in root tissues among crops ($F = 22.07$, $p < 0.001$) with lettuce accumulating more Pb in roots (terrace = 20.54 ± 6.74 mg kg^{-1}, basement = 27.49 ± 5.15 mg kg^{-1}) than all other crops (Figure 4). Crops grown at the basement site, where soil Pb was higher, had higher Pb root concentrations ($F = 8.72$, $p < 0.01$).

There were also differences in shoot Pb accumulation among crop types ($F = 4.49$, $p < 0.01$). Lettuce (terrace = 0.81 ± 0.25 mg kg^{-1}, basement = 0.49 ± 0.17 mg kg^{-1}) and carrot shoots (terrace = 0.73 ± 0.27 mg kg^{-1}, basement = 2.02 ± 0.33 mg kg^{-1}) accumulated more Pb than collards (terrace = 0.71 ± 0.15 mg kg^{-1}, basement = 0.37 ± 0.07 mg kg^{-1}). Plants grown at the basement site also accumulated more Pb in shoots than those grown at the terrace site ($F = 6.93$, $p = <0.001$) (Figure 5).

Pb accumulation in bean fruits was similar at the terrace and basement sites, 0.34 ± 0.03 and 0.35 ± 0.08 mg kg^{-1}, respectively.

3.4 | Influence of soil factors and AMF on Pb uptake

Pb uptake into roots was affected by plant identity, pH, and AMF. Plant identity affected Pb uptake into roots, as lettuce was associated greater Pb uptake ($F = 16.5$, $p < 0.001$). Higher soil pH was associated with less Pb uptake into the roots ($F = 6.2$, $p = 0.02$). AMF arbuscules were also significantly associated with greater Pb uptake into roots ($F = 7.7$, $p < 0.01$) (Table 2).

TABLE 2 Summary of linear regression coefficients and *p*-values for As and Pb uptakes into shoots and roots for each predictor.

	Pb uptake				**As uptake**			
	Shoots		**Roots**		**Shoots**		**Roots**	
Predictors	*Coefficient*	*p*	*Coefficient*	*p*	*Coefficient*	*p*	*Coefficient*	*p*
(Intercept)	−1.14	0.182	−0.33	0.384	0.21	0.812	0.54	0.26
Soil P	**−0.48**	**0.001**	−0.03	0.824	−0.1	0.504	−0.34	0.066
Soil OM	0.2	0.107	−0.18	0.227	0.09	0.517	−0.14	0.436
Soil pH	0.15	0.518	**−0.44**	**0.022**	**−1.06**	**0.001**	**0.59**	**0.015**
Al in shoots	**0.78**	**<0.001**	N/A	N/A	−0.23	0.112	N/A	N/A
Plant (carrots)	1.14	0.01	−0.19	0.678	0	0.995	−0.7	0.245
Plant (lettuce)	2.43	<0.001	1.8	0.007	−0.46	0.487	0.1	0.901
Total colonization	**0.7**	**0.009**	−0.01	0.984	0.5	0.099	−0.15	0.667
Proportion arbuscules	**0.27**	**0.024**	**0.36**	**0.013**	0.02	0.865	−0.06	0.733
Proportion vesicles	−0.03	0.79	−0.1	0.474	**−0.31**	**0.03**	−0.21	0.225
Model R^2, *RSME*	0.82, 0.51		0.71, 0.63		0.85, 0.61		0.50, 0.82	

Note: Positive coefficients indicate a positive relationship between a predictor and As or Pb uptake into a given tissue. Bold values indicate statistically significant effects *p* value < 0.05.

