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“Effect of lead contaminated soils on lycopene and mineral contents of tomatoes (*Solanum lycopersicum*)”

by

Andrea T. Amati

A Thesis Submitted in Partial Fulfillment
of the Requirement for the Degree of
Master of Science in Environmental Science

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Rochester Institute of Technology
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May 4, 2018

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Table of Contents

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ABSTRACT

Eating fruits and vegetables is beneficial to human health, not just because they provide essential nutrients and vitamins, but also because phytochemicals scavenge free radicals and can reduce the risk of developing cancer and other diseases. However, not all people have access to affordable fresh fruits and vegetables. People who live in "food deserts" often are limited to smaller stores where prices are higher and the quality and variety of fresh fruits and vegetables are scarce. Urban gardens have been proposed as a potential solution to the issue of food deserts and as a way to promote nutrition in low income communities. However, soil in urban gardens is contaminated with heavy metals including lead (Pb). This contamination may have an impact on the nutritional quality of urban crops, and thereby have significant implications on public health. The goal of this study is to determine if Pb concentrations in soil can have an impact on the nutritional quality of tomatoes, a very common crop grown in urban gardens. Tomatoes were obtained from local communities as well as grown in soils containing Pb concentrations ranging from 0 to 1600 mg of Lead (IV) Hydrogen Phosphate ($Pb(HPO_4)_2$). Nutritional quality was examined by measuring mineral content as well as lycopene contents of the tomato fruits. Lycopene is an important phytochemical that gives tomatoes their red color. The Pb concentration of the tomato fruit was below detection level regardless of soil type, organic matter, or soil Pb concentration. Soil Pb did significantly correlate to potassium, iron, and phosphorus in the greenhouse samples and phosphorus in the community samples. Our results also show that the Pb concentration of the tomato fruit is consistently below detection level regardless of soil type, organic matter, or soil lead concentration. This study also indicated that the tomatoes grown in urban gardens and tomatoes purchased commercially did not differ in the lycopene content.

LIST OF TABLES

Tables

Supplemental Table

LIST OF FIGURES

INTRODUCTION

Eating fruits and vegetables is beneficial, not just because they provide essential nutrients and vitamins, but also because they can reduce the risk of developing cancer and other diseases. In the U.S., cardiovascular disease is the leading cause of death with cancer as the second leading cause (American Cancer Society, 2015). Previous studies indicated that the consumption of nutritious tomatoes (*Solanum lycopersicum*) and tomato products has been associated with a lower risk of developing digestive tract and prostate cancer, as well as a lower rate of mortality due to cardiovascular disease (Dumas et al., 2003).

However, not all people have access to affordable fresh fruits and vegetables. Food desert is a term that is frequently used to describe locations where consumers lack access to affordable nutritious food – typically populated, low-income, minority-dominated urban neighborhoods with limited access to supermarkets (Chung and Myers, 1999; Hendrickson et al., 2006; Kaufman et al., 1997; Larsen & Gilliland, 2009; Walker et al., 2010; Wang et al., 2014). The U.S. Department of Agriculture (USDA) stated that if at least 500 people and/or at least 33% of the population live more than one mile from a supermarket or large grocery store, it will be considered a "food desert" or "low-access community" and for rural census tracts, the distance is more than ten miles (Ver Ploeg et al., 2009). People who live in food deserts often are limited to smaller stores where prices are higher and the quality and variety of fresh fruits and vegetables are low. Therefore, the disadvantaged population residing in a food desert may have harmful effects on their health and quality of life (Hendrickson et al., 2006; Larsen & Gilliland, 2009; Wang et al., 2014).

Urban gardens have been proposed as a source of fresh produce within urban food deserts. The number of community gardens and farmers' markets have increased gradually over

the past ten years. More people want to gain better access to affordable fresh fruits and vegetables other than supermarkets and grocery stores (Defoe et al., 2014; Wang et al., 2014). A community garden is a plot of land that a group of people uses to plant fruits and vegetables. It provides fresh produce for group members and other local residents. A farmers' market is a public and recurring gathering where farmers sell food that they produced directly to consumers. A farmers' market takes place either outdoors or indoors and there are booths, tables, and stands where farmers sell fresh fruits, vegetables, meats, dairy products, and prepared foods and beverages. Community gardens and farmers' markets are often added in urban neighborhoods as potential solutions to food desert problems (Larsen & Gilliland, 2009; Wang et al., 2014). At the same time, it can help people to develop healthier dietary habits and hence potentially reduce health risks. A study by Larsen and Gilliland (2009) validated that the addition of a farmers' market in a food desert increased the availability of healthy food and lowered the overall food costs in the neighborhood. They also discussed how the farmers' market gave people, especially low-income shoppers, a better variety of fresh fruits and vegetables that are affordable.

Despite growing interest in urban gardens, concern about ingesting trace elements from contaminated fruits and vegetables continues. Urban environments are typically found to be contaminated with substances like metals and persistent organic pollutants (POPs) as a consequence of human activities that include transportation, construction, manufacturing, incinerator emissions, and fossil fuel combustion (Martin & Griswold, 2009). As a result, urban garden soils can be moderately to severely contaminated by one or more metals such as lead (Pb), cadmium (Cd), mercury (Hg), and barium (Ba) (McBride et al., 2014).

This study will focus on Pb contamination only. Anthropogenic sources of Pb include leaded paint and gasoline, soldering/ammunition products, Pb-based pesticides, aerosols or dusts

from old smelters or mine sites, and abandoned car batteries (Defoe et al., 2014). Pb-contaminated soil particles are transferred constantly between air, water, and soil by natural chemical and physical processes. These processes include weathering, runoff, precipitation, dry deposition of dust, and stream/river flow (Agency for Toxic Substances and Disease Registry, 2012). When Pb is exposed to air and water, films of Lead (II) Sulfate ($PbSO_4$), Lead (II) Oxide (PbO), and Lead (II) Carbonate ($PbCO_3$) are formed. These films act as a protective barrier that slows or stops the corrosion process of the underlying metal (Agency for Toxic Substances and Disease Registry, 2012). Sunlight, air, and water can transform some Pb compounds into different forms of Pb, but the elemental lead (Pb^0) cannot be broken down.

Nearly all forms of Pb that are released to the soil are from anthropogenic sources and the largest source is from the atmospheric deposition process. Additional sources of Pb in dust and soil also include the weathering and chipping of Pb-based paint from buildings, bridges, and other structures (Agency for Toxic Substances and Disease Registry, 2012). The standard mean Pb concentration for surface soils worldwide averages 32 mg/kg and ranges from 10 to 67 mg/kg (Wuana & Okieimen, 2011). The U.S. Environmental Protection Agency (EPA) recommends growing vegetables in soil with a total level below 400 mg/kg (Traunfeld, 2013). Even at soil levels above 300 mg/kg, most of the risk is from Pb-contaminated soil or dust deposits on the plants rather than from uptake of lead by the plant.

Ionic lead (Pb^{2+}), PbO and hydroxides (OH^-), and lead-metal oxyanion complexes are the common forms of Pb that are released into the soil and water (Wuana & Okieimen, 2011). Once the Pb compounds reach the soil, they are transformed by chemical and biotic processes into adsorbed forms. The soil's ability to bind Pb depends on the pH, organic matter content, clay content, temperature, and the cation exchange capacity (CEC) of the soil (Chaney et al., 1989;

Kibria, et al., 2009; Seregin & Ivanov, 2001), as well as the concentration of metal ions and the species of the plant. However, Pb is known to have an extremely low solubility in the soil. Previous studies have shown that insoluble Pb phosphates, Pb carbonates, and Pb hydroxides form under ambient soil conditions and thus control the available Pb concentration in the soil. (Wuana & Okieimen, 2011; Cotter-Howells, 1996). As a result of the presence of several phosphate containing lead minerals, particularly pyromorphite, Pb phosphate was identified as a general weathering product of Pb minerals in anthropogenically contaminated soils (Cotter-Howells, 1996). The solubility of Pb minerals is pH dependent therefore, the more acidic the soil, the more soluble Pb becomes (Chaney et al., 1989). It is possible that small amounts of Pb may enter rivers, lakes, and streams when soil particles are moved by acidic rain (Agency for Toxic Substances and Disease Registry, 2012).

