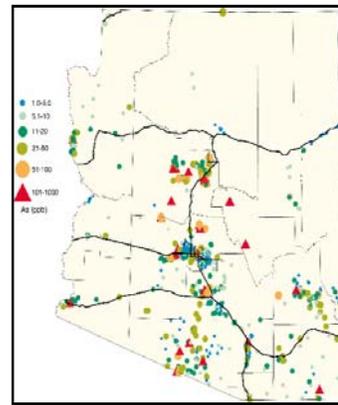


Arsenic in the Montezuma Well

Project Background

The goal of this project is to see if arsenic (As) is present within the Montezuma Well freshwater ecosystem, and if present, to determine if the amounts are increasing or decreasing as you move higher on the foodchain. Scientists know the water in Montezuma Well contains naturally occurring As that is derived from the minerals in the underlying aquifers.

Scientists are interested in how As behaves in the environment, and particularly in ecosystems, because it is a known toxin and carcinogen and is the source of recent tragic illness and death in some regions of the world, including Bangladesh and India.



Arizona Groundwater Arsenic Concentrations in Groundwater

What Will I Learn?

- Microwave sample digestion for As analysis
- Inductively Coupled Plasma Mass Spectrometry (ICP-MS)
- Use of Internal Standards
- Use of Excel spreadsheets and graphing techniques

Who, What, Why?

Who is this project for?

This research project is intended for chemistry majors who are interested in environmental analytical chemistry and in the technique of inductively coupled plasma mass spectrometry.

What will I do?

In this project, you will examine the bioaccumulation or biodiminution of As in a variety of samples from a freshwater ecosystem.

To Learn More

- Azcue, J.M.; Nriagu, J.O. Arsenic: Historical Perspectives. In *Arsenic in the Environment*; Nriagu, J.O., Ed. *Advances in Environmental Science and Technology Series Volume 26*; Wiley-Interscience: New York, 1994; pp 1-16.
- Cai, Y.; Georgiadis, M.; Fourqurean, J.W. Determination of Arsenic in Seagrass using Inductively Coupled Plasma Mass Spectrometry; *Spectrochim. Acta Part B* **2000**, *55*, 1411-1422.
- Konieczki, A.D.; Leake, S.A. Hydrogeology and Water Chemistry of Montezuma Well in Montezuma Castle National Monument and Surrounding Area, Arizona; U.S. Geological Survey Water-Resources Investigations Report **1997**, 97—4156.
- Matschullat, J. Arsenic in the Geosphere—A Review; *Sci. Total Environ.* **2000**, *249*, 297-312.
- Roychowdhury, T.; Tokunaga, H.; Ando, M. Survey of Arsenic and other Heavy Metals in Food Composites and Drinking Water and Estimation of Dietary Intake by the Villagers from an Arsenic-affected Area of West Bengal, India; *Sci Total Environ.* **2003**, *308*, 15-35.



Arsenic in Montezuma Well

Table of Contents

Project Overview cover, 4

This is a two-part description of your canned research project. The first part (the cover) is a somewhat flashy introduction to the project that highlights features of the project and lists readings in the research area. The second part places the work in context, emphasizing why the work is important and providing an overview of the project's purpose and methods.

Previous Work (text is also available in *As_PreviousWork.doc*) 5

This section describes work that has already been completed by previous students of your research group. Your project begins where their work ended. You will describe their previous work (together with your new work) in the Methods section of your paper. You can “cut and paste” sections of this text into the Methods section of your paper; hence, we have also included it as a Word document.

Methods 8

This section describes, step-by-step, the procedures that you will follow in conducting the canned research. It is written like a lab manual or lab notebook. Your goal will be to rewrite these procedures (combined with the previous work described above) in your Methods section, in a style appropriate for a journal article.

Data Analysis (all data are available in *As_Data.xls*) 16

This section describes how to analyze your data. Most of this work will not get reported in your final paper; however, it must be completed in order to arrive at your results. Specifically, this section describes how to construct the calibration curve, how to use the calibration curve to calculate As concentrations in your sample extracts, and how to convert “extract concentrations” to the more desirable “mass fractions”. You will also be directed to calculate averages and standard deviations, as appropriate.

Results 23

This section prompts you to consider the important features of your results, as well as how to present your data to an expert audience. This section is presented largely as a series of questions for you to consider. You will ultimately present your findings in your Results section using both text and one or more graphics (e.g., table or figure).

Discussion 23

This section prompts you to consider what your results mean, why they are important, and how they may apply to a larger context. This section is presented largely as a series of questions for you to consider. You will present the interpretations and implications of your research in your Discussion section.

Data Analysis Excel File Contents

A single Excel file (*As_Data.xls*) contains all the data needed to complete your project. Various sets of data are contained in separate worksheets within the file. The contents and names of individual worksheets are described below.

