Determination of Lead Levels in Soil and Plant Uptake Studies

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Abstract
In lieu of an abstract, below is the first paragraph of the paper.

Lead poisoning is a problem for many urban areas and Rochester is no exception. The large number of older homes and high traffic areas of a city as large as Rochester create a city with a high potential for lead poisoning. This paper presents research in which the soil from Rochester area homes was tested for lead content. The samples were digested using EPA Method 3050B section 7.5 and analyzed for lead by Flame Atomic Absorption Spectroscopy. The majority of the houses were found to have concentrations of lead higher than the EPA accepted values of 400 ppm for play areas and 1200 ppm for non-play areas. Plant uptake studies were conducted to identify plants that are able to remove lead from the soil and ones that are safe to consume when planted in lead contaminated soil. The results are preliminary and as such cannot yet be used to draw any conclusions.
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Amanda R. Lewis

Lead poisoning is a problem for many urban areas and Rochester is no exception. The large number of older homes and high traffic areas of a city as large as Rochester create a city with a high potential for lead poisoning. This paper presents research in which the soil from Rochester area homes was tested for lead content. The samples were digested using EPA Method 3050B section 7.5 and analyzed for lead by Flame Atomic Absorption Spectroscopy. The majority of the houses were found to have concentrations of lead higher than the EPA accepted values of 400 ppm for play areas and 1200 ppm for non-play areas. Plant uptake studies were conducted to identify plants that are able to remove lead from the soil and ones that are safe to consume when planted in lead contaminated soil. The results are preliminary and as such cannot yet be used to draw any conclusions.

Specific Aim and Significance

The goals of this work are to determine the levels of lead in soil from Rochester neighborhoods and to conduct plant uptake studies so as to identify plants that are able to remove lead as well as plants that can be planted in the garden for consumption.

This work is of significance because of a high incidence of lead poisoning in the Rochester area. Lead poisoning has been identified as a “silent epidemic” and “one of the most common pediatric health problems in the US today” (Mielke H. W., 1999). The most common mode of lead poisoning is through the ingestion of lead contaminated soil. The Environmental Protection Agency [EPA] defines lead contaminated soil as containing greater than 400 ppm lead in play areas and greater than 1200 ppm lead in non-play areas. To be considered lead contaminated the soil must be bare and uncovered, as this is when it poses the greatest likelihood of being ingested (CEHRC). While lead poisoning can affect anyone of any age it is most prevalent in children, especially those living in inner cities where there are a higher occurrence of houses painted with lead based paint and more traffic (Mielke H. R., 1998). Children are at greater risk because of increased hand to mouth behavior, higher respiratory rates and greater lead absorption in the intestine (Kelada, 2001). It is believed that children are ingesting on average between 50 and 200 mg of soil a day due to normal hand to mouth behavior (Oomen, 2003).

Lead poisoning has many effects on the various systems in the human body and is capable of disrupting multiple biological processes. Studies indicate that lead has an effect on 5-aminolaevulinic acid dehydratase [ALAD] (Kelada, 2001; Perez-Bravo, 2004; Warren, 1998), as well as metabotropic glutamate receptor 5. It is also believed that lead is mistaken by the body as calcium due to the similar charge (2+) (Konopka, 2003). Some common side effects of lead poisoning are cognitive deficits, anemia, lower IQ scores, an increase in impulsivity, an inability to pay attention and an increase in crime and aggressive behavior. Lead also has effects on the reproductive system (low sperm counts and increases in stillbirth and miscarriage), kidneys, liver and gastrointestinal tract (Kelada, 2001; Konopka, 2003; Perez-Bravo, 2004; Warren, 1998; Xu, 2009). The symptoms of lead poisoning mentioned are believed to occur as a result of chronic exposure to blood lead levels at or above 10 micrograms of lead per deciliter of blood [µg/dL], although multiple sources cite that symptoms can occur at concentrations lower than 10 µg/dL (Mielke H. W., 1999; Perez-Bravo, 2004; Xu, 2009). When blood lead levels reach concentrations greater than 20 µg/dL, chelation therapy may be implemented to reduce the bioavailability of the lead. Common chelation therapies are dimercaptosuccinic acid and calcium disodium EDTA (Keep Kids Healthy).