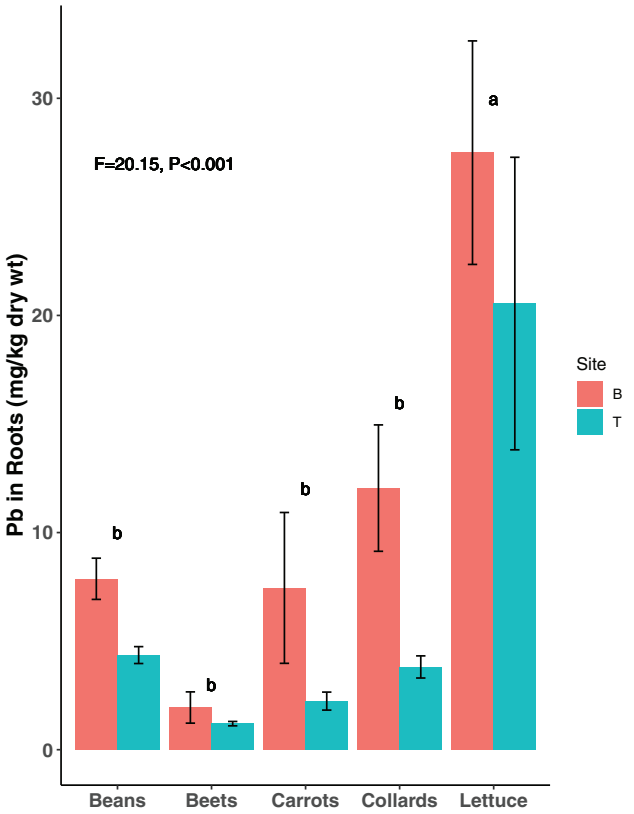

FIGURE 4 Dry weight Pb concentration in root tissue compared across five crop types grown on basement (B) and terrace (T) sites. Different letters indicate significant overall differences between crops at both sites combined. Test statistics for crop, site, and any interaction are given as text on the plot area. Error bars indicate + SE, $n = 25$ samples of three root systems form each of the five replicate plots.

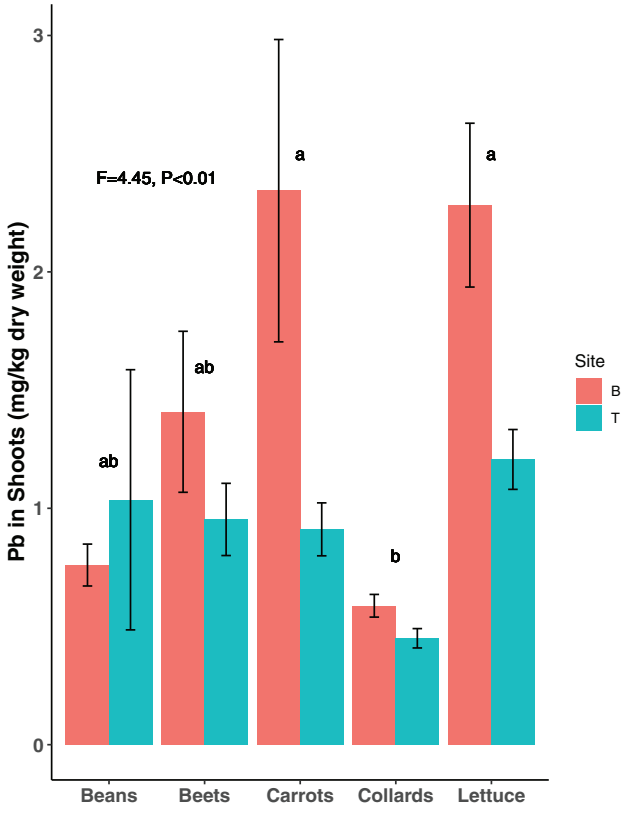

FIGURE 5 Dry weight Pb concentration (mg kg^{-1}) in shoot tissue compared across five crop types grown on basement (B) and terrace (T) sites. Different letters indicate significant overall differences between crops at both sites combined. Test statistics for crop, site, and any interaction are given as text on the plot area. Error bars indicate + SE, n = five samples, six pooled true leaves form each of the five replicate plots.

TABLE 3 As and Pb concentrations (mean ± 95% confidence interval mg kg^{-1}) for edible crop tissues, adjusted for fresh weight.