Worksheet Name	Contents	Description
CalData	ICP-MS area counts for Ge and As for calibration standards and blanks	You will use these area counts to calculate concentration and area-count ratios. These ratios will be used to plot a 4-point internal-standard calibration curve. Ultimately, the calibration curve will be used to calculate As concentrations in your SRMs and authentic sample extracts.
SRMData	ICP-MS area counts for Ge and As for two standard reference materials (SRMs), NIST 1571 (orchard leaves) and DORM-2 (dogfish muscle)	You will use these area counts to calculate area-count ratios. These ratios will be used in conjunction with your calibration curve to calculate As concentrations in your SRMs. Ultimately, these concentrations will be used to calculate SRM recoveries (%) to evaluate the accuracy of your experimental methods.
SampleData	ICP-MS area counts for Ge and As for your authentic sample extracts: Montezuma Well water, phytoplankton, <i>H. montezuma</i> , and <i>M. montezuma</i> .	You will use these area counts to calculate area-count ratios. These ratios will be used in conjunction with your calibration curve to calculate As concentrations in your authentic sample extracts. Ultimately, these concentrations will be used to calculate the mass fractions of As in the phytoplankton, <i>H. montezuma</i> , and <i>M. montezuma</i> .

Project Overview

Arsenic has been known for centuries to possess toxic properties. However, not all forms are toxic, and some organisms have developed mechanisms by which they tolerate high As concentrations. In fact, some organisms, such as the break fern, have been shown to accumulate high levels of As without detriment to the plant. Arsenic concentrations and behavior in the environment has been studied most commonly in marine environments and in anthropogenically contaminated environments, such as in mine tailings and smelters. The behavior of As in freshwater ecosystems with naturally elevated As levels is not well understood and has only been studied recently.

Montezuma Well is a naturally As-contaminated freshwater ecosystem in northern Arizona. Aqueous As levels are as high as 100 ppm (10 times the allowed EPA maximum contaminant level for drinking water). The ecosystem is unique, in that it contains several endemic species (species that are native to the Well and found nowhere else). In contrast to more complicated food webs, there is a rather simple foodchain in the Well that biologists have well characterized: phytoplankton → amphipod (*Hyallela montezuma*) → leech (*Motobdella montezuma*). This rather simple foodchain serves as a model system to study to see how As concentrations change as it moves up the foodchain (to higher trophic levels).

Purpose: Previous research has shown that As may either bioaccumulate (increase up the foodchain) or biodiminish (decrease up the foodchain), but few studies have examined a model freshwater ecosystem, such as Montezuma Well. The purpose of your Canned Research is to investigate whether As undergoes bioaccumulation or biodiminution in Montezuma Well.

Method. In this project, you will use microwave digestion to extract the As from Montezuma Well organisms (phytoplankton, *Hyallela montezuma*, and *Motobdella montezuma*). You will analyze the As concentration the extracts using ICP-MS. From there, you will perform calculations to determine the mass fraction of As in the organisms to decide whether bioaccumulation or biodiminution is occurring in the Well.

Previous Work

Research projects are generally long-term and involve many different students over several semesters or years. Your current research project is no exception, and you will be joining the project midstream. Previous members of your group have already collected the samples from Montezuma Well, stored and prepared them properly for digestion, and performed the acid-digestion protocol. Finally, they prepared the appropriate standard reference material (SRM) samples. Thankfully, these students wrote up their Methods section before they left (Excerpt 1). Additional important information is included in their lab notebook (Excerpt 2). You may copy the information in Excerpt 1 and include it in the experimental section of your journal article. Yes, that's right, you can *copy* it, but only on the condition that you *read and analyze* it and use it as a guide for writing your part of the experimental section. Feel free to ask your instructor questions about content in these excerpts that you do not understand.

Excerpt 1. Previous Work (text is also available in *As_PreviousWork.doc*)

Experimental Section

Well water, phytoplankton, *Hyalella montezuma* (an endemic amphipod), and *Motobdella montezuma* (an endemic leech) samples were collected from Montezuma Well in July, corresponding to the peak growing season in the well. Water was collected in 1-L acid-washed polyethylene bottles. Samples for total aqueous arsenic analysis were field-filtered (0.45- μm glass fiber filter) and acidified with trace metal grade nitric acid (Fisher Scientific), while the remaining samples were filtered according to EPA Method 445 for phytoplankton collection. Water samples for total arsenic analysis were stored at 4 °C prior to analysis. *H. montezuma* and *M. montezuma* were collected at the surface and at a depth of approximately 10 m, respectively, using a 254- μm plankton tow net. Invertebrate samples were rinsed with deionized distilled water, individually stored in plastic Ziplock bags, and immediately frozen in liquid nitrogen. Phytoplankton and invertebrate samples were freeze-dried (Emitech K-750X) and stored at -80 °C prior to analysis.

Invertebrate and phytoplankton samples were ground to pass through a 40-mesh screen. A composite subsample (~500 mg) of phytoplankton and each invertebrate was weighed into Teflon microwave digestion bombs (Milestone-Ethos 900) for acid digestion of samples. Digestion was performed similar to EPA Method 3051 using 9 mL HNO_3 (trace metal grade, Fisher Scientific) and 3 mL H_2O_2 (30%). Laboratory blanks, NIST Standard Reference Material 1571 (Orchard Leaves), and NRC Certified Reference Material DORM-2 (dogfish muscle) were digested for analytical quality control. Digests were diluted to appropriate concentrations with deionized, distilled water and filtered (0.45- μm Teflon) prior to analysis. All samples were run in triplicate.