One of the biological processes that lead has been found to have an effect on is N-methyl D-aspartate receptor [NMDAR] dependent long term potentiation [LTP]. NMDAR dependant LTP is a biological process which results in the creation of memory (Rager, 2008). Lead has been found to have an effect on metabotropic glutamate receptor 5 [mGluRS] whose function is necessary for the synaptic transmissions that result in the storage of memory. A study conducted by Xu et al. at Shanghai Jiao Tong University School of Medicine found that when cultured rat embryonic hippocampal neurons were exposed to a lead chloride solution growth was decreased in a dose-dependent manner. The neurons were also observed to have abnormal nuclei and soma as well as decreased axon and dendrite growth. The in vivo study, conducted at the same time, indicated that there was a decrease in mGluRS
messenger RNA [mRNA], which is believed to result in fewer mGluR5 (Xu, 2009). Since mGluR5 is necessary for NMDAR dependent LTP, a decrease in number of mGluR5 would result in less LTP and therefore less memory formation which could affect overall cognitive processes.

Another biological process which has been shown to be affected by lead is heme synthesis. Lead has inhibitory effects on three enzymes required for the synthesis of heme; 5-aminolaevulinic acid dehydratase [ALAD], coproporphyrinogen oidyase, and ferrochelatase. Lead has the greatest effect on ALAD. Heme is synthesized from two equivalents of 5-aminolaevulinic acid [ALA], which are combined by ALAD to form porphobilinogen [PBG]. It is believed that lead inhibits ALAD by binding to cysteine residues which zinc usually binds. Zinc is required for the catalytic activity of ALAD thus if lead binds in its place ALAD can no longer function properly (Warren, 1998). In addition to the prevention of zinc binding, lead also causes a change in the quaternary structure of ALAD, further ensuring that it will not function. The neurotoxicity of lead is believed to result from a buildup of ALA as a result of the inhibition of ALAD (Kelada, 2001). ALA resembles γ-aminobutyric acid [GABA], an inhibitory neurotransmitter (Nelson, 2005). The stimulation of GABA results in a larger inhibition of neurotransmissions causing fewer signals to be sent or received. This increase in inhibition could contribute to decreases in synaptic firings as well as neuron growth.

**Sources of Lead**

There are many uses of lead that have contributed to contamination of soil with lead for many years. Sources of lead range from lead shot, sinkers and jigs, pottery glazes, car batteries, industrial emissions and mining activity (Baird, 2005; Sharma, 2005). The two main sources of lead soil contamination are lead-based paint and leaded gasoline. Together, lead based paint and leaded gasoline have introduced 10 million metric tons of lead into our environment (Phytoremediation of Lead in Urban, Residential Soils).

**Reducing Threat of Lead Poisoning in Home**

Lead was used as an additive to paint from 1884 and until 1989 (Mielke H. R., 1998). Figure 1, above, indicates that the exterior of the house is the most frequent site of lead based paint (Goodrum). Weathering and natural deterioration of the paint on the external surface of a house causes the paint to chip, contaminating the soil around the exterior of the house. Other activities such as sanding and sandblasting during remodeling cause the paint to come off as dust which can also contaminate soil (CEHRC). The graph in Figure 2, below, shows that as the use of lead based paint was increasing, the use of leaded gasoline was on the rise (Mielke H. W., 1999). Lead was added to gasoline in the early 1920s to boost octane levels. Lead was banned as an additive of gasoline in 1996 after new technology became available which no longer required lead in gasoline. Although leaded gasoline was banned for most vehicle use it is still allowed for use by aircraft, race cars, and farm equipment (EPA). Approximately 75% of the lead used in leaded gasoline enters the atmosphere as a fine lead dust emitted from the exhaust pipe. The dust can then settle thereby contaminating the soil (Mielke H. R., 1998).