Plant	Site	Root As	Root Pb	Leaf As	Leaf Pb	Fruit As	Fruit Pb
Beans	B	NA	NA	NA	NA	0.02 ± 0.03	0.03 ± 0.02
Beans	T	NA	NA	NA	NA	0.05 ± 0.04	0.03 ± 0.01
Beets	B	0.02 ± 0.01	**0.23 ± 0.24**	0.13 ± 0.09	0.15 ± 0.1	NA	NA
Beets	T	0.05 ± 0.03	**0.14 ± 0.03**	0.09 ± 0.1	0.1 ± 0.05	NA	NA
Carrots	B	0.11 ± 0.16	**0.97 ± 1.25**	0.22 ± 0.1	0.26 ± 0.2	NA	NA
Carrots	T	0.08 ± 0.08	**0.29 ± 0.15**	0.08 ± 0.08	0.1 ± 0.03	NA	NA
Collards	B	NA	NA	0.03 ± 0.02	0.05 ± 0.01	NA	NA
Collards	T	NA	NA	0.06 ± 0.03	0.04 ± 0.01	NA	NA
Lettuce	B	NA	NA	0.02 ± 0.01	0.11 ± 0.05	NA	NA
Lettuce	T	NA	NA	0.04 ± 0.03	0.06 ± 0.02	NA	NA

Note: Numbers in bold indicate levels over the UN FAO recommended maximum levels in fresh produce (0.1 mg kg^{-1} for root and fruiting crops; 0.3 mg kg^{-1} for leafy greens).

Plant identity was also an important predictor of Pb uptake into shoots, with lettuce and carrots being associated with higher Pb uptake into shoots ($F = 10.8$, $p < 0.001$). Al concentration in shoot samples, included as a proxy for soil particles remaining on shoots after washing, was also a significant predictor of Pb in shoots ($F = 45.9$, $p < 0.001$). Higher soil P was associated with less Pb uptake into shoots ($F = 16.8$, $p < 0.001$). Greater total AMF colonization and arbuscules were also associated with greater Pb uptake into shoots ($F = 8.8$, $p < 0.01$ and $F = 6.2$, $p < 0.05$, respectively) (Table 2).

3.5 | Metrics related to potential health risks

Adjusting for fresh weight concentration, we compared Pb and As concentration in crops grown in this study with UN FAO–recommended maximum levels for fresh vegetables (The Codex, 2009) (Table 3). Carrot and beetroots had concentrations above the recommended safe levels for Pb in root crops (0.1 mg kg^{-1} fresh weight) at both the terrace (low contamination) and basement (high contamination) sites in this study. All leafy vegetables, including beet and carrot greens, fell below the recommended safe level for the Pb of 0.3 mg kg^{-1} fresh weight. Similarly, bean pods were below the threshold of 0.1 mg kg^{-1} fresh weight for Pb. The FAO does not provide recommended safe levels for As in fresh vegetables, but for reference, 0.35 mg kg^{-1} As is the threshold proposed for unpolished rice (The Codex, 2009).

We also calculated mean THQ and 95% confidence intervals for all edible crop tissues. All THQ ± 95% CI were less than 1 (Figure 6), indicating these crops are deemed safe for long-term consumption for average weight adults using this metric. The highest THQ values were consistently for As because of both its smaller *RfD* and its ability to be taken up in greater quantities by plants and transferred to aboveground tissues. In fact, the THQ values attributed to As were roughly an order of magnitude higher than those attributed to Pb, especially at the terrace site where soil As levels were more elevated.

4 | DISCUSSION

4.1 | Overall trends

In this study, we analyzed two urban farming sites contaminated with Pb and As: one assumed to be unsafe and one currently used for farming. Urban farmers and gardeners commonly make growing decisions based on the notion that plant uptake of toxic elements is proportional to soil toxic element concentrations (USEPA, 2020; Grubinger et al., 1993). However, our results highlight that factors, including crop type, soil pH, soil P, and AMF colonization, greatly influence toxic element uptake. Furthermore, these effects differed between As and Pb, presumably due to their different forms in soil with As as a mobile oxyanion and Pb as an insoluble cation. Furthermore, we found that the perceived safety of these crops depends on the metric used, with all crops perceived as safe according to THQ analysis, but root crops deemed unsafe according to recommended maximum levels set by the UN FAO.