Excerpt 2. Lab notebook summary of Montezuma Well sample collection and laboratory preparation of samples and standard reference materials (SRMs).

July 12, 2003 Montezuma Well, Sample Collection Trip MSR

M. Well water samples: collected and stored in cooler during transport to lab
 Bottle #1 = filtered (0.45- μ m Teflon) and acidified (1 mL HNO₃)
 Bottle #2 and #3 = non-filtered, non-acidified for phytoplankton analysis

Amphipod samples: ~2 g taken from Well water column, collected in Ziploc bag, dropped into N₂ (l)

Leech samples: ~10 leeches collected after sun-down from center of Well ~10 m depth.

July 15, 2003 Prep of M. Well samples MSR

Samples weighed out for microwave digestion:

Sample	Run #	Empty bomb (g)	bomb+Sample (g)	Sample (g)
Amphipod	1	114.2340	114.7298	0.4958
Amphipod	2	113.2840	113.7978	0.5138
Amphipod	3	114.0040	114.4798	0.4758
Leech	1	115.7782	116.2821	0.5039
Leech	2	114.7634	115.2557	0.4923
Leech	3	115.3342	115.8457	0.5115
Phytoplankton	1	114.8890	115.3816	0.4926
Phytoplankton	2	112.4531	112.9969	0.5438
Phytoplankton	3	112.6890	113.1716	0.4826
Orchard Leaves	1	113.8794	114.3807	0.5012
(NIST 1571: Orchard Leaves is certified @ 6.23 μg(As)/g(leaves))				
Orchard Leaves	2	114.2214	114.7511	0.5297
Orchard Leaves	3	113.1455	113.6541	0.5086
Dogfish tissue	1	115.8900	116.3792	0.4892
(NRC DORM-2 certified @ 18.0 μg(As)/g(tissue))				
Dogfish tissue	2	114.6648	115.1313	0.4665
Dogfish tissue	3	112.2971	112.7788	0.4817
Lab Blank	1	N/A	N/A	N/A
Lab Blank	2	“	“	“
Lab Blank	3	“	“	“

<ul style="list-style-type: none"> • 3 mL H₂O₂ and 9 mL HNO₃ added to all digestion bombs • microwaved using Ethos Program 3 for organic-matrix samples • let samples cool overnight, so they don't explode when opened 		
July 16, 2003	Dry-weight Measurements & Post-digestion Prep	MSR
<ul style="list-style-type: none"> • Bombs cool • Quantitatively transferred contents of each bomb into 25-mL volumetric flask, and diluted to volume with DDI (deionized, distilled H₂O) • Filtered each sample through 0.45-μm teflon filter (the ones in the HPLC drawer) and stored in (labeled!) 25-mL plastic centrifuge tube. • Store in fridge until tomorrow. 		

Research Steps

Research projects are complex and this Canned Research is no exception. It is generally useful to divide such a large project into smaller tasks. The **Research Steps** are designed to help you accomplish the smaller objectives. Unless assigned by your instructor, these steps are not required; however, following these steps will allow you to imitate the way many experts go about conducting research.

Understanding the Previous Work.

At this point in your Canned Research, it is important that you understand what the previous members of your research group have already accomplished.

- Read through Excerpt 1, and make a list of any terms or methods that you do not understand. Research these terms/methods using any resources available (e.g., textbooks, the internet, journal articles) to find out what they mean and how they are used.
- Find two or more peer-reviewed journal articles that describe procedures that are similar to those that were used by your previous group members. Reading the Methods section of these articles may help you understand the procedures better and will model the way experts write about these procedures for an expert audience. Based on these journal articles, read through Excerpt 1 and decide if there are any corrections, revisions, or changes you would like to make before you include it in your paper.

Methods

The methods for your canned research project are described below. Note the change in format. Excerpt 1 (Previous Work) was written as it might appear in a journal article. The information below is written as it might appear in a lab manual or lab notebook. Your goal will be to rewrite this information for an expert audience in a journal article.

I. Prepare ICP-MS As standard and Ge internal standard stock solutions

Reagents

Concentrated nitric acid (HNO₃) trace-metal grade, Fisher Scientific
Concentrated hydrogen peroxide (H₂O₂) (30%)
1000 µg/mL As certified standard (Spex Industries, Hoboken, NJ)
10 µg/mL Ge certified standard (Spex)

Equipment (all surfaces that contact samples and standards must be washed in 10% HNO₃)

Automatic micropipettes (50, 100, 200, 500, 1000 µL) with disposable plastic tips
Centrifuge storage tubes (25 and 50 mL)
Volumetric flasks (10 and 25 mL)

Procedure

1. Prepare 1000 µg/L As stock solution: Take 100 µL of 1000 µg/mL As SPEX standard and bring to volume with distilled, deionized water (DDI) in a 100 mL volumetric flask.
2. Prepare 100 µg/L As working stock solution: Take 1 mL of As stock solution (prepared in #1) and bring to volume with (DDI) in a 10 mL volumetric flask.
3. Prepare 100 µg/L Ge internal standard solution: Take 100 µL of 10 µg/mL Ge SPEX standard and bring to volume with DDI in a 10 mL volumetric flask.

