

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

References: Mielke, H. *American Scientist* 1999, 87-1, 62-73; Yarnell, A. *Chemical & Engineering News* 2006, Oct. 2, 47-49.

Experimental work to be done on March 5 and March 6 - 27
Notebook and Formal Report Due on April 3

INTRODUCTION

To prepare for this lab, please 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.

Before you start experimenting with your samples, develop a hypothesis as to what you expect your results will be for them – not so much the amount of lead they will contain, but the *trend* in lead concentrations across your collection of related samples. Record this hypothesis and the rationale behind it in your lab notebook, after your introduction.

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 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, H₂O₂, 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 beakers*, in the pouring spouts.)

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.** [Start heat at 10, reduce to 1 at 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 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.)
6. When the samples have cooled again, use a Pasteur pipet to add approximately 60 drops (*i.e.* ~ 3 mL) of 30%_{mass} H₂O₂, a few drops at a time, to each beaker. Adding the H₂O₂ slowly will minimize 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 (set to 10, then 1; again, do not boil) your sample to speed the oxidation by the H₂O₂. When the mixture stops effervescing, let it cool. Then add up to 50 additional drops of 30% H₂O₂ (*i.e.* ~ 2.5 mL) until additional effervescence is minimal.
7. Add 5 mL of concentrated HCl and then **slowly** add 10. mL of water. Replace the watch glass cover and reflux for 15 minutes, heating it up **slowly**. (Set to 3, then 1; Yes, you should still avoid boiling.)
8. 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. 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 complicate your calculations a bit. Don't forget that you did it!]

Standards Preparation

Your lab TA will have prepared a stock solution of Pb(NO₃)₂ by dissolving z g of lead(II) nitrate 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 should contain (very close to) 100.0 ppm Pb²⁺.

Important Notes:

- Wear gloves whenever you are working with lead-containing solutions.
- It is absolutely essential that you not contaminate or dilute the stock solution. Never insert a pipet or other glassware into a stock bottle, and never pour unused reagents back into stock bottles. When pipetting, dispense small portions of the reagent you need from the stock bottle repipet into a clean, dry beaker. If it is wet, it will dilute the standard! (The repipet will be set to deliver exactly 18.00 mL of the Pb²⁺ stock solution.)

1. Use the stock solution to make standards containing nominally 18, 10, 5, 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 "10 ppm" solution is actually 10.06 ppm.) Use these more precise concentration values when you construct your calibration curve.
2. Make 100 mL of a solution containing nominally 1 ppm Pb^{2+} . The absorbance of this solution will not only be plotted on your calibration curve, but 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 $\text{Pb}(\text{NO}_3)_2$ solution left over after you prepare your standards must go into a waste container.

INSTRUMENTATION PROCEDURE

During class on March 6, 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 then and one week before your report is due. Please come find me if you need help using the instrument.

You do not need to write down in your notebook anything from this part of the lab unless I tell you otherwise in the instructions.

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. 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 instrument). Our goal is to "aim" the 283-nm light directly onto the photomultiplier tube (PMT).
3. You may have to wait a moment if the D_2 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**.

5. Now, you will maximize the light intensity hitting the PMT. Do this by pressing the **ALIGN** button on the front control panel (a white key, on the bottom row – button "K"). 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 slowly 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 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.

7. 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 behind you.*
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 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.
9. **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/in², 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.
10. 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.*
11. 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.
12. 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.

13. 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

14. 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.
15. Aspirate your 18 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.
16. 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.
17. 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.
18. Finally, make six additional replicate measurements on your 1 ppm_{w/v} solution. Aspirate water (and re-zero) after every three measurements.

Shutdown procedure:

19. Run distilled water through the aspirator for a minute to rinse the burner head.
20. Stop the acetylene flow by pressing **OFF** on the left part of the machine.
21. Close the main valve (right side) on the acetylene cylinder completely.
22. Close the yellow "air" supply valve to the left of the AA.
23. Press in and hold the AIR button until the excess acetylene in the line has been vented.
24. Turn off the spectrometer by pressing the square red power button on the right side of the AA.
25. 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 or other documentation of your data analysis 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.* $\mu\text{g Pb}^{2+}/\text{mL}$ 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. 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 analyte solutions, convert your results in Steps 2 and 3 back to the original sample's $[\text{Pb}^{2+}]$ 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,
$$\text{mass Pb}^{2+} \text{ in sample} = \text{mass Pb}^{2+} \text{ in AA solution}$$

It therefore follows that

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

5. Convert each standard error in the $[\text{Pb}^{2+}]_{\text{sample}}$ 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 $[\text{Pb}^{2+}]$ 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 $y_{\text{blank}} = 0$.)

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. You are not required to use other references, but you are welcome to. State the hypothesis you developed around your soil samples (recorded in your lab notebook!), and the rationale for it at the time. 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. (Remember that if two measurement's 95% confidence intervals overlap, then any difference between the measurements may simply due to random error!) 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:

William L. Masterton; Emil J. Slowinski; Conrad L. Stanitski. *Chemical Principles*, 5th ed.; Saunders: Philadelphia, 1980; Chapters 10-11.

- **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:

Jeffrey A. Norman; Mary K. Montgomery. "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*. Stellos M. Tavantzis, Ed.; CRC Press: New York, 2001; Chapter 1.

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

Keith T. Kuwata; Rachel I Erickson; James R. Doyle. "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/qca7e/> (accessed 2/26/2009).

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.