4.2 | Crop type, toxic element accumulation, and crop safety

Overall, As and Pb accumulations in the crops tested here showed some consistent trends. Pb tended to accumulate more in roots, with root Pb concentrations roughly an order of

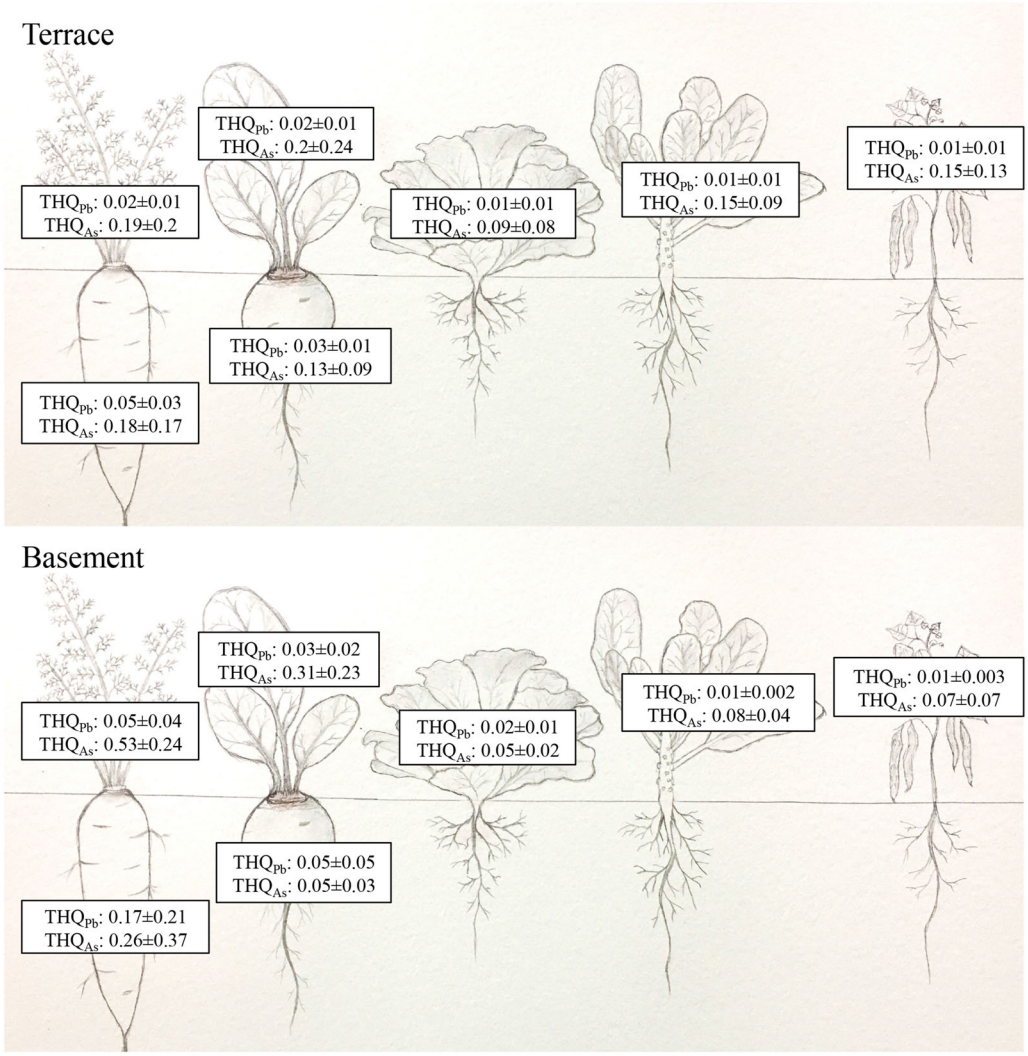

FIGURE 6 Target hazard quotients (THQ) for As and Pb in edible crops grown at the terrace and basement sites in this study. THQ values <1 indicate no risk of adverse health effects for average US weight adults consuming a USDA-recommended serving of a given vegetable 365 days year^{-1} for 70 years.