II. Prepare Calibration Standards

Using the working stock solutions, prepare 25 mL of a laboratory blank (LB) for each blank sample (B1, B2, B3) and 3 replicates each of 3 calibration standards (e.g., CS1-1, CS1-2, CS1-3; CS2-1, CS2-2, CS2-3, etc.), varying in As concentration from lowest (CS1) to highest (CS3). The blank and all standards will have the same Ge concentration (1 µg/L). Ge serves as an internal standard (IS).

Procedure

- 1. LB1 (LB2, LB3) (0 µg/L As):** Mix 2.5 mL of blank B1 (B2, B3) (see Work Others Did) and 250 µL of the Ge stock solution in a 25 mL volumetric flask. Bring to volume with DDI.
- 2. CS1 (1 µg/L As):** Mix 2.5 mL of blank (B1, B2, or B3) and 250 µL EACH of As working stock solution and Ge stock solution in a 25 mL volumetric flask. Bring to volume with DDI. Repeat for two additional reps of CS1.
- 3. CS2 (3 µg/L As):** Mix 2.5 mL of blank, 750 µL of As working stock solution, and 250 µL of Ge stock solution in a 25 mL volumetric flask. Bring to volume with DDI. Repeat for two additional reps of CS2.
- 4. CS3 (5 µg/L As):** Mix 2.5 mL of blank, 1250 µL of As working stock solution, and 250 µL of Ge stock solution in a 25 mL volumetric flask. Bring to volume with DDI. Repeat for two additional reps of CS3.

III. Obtain a 4-point calibration curve for As

Instrumentation: The instrument you will use is a VG Axiom MC ICP-MS (Thermo Elemental) with ultrasonic nebulizer and water-cooled condenser (Model U-5000AT, Cetac). The ICP-MS will be operated in peak-hopping mode, measuring counts at m/z 74 (Germanium) and 75 (Arsenic).

Procedure

1. Run a 2% HNO₃ rinse solution to check for any evidence of background contamination. Set the capillary tube (that leads to the nebulizer) into rinse solution. Acquire 3 scans on the rinse solution. Counts at both m/z 74 and 75 should be low, indicating low background contamination/interference. Three scans = 1 run.
2. Obtain 3 scans (1 run) on LB1. The counts for m/z 75 should be low ($\sim < 100,000$) (because there should be very low As concentration in the blank). The counts m/z 74 (Ge internal standard) should show considerable counts ($\sim 500,000$), because it is present at 1 µg/L in all blanks and samples.
3. Repeat #2 on each of the LBs.
4. Collect 3 scans (1 run) of each of the calibration standards, beginning with C1 replicates and ending with C3 replicates. For each replicate, you will obtain the average area counts for three scans for each m/z peak. Thus, you will have collected 12 run-averages (3 cal standards x 3 replicates each + 3 blanks).
5. The data acquired from steps 1-4 are shown below (Figure 1) and are available in the *CalData* worksheet in the *As_Data.xls* file.

Figure 1. Arsenic calibration data using Ge as an internal standard.

Sample	Run #	Average m/z 74 counts (Germanium)	Average m/z 75 counts (Arsenic)
Blank	1	480162	25381
Blank	2	526916	25957
Blank	3	625638	30037
CS1	1	731561	315517
CS1	2	722842	318090
CS1	3	734161	338092
CS2	1	924401	1147681
CS2	2	799111	1003642
CS2	3	812725	1042083
CS3	1	863237	1767640
CS3	2	884584	1570982
CS3	3	882060	1655517

Research Steps

Understanding the Methods.

It is important that you understand the methods that are being used in your canned research. Until you understand the instruments and procedures, it will be very difficult to understand the data.

- Read through the procedures and instrumentation described above, and make a list of any terms, procedures, or instrumentation that you are not familiar with. Research these terms/procedures/instruments using any resources available (e.g., textbooks, the internet, journal articles).
- Be sure that you understand the following terms, including why they are important for your project :
 - certified standard
 - internal standard
 - laboratory blank
 - calibration curve
 - inductively coupled plasma mass spectrometry (ICP-MS)
 - standard reference material (SRM)
- Find two or more peer-reviewed journal articles that describe procedures that are similar to those that used in your Canned Research. Reading the Methods section of these articles may help you understand the procedures better and will model the way experts write about these procedures for an expert audience. Based on these journal articles, read through the methods described above and jot down any additional questions/terms you have. Research the answers to your questions.