![Figure 1: Frequency of lead presence in a typical home (Goodrum).](image1)

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![Figure 2: Lead use in paint and gasoline from 1910 to 1990 (Mielke H. W., 1999).](image2)

**Figure 2: Lead use in paint and gasoline from 1910 to 1990 (Mielke H. W., 1999).**

There are multiple strategies that can be implemented to decrease the potential of lead poisoning through contaminated soil. The traditional way to prevent lead poisoning from contaminated soil is through the removal of contaminated soil. The contaminated soil is transported to a storage site where it is usually buried and new soil is spread in its place (Butcher, 2009). This process can be very expensive, requiring up to of $1,000,000 per acre of soil (Raskin, 1997). Other strategies involve creating a barrier to the soil by either planting grass or shrubs in the soil, adding clean soil over the contaminated soil, or implementing physical barriers such as gravel.
or mulch. These strategies are less expensive than removing the soil, but still leave the potential that the contaminated soil will be exposed in the future (The Lead Group). One strategy which has shown promise as a cheap, permanent solution to lead contaminated soil is phytoremediation.

**Phytoremediation**

Phytoremediation is the use of green plants to remove pollutants from the environment or render them harmless (Raskin, 1997; Weller, 2000). The observation of certain wild plants growing in areas contaminated by metals, lead to the belief that plants could be used to concentrate the contaminants, thereby decontaminating the soil. This form of remediation is cheaper than removing and replacing the contaminated soil, and it has a more permanent effect than creating a barrier to the soil. Over time plants would continue to accumulate lead until the soil was no longer contaminated (Raskin, 1997). For maximum effect it is best to use plants which are hyperaccumulators for lead. Hyperaccumulators are plants which have a large biomass and an increased ability to accumulate certain contaminants (Phytoremediation of Lead in Urban, Residential Soils; Weller, 2000). Some known hyperaccumulators for lead are Indian mustard, Corn, Ragweed, Turnips, Sunflowers, Broccoli and Pennycress (Phytoremediation of Lead in Urban, Residential Soils; Raskin, 1997). Since lead is able to complex with multiple things in the soil, such as organic matter, there are few hyperaccumulators for lead; however measures can be taken to increase the accumulation of lead. Adding chelating agents such as EDTA helps to solubilize the lead for increased plant uptake. Lowering the pH of the soil also acts to increase the solubility of the lead in the soil for better plant uptake (Butcher, 2009). This project will use plants common to the Rochester area, to test for the accumulation of lead with the goal of identifying plants that can be used to remove lead from the soil as well as plants that do not accumulate lead which can be consumed.

**Method**

**Analysis of soil and plant samples for lead**

The testing in the South Wedge and Highland Parkway neighborhoods was done in conjunction with a Service Learning project for Spring 2009-CHEM 316L Analytical Chemistry II laboratory courses.

**Collection of Soil Samples**

For each house where samples were collected, the permission of the homeowner was first obtained. Samples were only collected from exposed, uncovered soil. Each sample was collected using a plastic measuring cup, stored in a plastic zip-lock bag and labeled. The measuring cup was washed with a dilute soap solution then rinsed with distilled water after each collection to prevent cross contamination. At each house four samples were collected from various points in the yard. The samples were collected in the following areas unless indicated otherwise: one sample was collected from the front of the house close to the road, the next sample was collected from the front of the house close to the house, the third sample was collected from behind the house close to the house, and the fourth sample was collected from behind the house on the opposite side of the yard. The samples were brought back to the lab and stored in drawers until further testing could be run.

**Planting**

For this project the following plants were chosen to be planted: Southern Giant Curled Mustard, Ruby Queen Beets, Scarlet Nantes Carrots, California Wonder PS Peppers, Vates, Short Stem Collards, Ashley Cucumbers, Bush Blue Lake Beans, Danvers Carrots, FA Broadleaf Mustard, and Southern Collards. The plants were chosen to provide a range of types of vegetables: root vegetables, leafy vegetables, as well as fruiting vegetables. Two varieties of each type of vegetable were planted to provide an added level of comparison. These plants were also chosen to represent the types of vegetables commonly grown by homeowners in the Rochester area. The plants were planted in plastic garden six-packs. Two six-packs were used for each plant, one labeled control and one labeled spiked. The control plants were watered, everyday, using water from the tap in the lab. The spiked plants were watered using a 1000 ppm lead nitrate solution prepared from lead(II) nitrate (lead(II) nitrate, 99+, A.C.S. reagent, Sigma-Aldrich, USA) and water from the tap in the lab. The spiked plants were watered with this solution every-other day to build up the concentration of Pb²⁺ in the soil. On opposing days the spiked plants were watered with water from the tap in the lab.