magnitude higher than shoot concentrations in all crops. This is consistent with previous findings and consensus in the literature that Pb uptake is restricted mostly to roots in most plants, perhaps due to the barrier of the endodermis and Casparian strip, which largely excludes toxic elements such as Pb from entering root vascular tissue (Ricachenevsky et al., 2018).

As, on the other hand, was found in greater proportions in shoots versus roots for some crops in our study, indicating more efficient translocation to aboveground tissues likely because of the chemical similarly of arsenate and phosphate leading to absorption via phosphate transporters (Singh et al., 2012). However, total arsenic has also been reported to accumulate in roots as opposed to shoots in other studies (He & Lilleskov, 2014; Meharg, 1994; Singh et al., 2017), indicating a host-dependent effect. This crop effect is consistent with our findings where carrots and beans accumulated more As in shoots than collards and lettuce, in this case to the benefit of the consumer of these leafy greens.

Despite lower levels of toxic elements found in carrots and beetroots in our study compared to other crops, both of these root crops were deemed unsafe when compared against UN FAO recommendations. THQ analysis, on the other hand, did not indicate beets and carrots were unsafe. There are considerable assumptions in calculating THQ, including average adult bodyweight, days per year the commodity is consumed, and, perhaps most importantly, the reference dose. It should be noted that the US EPA no longer reports a reference dose for Pb as more recent studies show that no safe level really exists (USEPA, 2020). Our calculations were based on the reference dose of 0.004 mg kg body weight^{-1} day^{-1}, which is most commonly used (e.g., Aendo et al., 2019; Nag & Cummins, 2022; Zhuang et al., 2009). Both metrics used

showed that the risk associated with consuming leafy greens, however, is quite low. This finding could indicate that the mechanisms that prevent Pb uptake (Ricachenevsky et al., 2018) are robust enough in these soils to largely allow for the safe consumption of aboveground crop tissue as long as surface contamination is controlled, as shown by Egendorf et al. (2021), despite these soil levels of contamination. However, cumulative exposure to these toxic elements from other sources such as soil/dust ingestion and indoor contamination as well as the health benefits of gardening and consuming fresh produce must also be considered if one were to make a holistic choice about whether or not to utilize a soil with elevated toxic elements.

4.3 | Influence of soil factors on uptake of toxic elements

Aside from differences in plant identity, the uptake of toxic elements was also strongly influenced by soil factors in our study. Higher levels of soil P were associated with less uptake of Pb to shoot tissues, which is to be expected if P and Pb form compounds such as pyromorphite that are very immobile and not bioavailable (Laperche et al., 1997; Soares & Siqueira, 2008). The influence of soil P on Pb uptake is likely more important at lower soil P concentrations, which could have been the case at the basement site where pseudototal Pb was as high as P (exchangeable P was also in a suboptimal range for vegetable production). In this case, the use of organic P fertilizers and/or extra compost, which often includes more than enough P when applied for sufficient N and K, could potentially decrease Pb uptake into crops as is often recommended as a best management practice. Soil pH was only related to Pb uptake into roots and not into shoots and is likely related to the effect of pH on P and other nutrient availability and complexes formed with Pb, perhaps limiting mobility in the rhizosphere.

