PHYS 599 Final Report
Lead Isotopic Composition in the Human Body

Student: Siegfried Peschke
Supervisor: Michael Wieser

1 Abstract

Radon gas is a carcinogen and also the second leading cause of lung cancer in Canada [1]. The radon atoms come from ^{238}U deposits deep in the soil. Once in the body, the radon further decays into ^{210}Pb, a daughter product with a long half-life (22 years), making it a potentially strong indicator for radon gas exposure in biological material [2][3]. In this endeavour, experimental procedure must be refined in order to reliably measure the tiny concentrations of ^{210}Pb (10^{-16}g/gram of nail). Thus my project is to develop, test, and assess the experimental procedure used in quantitatively extracting stable lead isotopic content from human biological samples. Through the analysis of human nail samples, we found areas of improvement to the experimental procedure which resulted in greater lead recovery and accuracy of the procedure. The ion exchange acids and the thermal ionization mass spectrometer's heating process were the changes with greatest impact. Changing to an ion exchange procedure which utilized only HCl over the combination of HBr and HCl combined with careful evaporation techniques resulted in a 2726% decrease of total lead contamination between the samples from sets 1 and 2 where the change was made. Areas of innovation with the thermal ionization mass spectrometer's procedure focus on the heating time, as preferential evaporation of lighter elements is an issue. Consistently fast heating time will result in greater retention of the lead content. More work must be done in the effort to reliably measure ^{210}Pb in biological material.

Contents

2 Introduction

2.1 Background

The motivation for this project comes from radon gas exposure to humans being the second leading cause of lung cancer in Canada next to smoking [1]. Humans are exposed to radon gas in their own homes. The radon gas comes from uranium ore deposits deep in the soil, which decay into the gas, which then leaks through the soil and into the basements of homes. Once in your home, the radon gas exists in the air and survives with a half life of 3.8 days, entering the body through the lungs. ^{222}Rn decays into ^{218}Po through alpha decay. Once in the body, the high energy alpha particles that are released from this decay collide with neighbouring cells and cause damage to the DNA, which can result in a cancerous mutation. Those concerned with being at risk need methods to test for exposure.

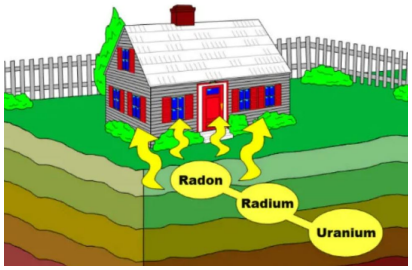

Figure 1: Radon gas depicted leaking through the soil into homes.

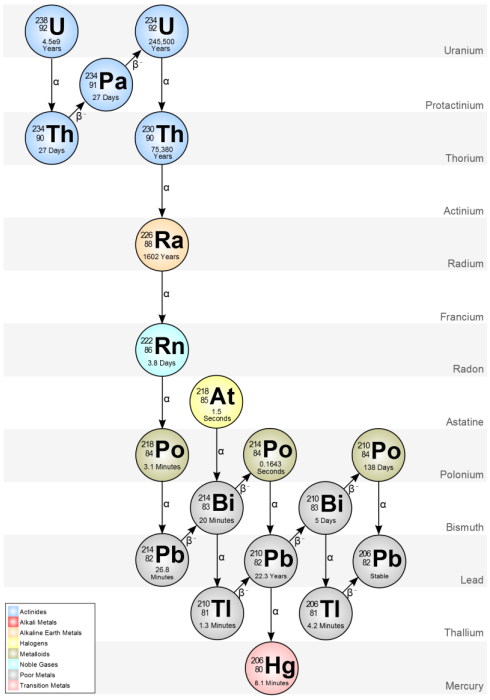

Figure 2: The decay chain of ^{238}U highlighting ^{222}Rn and ^{210}Pb.

In order to test for radon gas exposure, we look further down the decay chain of ^{238}U at the daughter products of ^{222}Rn for potential indicators [2][3]. Of all the daughter products, only two have half lives longer than a few minutes. ^{206}Pb, the stable isotope at the end of the decay chain, and ^{210}Pb, with a half life of 22 years. Since ^{210}Pb has such a long half life, the ^{206}Pb content is extremely low and as a result, ^{206}Pb from radon decay will be indiscernible from the abundance of environmentally sourced ^{206}Pb (24.14%). Thus ^{210}Pb is the only candidate for indicating radon gas exposure. Therefore reliably measuring ^{210}Pb content in human tissue is the long term objective of the project.