Before proceeding with your Methods, it is wise to check the calibration data you've just collected. If you simply assume that all is well, you may delay catching a problem either with your laboratory method, the instrument performance, or in your data analysis procedures.

Go to the Calibration Curve subheading of the Data Analysis section (page 15) and learn how to analyze your calibration data.

IV. Prepare Digested Samples for ICP-MS Analysis

Procedures

Samples must be diluted and have the IS added prior to ICP-MS analysis.

1. Pipet 1 mL of EACH digested sample (M. Well organisms and SRMs) into separate 10 mL volumetric flasks. Add 100 μ L of Ge solution to each flask. Bring to volume with DDI.

Final acid % left in diluted digest is:

$$\frac{9\text{mL (HNO}_3\text{)}}{25\text{mL (digest)}} \frac{1\text{mL (digest)}}{10\text{mL (diluted digest)}} \times 100\% = 3.6\% \text{ (HNO}_3\text{ in diluted digest)}$$

$$\frac{3\text{mL (H}_2\text{O}_2\text{)}}{25\text{mL (digest)}} \frac{1\text{mL (digest)}}{10\text{mL (diluted digest)}} \times 100\% = 1.2\% \text{ (H}_2\text{O}_2\text{ in diluted digest)}$$

V. Prepare Water Samples for ICP-MS Analysis

Water samples need to have IS added and be matrix-matched to the calibration standards in terms of the amount of acid(s). (We already made sure the blanks, calibration standards, and organism samples were matrix-matched above, because we prepared them all with the same ratio of blank/sample to total diluted volume. Remember, the blanks and samples were digested by the same method and contain the same amount of the two acids; therefore, 1 mL sample diluted to 10 mL contains the same acid concentration as 2.5 mL blank diluted to 25 mL. So, now, make up the water samples such that they have the same acid concentration as everything else (see IV above for calculation of acid concentration). Those samples have 3.6% HNO₃ and 1.2% H₂O₂, so to prepare 10 mL water samples.

$$3.6\% \text{ of } 10 \text{ mL is: } 0.036 (10 \text{ mL}) = 0.360 \text{ mL} = 360 \mu\text{L (amount of HNO}_3\text{ needed)}$$

$$1.2\% \text{ of } 10 \text{ mL is: } 0.012 (10 \text{ mL}) = 0.120 \text{ mL} = 120 \mu\text{L (amount of H}_2\text{O}_2\text{ needed)}$$

Procedure

1. Pipet 5 mL of water from Bottle #1, 100 μ L Ge solution, 360 μ L HNO₃, and 120 μ L H₂O₂ into a 10 mL volumetric flask. Bring to volume with DDI.
2. Repeat #1 for two additional aliquots of Bottle #1 water. Label these three samples W1, W2, and W3

VI. Run your SRMs using ICP-MS**Procedure**

1. Run a 2% HNO₃ rinse solution to check for any evidence of background contamination. Counts at both m/z 74 and 75 should be low, indicating low background contamination/interference. Three scans = 1 run.
2. Obtain 3 scans (1 run) on each digested NIST 1571 replicate (O1, O2, O3).
3. Repeat step 2 for the NRC DORM-2 digests (D1, D2, D3).
4. For each replicate, you will obtain the average area counts for three scans for each m/z peak. Thus, you will have collected 6 run-averages (2 SRMs x 3 replicates each).

The data acquired from steps 1-4 are shown below (Figure 2) and are available in the *SRMData* worksheet in the *As_Data.xls* file.

Figure 2. Arsenic SRM data using Ge as an internal standard.

Sample	Run #	Average m/z 74 counts (Germanium)	Average m/z 75 counts (Arsenic)
orchard leaves	1	78541	336873
orchard leaves	2	75698	337821
orchard leaves	3	80123	337556
dogfish	1	52137	701238
dogfish	2	53375	700139
dogfish	3	57671	702954



It is a good idea to analyze your SRM data before moving on to analyzing your Montezuma Well samples, because your SRMs give an indication of the accuracy of your method.

Go to the Standard Reference Materials subheading of the Data Analysis section (page 17) and learn how to analyze your SRM data.

VII. Run authentic samples using ICP-MS**Procedure**

1. Run a 2% HNO₃ rinse solution to check for any evidence of background contamination. Counts at both m/z 74 and 75 should be low, indicating low background contamination/interference. Three scans = 1 run.
2. Obtain 3 scans (1 run) on each digested phytoplankton replicate.
3. Repeat step 2 for the *H. montezuma* and *M. montezuma* extract replicates, as well as for the M. Well water replicates.
4. For each replicate, you will obtain the average area counts for three scans for each m/z peak. Thus, you will have collected 9 run-averages (3 samples x 3 replicates each).

The data acquired from steps 1-4 are shown below (Figure 3) and are available in the *SampleData* worksheet in the *As_Data.xls* file.

Figure 3. Arsenic sample data using Ge as an internal standard.

