Experiment 3: Determination of Lead in Soil by Atomic Absorption Spectroscopy


Experimental work to be done on March 4 and March 5 - 26
Notebook and Formal Report Due on April 1

INTRODUCTION

To prepare for this lab, please go to the course web site and 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.)

You will be reporting on your work from this experiment in a formal written presentation, modeled after a paper in an analytical chemistry journal. However, this is no time to let up on your lab notebook technique! You will find a good lab notebook essential to writing a good formal report, and I will be looking at your lab notebook as I read through your formal report.

SAMPLE COLLECTION

Because it is still cold, and the soil around here is mostly still frozen, last fall Rob Rossi collected samples for you to work with in this experiment, rather than asking you to do so yourself. However, it is still important for you to understand the details of the sample collection process. Each group will be given a family of three related soil samples to analyze. These will come from related locations, or soil depths, and consist of outdoor soil at reasonable risk for lead contamination. You'll need about 2 g of dirt (excluding vegetation, rocks, etc.) per sample. This will be dried at 110°C in the oven in Olin-Rice 380, in preparation for your arrival. Record in your lab notebook where each of your soil samples come from, after your introduction. A significant part of your report will involve comparing expected and measured trends in lead mixing ratios.

WET CHEMISTRY EXPERIMENTAL PROCEDURE

To be efficient, please do the Sample Digestion and Standards Preparation steps in parallel

Sample Digestion (adapted from an Environmental Protection Agency protocol)

1. On an analytical balance, weigh out approximately 2 grams of each of your three soil samples into separate, labeled, clean, and dry 100-mL beakers. The samples need not be exactly 2.000 g, but you should record whatever the weight is to limit of the balance’s precision. In no case should you use more than 2.5 g per beaker, though. Make sure that – as much as possible – you transfer only soil: leave behind any grass, rock, etc. when weighing out your samples.

2. Put on gloves, and throughout the rest of the sample digestion procedure, wear them! Avoid exposing your skin to concentrated acids or to the fumes generated in this process!
3. **In a hood**, add to each beaker **first** 5 mL water, and **then** 5 mL concentrated HNO₃. **THE ORDER IS IMPORTANT** – **ADD ACID TO THE WATER**, not vice-versa. Mix each slurry with the bare glass end of a different stirring rod 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, to reduce gas loss. To avoid sample loss and cross-contamination, **leave your stirring rods in the pouring spouts of the beakers.**)

4. Heat all samples together on one hotplate until they are refluxing (that is, until vapor is condensing on the bottom of the watch glass and dripping back down into the beaker), and keep them at reflux for 10 min. **Do not allow any of your samples to boil over.** (Start the heat at 10, and reduce to 1 at the first sign of boiling.)

5. Remove the samples from the hotplate and let them cool until they can be safely handled. Add another 5 mL of concentrated HNO₃ to each, replace the watch glasses, and reflux for another 10 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. You do not want to exceed 50 mL total volume by the end of this procedure.)

6. Add 5 mL of concentrated HCl and then **slowly** add 10 mL of water. (At this point, you do not need to wait until the samples are cool.) Replace the watch glass cover and reflux for 15 minutes, heating it up **slowly.** (Start the heat at 3, and then reduce to 1 at the first sign of boiling.)

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!) Hold the beaker upside down at an angle over the funnel and squirt it with a fine stream of DI water in order to rinse as much of the remaining solids into the funnel as possible – but do not overshoot the mark. Use what rinse volume you might have left to try to wash the yellow color out of the filter paper and into the volumetric flask.

8. When the solution has cooled to room temperature, dilute to the mark and then commence obsessive-compulsive behavior (**i.e.** invert each of your three flasks at least 13 times to mix). [If you mistakenly overshoot, you can recover by quantitatively transferring everything into a 100 mL volumetric flask...but it will change your calculations a bit. Don't forget that you did it!]