Soils that are contaminated with heavy metals may cause health hazards and risks to humans through direct ingestion or contact with the soil (Wuana & Okieimen, 2011). No level of Pb is considered necessary nor beneficial to the body and there is no "safe" level of exposure (Flora et al., 2012). Pb may be found on plant surfaces as a result of atmospheric deposition and its presence in plant tissues is from the uptake from the soil and leaf surfaces (Agency for Toxic Substances and Disease Registry, 2012). Pb is responsible for diverse direct and indirect effects on plant growth and metabolism (Wang et al., 2007). The symptoms of Pb toxicity are visible, with indications of stunted growth, chlorosis and blackening of root systems (Godbold and Kettner, 1991).

Numerous studies have examined the relationship between the concentration of heavy metals in garden-raised foods, especially for Pb and Cd. Xian (1989) and Jorhem et al. (2000) stated that the Pb levels in vegetables were strongly influenced by the Pb level in the soil. Often,

the level of Pb in plants is similar to the level of Pb found in the environment (Kibria et al., 2009). There are no health-based standards for heavy metals in fruits and vegetables in the U.S. so most of the studies used European Union's (EU) food standard for Pb in tomatoes, which is 0.1 mg/kg. McBride et al. (2015) suggested that tomatoes and possibly a number of other fruit crops could be grown in substantially Pb-contaminated soils up to 1000 mg/kg without exceeding health-based standards.

However, there is very little evidence on how metal contamination in the soil impacts nutritional quality of crops grown in contaminated soils. This study examines how Pb contamination impacts the nutritional value of a common urban agricultural crop, tomatoes. Tomatoes were obtained from local communities in Rochester, N.Y. as well grown in soils containing Pb concentrations ranging from 0 to 1600 mg of Lead (IV) Hydrogen Phosphate ($Pb(HPO_4)_2$). Nutritional quality was examined by measuring mineral content as well as lycopene contents of the tomato fruits.

Minerals are essential nutrients that people need to stay healthy and they have vital roles in several body functions and development. Such minerals, including potassium (K), calcium (Ca), and magnesium (Mg), can be found in tomatoes. Initially, plants absorb nutrients through their roots. Then, the nutrients are transported from the roots to the shoots via xylem and phloem transports (Van Goor & Wiersma, 1974). There often will be a competition that occurs between ions with similar size, valency, and ion charge. K, Ca, and Mg ions are fairly similar in size and charge, and the xylem/phloem transports cannot tell the difference between the ions (Fageria, 2001). Thus, both transport sites will accept either ion regardless of which ion was meant for that site. K and Ca have stronger binding strengths than Mg and they both will out-compete Mg easily at exchange sites (Malvi, 2011).

Various studies have shown that the uptake of excessive Pb decreased the concentration of K and Ca ions in crops, such as winter wheat (Trivedi & Erdei, 1992), eggplants and tomatoes (Khan & Khan, 1983), and amaranth (Kibria et al., 2009). The influence of Ca in the accumulation of particular ions from solutions might act as a stimulator, but often it is an inhibitor, especially for heavy metals (Garland & Wilkins, 1981). Khan and Khan (1983) concluded that the concentration of Ca, K, and Mg ions decreased significantly with higher doses of Pb. Furthermore, Pb is known to interfere with mineral nutrients of seedlings and plants, hence causing deficiencies or adverse ion distribution within the plant (Khan & Khan, 1983; Malkowski et al., 2002; Trivedi & Erdei, 1992). In Walker et al (1977), Pb decreased the uptake of Ca, Mg, K, and phosphorus ions (P) in maize (*Zea mays*), and in Burzyński & Grabowski (1984) and Burzyński (1987), Pb decreased the uptake of K, Ca, Mg, Fe, and Nitrate (NO_3) in cucumber (*Cucumis sativus*) seedlings. In addition, the results in the Kibria, et al. (2009) study indicated that Pb application significantly decreased the shoot and root weight of *Amaranthus gangeticus* and *Amaranthus oleracea*. Comparing with the control, the shoot and root weight of *A. gangeticus* were reduced by 28 and 53% and *A. oleracea* 46 and 37%. In conclusion, Ca is impacted by Pb and that impacts the growth of plants, but it is not known whether it has an impact on the fruits and vegetables that we eat.

Tomatoes contain large quantities of the phytochemical lycopene. Experimental studies have shown that lycopene exhibits antioxidant activities, including suppressing the proliferation and growth of cancer cells (Shi & Le Maguer, 2000). The most common sources of lycopene are found in red fruits and vegetables, including tomatoes (Rao & Agarwal, 2000). Several studies concluded that the largest concentrations of lycopene were found in the skin and outer pericarp of the tomato (Shi & Le Maguer, 2000). Lycopene is found in the chloroplasts of tomatoes and

can be located among the thylakoid membranes (Shi & Le Maguer, 2000). The color change in pigmentation occurs during tomatoes' ripening process after an accumulation of lycopene within the plastids and the chlorophyll disappears (Paiva et al., 2015). The chloroplasts of the mature green tomato change into chromoplasts, which accumulate lycopene in membrane-bound crystals and give off the color red (Fraser et al., 1994). One of the functions of lycopene is to absorb light during photosynthesis, in order to protect plants against photosensitization (Shi & Le Maguer, 2000). As a carotenoid, lycopene is a valuable antioxidant and free radical scavenger that has been suggested to prevent carcinogenesis and atherogenesis by protecting vital biomolecules including lipids and DNA (Rao & Agarwal, 2000).

The lycopene content of tomatoes varies with the variety and increases with fruit ripening (Rao & Agarwal, 2000). Also, lycopene might depend on genetic and environmental factors (temperature and light), agricultural techniques used (water availability, nutrient availability, and date of harvest), and the post-harvest storage conditions (Dumas et al., 2003). The synthesis of lycopene was shown by Dumas et al. (2003) to be completely inhibited at 32ºC and temperatures of 30-35ºC significantly reduced the content of lycopene in fresh tomatoes. The temperatures 22-25ºC were identified as favorable temperatures. Lycopene content also has been correlated with Ca concentration in the tissues of the plant. Excess Ca in the soil can inhibit K absorption due to competition between both ions. In a study by Paiva et al. (2015), the Ca levels in tomatoes increased with increasing Ca concentration in the nutrient solution. But Mg and K levels decreased with increasing Ca concentration in the nutrient solution. Due to the competition between ions, excess Ca in the root environment may have inhibited the absorption of other cations, including copper (Cu) and iron (Fe) (Dumas et al., 2003; Paiva et al., 2015).

Since Pb can impact Ca/K and these ions have been found to influence lycopene, it may indicate that Pb impacts lycopene production in tomato plants. Yet, there is insufficient data on whether Pb in the soil impacts lycopene contents in tomatoes. A greenhouse study was conducted on tomato plants grown in soils with different Pb concentrations to determine whether Pb has any impact on lycopene content in tomatoes. Tomatoes were grown in soils with known amounts of Pb and the lycopene content was examined. A tomato and soil sample was collected from several different gardens in Rochester, NY. The soil samples were examined for Pb concentration and the tomato samples were measured for its lycopene content. The greenhouse and Rochester tomatoes' lycopene content was compared with the lycopene content of tomatoes that were bought from various grocery stores and a farmers' market as well. It was hypothesized that the mineral and lycopene content in all three groups of tomatoes would decrease when grown with highly Pb-contaminated soils.