Sample	Run #	Average m/z 74 counts (Germanium)	Average m/z 75 counts (Arsenic)
amphipod	1	67854	640237
amphipod	2	70152	601317
amphipod	3	62122	607589
leech	1	88975	11921753
leech	2	93854	13545238
leech	3	85132	11423546
phytoplankton	1	102532	387367
phytoplankton	2	105621	407846
phytoplankton	3	98213	412276
water	1	845266	32634560
water	2	854584	33854613
water	3	839433	30622997



You've now completed your methods. It is time to analyze your authentic sample data before moving on to the Results section.

Go to the Authentic Samples subheading of the Data Analysis section (page 19) and learn how to analyze your Montezuma Well data.

Data Analysis

Data analysis (calculations) is an important step in conducting research, a step that links the raw data collected to the final meaningful results that are presented in journal articles. Your data analysis will include the following steps:

- I. Prepare an internal standard calibration curve for arsenic
- II. Process Standard Reference Material data
 - A. Calculate the arsenic concentrations in the SRM extracts
 - B. Convert SRM extract As concentrations ($\mu\text{g/L}$) to As mass fractions ($\mu\text{g/g}$).
 - C. Calculate SRM % recoveries, mean % recovery, and % recovery standard deviation.
- III. Process Authentic Sample data
 - A. Calculate the arsenic concentrations in the authentic M. Well sample extracts
 - B. Convert authentic sample extract concentrations ($\mu\text{g/L}$) to As mass fractions ($\mu\text{g/g}$).
 - C. Calculate mean sample mass fractions, mean mass fractions, and standard deviations for authentic samples.

Research Steps

Rigorous Data Analysis.

It is important that you understand the calculations that lead to the final results presented in your paper.

- Read through the procedures/equations described in each section below, and make a list of questions you may have. Research these questions using any resources available.
- Often students first attempt to use calculators to analyze their data. We discourage this approach and recommend that you use a spreadsheet program (Excel) instead. Although the calculator-approach may seem easier at first, in the long run calculators are time-consuming and error-prone. For example, jotting down numbers and using a calculator for multi-step calculations often leads to rounding errors. Moreover, calculations “by hand” do not provide a track record of your steps. By using a spreadsheet, you have an electronic record of your calculations; the spreadsheet is easily corrected and can serve as a permanent record of your data analysis.
- The Excel file *As_Data.xls* contains sample calculations within it that are specific to that step in your data analysis.
- This canned research project assumes that you have already used Excel and are familiar with using formulas and preparing graphs. If you are a beginner, try using the built-in Excel help or ask a friend or instructor to walk you through the spreadsheets. It will not take long before you realize how much easier it is to use Excel than a calculator!

I. Calibration Curve.

You will be working directly with the calibration data contained in the *CalData* worksheet of the *As_Data.xls* file. This section describes the concept behind using an internal standard calibration curve, as well as the equations you will use to construct your curve.

Within a range of concentrations (denoted as the linear range), the concentration of As will be proportional to its integrated ICP-MS peak area (counts) (eq 1). Similarly, the concentration of the internal standard (IS), Ge in this case, is proportional to its integrated peak area (counts) (eq 2).

$$[\text{As}] \propto \text{Peak Area}_{\text{As}} \quad \mathbf{1}$$

$$[\text{Ge}] \propto \text{Peak Area}_{\text{Ge}} \quad \mathbf{2}$$

Due to instrumental variability, taking a ratio of areas is more reproducible than using absolute areas of individual compounds. One of the causes of this variability is that only a tiny fraction of the analyte atoms are properly vaporized in the injector, atomized in the plasma, carried through the MS, and properly detected. The % of atoms actually detected is sensitive to changes in temperature, pressure, flowrate, and the electronics in the detector. For example, let's take the following hypothetical duplicate runs (same sample run twice).

Run 1: Peak Area_{As} = 333,000

Run 2: Peak Area_{As} = 487,003 counts

A change of ~32%!

If, however, we take the ratio of As peak area to the Ge peak area, for which the concentration never changes (1 ug/L in our case), we have the following hypothetical scenario:

Run 1: Peak Area_{As} = 333,000 counts

Peak Area_{Ge} = 1,250,000 counts

Run 2: Peak Area_{As} = 487,003 counts

Peak Area_{Ge} = 1,828,089 counts

For whatever reason, a smaller fraction of atoms was detected in Run #1. If we assume that because As and Ge are similar in atomic weight, they behave similarly in response to instrumental conditions, when fewer As atoms are detected, so are fewer Ge atoms. When we take the ratios for a given run . . .

$$\begin{array}{l} \text{Run \#1} \\ \text{Run \#2} \end{array} \quad \frac{\text{Peak Area}_{\text{As}}}{\text{Peak Area}_{\text{Ge}}} = \frac{333,000}{1,250,000} = 0.2664$$

$$\frac{\text{Peak Area}_{\text{As}}}{\text{Peak Area}_{\text{Ge}}} = \frac{487,003}{1,828,089} = 0.2664$$