**Collection of Plant Samples**

When the plants were believed to have matured a sample of the edible portion of the plant was collected. A sample was collected from both the
control plant and the spiked plant, if both were available. The soil that each plant was grown in was also collected. The soil was collected using a spatula, rinsed between collections of soil from each plant. The soil was collected in a piece of wax paper, folded over and sealed with tape.

Digestion of Soil and Plant Samples

Each sample collected was digested following EPA method 3050B section 7.5. Initially two different glassware set-ups were used for the digestion. The first set-up used a 100 mL round-bottom flask with a reflux condenser, heated in a heating mantel. The second set-up used a 250 mL beaker with a watch glass, heated on a heating plate. The samples, after digestion, were stored in plastic bottles with screw-cap tops and stored in the refrigerator for later analysis. Following the digestion, the glassware used was washed with a 4.0 M solution of nitric acid to remove any lead which may have leached into the glass.

In addition to the samples collected a method blank and matrix spike were also made. The procedure for the method blank is the same as the digestion (EPA Method 3050B section 7.5) with the only change being a lack of sample. The purpose of the method blank is to determine if there is any source of contamination from the reagents or glassware. For the matrix spike a sample of soil was obtained from the original source of soil used for planting. The sample was spiked with 3.0 mL of 1000 ppm lead nitrate solution. The sample was then digested following EPA Method 3050B section 7.5. The purpose of the matrix spike is to determine the efficiency of the digestion procedure.

Flame Atomic Absorption Spectrometry

Calibration standards were made using lead standard (lead atomic absorption standard solution, 1002 μg/mL, Sigma-Aldrich, USA) and diluted to various concentrations using a 1 wt% HNO₃ solution prepared using nitric acid (nitric acid, A.C.S. reagent, 70%, Sigma-Aldrich, USA). The concentrations used were 75 ppm, 150 ppm, and 250 ppm. The 1 wt% HNO₃ solution was used as the blank calibration standard for the calibration curve. Using a Buck Scientific Flame Atomic Absorption Spectrometer (FAAS), each standard and sample were measured in replicate.

For samples collected in September 2009 through November 2009, calibration standards were made to have a concentration of 50 ppm, 100 ppm, 250 ppm, and 500 ppm following the procedure detailed previously. The samples collected during those months as well as the standards prepared at that time were analyzed in triplicates using a Perkin Elmer FAAS at Nazareth College.

Data Analysis

The slope and intercept value from each calibration curve was used to calculate the concentration of each sample from the average absorbance value. The concentration of the blank \(C_{\text{blank}}\) was calculated by subtracting the intercept of the calibration line \(b_0\) from the absorbance of the blank \(A_{\text{blank}}\) and dividing by the slope of the calibration line \(b_1\) as shown in the following equation

\[
C_{\text{blank}} = \frac{A_{\text{blank}} - b_0}{b_1}
\]

The concentration of the sample was calculated the same way as the blank except the absorbance of the sample \(A_{\text{sample}}\) replaces the absorbance of the blank. The corrected concentration of the sample \(C_{\text{CT}}\) was calculated by subtracting the concentration of the blank from the concentration of the sample as in the equation

\[
C_{\text{CT}} = (C_{\text{sample}} - C_{\text{blank}})
\]

The concentration of lead in the sample \(Q_{\text{b}^+}\) was calculated by multiplying the corrected concentration of the sample by the volume of the volumetric flask and dividing by the mass of the sample \(M_s\) as in the equation

\[
Q_{\text{b}^+} = \frac{C_{\text{CT}} \times 100 \text{ mL}}{M_s}
\]

The concentration of the lead in the sample was reported as part per million.

Results and Discussion

The results presented below are very preliminary and have yet to be duplicated unless indicated, by an asterisk.

House Soil Analysis Results

The values from the equation of the best fit line of the calibration curve in Figure 1 was used to calculate the concentration of lead for each sample listed in Table 1 and Table 2.