2.2 The Project

Since the concentration of ^{210}Pb in the body is so little, on the order of 10^{-16} grams per gram of nail, an experimental procedure must be refined enough in order to reliably measure ^{210}Pb concentrations in the body. Standard procedure for the determination of lead isotopic content in biological tissue exists, but is not currently sensitive enough measure such low amounts. The amount of ^{210}Pb in the biological material is roughly 0.05% that of the stable isotopes of lead. Thus my project is to develop, test, and assess the experimental procedure used in quantitatively extracting stable lead isotopic content from human biological samples.

I will present an operating procedure for determining lead isotopic composition in human nail samples. A few changes made to the experimental procedure between two data sets revealed promising results. These changes will be outlined in detail later on. There had been two more data sets planned but due to COVID-19, these were unable to be processed.

3 General Experimental Procedure

3.1 Acid Digestion of Biological Sample

The biological matrix in the nail samples is digested using inorganic acids. The result is a solution which contains an array of elements including the lead content originally in the sample. This is the first step in isolating the lead content from the biological tissue.

The acid digestion we opted for was the combination of HNO_3 and H_2O_2 as this method was shown to be optimal on human hair and nail samples as well as other biological tissues [4][5]. We begin by taking a Teflon beaker and measuring its mass, then the beaker mass including the dry nail sample. Then $10mL$ of MQ H_2O is added, measure the final mass of the beaker and solution. These values are important for calculating the concentration of the lead content found in the sample. The following are the steps for the acid digestion procedure:

1. Add $2mL$ of ultra pure water to the sample. [6]

   Serves as a reagent.

2. Sonic bath for 30 minutes at 70°C. [6]

   The energy facilitates and accelerates some steps, such as dissolution, fusion and leaching, among others.

3. Decant and evaporate remaining water in the beaker.

4. Add $2mL$ of double-distilled HNO_3.

5. Add $500\mu L$ of 30% H_2O_2.

6. Let the acid sit for 48 hours.

Now the samples are prepared for isotope dilution.

Figure 3: Samples of set 2 in teflon cylinders in the sonic bath.

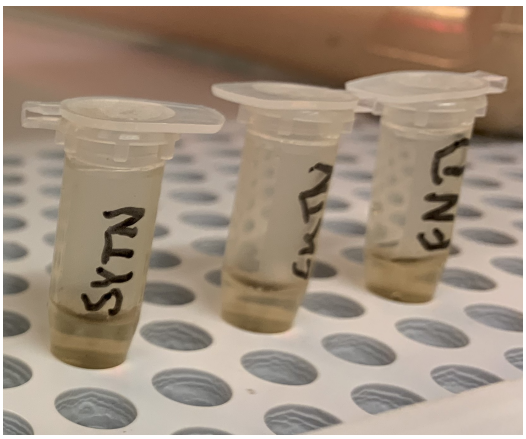

Figure 4: Samples from set 1 in plastic cylinders after digestion.

3.2 Isotope Dilution Mass Spectrometry

Isotope dilution analysis (IDA) is an analytical technique for determining the isotopic composition of a sample and can be used in conjunction with mass spectrometry in varying fields. Becker finds IDA is the best analytical technique for the determination of radionuclides, including lead. "IDA involves the measurement of isotopes of the same element, thus eliminating differences in chemical behavior. A significant advantage of IDA is that during the entire analytical procedure, including sample preparation, analyte separation, and enrichment, no quantitative recovery of analyte is necessary once the spike and sample have been equilibrated. This characteristic feature makes the technique more stable with respect to errors during all the chemical processing steps in comparison to other element analytical techniques" [7].