The same ratio!! Thus, the use of an internal standard minimizes the effect of instrument variability from run-to-run. Thus, for our calibration curve, we use this ratio, rather than just the peak area of As. We can say the ratio of As and Ge concentrations is proportional to their peak area ratio. Mathematically,

$$\frac{[\text{As}]}{[\text{Ge}]} \propto \frac{\text{Peak Area}_{\text{As}}}{\text{Peak Area}_{\text{Ge}}} \quad \mathbf{3}$$

Eq. 3 can be rearranged to solve for the concentration of As (eq 4).

$$[\text{As}] \propto \frac{\text{Peak Area}_{\text{As}}}{\text{Peak Area}_{\text{Ge}}} \times [\text{Ge}] \quad \mathbf{4}$$

And the proportionality can be converted into an equality through the use of a proportionality constant, the response factor (RF) (Eqn. 5)

$$[\text{As}] = RF \times \frac{\text{Peak Area}_{\text{As}}}{\text{Peak Area}_{\text{Ge}}} \times [\text{Ge}] \quad \mathbf{5}$$

Equation 5 is the equation of a line ($y = mx + b$), where RF is the slope (m). The RF can be determined by plotting y (the known As calibration standard concentrations) versus x (the ratio of As and Ge peak areas multiplied by the known Ge concentration (1 $\mu\text{g/L}$)). The intercept b should ideally equal zero, corresponding to $[\text{As}] = 0$; however, typically a non-zero value is found representative of background noise in ICPMS.

$$y = m \quad x \quad + b$$

$$[\text{As}] = RF \times \frac{\text{Peak Area}_{\text{As}}}{\text{Peak Area}_{\text{Ge}}} \times [\text{Ge}] + \text{noise} \quad \mathbf{6}$$

Thus, equation 6 is used to construct your internal standard calibration curve. Once you've plotted the line, you need to add a bestfit line (complete with equation and r^2) to your graph. Your r^2 should be >0.99 , indicating good linearity and proper calibration. If this criterion is met, you can confidently proceed with your sample analysis.

II. Standard Reference Materials (SRMs)

A. Calculating As Concentrations

Once you have your calibration equation (the bestfit line from the Calibration Curve), you can use it to solve for the unknown As concentrations (y-value) in your SRMs. You simply plug in the x-value, which is the peak area ratio for the sample of interest multiplied by the Ge concentration (1 µg/L).

Keep in mind that equation 6 reveals that, $y = [\text{As}]$ and $x = \frac{\text{Peak Area}_{\text{As}}}{\text{Peak Area}_{\text{Ge}}} \times [\text{Ge}]$.

Now, let's imagine the following:

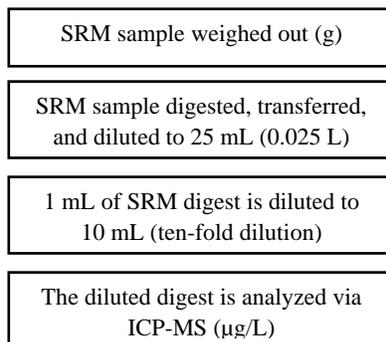
- Your bestfit line equation is: $y = 3.9034x - 0.0123$
- Your ICP-MS run for a sample of interest yielded As area counts = 9,875,892 and Ge area counts = 2,564,218, while using $[\text{Ge}] = 1 \mu\text{g/L}$

Go ahead and try calculating the $[\text{As}]$ for this sample. If you calculate 15.02 µg/L, you're on track. Use this approach to calculate the concentrations of As in your SRMs.

B. Converting Concentrations to Mass Fractions

Recall the purpose of analyzing the certified reference materials: to verify the accuracy and appropriateness of the experimental methods, including weighing, digesting, transferring, pipetting, filtering, ICP-MS analysis, etc. Thus, if the *measured* SRM concentrations are close to the *certified* concentrations, you are assured of and demonstrate for readers the analytical accuracy of your method.

Remember, however, that the concentration of As certified is in units of µg/g (µg of As per g of SRM material), while the *measured* concentration obtained from the ICPMS is in units of (µg of As per L of solution) after all the dilutions steps. Thus, you cannot compare µg/L in diluted/treated sample to µg/g of original certified sample – this would be comparing apples to oranges! Thus, you will need to convert all concentrations (µg/L) to mass-fraction units of µg/g. The mathematical conversion needs to take into account all the steps the sample underwent in the course of the analysis. Specifically, the steps were:



Because you know the final concentration in $\mu\text{g/L}$, you need to work backwards to get back to units of $\mu\text{g/g}$. In essence, we work, mathematically, backwards through the steps above. In other words, the equation is:

$$\frac{[\text{As}] \left(\frac{\mu\text{g}}{\text{L}} \right) \times DF \times Vol}{\text{Sample Mass}} = \text{mass fraction } (\mu\text{g} / \text{g}) \quad 7$$

where:

$[\text{As}]$ = arsenic concentration ($\mu\text{g/L}$)

DF = dilution factor

Vol = the digest volume

Sample Mass = the mass of SRM you weighed out

Let's imagine the following:

- Your SRM concentration, as analyzed by ICP-MS, is $11.908 \mu\text{g/L}$
- The SRM digest was 50 mL and then diluted 5-fold prior to analysis
- You originally digested 0.245 g of an SRM certified to be $5.43 \mu\text{g/g}$ in As

Go ahead and calculate the mass fraction of As in this particular SRM sample. If you calculate $12.151 \mu\text{g/L}$, you're on track. Use this approach to calculate the mass fractions of As in your SRMs.

