

Experiment 3: Atomic Absorption Determination of Lead in Soil Samples

References: Mielke, H. *American Scientist* **1999**, 87, 62-73.

Yarnell, A. *Chemical and Engineering News* **2006**, October 2, 47-49.

Experimental Work on March 1 and March 2-23
Notebook and Formal Report Due on March 30 by 4 p.m.

Introduction

- (1) Read the *American Scientist* article by former Macalester professor Howard Mielke on the prevalence and danger of lead in urban soil. (Also read the article from *Chemical and Engineering News* on how Hurricane Katrina changed Mielke's life.)
- (2) **Before lab on March 1, your team should collect three soil samples for analysis.** Think about interesting places to investigate, such as along Snelling Ave. or in the gardens of old homes. Do not use indoor samples (such as the potting soil inside Olin-Rice) that are not expected to contain any lead! Note the approximate distances between the samples—remember that the goal is to probe trends in Pb concentration. You need ~2 g of dirt per sample. If your samples have vegetation, rocks, etc., collect more than 2 g. Confine your sampling to the soil surface.
- (3) **I strongly recommend that you dry your samples before you digest them in lab on March 1.** Use the oven in Olin-Rice 380.

Lab Work on March 1 (document both procedure and observations in your team's notebook)

Sample Digestion (adapted from an Environmental Protection Agency procedure)

- (1) Transfer each of your ~2 g soil samples to a 100-mL beaker. Weigh each sample to the nearest 0.001 g on an analytical balance. The samples need not be exactly 2.000 g, but record whatever the weight is to three decimal places. Do not use (much) more than 2 g per beaker. However, this should be 2 g of soil; remove as much grass, rock, etc. as possible before weighing.
 - For the rest of the procedure, wear gloves and handle glassware with tongs to avoid getting concentrated HNO₃ or H₂O₂ on your skin!
 - Be careful about the amounts of liquid you add! The total volume in each beaker should not exceed 50 mL before Step 7!
 - Remember that it is good, standard chemical practice to pour out small portions of reagents for your team from the stock bottles. Do not risk contaminating the stock bottles by inserting pipets or other glassware. Also, never pour unused reagent back into stock bottles.
- (2) **In a hood**, add to each beaker 5 mL water, and then 5 mL concentrated HNO₃. Mix each slurry and cover each beaker with a non-ribbed watch glass placed concave up. (We want to nearly seal the top of the beaker with the watch glass.) Heat all samples until they are refluxing (that is, when vapor is condensing on the bottom of the watch glass and dripping back down into the beaker) for 10 min. **Do not allow your sample to boil.**

- (3) Allow each sample to cool to room temperature, add another 5 mL of concentrated HNO_3 , replace the watch glass, and reflux for another 25 min. (If there appears to be material condensed on the bottom of the watch glass, you may rinse the material back into the beaker with a minimum amount of water.)
- (4) Repeat Step 3 to ensure complete oxidation.
- (5) When the samples have cooled for the third time, add approximately 60 drops (*i.e.* ~3 mL) of 30% H_2O_2 **a few drops at a time** to each beaker. **Adding the H_2O_2 slowly minimizes effervescence. This minimizes sample loss, and more importantly, potential damage to your skin!** Cover each beaker with a watch glass and return it to the hot plate. Heat (again, **do not boil**) your sample to start a reaction with the H_2O_2 . When the mixture stops effervescing, let it cool. Then add up to 50 additional drops of 30% H_2O_2 (*i.e.* ~2.5 mL) until additional effervescence is minimal.
- (6) Add 5 mL of concentrated HCl and **slowly** add 10 mL of water, replace the watch glass cover, and reflux for 15 minutes. (Yes, you should still avoid **boiling**.) After cooling, dilute to ~40 mL total volume with water (unless you are already at or above 40 mL).
- (7) Finally, filter each solution through qualitative filter paper into a 50-mL volumetric flask. (If you were careful with your volumes, you will not be overshooting the marks here!) Dilute to the mark and then commence obsessive-compulsive behavior (*i.e.* invert each of your three flasks at least 20 times to mix).