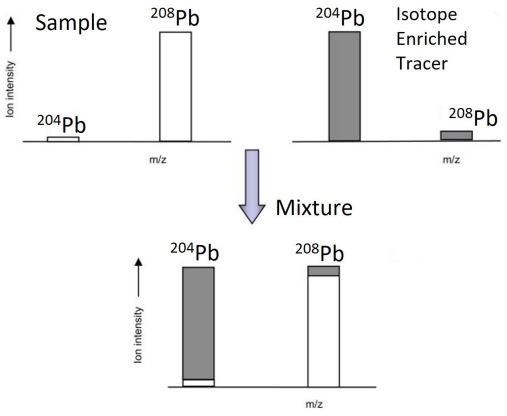

Figure 5: A figure depicting the isotope dilution, spiking with ^{204}Pb.

Two candidates for spiking isotopes were ^{204}Pb and ^{205}Pb. We selected ^{204}Pb as our spiking isotope, however since ^{205}Pb is a trace level isotope and not naturally abundant, it would have been a better spiking isotope. ^{204}Pb was selected due to accessibility. ^{204}Pb has a relative abundance of 1.5%, which means the amount within the nail samples is relatively low compared to the other stable isotopes of lead. So it is still a reasonable candidate to spike with. As such we will artificially inject $200\mu L$ of a ^{204}Pb enriched solution in order to allow calculation of the amounts of the other stable isotopes.

The resulting analytical analysis follows. In order to calculate the isotopic amounts, we first have to make a few considerations. Since the spike solution utilized in the isotope dilution is not uniquely ^{204}Pb, we must account for the amount of each isotope introduced with the spike in our calculations. This leads to the following equation which dictates the amount of an isotope introduced with the spike.

$$Amnt_{sp} = \frac{m_{sp} * N_A * \%Amnt_{sp} * conc_{sp}}{W_{sp}} \tag{1}$$

where m_{sp} is the mass of the spike introduced, N_A is Avogadro's number, $\%Amnt_{sp}$ is the relative abundance percentages of the isotopes in the spike solution, $conc_{sp}$ is the spike concentration, and W_{sp} is the atomic weight of the spike.

The amount of an isotope in a sample is given by

$$Amnt_{sam} = \frac{m_{sam} * N_A * \%Amnt_{sam}}{W_{sam}} \tag{2}$$

where m_{sam} is the mass of the $800\mu L$ sample, N_A is Avogadro's number, $\%Amnt_{sam}$ is the relative abundance percentages of the isotopes in the sample, and W_{sam} is the atomic weight of the sample.

Since lead content in the nail is introduced due to environmental source, we assume the relative abundance ratios in the nail samples follow those naturally occurring in nature [8]. These are as follows

- ^{204}Pb: 1.43%
- ^{206}Pb: 24.14%
- ^{207}Pb: 22.08%
- ^{208}Pb: 52.35%

and the atomic weights are calculated using

$$W = \sum W_i * \%Amnt$$

where W_i are the individual atomic weights of the isotopes and $\%Amnt$ is the corresponding relative abundance ratio either in the spike or sample.

Then, since the lead content in the sample is either directly from the sample or introduced from the spike, using the ratio of ^{206}Pb to ^{204}Pb we derive the following equation

$$\left(\frac{206}{204}\right)_m = \frac{206_{sam} + 206_{sp}}{204_{sam} + 204_{sp}} \tag{3}$$

$$\left(\frac{206}{204}\right)_m (204_{sam} + 204_{sp}) = (206_{sam} + 206_{sp})$$

$$\left(\frac{206}{204}\right)_m 204_{sam} - 206_{sam} = 206_{sp} - 204_{sp}\left(\frac{206}{204}\right)_m$$

Then substituting in equations (1) and (2) we get

$$\left(\left(\frac{206}{204}\right)_m - \frac{\%206_{sam}}{\%204_{sam}}\right)\frac{\%204_{sam}\ m_{sam}}{W_{sam}} = \frac{\%204_{sp} m_{sp}\ conc_{sp}}{W_{sp}}\left(\frac{\%206_{sp}}{\%204_{sp}} - \left(\frac{206}{204}\right)_m\right)$$

$$m_{sam} = \frac{W_{sam}}{W_{sp}}\frac{\%204_{sp}}{\%204_{sam}} m_{sp}\ conc_{sp}\frac{\left(\frac{\%206_{sp}}{\%204_{sp}} - \left(\frac{206}{204}\right)_m\right)}{\left(\left(\frac{206}{204}\right)_m - \frac{\%206_{sam}}{\%204_{sam}}\right)} \tag{4}$$

which leaves us with an equation to calculate the total mass of lead isotopic content in the nail sample. We then apply the relative abundance ratios assumed earlier to calculate individual isotopic amounts.