Figure 1: Calibration Curve for House Samples
The majority of the values in Table 1 are above the EPA standard for play areas, 400 ppm, and almost half are above the EPA standard for non-play areas of the yard, 1200 ppm. The values also show a trend that in each section of the yard, both the front and the back, there is a higher concentration of lead closer to the house. A possible explanation for this is that since the houses are not located on a road with heavy traffic the contributing factor for soil contamination with lead is from paint on the exterior of the house. The majority of values also show a trend towards having a higher concentration near the front of the house. This could possibly be because the front of a house is more likely to get painted. If there are more layers of lead-based paint on the front of the house when it deteriorates more is able to come off and contaminate the soil around the front of the house.

The values in Table 2 support the trend that the majority of the soil tested contained a concentration of lead higher than the EPA standard of 400 ppm for play areas. However there are a lower percentage of samples above the EPA standard for non-play areas, 1200 ppm. Table 2 also presents information on five gardens at different homes. Of the five gardens, three are above the EPA standard for play areas and one is above the EPA standard for non-play areas. The high concentrations of lead in these gardens are of concern should vegetables be planted in them for consumption. Discovery of such high concentrations of lead in the soil tested from the two...
neighborhoods prompted plant uptake studies to determine what plants could be used to remediate the lead and what plants can be safely planted in contaminated soil without risk of transferring the lead during consumption of the plant.

Quality Control

Method Blank

The values from the equation of the best fit line of the calibration curve in Figure 2 were used to calculate the concentration of lead for the method blank. The method blank was found to not have a detectable level of lead. This indicates that the procedure used for the digestion of the neighborhood and plant uptake study samples does not introduce any contamination to the samples.

Matrix Spike

The values for the matrix spike have not yet been determined due to a limited access to a functioning FAAS.

Plant Uptake Study Results

The values from the equations of the best fit lines of the calibration curves in Figures 2-5 were used to calculate the concentration of lead for each sample, as indicated by the title of the graph, listed in Table 3.

**Figure 2: Calibration Curve for Bush Blue Bean Samples 7/27/09**

**Figure 3: Calibration Curve for Bush Blue Bean Samples 8/17/09**

**Figure 4: Calibration Curve for Vates, Short Stem Collard Samples 8/13/09**

**Figure 5: Calibration Curve for Samples Collected 9/25/09 – 11/1/09**
The first plant tested in the plant uptake study was Bush Blue Lake beans. The spiked plant showed an uptake of lead resulting in a lead concentration of 2000 ppm. However, since the soil was not tested with the plant the result is invalid. Without the concentration of lead in the soil it cannot be determined if the value has any significance. From this result, it was determined that all testing should include both a plant and soil sample from the spiked and control plant. When the Bush Blue Lake beans were retested on 8/17/09 the results show that although the spiked soil had a lead concentration of 6000 ppm, there was not a detectable level of lead in the plant. This contradicts the original test of Bush Blue Lake beans which indicated a high concentration of lead in the spiked plant. Due to the mixed results it cannot yet be determined if Bush Blue Lake beans are successful at accumulating lead in the beans of the plant.

The second plant tested was Vates, Short Stem collards. Both the spiked plant and soil were found to have a concentration of lead. The spiked plant showed an uptake of lead less than the lead concentration in the soil. The control plant and soil were also found to have a concentration of lead. This was not to be expected, as the plant and soil were not directly exposed to lead. A possible explanation is that water used to water the spiked plants was accidentally splashed on the control plant tested. In future plant uptake studies solid lead (II) nitrate will be used to spike the soil before planting as to eliminate the necessity of continued exposure to lead, which has a higher chance of contaminating control plants. The control plant was also found to have a higher concentration of lead than that found in the soil. It is believed that this resulted from an accumulation of lead on the outer surface of the plant as a result of splashing with lead water. To correct this possible source of contamination all plant, spiked and control, are rinsed in tap water previous to being tested. Since homeowners are encouraged to wash all produce before consumption, it is a reasonable expectation that lead on the outer surface of a plant would not generally be consumed as it would be washed off before being ingested. Results of a repeat test for the Vates, Short Stem collards on 9/25/09 showed that only the spiked soil was found to have a high concentration of lead. The concentration of lead in the spiked soil is greater for the test conducted on 9/25/09 than on 8/31/09, consistent with a continued exposure to lead through lead water. The spiked plant was found to have no accumulation of lead, which refutes the original testing of the collards. The control plant and control soil were found to not have any lead. For the