MATERIALS AND METHODS

Soil Characteristics

Soils used in this experiment were collected from a farm in the town of Honeoye Falls, NY. The site was chosen due to its distance from industrial centers and human structures that might have caused Pb contamination. Subsurface soils were sampled at a depth of 0.3 – 0.7 meters. The Pb concentration of the soil was below detection. The soils were obtained specifically from the subsurface because it has very low organic matter and it is not common for Pb to be transported that far (Craul, 1985). During construction in urban areas, B- horizon soils are often used as fillers to level the site (Scheyer and Hipple, 2005). This specific subsurface soil was intended to simulate urban soils in this study. Upon arrival, a 2-mm sieve was used to filter out rocks from uncontaminated soils.

Measurement of Soil pH

The soil pH was measured by using a 0.01 M Calcium Chloride ($CaCl_2$) stock solution and a glass pH electrode (Sparks, 2003). First, 0.147 g of Calcium Chloride Dihydrate ($CaCl_2 \cdot 2H_2O$) was weighted and dissolved in 100 mL of distilled water. Next, a suspension of soil was prepared by mixing 10 g of soil and 10 mL of 0.01 M $CaCl_2$ solution. The suspension was stirred vigorously before it was left to stand. After 10 minutes, the suspension was stirred again and the pH level of the soil was measured with a Hach portable pH meter, Model HQ40d. The probe was calibrated with pH 7.00 and 10.00 buffers. Three replicates were performed and the mean pH was 7.20.

Soil Particle Analysis

Particle size analysis was performed to characterize the size distribution of soil particles using sieves number 40, 70, 100, and 120. The hydrometer analysis method was used in this study for particles smaller than 0.075 mm (Bouyoucos, 1962). Based on the results (Table 1), it can be concluded that the soils are in the silt loam class (Table 1).

Table 1. Chemical characteristics of collected soils before lead treatment.

pH	Organic Matter (%)	Particle Size (%)
7.20	1.56	40.2 Sand 58.4 Silt 1.4 Clay

Total Organic Matter

Soil organic matter was estimated by using the loss on ignition procedure. A soil sample was dried in a VWR® forced air oven at 105ºC for 24 hours. Next, 5 g of soil were obtained from the oven-dried soil and placed in a Neytech Vulcan Model D-1750 muffle furnace at 360ºC for 2 hours. The soil sample was weighed after it was removed from the furnace. To find the total organic matter in the soil sample, the difference in mass between the dry soil before and after the furnace is equal to the mass of organic matter present in the sample. It was calculated that the sample had a total of 1.56% organic matter (Table 1). This draws a parallel with the soils found in urban gardens because they consist of subsurface soil, which has a low organic matter content.

Greenhouse Study

First, the soils were ground and sieved to >2mm. Next, 20 kg of soils were added to 5-gallon buckets. The buckets were spiked with seven different concentrations of Lead (IV) Hydrogen Phosphate ($Pb(HPO_4)_2$). Three replicates were prepared for each of seven different concentrations, bringing the sample size to a total of 21 buckets. The soil was treated specifically

with $Pb(HPO_4)_2$ because this Pb compound is the closest to the type of Pb that is found in the environment (Howells 1996). The seven concentrations were: 0, 50, 100, 200, 400, 800, and 1600 mg Pb/kg soil. Soils were left to equilibrate for approximately 9 weeks. No organic matter was used in this experiment.

After planting beefsteak tomato seeds in 21 mini-square plastic pots filled with perlite, the seedlings were left to grow until they each reached the five-leaflet stage. The seedlings were removed from the pots and re-planted in the 5-gallon buckets filled with Pb-contaminated soils. The tomato plants received the necessary nutrients from a modified half-strength Hoagland nutrient solution (Table 2; Hoagland and Arnon, 1950). The pH level was adjusted using 1 M Nitric Acid (HNO_3) or 1 M Sodium Hydroxide (NaOH) to 5.6 - 6.0. Each bucket received 250 mL of the nutrient solution twice a week along with ~250 mL of water daily. The tomato plants were kept under artificial lights, which were left on for 18 hours daily. Plants were artificially pollinated approximately 8 weeks after the seedlings were planted.

Table 2. Hoagland solution recipe used in the greenhouse study.

	Formula	**Molecular Weight**	**Per liter of solution**
Macronutrient stock solution			
Potassium nitrate	KNO_3	101.1032 g/mol	82.1013 g
Calcium nitrate tetrahydrate	$CaNO_3 \cdot 4H_2O$	236.15 g/mol	118.08 g
Dipotassium phosphate	K_2HPO_4	174.2 g/mol	43.55 g
Magnesium sulfate heptahydrate	$MgSO_4 \cdot 7H_2O$	246.466 g/mol	61.6175 g
Micronutrient stock solution			
Boric acid	H_3BO_3	61.83 g/mol	0.2844 g
Manganese (II) chloride tetrahydrate	$MnCl2 \cdot 4H_2O$	197.91 g/mol	0.0990 g
Zinc chloride	$ZnCl_2$	287.56 g/mol	0.0273 g
Cupric sulfate	$CuSO_4 \cdot 5H_2O$	159.602 g/mol	0.0499 g
Ammonium molybdate	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	196.01 g/mol	0.1236 g
Ferric EDTA	$C_{10}H_{13}FeN_2O_8$	345.065 g/mol	16.51725 g

All of the tomatoes were picked from each bucket eight weeks after pollination. After harvesting, the tomatoes were cut in half. The first half was stored in a sterile 18-oz Nasco Whirl-Pak® bag and placed in the freezer, while the other half was analyzed immediately for its lycopene content.

Market Basket Study

While the greenhouse tomatoes were growing, 37 non-organic beefsteak tomatoes were collected from four different grocery stores and a farmer's market. Then the tomatoes were cut in half. The first half was stored in a sterile 18-oz Nasco Whirl-Pak® bag and placed in the freezer, while the other half was analyzed immediately for its lycopene content.

Community Garden Study

A tomato and soil sample were collected from 25 different gardens located in Rochester, NY. All 25 tomatoes were of the same variety as the greenhouse and market basket tomatoes. Next, the tomatoes were cut in half. The first half was stored in a sterile 18-oz Nasco Whirl-Pak® bag and placed in the freezer, while the other half was analyzed immediately for its lycopene content. The soil samples were oven-dried at 105ºC for 24 hours and filtered with a 2-mm sieve. The samples were stored in a plastic container until the analysis for the concentrations of Pb was performed.

ANALYTICAL METHODS

Lycopene Extraction

First, the tomato was blended and 100 mL of tomato purée was transferred to a screw-cap tube. Several blank samples were also prepared with 100 mL of water instead of tomato purée. Next, 8 mL of 2:1:1 Hexane:Ethanol:Acetone (HEA) solution was added to the tubes before it was capped. Then the tubes were vortexed and incubated out of bright light. After 30 minutes of incubation, 1 mL of water was added to all tubes and the tubes were vortexed once again. The tubes were incubated for an additional 10 minutes to allow phases to separate and all air bubbles to disappear. The lycopene analysis was performed using a Shimadzu Ultraviolet (UV) Spectrophotometer, Model UV-1800 at 503 nm. A glass cuvette was rinsed with the upper layer from one of the blank samples. After discarding the upper layer, a fresh blank was used to zero the spectrophotometer. A second glass cuvette was filled with the puréed tomato-HEA sample and the lycopene content was measured. Lycopene levels in the hexane extracts were calculated using the formula: (A503 x 537 x 8 x 0.55)/(0.10 x 172), where 537 g/mole is the molecular weight of lycopene, 8 mL is the volume of mixed solvent, 0.55 is the volume ratio of the upper layer to the mixed solvents, 0.10 g is the weight of tomato added, and 172 mM^{-1} is the extinction coefficient for lycopene in hexane (Alda et al., 2009).