In order to calculate the total lead concentration of the sample the following formula was used

$$\frac{m * T}{m_N * V} \tag{5}$$

where m is the mass of the $800\mu L$ sample, T is the total mass of lead content found in the sample, m_N is the mass of the nail sample before acid digestion, and V is the volume of sample digest analyzed (in our case, $800\mu L$).

3.3 Isotope Dilution - Spiking

We begin the spiking procedure by measuring the mass of an empty teflon cylinder. Add $800\mu L$ of the sample solution to the cylinder and measure the combined mass. Add $200\mu L$ of ^{204}Pb saturated solution and measure the combined mass. Our ^{204}Pb solution had the following abundance ratios of the stable lead isotopes

- ^{204}Pb: 70.91%
- ^{206}Pb: 12.68%
- ^{207}Pb: 6.42%
- ^{208}Pb: 9.96%

Now the sample is spiked and ready for ion exchange.

3.4 Isolation of Lead Content - Ion Exchange

Following the ^{204}Pb spike, we utilize ion exchange resins to isolate the lead content from solution. These resins are tiny porous microbeads, designed to have an affinity for a certain element or elements, and work by exchanging an ion from its surface for the desired ion, trapping it the desired ion to its surface [9]. Typically these resins are used to clean the drinking water we have, by exchanging harmful chemicals for non-harmful ones. But they can also be used for metal separation, which with the conjunction of acids, allows us to separate out the lead content from the sample solution.

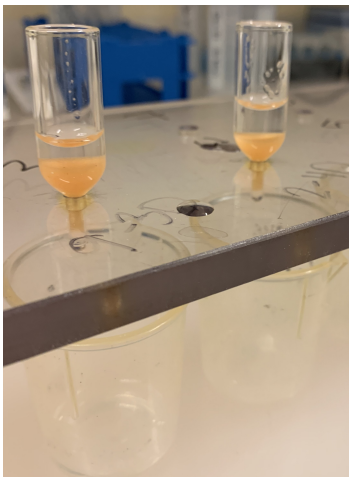

Figure 6: Experimental setup after step 2 of the second ion exchange procedure.

Two separate ion exchange procedures were used to prepare sample sets 1 and 2. A change was made due to a large amount of lead contamination measured in the blank sample from the first data set, shown in table 1.

3.4.1 First Ion Exchange Procedure

1. Convert samples to bromides using $200\mu L$ conc. HBr ($30\mu L$)

   Functions as a reagent, displacing the HNO_3 from acid digestion and placing the lead in the chemical form required for ion exchange.

2. Dissolve sample in $500\mu L$ of 0.5M HBr

3. Prepare and fill open glass columns with 2/3 full with water to hold samples.

   These columns need a frit, a porous piece at the bottom allowing for the slow drip through of the solution held inside.

4. Add $250\mu L$ of anion exchange resin to glass column.

5. Preclean the resin with $1.0mL$ MQ H_2O.

   follow up with 2x $750\mu L$ of 6M HCl.

   followed by $750\mu L$ of MQ H_2O.

   This removes any lead contamination from the ion exchange resin.

6. Precondition the resin with $0.5mL$ of 0.5M HBr.

   Preconditioning converts the resin environment to that required to make the element of interest bind to the resin.

7. Load sample into glass column.

8. Rinse with 2x $1ml$ 0.5 HBr.

   Once the sample was loaded, the lead content is isolated and trapped in the resin. Thus rinsing flushes out all unwanted elements.

9. Elute into a collecting teflon beaker with $0.4ml$ conc. HCl.

   Eluting simply removes the lead content from the surface of the resin microbeads.

10. Evaporate in an evaporation chamber eluted solution overnight.

    Evaporation results in a dry lead sample.

For the second sample set, we decided to change to an ion exchange method which utilized only one acid. These changes will be discussed later.