C. Calculating SRM Recoveries

Now, that you've calculated the mass fractions of As in your SRMs, you can compare the *measured* and *certified* mass fractions; in other words, you can calculate SRM recoveries.

The percent recovery is defined as: $\frac{\text{MeasuredSRM}[\text{As}]}{\text{CertifiedSRM}[\text{As}]} \times 100\%$

Given that the SRM you considered above has a certified mass fraction of $14.521 \mu\text{g/g}$, calculate the percent recovery. If you calculate 83.7%, you're on track. Use this approach to calculate the % recovery of As in your SRMs. For each SRM calculate the following:

- % recovery for each SRM replicate
- the mean (average) % recovery for the three replicates of each SRM
- the standard deviation in the % recovery for the three replicates of each SRM

Recoveries close to 100% indicate the accuracy of your analysis. However, there are a number of reasons why recoveries may deviate from 100%. If appropriate, research the reasons for high or low recoveries. Percent recovery standard deviations are expected to be less than 10%.

III. Authentic Samples

A. Calculating As Concentrations

Once you have your calibration equation (the bestfit line from the Calibration Curve), you can use it to solve for the unknown As concentrations (y-value) in your authentic samples. This is the same procedure you used to calculate As concentrations in your SRMs. See Data Analysis III. SRMs section A. Use this approach to calculate the concentrations of As in your Montezuma Well samples.

B. Converting Concentrations to Mass Fractions

Just as you did for the SRMs, you must now convert the As concentrations obtained on the extracts via ICP-MS into mass fractions (μg of As per g of dry-weight organism). The reason for performing the conversion on the authentic samples, however, is different from the reason we gave for the SRMs. Recall, that for the SRMs, we wanted to compare the *certified* value (given in $\mu\text{g/g}$) to the *measured* value; we needed both values in the same units to do so. For the authentic samples, there are no certified values, and yet it is still desirable to convert into mass fraction units. Why? The measured As concentrations in the extracts you prepared have as much to do with how you chose to prepare your samples (e.g., how much organism you digested, to what volume you diluted the extracts) as they do with the actual concentrations that are found in the organisms in their natural environment (precollection, predigestion, predilution, preanalysis). Thus, if you had chosen to digest twice as much phytoplankton, your resulting As concentration would be double. These method-specific values are of little interest to other scientists and do not allow others to make comparisons between a variety of organisms examined in a variety of studies. The units of mass fraction avoid these problems by expressing the value in the amount (mass) of As present per amount of organism (mass).

Although the reason for converting these units has changed, the procedure for doing so hasn't. Refer back to Data Analysis II. B. Converting Concentrations to Mass Fractions for help. Then proceed to do the following sample problem.

Let's imagine the following:

- Your phytoplankton concentration, as analyzed by ICP-MS, is $18.544 \mu\text{g/L}$
- The SRM digest was 25 mL and then diluted 10-fold prior to analysis
- You originally digested 0.458 g of the phytoplankton

Go ahead and try using Equation 7 calculate the mass fraction of As in this particular phytoplankton sample. If you calculate $10.122 \mu\text{g/L}$, you're on track. Use this approach to calculate the mass fractions of As in all of your authentic samples.

For each sample calculate the following:

- Mass fraction of As in each sample

- the mean (average) mass fraction of As for the three replicates of each sample
- the standard deviation of mass fractions for the three replicates of each sample

The standard deviations in your mass fractions are expected to be within 10% of the mean, indicating a reasonable degree of reproducibility. If appropriate, research reasons why analyses are never perfectly reproducible.

Results

Once the numbers have been crunched, it is time to think about your data. Use the following questions to guide your presentation of the data.

- What story do the data tell about As levels in the Montezuma Well foodchain? What will be the “take-home” message you want to make? How can you present the data (consider tables, figures, and text) to emphasize that take-home message?
- Which data will you present (e.g., concentrations, mass fractions, individual replicate values, averages, standard deviations)? What do you think is most important and useful for the reader?

Discussion

How will you discuss your results? Consider the following guidelines as you write the discussion of your paper.

- Were the data consistent? If so, what trends can you discern? Highlight these in your discussion.
- How did your findings compare to literature findings? What might be some reasons for the similarities and differences between your study and literature studies. How relevant do you think these results will be to other foodchains, other ecosystems?
- How do you think the information obtained in your study may be important in a larger context. What might be the implications or applications of your findings?

**REMEMBER THESE DATA, ALTHOUGH BASED ON PRELIMINARY
RESULTS, ARE FICTITIOUS!**

HAVE FUN WITH YOUR INTERPRETATIONS

BUT DON'T TAKE THEM TOO SERIOUSLY!