Elemental Analysis

The samples from the freezer were cut into smaller pieces, placed on a watch glass and oven-dried at 65ºC for 72 hours, and then ground with a mortar and pestle. The dried, ground samples were sent to Cornell Nutrient Analysis Laboratory at Cornell University for elemental analysis by acid digestion and Inductively Coupled Plasma Optical Emission Spectrophotometry (ICP-OES).

Quantifying Lead Concentration in Urban Soils

Total soil Pb was determined using HNO_3 digestion of soils sampled after harvest (U.S. Environmental Protection Agency, 1996). First, the samples were ground in a coffee grinder. Then, 1 g of soil was added to a glass tube containing a mixture of 1.5 mL concentrated HNO_3 and 6 mL concentrated Hydrochloric acid (HCl). To allow the acids to react, the samples were left alone for 20 minutes. The samples were then placed in a heating block and heated to reflux for 15 minutes. Next, the digests were filtered through a Whatman Grade 42 filter paper and filtrates were collected in a 250mL Nalgene® wide mouth sample laboratory bottle. Afterwards, the filter papers were washed with 5 mL 95ºC HCl and 20 mL of distilled water. The washings were collected in the same bottle and brought up to 100 mL with distilled water. The concentrations of Pb in the final solutions were determined by using a Flame Atomic Absorption Spectrophotometer (FAAS).

A Perkin Elmer Atomic Absorption Spectrometer, Model AA Analyst 200 was used to analyze the 25 soil samples that underwent the acid digestion process. The wavelength was set at 283.31 nm. Standards were prepared using a 3% HCl stock solution and Lead Nitrate ($Pb(NO_3)_2$) solids. The standards were used to calibrate the FAAS at 0.2, 0.5, 1, 2, 5, and 10 mg/kg.

Multiple Regression Analyses

A multiple regression analysis was conducted to examine how the mineral concentration of tomatoes and the soil Pb concentration impacted the lycopene content in the fruit. The greenhouse and community garden data ware examined independently. The multiple regression analyses were executed using the data analysis package in Microsoft Excel ©. Elements that produced coefficients of zero in the regression analysis were removed from analysis until a final model was produced.

RESULTS

Lycopene Content

A Kruskal-Wallis test was performed on the average lycopene levels of all nine groups (Figure 1). The result of the Kruskal-Wallis test determines if the differences between two or more groups of tomatoes and their average lycopene levels are statistically significant. As a result, the p-value was 0.433. Since the p-value is greater than 0.05, the differences between the average of lycopene levels in all nine groups of tomatoes is not statistically significant.

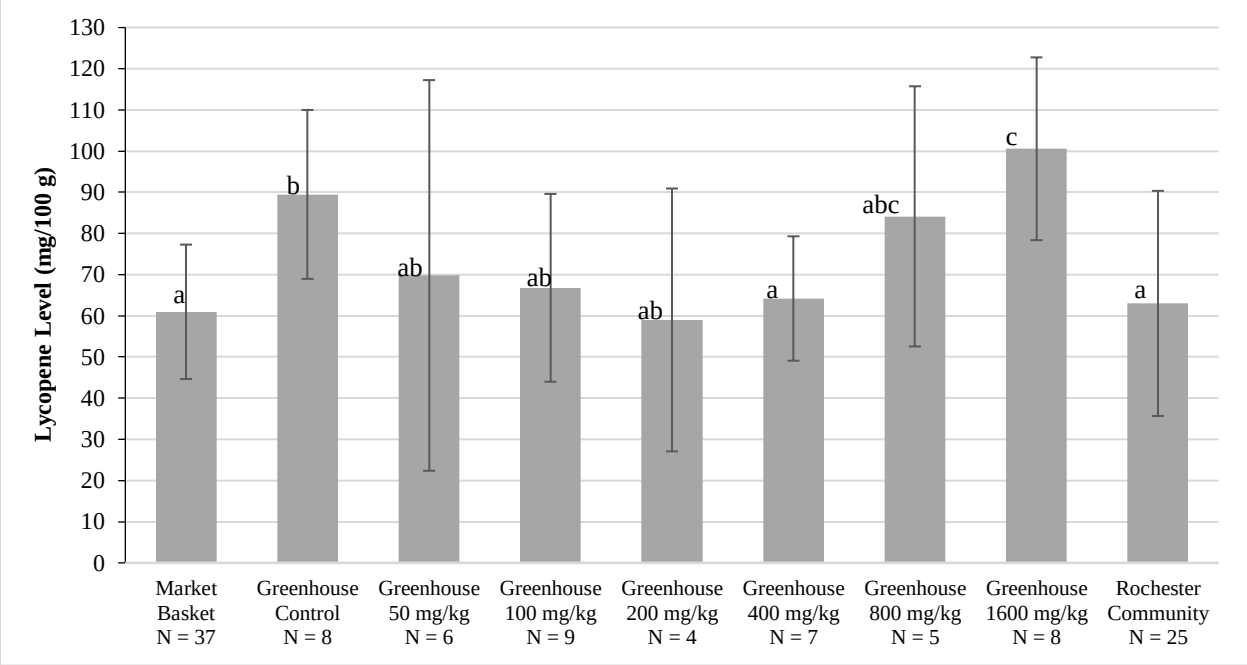


Figure 1. Average lycopene levels in market basket tomatoes, control and Pb-contaminated tomatoes from the greenhouse, and urban tomatoes with sample size (N) and standard deviation. Columns with different letters indicate significant differences between types of tomatoes according to two-sample t-test ($P < 0.05$).

Next, a two-sample t-test was used to compare whether the difference between the nine average lycopene levels are statistically significant (Figure 1). First, the p-value for comparing the market basket tomatoes with the greenhouse control tomatoes was 0.008. With the p-value

being less than 0.05, the difference between market basket tomatoes' average lycopene level with the average lycopene level of greenhouse control tomatoes is statistically significant. Next, the p-value for comparing the market basket tomatoes with the greenhouse tomatoes grown in 1600 mg Pb/kg soil was 0.002. Since the p-value is less than 0.05, the difference between market basket tomatoes' average lycopene level with the greenhouse tomatoes grown in 1600 mg Pb/kg soil is statistically significant. After comparing the greenhouse control tomatoes with the greenhouse tomatoes grown in 400 mg Pb/kg soil, the resulting p-value was 0.025. Therefore, the difference between greenhouse control tomatoes' average lycopene level with the greenhouse tomatoes grown in 400 mg Pb/kg soil is statistically significant with their p-value being less than 0.05. The greenhouse control tomatoes' average lycopene level was compared with the average lycopene levels of tomatoes grown in Rochester. The p-value was 0.014, which means the difference between the greenhouse control tomatoes' average lycopene level and the average lycopene level of tomatoes grown in Rochester is statistically significant. After that, the greenhouse tomatoes grown in 1600 mg Pb/kg soil was compared with the greenhouse tomatoes that were grown in 100 mg Pb/kg soil. With the p-value of 0.012 being less than 0.05, the difference between the greenhouse tomatoes grown in 1600 mg Pb/kg soil's average lycopene level and the average lycopene level of the greenhouse tomatoes grown in 100 mg Pb/kg soil is statistically significant. The greenhouse tomatoes grown in 1600 mg Pb/kg soil was compared with the greenhouse tomatoes that were grown in 400 mg Pb/kg soil and the resulting p-value was 0.004. The p-value was less than 0.05, therefore the difference between the average lycopene level of greenhouse tomatoes grown with 1600 mg Pb/kg soil with the greenhouse tomatoes grown 400 mg Pb/kg soil is statistically significant. Lastly, the p-value for comparing the greenhouse tomatoes grown in 1600 mg Pb/kg soil and the tomatoes grown in Rochester was

0.002. Since the p-values were less than 0.05, the difference between the average lycopene level of greenhouse tomatoes grown with 1600 mg Pb/kg soil with the average lycopene level of tomatoes grown in Rochester are statistically significant.