3.4.2 Second Ion Exchange Procedure

1. Prepare and fill open glass columns with 2/3 full with water to hold samples.

   These columns need a frit, a porous piece at the bottom allowing for the slow drip through of the solution held inside.

2. Add $250\mu L$ of cation exchange resin.

3. Preclean resin with 2x $1mL$ of 2M HCl.

   This removes any lead contamination from the ion exchange resin.

4. Precondition with 2x $500\mu L$ of 0.2M HCl.

   Preconditioning converts the resin environment to that required to make the element of interest bind to the resin.

5. Reconditioning samples with $500\mu L$ of 0.2M HCl.

   Reconditioning optimally increases the solution acid strength to for the lead isotopes to ion exchange with the resin.

6. Load samples into column.

7. Rinse with 2x $1mL$ of 0.2M HCl.

   Once the sample was loaded, the lead content is isolated and trapped in the resin. Thus rinsing flushes out all unwanted elements.

8. Elute with $1ml$ of 2M HCl.

   Eluting simply removes the lead content from the surface of the resin microbeads.

9. Evaporate eluted solution overnight.

   Evaporation results in a dry lead sample.

3.5 Thermal Ionization Mass Spectrometry

Once the lead content is separated from the sample solution, isotopic analysis is done through thermal ionization mass spectrometry (TIMS) [10]. TIMS is used to measure the lead isotopic abundance ratios. The isotopic ratios allow for the determination of the lead isotopic amounts and overall lead concentration in the original sample as allowed by IDMS. The sample is initially prepared and loaded onto a filament capable of carrying current. When a current is ran through the filament, due to inherent resistance, the filament heats up.

Here metal selection for the filament is important. Two key considerations dictate which metals are the best candidates. High melting point is the first. The evaporation temperatures of heavy metals are non-trivial, the boiling point of lead being 2022 K. So metals such as tantalum (Ta), tungsten (W), platinum (Pt) or rhenium (Re) with melting points of 3290 K, 3695 K, 2041 K, and 3459 K respectively. One can also bring down the boiling point of the sample, to enable greater selecting of metals for the filament, in our case, $2\mu L$ of silica gel is used to dissolve the dry lead sample and place it onto a filament. The silica gel lowers the evaporation temperature of the lead to approximately 1425 K.

The second key consideration for metal selection of the filament is the electron affinity of the metal. Metals with high electron affinity allow for easier ionization of the isotopes once they have evaporated. Two factors influence the electron affinity of an element, atomic size and nuclear charge. Larger atoms fill their inner shells with electrons, and outermost shells get filled when increasing in proton number through the periodic table. Nuclear charge also influences affinity since positively charged elements exhibit an attractive electromagnetic force with electrons. These factors also influence the selection of the aforementioned preferred metals for filaments in TIMS analysis. In this experiment, rhenium filaments were used.

TIMS utilizes a magnetic sector mass analyzer to separate the ions based on their mass to charge ratio. The filament is heated, and at roughly 1425 K the lead atoms are evaporated since they have reached the boiling point set by the silica gel. Then the lead isotopes lose an electron to the filament, thus ionizing the atom. The ions are accelerated through an electric potential difference and are focused into a beam by electrostatic lenses. The ion beam then passes through a magnetic field where, due to the Lorentz force, it is partitioned into separate ion beams based on the ion's charge/mass ratio. These mass-resolved beams are directed into a detector where it is converted into voltage. The voltage detected is then used to calculate the isotopic ratio. The procedural outline for executing this analysis may vary depending on software and equipment. In the results of the IDMS and TIMS analysis, one receives isotopic abundance ratios, which for example, is the ratio of number ^{208}Pb isotopes to the number of ^{204}Pb isotopes.

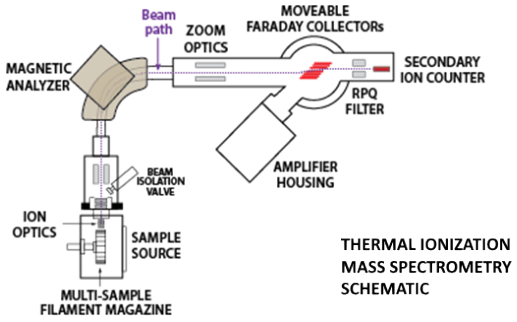

Figure 7: A general schematic of a thermal ionization mass spectrometer.