**Standards Preparation**

There will be a Pb²⁺ stock solution prepared by dissolving z g of Pb(NO₃)₂ lead(II) into a 1 L volumetric flask containing 1% mass nitric acid. The exact value of z will be given in lab, but the stock solution will contain (very close to) 100.0 ppm Pb²⁺. (Strictly speaking, “ppm” here means μg Pb²⁺/mL solution. However, since the density of the solution is very close to 1.000 g/mL, “ppm” also refers to a ratio of masses: μg Pb²⁺/g solution.) **Write z down in your notebook!**

**Important Notes:**

- Wear gloves whenever you are working with lead-containing solutions.
- The repipet will be set to deliver exactly 15.00 mL of the Pb²⁺ stock solution.
• The repipet will be set to deliver exactly 15.00 mL of the Pb²⁺ stock solution. The stock solution can therefore be dispensed directly into a 100.00-mL volumetric flask to prepare the 15-ppm standard. Preparing the less concentrated standards will require you first to dispense stock solution into a clean, dry beaker. (A wet beaker will dilute the analyte!)

1. Use the stock solution to make standards containing nominally 15, 10, 5, 2, and 1 ppm Pb²⁺. Prepare 100.00 mL of each standard. (Please do not round off the concentrations of your standards to the nearest 1 ppm when you construct your calibration curve later. For example, if the stock solution is 100.6 ppm, your “10 ppm” solution is actually 10.06 ppm.)

2. The nominally 1 ppm Pb²⁺ standard will do statistical “double duty.” First, the absorbance of this solution will be plotted on your calibration curve. Second, its standard deviation will also be used to determine the method’s limit of detection and limit of quantitation.

WASTE DISPOSAL: All washings from the soil digestion glassware can go down the drain. Any Pb(NO₃)₂ solution left over after you prepare your standards must go into a waste container.

INSTRUMENTATION PROCEDURE (Do not write down what you do or observe here in your notebook, unless the instructions explicitly tell you to do otherwise.)

During class on March 5, I will demonstrate how to use the Buck 211 Atomic Absorption (AA) Spectrometer in Olin-Rice 386. Your team will sign up for a two hour time slot to make measurements this machine, sometime between March 5 and one week before your report is due. Please come find me if you need help using the instrument.

Setting Up the AA Software

Do the following steps before you retrieve your standards and analyte solutions—you want to maximize the warm-up time for the instrument's hollow cathode lamp.

1. Put on your safety goggles and a pair of disposable gloves. (The gloves are necessary because you will be working with lead and acids!) 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. Make sure that it is set to 7 Å. The other knob controls the wavelength passed to the detector. Pb atoms absorb most intensely at ~283 nm. **Do not touch the dial; it has already been set to **$\lambda_{\text{max}} \approx 283$ nm.**

3. You may have to wait a moment if the D₂ lamp is set to be used. Once it is done, near the top right corner of the screen, below "LAMP #", it should read “D2 Bkg Comp On”. If it reads “Off” instead, press the BKGND button on the machine once to turn it on.

4. At the very top right corner of the screen there will be an indication of which lamp has power applied to it. We want to use Lamp 1, so if it doesn't already read that, press the SEL button on the control panel (button "L"), which will change the lamp
number. Keep pressing SEL until "LAMP 1" appears in the upper right hand corner, then press ESC.

I will have already done the next step: Maximize the light intensity hitting the detector by pressing the ALIGN button on the front control panel (a white key, on the bottom row – button "K"). There will be two bars, each over a number line, and above that a value labeled “Energy” and “Bkg Energy”. The goal is to maximize the rightward deflection of the “Sample” bar and the size of the energy (they increase in tandem). To do this, turn the wavelength knob on the side of the machine slowly in either direction until the energy is at its maximum. Typically, the optimal position is ~1 nm different from $\lambda_{\text{max}}$ – the wavelength dial is only approximate.

5. Press the A/Z button to autozero the machine at its maximal energy and to lock in the new alignment. Wait until the screen returns to the main, initial “Active Analysis” screen.

Preparing the AA for Use

You should now retrieve your standards and analyte solutions, moving them to near the AA.