Tomatoes grown in soils with a Pb concentration between 50-200 mg/kg produced a decrease in the lycopene content of the tomatoes (Figure 2). A one-way analysis of variance (ANOVA) test was performed on the lycopene content of tomatoes grown at the different soil Pb concentrations in the greenhouse tomatoes. The p-value for the data was 0.1292, indicating that there is insufficient evidence to state that all the concentrations of lycopene are different for tomatoes grown at increasing soil Pb concentrations. Pearson's correlation was also performed on lycopene levels versus Pb concentration of the greenhouse tomatoes. The Pearson coefficient (r) was -0.896 and the p-value was 0.104. The negative Pearson's coefficient shows the strong negative relationship between both variables. As the soil Pb concentration increases, the lycopene content decreases (Figure 2). The p-value is greater than 0.05, which tells us that the correlation is not statistically significant. A similar trend was observed for tomatoes grown in local community gardens. Figure 3 illustrates us that there is no relationship between the soil Pb concentration and the lycopene levels in tomatoes that were grown in urban areas around Rochester.

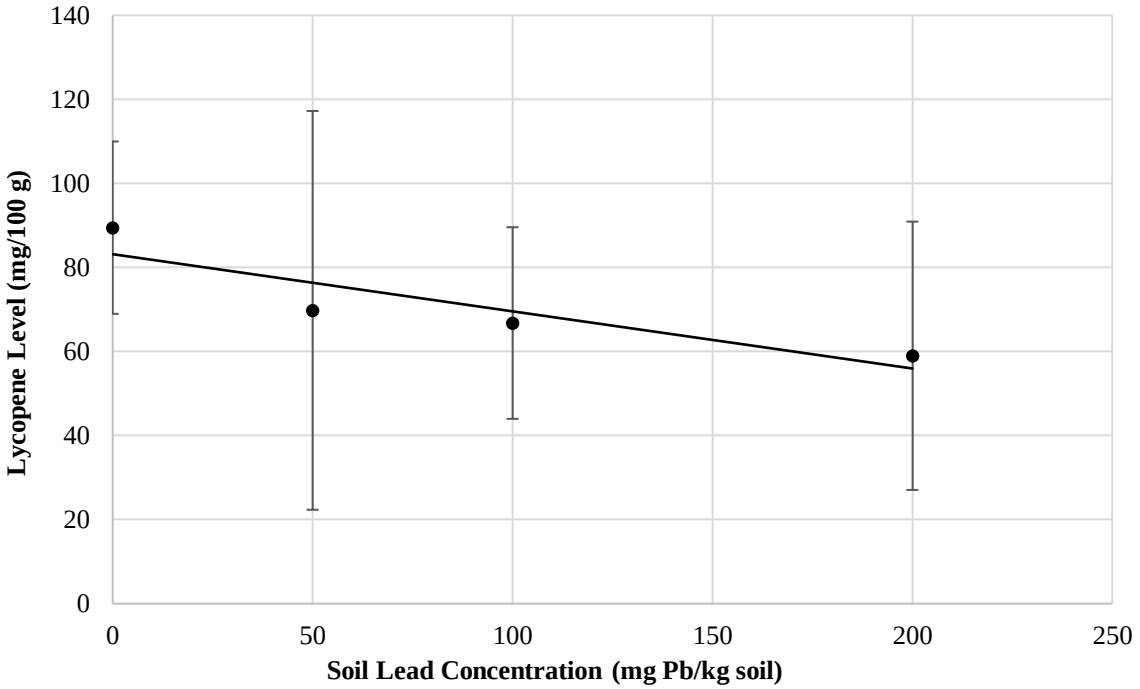


Figure 2. Average lycopene levels of tomatoes grown in the greenhouse with 0, 50, 100, and 200 mg Pb/kg soil. The coefficient of determination (R^2) is 0.80205.

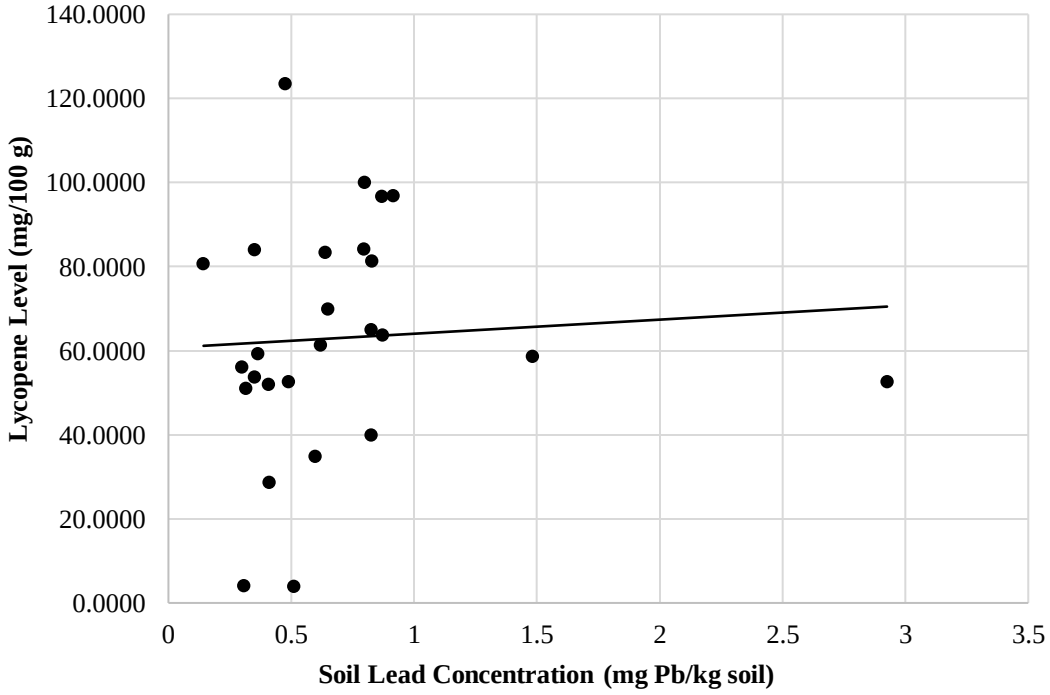


Figure 3. Lycopene levels of tomatoes grown in Rochester, NY. The coefficient of determination (R^2) is 0.00416.

Mineral Content

As a result of the elemental analysis that was performed by Cornell Nutrient Analysis Laboratory, Tables 3 and 4 show that K and P had the highest concentrations in the greenhouse and Rochester tomatoes, while Ca, Mg, and sulfur (S) had the second highest concentrations. However, Pb was found to be below the detection limit for all of the tomato samples.

Table 3. Average elemental levels (mg/kg) of tomatoes grown in greenhouse with 0, 50, 100, 200, 400, 800, and 1600 mg Pb/kg soil and tomatoes grown in Rochester, NY.