Specific procedural instruction will vary depending on software on the spectrometer. As such, none is presented here as the general blueprint for the determination of isotopic abundance ratios is outlined above.

4 Results

Two separate sets of data were analyzed where changes were made to the standard procedure between them. This was to test the result of modifications to the procedure. The amounts of the lead isotopes have been calculated for, as well as the concentration of total lead in the various samples, which are shown in the tables below.

4.1 Data Set 1

Sample	^{204}Pb (pg)	^{206}Pb (pg)	^{207}Pb (pg)	^{208}Pb (pg)	Conc. ($\mu g/mg$)
Blank	19	324	297	704	-
SY-TN	3	47	43	102	6.9
CK-FN	14	233	213	504	25.8
CK-FN-D	14	229	210	497	25.4

Table 1: Lead isotopic amounts and concentration of samples from sample set 1.

4.2 Data Set 2

Sample	^{204}Pb (pg)	^{206}Pb (pg)	^{207}Pb (pg)	^{208}Pb (pg)	Conc. ($\mu g/mg$)
Blank	1	12	11	25.8	-
JT-FN	3	52	48	113	5.4
JT-FN-D	3	52	47	112	5.3

Table 2: Lead isotopic amounts and concentration of samples from sample set 2.

5 Analysis

The results of the first sample set exposed a few areas for improvement in the experimental procedure. The first is the extremely high amount of lead content in the blank sample shown in table 1. Ideally, these values should be zero so we knew there was a problem. Contamination during isotope dilution and ion exchange seemed to be the cause so adjustments were made to the experimental procedure of the second sample set.

Changes during isotope dilution and ion exchange were as follows; Teflon cylinders were used to hold the samples in data set 2 over the plastic cylinders from data set 1. This was done in order to reduce contamination of the sample. At every evaporation stage of set 2, an evaporation chamber was used over the evaporation lamp. Again, with the hope of reducing contamination. The most important change was switching from using a combination of HBr and HCl as the acids during ion exchange to low molarity HCl. This was believed to have the effect of reducing lead content in the blank sample by eliminating HBr, which was believed to be less clean than HCl, and the low molarity results in less chloride complexes formed between the HCl and Pb isotopes. These changes resulted in a 2726% drop in lead content between the blank samples of data sets 1 and 2, which indicates the precautionary measures combined with the low molarity HCl ion exchange procedure result in a significant improvement to the experimental procedure.

Separately, analysis of the values for duplicate samples in both data sets reveals great reproducibility of results. This reinforces the effectiveness of the current experimental procedure. It should be noted that it would be helpful to test the experimental procedure against samples with known isotopic composition in order to test for accuracy.

However, a source of error influencing both accuracy and precision, derives from a phenomena known as preferential evaporation. We assume the sample and the filament are in thermal equilibrium. Thus from the Saha ionization equation, we find the rates of evaporation are proportional to the mass of the isotope, the lighter the ion, the faster the evaporation rate. Due to this, the isotopic abundance ratios bias the heavier elements the longer evaporation is occurring.

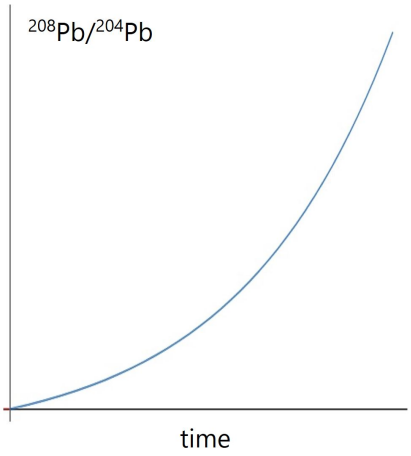

Figure 8: Example of preferential evaporation showing the ^{208}Pb to ^{204}Pb ratio favouring the heavier isotope over time once the filament reaches the evaporation temperature of the element.