Colonization by AMF was positively related to Pb uptake in our study with both total colonization intensity and proportion arbuscules leading to more Pb uptake into shoots, whereas proportion arbuscules (but not total colonization) were positively related to Pb uptake into roots. Contrary to some studies that show a protective effect of AMF colonization for plant hosts (Alvarado-López et al., 2019; Riaz et al., 2021), AMFs in this case were contributing to greater plant Pb. We suggest that this could be due to one or more of the following factors: (1) Greater AMF colonization effectively extends the root absorptive area and, as a result, mines a greater amount of nutrient and toxic elements from the surrounding soil. There is evidence that plants used for phytoextraction can be more effective when AMF is more abundant (Chen et al., 2006; Huang et al., 2018; Li et al., 2014); (2) due to the P-limited nature of these soils, AMF could be involved in solubilizing complexes that include both P and Pb, effectively freeing more Pb for uptake by plants; (3) AMF, like many fungi, could have a general affinity for accumulating toxic elements on hyphae, and this could vary by fungal identity. For example, Sudová and Vosatká (2007) showed that both P and Pb accumulated in root segments that were highly colonized by two pollution-adapted strains and one reference strain of *Glomus intraradices* N.C. Schenk & G.S. Sm (= *Rhizophagus intraradices* (N.C. Schenk & G.S. Sm) C. Walker & A. Schüßler). All three strains in that study seemed to accumulate Pb, but the reference strain was more negatively affected by the substrate Pb concentration, which was quite high. Given that AMF inoculants are popular and show various other benefits when used in highly disturbed soils (Balacco et al., 2023; Delavaux et al., 2017), more work is needed to ascertain how the management of AMF by encouraging local populations or introducing new strains could play a role in crop safety.

The opposite effect of AMF colonization was seen for As uptake, with greater proportion of vesicles related to less As uptake into shoots. Previous studies have found that AMF increase nutrient availability and help plants grow larger in high As soils (Chen et al., 2006; Huang et al., 2018; Li et al., 2014), but these studies did not report on AMF affecting where As accumulates in tissues. Dong et al. (2008) and Wang et al. (2008) both reported greater accumulation of As in roots of inoculated versus non-inoculated agricultural forage plants. Our finding of the relationship between vesicle storage structures and less As uptake into shoots suggests that this could be one mechanism by which AMF improve both plant health and crop safety. Although there is some evidence of other toxic elements such as Pb accumulating in these fungal storage structures (Alvarado-López et al., 2019; Riaz et al., 2021), to our knowledge, this interaction has not yet been reported for As. It is important to note that the soils in our study had As levels that were elevated but not nearly as high as are often used in other experiments (He & Lilleksov, 2014), which could influence this relationship.

5 | CONCLUSION

This study highlights the importance of soil factors and crop selection for determining plant uptake of As and Pb in a real-world context that can be useful to minimize risks and increase confidence surrounding urban growing on soils with elevated levels of toxic elements. We conclude that pH, soil P, and AMF are important predictors of toxic element uptake, along with crop type and edible tissues consumed. Furthermore, this study adds field-based empirical evidence that Pb and As behave very differently in the plant–fungus–soil system and will thus be uniquely influenced by soil management practices and crop choice. To our knowledge, this is the first report of a relationship between AMF vesicles and less As uptake into shoots of crop plants; however, we also show the positive

relationship between AMF and Pb uptake into these same plants. Future work could investigate further the importance of AMF in urban crop–soil systems.

AUTHOR CONTRIBUTIONS

Logan Bowdish: conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; writing–original draft; writing–review & editing. **Adalie Duran**: conceptualization; data curation; investigation; methodology; writing–review & editing. **Justin Richardson**: conceptualization; data curation; formal analysis; investigation; methodology; resources; writing–review & editing.**Eric Vukicevich**: conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; Software; supervision; Validation; Visualization; writing–original draft; writing–review & editing.

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CONFLICT OF INTEREST

The authors have no relevant financial or nonfinancial interests to disclose.

DATA AVAILABILITY STATEMENT

Data and code used to analyze data are available for viewing on the Open Science Framework at the following link: https://osf.io/3je2q/?view_only=b3212c820e754e72bdfb59531f861885.

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