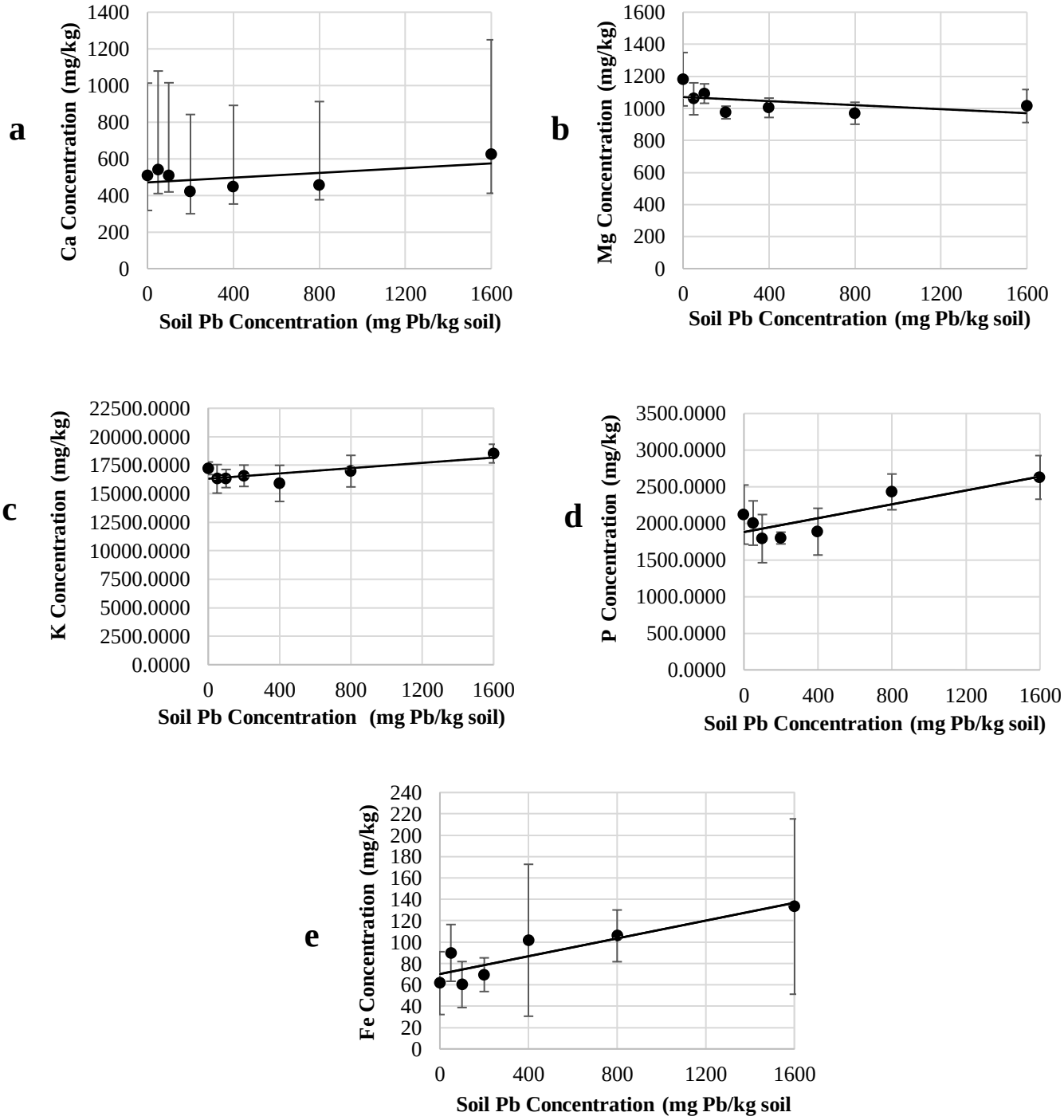
	Control 0 mg/kg	**50 mg/kg**	**100 mg/kg**	**200 mg/kg**	**400 mg/kg**	**800 mg/kg**	**1600 mg/kg**
Al	4.80	4.25	5.71	6.80	7.27	19.44	34.40
As	0.08	0.08	0.07	0.10	0.07	0.08	0.05
B	23.64	23.95	24.50	23.35	24.54	23.46	25.06
Ba	0.15	0.15	0.16	0.10	0.21	0.60	0.86
Ca	506.46	539.50	507.42	420.75	445.67	456.14	624.76
Cd	0.16	0.10	0.14	0.18	0.19	0.18	0.21
Co	0.11	0.13	0.12	0.13	0.11	0.18	0.20
Cr	0.64	0.57	0.78	0.85	0.76	1.08	1.16
Cu	6.30	5.65	5.24	4.95	5.59	5.38	4.65
Fe	61.58	89.85	60.23	69.45	101.69	105.82	133.24
K	17231.73	16308.58	16331.74	16575.95	15907.00	16982.64	18524.44
Mg	1181.96	1059.63	1092.42	974.43	1003.74	969.56	1014.73
Mn	8.08	7.87	7.72	6.95	7.74	7.84	8.80
Mo	0.79	0.78	0.78	0.80	0.86	0.88	0.98
Na	210.36	240.42	177.08	174.88	191.41	168.88	176.74
Ni	0.34	0.32	0.31	0.35	0.49	0.58	0.59
P	2119.95	2004.55	1791.57	1799.38	1886.73	2429.32	2627.13
Pb	0	0	0	0	0	0	0
S	1143.18	1150.25	1127.41	1127.38	1174.70	1232.28	1222.18
Si	9	9.27	8.69	9.3	11.2	12.08	11.35
Sr	0.33	0.33	0.31	0.20	0.23	0.30	0.48
Ti	0.06	0.08	0.11	0.13	0.20	0.42	0.38
V	0	0	0	0	0	0	0
Zn	14.51	14.15	12.78	13.28	19.43	22.92	21.90

Table 4. Average elemental levels (mg/kg) of tomatoes grown in Rochester, NY.

	mg/kg
Al	5.84
As	0.08
B	28.74
Ba	0.48
Ca	1007.01
Cd	0.13
Co	0.12
Cr	0.37
Cu	7.09
Fe	24.46
K	21510.04
Mg	1561.45

	mg/kg
Mn	9.05
Mo	0.78
Na	203.79
Ni	0.16
P	3786.18
Pb	0
S	1556.28
Si	11.37
Sr	2.19
Ti	0.03
V	0
Zn	17.38