An attempted solution to this problem was to automate the heating of the filament and then conduct measurements after the same elapsed time as there would be consistency with the amount of preferentially evaporated lighter isotopes. In theory, this means measuring between the same two points along the curve with respect to time, increasing accuracy or reproducibility of measurements. However, when applied to the second data set, although consistently 25 minutes long, the heating time was significantly longer than when human controlled. Measurements should be conducted as close to the evaporation start time as possible. This way, measurements are conducted closest to the point of true isotopic abundance at the origin of figure 8. Furthermore, due to this extra time under high heat, many samples did not register any lead content. The extremely long time of the automated heating procedure was assumed to have burned the samples off the filament before measurement had begun. This also happened to two of the samples from the first set, likely due to inexperience handling equipment. There is room for finding ways of preventing the lead from being burned off before measurement. It is also possible that the target 1425 K temperature for the filament was too high. A lower target temperature may be closer to the evaporation temperature of the lead isotopes with the silica gel and may result in greater retention of the lead content thus increasing relative precision.

The biological samples themselves were also an area for improvement. For better comparison between sets of data, where the experimental procedure is modified, we want to use samples sourced from the same person. For example, it is not possible to compare data for consistency or improvement of precision between SY toe nail and CK finger nail in the data above. To solve this I planned to use my own samples of nails and hair for two consecutive data sets. This would allow for better comparison of experimental procedures by eliminating variation in lead content between different people. Unfortunately there was not sufficient time to implement this idea due to COVID-19.

References

[1] Chen, J.; Moir, D.; Whyte, J.; Canadian population risk of radon induced lung cancer. *Radiation Protection Dosimetry* **2012**, Vol. 152 (1-3), pp 9-13. https://doi.org/10.1093/rpd/ncs147

[2] Keith, S.; Doyle, J.; Harper, C.; *Toxicological Profile for Radon*, Agency for Toxic Substances and Disease Registry **2012**; pp 15-18. https://www.ncbi.nlm.nih.gov/books/NBK158780/#S23

[3] Murray, R.; Holbert, K.; Radioactivity. *Nuclear Energy*, 7; Butterworth-Heinemann, **2015**; pp 31-46. https://doi.org/10.1016/C2012-0-02697-X

[4] Mohammed, E.; Mohammed, T.; Mohammed, A.; Optimization of an acid digestion procedure for the determination of Hg, As, Sb, Pb and Cd in fish muscle tissue. *ScienceDirect* **2017**, Vol. 4, pp 513-523. https://doi.org/10.1016/j.mex.2017.11.006

[5] Ishak, I; Rosli, FD; Mohamed, J; Mohd Ismail, MF; Comparison of Digestion Methods for the Determination of Trace Elements and Heavy Metals in Human Hair and Nails; *Malays J Med Sci.* **2015**; Vol. 22 (6), pp 11–20. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5295749/

[6] Kazi, T; Jamali, M; Arain, M; Afridi, H; Jalbani, N; Sarfraz, R; Ansari, R; Evaluation of an ultrasonic acid digestion procedure for total heavy metals determination in environmental and biological samples; *Journal of Hazardous Materials* **2009**; Vol. 161 (2-3), pp 1391-1398. https://doi.org/10.1016/j.jhazmat.2008.04.103

[7] Becker, J; L'Annunziata; Inorganic Mass Spectrometry of Radionuclides Handbook of Radioactivity Analysis, 3rd Edition; *Academic Press* **2012**; pp 833-870. https://doi.org/10.1016/B978-0-12-384873-4.00013-X

[8] Rosman, KJR; Taylor, PDP; Isotopic compositions of the elements 1997 (Technical Report); *Pure and Applied Chemistry* **1998**; Vol. 70 (1), pp 217-235. https://doi.org/10.1351/pac199870010217

[9] What is ion exchange resin and how does it work?; *Samco Tech*; Nov 28, **2017**. https://www.samcotech.com/ion-exchange-resin-work-process/

[10] Doucelance, R; Manhes, G; Reevaluation of precise lead isotope measurements by thermal ionization mass spectrometry: comparison with determinations by plasma source mass spectrometry; *Chemical Geology* **2001**; Vol. 176 (1-4), pp 361-377. https://doi.org/10.1016/S0009-2541(00)00409-5