Standards Preparation

Your TA will have prepared a stock solution of lead (II) nitrate containing (very close to) 100.0 ppm Pb^{2+} . (Remember that this is a mass/mass ratio). This works out to be 0.1598 g $\text{Pb}(\text{NO}_3)_2$ in exactly 1 L of solution. The exact concentration will be reported that morning. Please note:

- Wear gloves whenever you are working with lead-containing solutions.
 - It is good, standard chemical practice to pour out small portions of Pb stock solution for your team. Do not risk contaminating the stock bottles by inserting pipets or other glassware. Also, never pour unused reagent back into stock bottles.
- (1) Use the stock solution to make standards containing 20, 10, 5 ppm, and 2 ppm Pb^{2+} . Prepare 100 mL of each standard. Note the concentration of each solution to four significant figures. (For example, if the stock solution is 100.6 ppm, your “20 ppm” solution is actually 20.12 ppm.) Use the more precise values of the concentrations when you construct your calibration curve.
 - (2) Make 100 mL of a solution containing 1 ppm Pb^{2+} . The absorbance of the 1-ppm solution will not be plotted on your calibration curve, but rather its standard deviation will be used to determine the limit of detection and the limit of quantitation of the instrument.

WASTE DISPOSAL: All washings from the soil digestion glassware can go down the drain. Any $\text{Pb}(\text{NO}_3)_2$ solution left over after you prepare your standards must go into a waste container.

Lab Work on March 2-23 (you do not have to document your procedure, but do document your observations)

Analysis by Atomic Absorption Spectrometry

On March 2 (*i.e.*, during class), I will demonstrate how to use the Buck Atomic Absorption Spectrometer in Olin-Rice 386. Each team will sign up for a two-hour time slot to make measurements on your solutions sometime between then and one week before your report is due. Please come find me or Rob Rossi if you need help using the instrument.

Directions for Using the Buck 211 Atomic Absorption Spectrometer (AA)

Setting up the AA software:

1. Put on your safety goggles and a pair of disposable gloves! Next, push in the square red power button (on the right side of machine) on the AA. You may assume that the Pb hollow cathode lamp has already been installed and properly aligned.
2. On the right side of the machine are two knobs. One controls the slit width, and can only be set to one of three numbers. Set it to 7 Å. The other knob controls the AA's detector wavelength. Pb atoms emit and absorb most intensely at ~283 nm. Set the dial to this number. (Note that the wavelength control knob actually adjusts the angle of the diffraction grating in the machine). Our goal is to "aim" the 283-nm light directly onto the photomultiplier tube (PMT).
3. At the top right corner of the screen, below "Lamp 1", it should read "D2 Bkg Comp On". If it reads "off" instead, press the **BKGND** button on the machine.

Do the following three steps before you retrieve your standards and analyte solutions—you want to maximize the warm-up time for the instrument.

4. Press the **SEL** button on the front control panel (bottom right key). You may have to wait a moment for the machine to warm up. The top right of the screen should then say LAMP 1, 2, or 3. Pressing the **SEL** button again will change the lamp number. Continue to press **SEL** until the screen reads LAMP 1. Then press **ESC**.
5. Next, you will maximize the light intensity hitting the PMT. Do this by pressing the **ALIGN** button on the front control panel. There will be 2 bars, each over a number line, and above that a value labeled "Energy" and "Bkg Energy". The goal is to make the "Sample" bar read as high a positive value as possible, and thus make the "Energy" number as high as possible (they increase in tandem). To do this, turn the wavelength knob on the side of the machine in either direction until the energy is at its maximum. Do not be surprised if the optimal position is a few nm different from the nominal λ_{\max} – the wavelength dial is only approximate. In your notebook, record the wavelength and energy readings obtained once the energy has been maximized.
6. Press the **A/Z** button to autozero the machine at its maximal energy. Wait until the screen returns to the main, initial "Active Analysis" screen.

Preparing the AA for use:

7. Your standards and analyte solutions should be retrieved before moving beyond this step. Immerse the AA's thin Teflon aspirator tube in a 250-mL Erlenmeyer flask full of distilled water. Keep this tube immersed in liquid while the flame is on – that is, if the AA sucks all the water out of this flask, be sure to re-fill it within a few seconds; don't let the flame continue to burn for very long (15 seconds) without aspirating a liquid.
8. Open the yellow air jet along the wall to your left, which is connected to a filter system via a braided nylon hose. (It says "Apollo" on it, and is open when the handle points toward the back wall.) Confirm that the black and red gauge connected to the filter in the air line (above and to the right) indicates that the line pressure is between 60 and 70 psig.
9. Open the main valve (directly on top of the cylinder) of the acetylene cylinder behind the AA bench completely. **The cylinder should not be used if the cylinder pressure (right-hand gauge) has dropped below 50 psig (lb/in²)**, as too much acetone will be extracted (acetylene is dissolved in acetone to prevent explosions). [If the pressure is near 50 psig, please inform Professor Kuwata]. The line pressure (left dial) should be between 13 and 14 psig (and definitely not more than 15 psi). Unscrewing the large black knob of the regulator will lower the line pressure, but only if you press in the white **AIR** button on the AA to release some of the gas pressure.
10. On the left part of the machine, press and hold **AIR** (bottom right button). The columns for air ("oxidant") and acetylene ("fuel") should both read near 5 on the flow tube bobs below. If the "fuel" is too low, adjust with the "Fuel Adjust" knob below. Before igniting the flame, wait 1 minute after pressing **AIR**.
11. To ignite the flame, press and hold **ON** for 5-8 seconds while repeatedly pressing the trigger for the old red and white ignitor above the burner head. (**If you ever need to extinguish the flame quickly, press OFF at any time.**) Water should begin to be aspirated.
12. Make sure you are always aspirating some liquid whenever the flame is on. Otherwise you risk overheating and damaging the burner. Re-fill the 250 mL flask with deionized water as needed. The nearest DI water tap is at the sink BEHIND you!
13. Check that the flow rates of "air" remains close to 5 (as measured by the flow tube bob), and turn the "FUEL ADJUST" knob to lower the fuel flow rate to 3. The flame should go from being bright yellow to a dull orange, remaining bright blue at the very bottom.

General measurement procedure:

14. If the absorbance reading on the screen is more than ± 0.003 away from zero, press the **A/Z** button again.
15. *Do not aspirate standards or samples before the machine has aspirated pure water for 5 minutes.* Aspirate your 20-ppm Pb standard by quickly moving the aspirator tube from the water to the standard. Press **READ** to integrate the value for the absorbance. The screen should then display a constant absorbance value. Then aspirate water for at least a minute.
16. Repeat this procedure for each of your other standards, and each of your analyte solutions. You should cycle through all of the solutions at least three times. Re-zero the display with the blank solution (distilled water) after each solution.
17. Finally, make nine measurements on your 1-ppm solution. Aspirate water (and re-zero if necessary) after every three measurements.

Shutdown procedure:

18. Run distilled water through the aspirator for a minute to rinse the burner head.
19. Stop the acetylene flow by pressing **OFF** on the left part of the machine.
20. Close the main valve (right side) on the acetylene cylinder completely.
21. Close the yellow “air” supply valve to the left of the AA.
22. Turn off the spectrometer by pressing the square red button on the right side of the AA.
23. Clean up after yourself! Remove all of your solutions, and clean up any spills.

Data Analysis

All of the following calculations should be presented in your Excel spreadsheet. Be sure to e-mail me a copy of your spreadsheet, and to include a copy of your spreadsheet and your calibration curve in your formal report (details below):

1. Use the spreadsheet you constructed for Problem Set 3 to determine the equation of your calibration curve. Your x-axis will be ppm Pb^{2+} (i.e. $\mu\text{g Pb}^{2+}/\text{g}$ solution). Fit one line to all your calibration data. This will maximize the precision of your determination. Do not include the 1-ppm readings in your calibration curve.
2. Use the curve to determine $[\text{Pb}^{2+}]$ (in ppm) in each of your three analyte solutions.
3. For each of your three analyte solutions, determine s_x , the standard error in $[\text{Pb}^{2+}]$, by evaluating Equation (4-27) on p. 71 of Harris. You do not have to also determine s_x by propagation of s_m , s_b , and s_y .
4. For each of your three samples, convert the $[\text{Pb}^{2+}]$ and its standard error determined in Steps 2 and 3 (which are per 50.00 mL of solution) to $[\text{Pb}^{2+}]$ and its standard error per gram of that soil sample. Then convert each standard error to a 95% confidence interval by multiplying by the value of Student's t for $n - 2$ degrees of freedom, where n is the number of points on your calibration curve. Note that the difference in $[\text{Pb}^{2+}]$ for two samples is statistically significant if their 95% confidence intervals do not overlap.
5. Calculate the standard deviation in the nine measurements you made on the ~1-ppm solution. Use this to determine the signal and concentration limits of detection and limits of quantitation, assuming that the absorbance of the blank is given by the y-intercept. (Yes, as I discussed in class on February 12, this is generally a bad idea! However, based on data from last year's class, I found that assuming $y_{\text{blank}} = b$ leads to more reasonable detection limits than assuming $y_{\text{blank}} = 0$.)