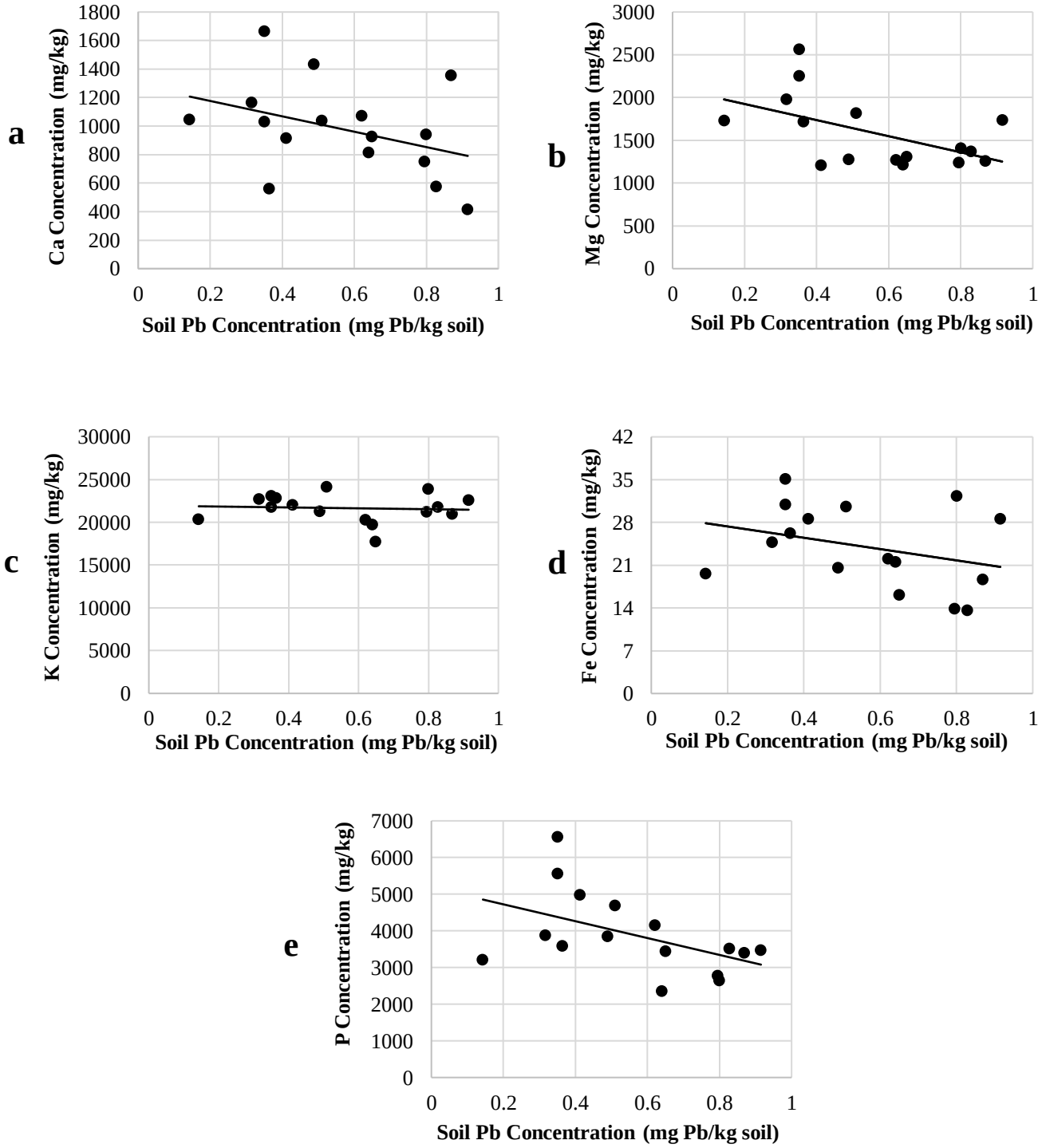
Pearson's correlation test was performed on the relationships between soil Pb concentration and the elements Ca, P, K, Fe, and Mg in greenhouse tomatoes. As a result, K, Fe, and P showed to be statistically significant with the p-value of 0.007, 0.017, and 0.042, respectively. Next, the Pearson's correlation coefficient (r) of Fe, P and K exhibited a positive correlation with the soil Pb concentration with the r of 0.891, 0.845, and 0.773 respectively. This suggests that as the Pb concentration in soil increases, the concentration of Fe (Figure 4b), P (Figure 4d) and K (Figure 4e) in tomatoes increases.



Figures 4a-e. Concentrations of calcium (Ca), magnesium (Mg), potassium (K), iron (Fe) and phosphorus (P) in tomatoes grown in the greenhouse with 0, 50, 100, 200, 400, 800, and 1600 mg Pb/kg soil. The coefficients of determination (R^2) are 0.29552, 0.59744, 0.22944, 0.79301, and 0.71356 respectively.

Pearson's correlation test was also performed on the relationships between soil Pb concentration and the following elements: Ca, P, K, Fe, and Mg in tomatoes that were collected from the community gardens in Rochester. Only P was found to be statistically significant with the p-value of 0.048. The Pearson's correlation coefficient (r) of P was -0.471, which tells us that the relationship is negatively correlated. As the Pb concentration in soil increases, the concentration of P in tomatoes decreases (Figure 5e).

No correlation was found for the following elements: aluminum (Al), arsenic (As), boron (B), Ba, Cd, cobalt (Co), chromium (Cr), Cu, manganese (Mn), molybdenum (Mo), N, nickel (Ni), Pb, sulfur (S), silicon (Si), strontium (Sr), titanium (Ti), vanadium (V), and zinc (Zn) with the soil Pb concentration. Therefore, the Pb concentration in the soil does not appear to have an impact on these elements.



Figures 5a-e. Concentrations of calcium (Ca), magnesium (Mg), potassium (K), iron (Fe), and phosphorus (P) in tomatoes grown in Rochester, NY. The coefficients of determination (R^2) are 0.1462, 0.2852, 0.0055, 0.1035, 0.2348 and respectively.

The results of the regressions analysis for the community garden and greenhouse tomatoes are shown in Table 5 and 6, respectively. For the greenhouse tomatoes, the multiple regression gave a model in which Mg, Mn, Na and P combined to significantly predict the lycopene concentration (Significance F = 0.01, R^2=0.992). As in the community garden tomatoes, P was contributed the most the fit of the model (p-value = 0.021). Mg was also a significant predictor of lycopene concentration of the tomato in the greenhouse model. For the community garden tomatoes, the concentrations of Ca, Mg, Mn, Na and P produced a model that significantly predicted the lycopene content of the tomato (Significance F=0.0486, R^2 =0.595). In particular, the P concentration of the tomato was the most significant predictor of the lycopene content in the community garden tomatoes (p-value = 0.007). Na concentration of the tomato was also a significant predictor of lycopene content (p-value = 0.026).

Table 5. Multiple regression analysis results for tomatoes grown in the greenhouse with 0, 50, 100, 200, 400, 800, and 1600 mg Pb/kg soil.

Regression Statistics	
Multiple R	0.995974435
R Square	0.991965075
Adjusted R Square	0.975895225
Standard Error	2.373863124

ANOVA

	df	**SS**	**MS**	**F**	**Significance F**
Regression	4	1391.412491	347.8531226	61.72833433	0.01600529
Residual	2	11.27045226	5.635226131		
Total	6	1402.682943			

	Coefficients	**Standard Error**	**t Stat**	**P-value**
Intercept	-104.1041629	17.29222949	-6.020285753	0.026499103
Mg	0.087540505	0.017852799	4.903461157	0.039163553
Mn	1.489267498	3.799407625	0.391973604	0.732902432
Na^+	-0.069538334	0.044791215	-1.552499407	0.260735891
P	0.043332842	0.006340649	6.834133562	0.020746828

Table 6. Multiple regression analysis results for tomatoes grown in Rochester, N.Y.

Regression Statistics	
Multiple R	0.771552097
R Square	0.595292638
Adjusted R Square	0.411334747
Standard Error	15.35531508

ANOVA

	df	**SS**	**MS**	**F**	**Significance F**
Regression	5	3815.044054	763.0088108	3.236026643	0.048618636
Residual	11	2593.642712	235.7857011		
Total	16	6408.686766			

	Coefficients	**Standard Error**	**t Stat**	**P-value**
Intercept	173.2771951	38.32626125	4.52110875	0.000870455
Ca	-0.012549525	0.013067169	-0.960385863	0.357496168
Mg	-0.022081618	0.03460093	-0.63817991	0.53641614
Mn	5.896904811	3.227297201	1.827196085	0.094900854
Na^+	-0.112582897	0.043990753	-2.559240039	0.026554526
P	-0.023031047	0.006673245	-3.451251354	0.005416082

DISCUSSION

Lycopene Content

The market basket and community garden studies indicated that the tomatoes grown in urban gardens and tomatoes purchased commercially did not differ in lycopene content (Figure 1). This indicates that tomatoes grown in urban gardens are an equivalent source of lycopene. In the greenhouse study, the lycopene content significantly decreased between the control and 50 mg Pb/kg soil tomato samples and it continued to decrease between the 50, 100, and 200 mg Pb/kg soil samples (Figure 2). Yet, the greenhouse tomatoes grown in 1600 mg Pb/kg soil had significantly greater lycopene than all other tomatoes (Figure 1). For the Pb levels higher than 200 mg/kg in the greenhouse study, the impact of Pb on the lycopene level is mitigated, which is likely a result of the high Pb level exceeding the solubility of Pb in the soil, making it less bioavailable for the tomato plants.