6. Immerse the AA’s thin Teflon aspirator tube in a 250-mL beaker 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 beaker, be sure to re-fill it within a few seconds. Do not let the flame continue to burn for any longer than 15 seconds without aspirating a liquid, or you risk overheating and damaging the burner. The nearest DI tap is in the other instrument bay in the lab.

7. 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 is parallel to the 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.

8. COMPLETELY open the main valve (directly on top of the cylinder) of the acetylene cylinder behind the AA bench. The AA should not be used if the cylinder pressure (right-hand gauge) has dropped below 50 psig (lb/in$^2$, relative to the atmosphere), as too much acetone will be extracted (acetylene is dissolved in acetone, to prevent explosions and reduce the tank pressure). If the pressure is getting near 50 psig, please inform me or Rob Rossi!] The line pressure (left dial) should be between 13 and 14 psig (and definitely not more than 15 psig). Unscrewing the large black knob of the regulator will lower the line pressure, but only if you press in and hold the white AIR button on the front of the AA to release some of the gas pressure.

9. On the left part of the instrument, press and hold AIR (bottom right button). The flow meter for air (“oxidant”) should read about 5.5. Adjust the acetylene (“fuel”) flow rate to about 6 with the “Fuel Adjust” knob. If you don’t do this, the flame will be very hard to light.

10. Wait 20 seconds after pressing AIR. To ignite the flame, repeatedly press the trigger of the red and white ignitor while holding its tip above the burner head and pressing ON. If you ever need to extinguish the flame quickly, press OFF at any time. Water should begin to be aspirated as the flame lights up.
11. **Again, make sure you are always aspirating some liquid whenever the flame is on.** Re-fill the 250 mL beaker with deionized water as needed.

12. Check that the flow rate of “air” remains a bit above 5 (as measured by the flow tube bob), and turn the "FUEL ADJUST" knob to lower the fuel flow rate to about 3. The flame should go from being bright yellow to a very dull orange, remaining bright blue at the very bottom.

**General Measurement Procedure**

13. Lighting the flame will change the absorbance. Once you have aspirated pure water for at least 1 minute, press the A/Z button to correct it back to zero.

14. Aspirate your 15 ppm Pb standard by quickly moving the aspirator tube from the water to the standard. *Wait at least five seconds*, then press READ to integrate the value for the absorbance. The screen should then display a constant absorbance. You can return the aspirator to the water once the screen says "Ready": your reading will be retained on the screen.

15. Aspirate water for at least twenty seconds, to flush out any residual solution from the previous measurement from the nebulizer, aspirator, and burner head. You can record your absorbance measurement while you wait. When you are ready to take your next reading, but before you remove the aspirator from the DI water, hit the A/Z button again to re-zero the instrument and leave the aspirator in the DI water until the absorbance reading starts to update again.

16. Repeat this sampling 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.

17. Finally, make six additional replicate measurements on your 1 ppm solution. Aspirate water (and re-zero) 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. Press in and hold the AIR button until the excess acetylene in the line has been vented.

23. Turn off the spectrometer by pressing the square red power button on the right side of the AA.

24. 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. (You are not required to present any sample calculations, other documentation of your data analysis, or any conclusion in your notebook.) 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. Adapt the spreadsheet you used for Problem Set 3 and Experiment 2 to determine the equation of your calibration curve. Your x-axis will be ppm Pb\(^{2+}\) (i.e. \( \mu g Pb^{2+}/mL \) solution). Fit one line to all your calibration data. This will maximize the precision of your determination. Include all nine 1 ppm readings in your calibration curve.

2. Calculate the average absorbance for each of your three analyte solutions, and use the equation of your curve to determine [Pb\(^{2+}\)] (in ppm) for each solution.