### Table 3: Concentration Values for the Plant Uptake Studies

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample</th>
<th>Concentration Plant Spiked</th>
<th>Concentration Soil Spiked</th>
<th>Concentration Plant Control</th>
<th>Concentration Soil Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>7/27/09</td>
<td>Bush Blue Lake Beans</td>
<td>2 x 10^4 ppm</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
<tr>
<td>8/17/09</td>
<td>Bush Blue Lake Beans</td>
<td>Not detected</td>
<td>6 x 10^3 ppm</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
<tr>
<td>8/31/09</td>
<td>Vates, Short Stem Collards</td>
<td>1 x 10^3 ppm</td>
<td>1 x 10^1 ppm</td>
<td>4 x 10^3 ppm</td>
<td>3 x 10^3 ppm</td>
</tr>
<tr>
<td>9/25/09</td>
<td>Vates, Short Stem Collards</td>
<td>Not Detected*</td>
<td>2.000 x 10^4 ppm*</td>
<td>Not Detected*</td>
<td>Not Detected*</td>
</tr>
<tr>
<td>10/9/09</td>
<td>California Wonder PS Peppers</td>
<td>Not Detected*</td>
<td>1.166 x 10^4 ppm*</td>
<td>Not Detected*</td>
<td>Not Detected*</td>
</tr>
<tr>
<td>10/23/09</td>
<td>Southern Giant Curled Mustard</td>
<td>Not Detected*</td>
<td>9.487 x 10^3 ppm*</td>
<td>Not Detected*</td>
<td>Not Detected*</td>
</tr>
<tr>
<td>11/1/09</td>
<td>Scarlet Nantes Carrots</td>
<td>Not Detected*</td>
<td>8.173 x 10^3 ppm*</td>
<td>Not Detected*</td>
<td>Not Detected*</td>
</tr>
<tr>
<td>11/1/09</td>
<td>Danvers Carrots</td>
<td>Not Detected*</td>
<td>Not Detected*</td>
<td>Not Detected*</td>
<td>Not Detected*</td>
</tr>
</tbody>
</table>

* - averaged value (conducted on the same day)
plant this could indicate that the original concentration of lead was in fact a result of lead on the surface of the plant.

The remaining plant uptake study results found that only the spiked soil samples had a concentration of lead. Since none of these plants were retested no conclusion can be made as to whether or not the plants are capable of accumulating lead. The last two results are for two varieties of carrots. The two varieties were tested since neither variety had a counterpart to test. The only available Scarlet Nantes carrots were spiked and the only available Danvers carrots were control. The two varieties cannot be compared as they are two different subspecies of carrots. Since only a few results have indicated plant uptake of lead future work will include digesting an entire plant as well as testing a core sample. The purpose of the digestion of the entire plant is to determine if the lead is accumulating in another area of the plant which is not considered edible. The purpose of the core sample is to determine how far the lead from the lead(II) nitrate solution, used to water the spiked plants, has penetrated the soil.

Conclusion
The results from both the neighborhood sampling and the plant uptake studies are very preliminary. Most results have yet to be duplicated. Also possible sources of contamination in the plant uptake studies have yet to be resolved.

Acknowledgments
I would like to thank Nazareth College for the use of their Flame Atomic Absorption Spectrometer. I would like to acknowledge Drs Kimberly Chichester, Maryann Herman, and Irene Kimaru, my research advisors, for all the help and guidance they have provided me on this project. I also wish to acknowledge Colleen Dugan and Emily Milgate for working on the project with me; Colleen worked on the digestions and the plant uptake study, Emily worked to determine the effects of lead on plant growth. I would like to thank Lynn Donahue, the program director for Service Learning projects, and the Spring 2009-CHEM 316L Analytical Chemistry laboratory courses, for making contact with the houses in the Rochester neighborhood and helping to collect samples, respectively.

References