The multiple regression analysis suggested that the lycopene content of the tomato was not correlated with Ca as suggested by Dumas et al. (2003) and Paiva et al. (2015). Both the greenhouse study (Figure 3) and the multiple regression analysis (Tables 4 and 5) showed that soil Pb was not a significant predictor of lycopene concentration in either the greenhouse or community garden tomatoes. However, the models produced by the multiple regression analysis for both the greenhouse experiment and the community tomatoes showed a significant relationship between P and lycopene concentrations in the tomato. The results of this study showed that P concentration of the tomato was significantly related to the soil Pb concentration. This suggests that soil Pb is having an indirect impact on the lycopene concentration of tomatoes across soil types. This impact may be mediated by phosphorus and requires further analysis to determine the potential mechanisms by which lead concentration of the soil may impact lycopene synthesis.

Mineral Content

Khan and Khan (1983) concluded that the concentration of Ca, K, and Mg ions decreased significantly with higher doses of Pb. In Walker et al (1977), Pb decreased the uptake of Ca, Mg, K, and P in *Zea mays*, and in Burzyński & Grabowski (1984) and Burzyński (1987), Pb decreased the uptake of K, Ca, Mg, Fe, and NO_3 in cucumber (*Cucumis sativus*) seedlings. Excess Ca in the soil can inhibit K absorption due to competition between both ions. Mg and K levels have been shown to decrease as Ca concentration increases simultaneously in nutrient solution. Due to the competition between ions, excess Ca in the root environment may have inhibited the absorption of other cations, including Cu and Fe (Dumas et al., 2003; Paiva et al., 2015). The results of our experiment showed that the soil Pb did not correlate to changes in Ca and Mg in either the greenhouse or community garden tomatoes. In the community garden study, the concentrations of K increased as the soil Pb concentration increased, while Mg showed a decrease. This could be a result of the greenhouse soil having very little organic matter and clay content for these trace elements to bind to. The greenhouse tomatoes were grown with soil that had a pH of 7.2, which was not acidic enough for Pb to be soluble (Table 1). Tomatoes thrive in soil with the pH range of 6 - 6.9, but the pH range of the half-strength Hoagland nutrient solution (Table 2) was kept between 5.6 – 6.0 in order to keep P and Ca from precipitating.

Pb has been shown to compete with Ca, which would suggest that soil Pb would impact the uptake of K, Mg, Fe, Ca and Cu (Kibria et al., 2009). However, the soil Pb content was not found to be related to the concentration of these nutrients in the tomatoes in this study. But in the multiple regression analyses, soil Pb did significantly correlate to K, Fe, and P in the greenhouse samples and P in the community samples (Figures 5b-e). This could mean a few things: the P concentration in the nutrient solution might have caused the insolubility of Pb in the soil (Cotter-

Howells, 1996), it is possible that the half-strength Hoagland nutrient solution (Table 2) consisted of an excessive concentration of Ca and Mg, or giving the greenhouse tomatoes the nutrient solution twice a week was too frequent, or the composts and amendments that were used in the community garden study had high P content, which have been found to bind soil Pb and make them less mobile in soil (Cotter-Howells & Caporn, 1996; Defoe et al., 2014),

Four different studies stated that Pb levels in vegetables were strongly influenced by the Pb level in the soil (Xian, 1989; Jorhem et al., 2000, Malkowski et al., 2002; McBride et al., 2015). But this did not occur in the greenhouse and community garden studies. Our results show that the Pb concentration of the tomato fruit are consistently below detection level regardless of soil type, organic matter, or soil Pb concentration. The results might contain some anomaly. Overall, the results of this study indicated that tomatoes grown in lead contaminated soils were not as nutritious . The concentration of K and Ca were much lower in the greenhouse tomatoes and the concentration of P was higher in the community garden tomatoes. Nevertheless, the results of this study suggest that variety of tomatoes grown in lead contaminated areas is safe to eat and confirms previous work from McBride et al. (2015) regarding the risk of eating tomatoes.

CONCLUSION

Community gardens as a potential solution to food deserts is a feasible idea. But it might not seem like it is a good idea, especially knowing that our urban environment is contaminated with heavy metals and POPs from human activities. The results of this study did not agree with the findings of Kibria et al. (2009); the level of Pb in tomatoes was not similar to the level of Pb in the soil. The results suggest that the Pb concentration of tomatoes will be found below detection level regardless where the tomatoes came from and the amount of Pb concentration in the soil. Along with the evidence that P significantly predicted the lycopene concentration in tomatoes, more focus should be shifted on environmental factors and the possibility of using P as an amendment for soil remediation. Additional work is needed to explore the relationship between Pb, P, and lycopene. Furthermore, P may be mediating the impact of soil Pb on lycopene or the change in P of the tomatoes may be a symptom of a more complex mechanism. If urban gardens are used as a potential solution to food desert problems, additional research on how the soil contamination might impact the nutritional value of urban crops is needed.

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APPENDIX

Supplemental Table

Table S-1. Location of tomatoes grown in Rochester, N.Y. and the type of soils they were grown

#	Sample	Location (Zip Code)	Type of Soils
1	RIT Garden	Henrietta (14623)	Grown in the ground
2	Winbourne Road	Brighton (14619)	Grown in a raised bed with compost
3	Trafalgar Street	Brighton (14619)	Grown in the ground then transferred to a large pot with compost
4	Rearview Drive	Henrietta (14623)	Grown in the ground with enhanced soil and mulch
5	North Winton Village	Rochester (14609)	Grown in a raised bed with organic soil and fertilizer
6	Big Ridge Road	Spencerport (14559)	Grown in a raised bed with a mixture of store-bought peat moss, top soil, manure fertilizer, and mulch
7	Sylvan Road	Brighton (14618)	Grown in a raised bed with organic soil
8	Maxson Street	Irondequoit (14609)	Grown in the ground
9	Kemphurst Road	Greece (14612)	Grown in the ground, amended the soil with compost.
10	Glenhill Drive	Brighton (14618)	Grown in the ground, used 10-10-10 fertilizer before planting
11	Hazelwood Terrace	Irondequoit (14609)	Grown in a raised bed with store-bought soil
12	Madison Street	Rochester (14608)	Grown in the ground
13	Genesee Park Blvd	Brighton (14619)	Grown in a raised bed, added compost and amended the soil with fertilizer at the beginning of growing season
14	Garfield Ave	East Rochester (14445)	Grown in the ground with uncultivated and unfertilized soil
15	E. Main Street	Irondequoit (14609)	Grown in a raised bed with organic potting soil and mushroom compost
16	Beckwith Ave	Scottsville (14546)	Grown in a raised bed with a mixture of organic potting soil and mushroom compost
17	Commonwealth Road	Brighton (14618)	Grown in a raised bed with a mixture of compost, manure, and ground soil
18	Linden Street	Brighton (14620)	Grown in a pot with a topsoil
19	Chili Road	Chili (14624)	Grown in the ground
20	Saddleback Trail	Chili (14624)	Grown in a raised bed with soil consisting brewers compost, mushroom compost, and ground soil
21	Winbourne Road	Brighton (14619)	Grown in the ground
22	Eden Lane	Greece (14626)	Grown in a raised bed with a 50/50 mixture of sifted local top soil and compost. The bed is tilled with 40 pounds of mushroom compost.
23	Pelham Road	Brighton (14610)	Grown in the ground with compost and mulch chips
24	Wood Duck Run	Spencerport (14559)	Grown in the ground
25	Winbourne Road	Brighton (14619)	Grown in a raised bed with potting soil and compost