3. For each of your three analyte solutions, determine \( s_x \), the standard error in [Pb\(^{2+}\)], by evaluating Equation (4-27) on p. 71 of Harris. Use the \( s_y \) from LINEST, not the standard deviation in the absorbances of each sample. 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 analyte solutions, convert your results in Steps 2 and 3 back to the original sample’s [Pb\(^{2+}\)] and its standard error. Think about this conversion as a dilution factor problem. Assuming that all of the lead in a given soil sample ended up in a solution whose absorbance was measured,

   \[
   \text{mass Pb}^{2+} \text{ in sample} = \text{mass Pb}^{2+} \text{ in AA solution}
   \]

   It therefore follows that

   \[
   ([\text{Pb}^{2+}]_{\text{sample}})(\text{total mass of sample}) = ([\text{Pb}^{2+}]_{\text{solution}})(\text{total mass of AA solution})
   \]

5. Convert each standard error in the [Pb\(^{2+}\)]\(_{\text{sample}}\) 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 [Pb\(^{2+}\)] for two samples is statistically significant if their 95% confidence intervals do not overlap.

6. Calculate the standard deviation in the nine measurements you made on the ~1-ppm solution. Use this to determine the instrumental signal (\( y \)) and concentration (\( x \)) limits of detection and quantitation, assuming that the absorbance of the blank is given by the y-intercept of your calibration curve:

   \[
   y_{LOD} = b + 3s \quad y_{LOQ} = b + 10s
   \]

   \[
   x_{LOD} = \frac{y_{LOD}}{m} \quad x_{LOQ} = \frac{y_{LOQ}}{m}
   \]

(Yes, as we discussed in class earlier this semester, using the y-intercept in this way is usually a bad idea! However, based on data from previous years, Rob Rossi and I have found that assuming \( y_{\text{blank}} = b \) leads to more reasonable detection and quantitation limits than assuming \( y_{\text{blank}} = 0 \), which is the value we sought whenever
we zeroed the spectrometer.) Use the same dilution factor employed in Step 4 to convert the instrument’s \( x_{\text{LOD}} \) and \( x_{\text{LOQ}} \) to values that apply to the original soil samples. That is, calculate the lowest concentrations of lead in the original soil samples that you would be able to detect and quantify reliably.

**FORMAL REPORT GUIDELINES**

Your formal written report should contain each of the following sections, in this order: Title, Abstract, Introduction, Procedure, Results and Discussion, Conclusions, References, Appendix.

Assume your reader is a student taking analytical chemistry who is familiar with (but somewhat forgetful about) atomic absorption spectroscopy, and who has not done this particular experiment.

1. The **Title** should be specific and descriptive: identify what you were looking into and what method you employed in doing so, being specific as possible while remaining brief.

2. The **Abstract** should provide a less-than-250-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.

3. The **Introduction** should summarize the background and theory for your experiment. What have you analyzed, and why? Briefly discuss some of the key ideas from Mielke’s paper. Based on Mielke’s work and the Sampling Details provided by Rob Rossi (on the course web page), state a reasonable hypothesis for the trend in lead levels in your soil samples. You should also briefly discuss the basic concepts underlying atomic absorption spectrometry, using Harris as a reference.

4. 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. You do not need to include drawings of any apparatus used in the experiment unless you feel it will aid your discussion.

5. The **Results and Discussion** section presents the key numerical results—the concentration (in ppm) of lead in each of your soil samples, the 95% confidence intervals of the concentrations, 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, discussing whether or not your hypothesis is supported. (Remember that if two measurement’s 95% confidence
intervals overlap, then any difference between the measurements may simply due to random error!)
Finally, discuss potential sources of both random and systematic error, their likely importance, and how they might be reduced.

6. Your report’s Conclusion section 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 (how could the experimental protocol be realistically modified to obtain better results), and what hypothetical future experiments might be useful or interesting.

7. References: You must cite all sources you have used, except for course handouts. Sources you should cite include 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 section. Follow (a slight modification of) 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:


- 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:


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


- Web Sites: Cite their URL. Also note the last day you accessed the site. For example: http://bcs.whfreeman.com/qca7e/ (accessed 2/22/2010).

8. Appendix: Put here a copy of your spreadsheet and a full-page copy of your calibration curve. You may also include any additional information that you feel will be useful to the reader, but too detailed to be included in the main body of your paper.

Experiment 3 will be worth 50 points, twice the point value of the other experiments. I will evaluate the quality of your notebook and experimental results, the accuracy of your data analysis, and the scientific and writing quality of your formal report.