Formal Report

Your written report should contain each of the following sections, in the order given: Title, Abstract, Introduction, Procedure, Results and Discussion, Conclusions, References, Appendix. Assume your reader is a student taking analytical chemistry who is familiar with atomic absorption spectroscopy, but who has not done this particular experiment.

The **Title** should be specific and descriptive.

The **Abstract** should provide a less-than-150-word summary of the entire work: the purpose, procedure, key results, and their significance should all be **briefly** addressed in this essential part of your report. The Abstract is not the place to introduce the experiment or describe the underlying principles in any detail. Stated in another way, the paper really begins with the Introduction, not the Abstract. Most scientists write the Abstract after they have written the rest of the paper, since it *summarizes* the work described. Never present material in the Abstract that you have not also presented somewhere in the main body of the report.

The **Introduction** should describe the specific goals of your experiment. What have you analyzed, and why? Briefly discuss some of the key ideas from Mielke's paper. You are not required to use other references, but you are welcome to. State the hypothesis you were testing

with the soil samples you chose. You should also briefly discuss the theory underlying your analytical method, using Harris as a reference.

The **Procedure** should provide a concise description of how the experiment was *actually* conducted. Note important observations (especially events that likely introduced error) and highlight any deviations from the instructions in the handout. You do not need to include drawings of any apparatus used in the experiment unless you feel it will aid your discussion.

The **Results and Discussion** section presents the key numerical results—the concentration (in ppm) of lead in each of your soil samples, their 95% confidence intervals, and the detection and quantitation limits. Briefly describe how these values were obtained, making reference to your spreadsheet and calibration curve, which should appear in the **Appendix**. Note if any (or all!) of your samples have $[\text{Pb}^{2+}]$ concentrations below the detection or quantitation limits. Interpret any trends in your soil measurements. (Remember that if two measurement's 95% confidence intervals overlap, then any difference between the measurements is due only to random error!) Discuss potential sources of both random and systematic error, and their likely importance.

Your report's **Conclusions** should summarize what you have accomplished in the experiment. Unlike the **Abstract**, the conclusion need not recapitulate every part of the paper. This section also should contain reflections on anything you would do differently if you had to repeat the experiment, and what hypothetical future experiments would be useful or interesting.

References: You must cite all sources you have used except for course handouts. This includes Mielke's *American Scientist* article and your textbook. Insert a superscript number the first time you cite a particular reference, and always use the same superscript number whenever you cite the same source in your report. Instead of using footnotes, collect all citations in this final section. Follow the American Chemical Society's conventions:

Books without Editors: Author 1; Author 2; Author 3; Author 4. *Book Title*, number of ed.; Publisher: Place of Publication, Year; Number of Chapter(s) Cited. For example,

Masterton, W. L.; Slowinski, E. J.; Stanitski, C. L. *Chemical Principles*, 5th ed.; Saunders: Philadelphia, 1980; Chapter 23.

Books with Editors: Author 1; Author 2; Author 3; Author 4. Chapter Title. In *Book Title*, number of ed.; Editor 1; Editor 2, Eds.; Publisher: Place of Publication, Year; Number of Any Specific Chapter(s) Cited. For example,

Montgomery, M.; Norman, J. RNAi and Cosuppression: Double-stranded RNA as an Agent of Sequence-Specific Genetic Silencing in Animal and Plants. In *Molecular Biology of Double-stranded RNA: Concepts and Applications in Agriculture, Forestry, and Medicine*. Tavantzis, S., Ed.; CRC Press: New York, 2001; Chapters 3-5.

Articles: Author 1; Author 2; Author 3. Title of Article. *Name of Journal* **Year**, *Volume*, Beginning – Ending Page. For example,

Kuwata, K. T.; Erickson, R. I.; Doyle, J. R. Improved Interatomic Potentials for Copper and Aluminum Sputter Atom Transport Simulations. *Nuclear Instruments and Methods in Physics Research B* **2003**, *201*, 566-570.

Web Sites: Cite their URL. Also note the last day you accessed the site. For example,

<http://bcs.whfreeman.com/qca/> (accessed 2/17/2005).

Grading

This experiment will be worth **50 points** (or twice the value of a regular experiment), earned in the following ways:

8 points: Record-keeping and other notebook mechanics

2 points: Precision of calibration curve

40 points: Quality of your paper, and the correctness of your calculations and data analysis.