Answers in Ashes: Elemental Analysis of Cremains by ICP-OES

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ABSTRACT

Cremation has always been an alternative to burial for the final disposal of human remains. Due to the intense heats of retorts, cremated human remains (cremains) have only been distinguished as human by the bone fragments that remain, as all organic matter is destroyed following cremation. Traditional methods which depend on visual analysis of bone fragments can be unreliable as human bone fragments can be intermingled with non-human powders and ashes. To improve the current methods of forensic anthropology, elemental analysis of the powdery ash of cremains was performed by inductively-coupled plasma optical emission spectrometry (ICP-OES) followed by multivariate statistical analysis. Samples consisted of both known and unknown cremains donated to the Office of the Hamilton County Medical Examiner (Chattanooga, TN), including cremains from reputable crematories, concrete, and prepared mixtures of cremains and concrete. Three 1.0-gram samples of each specimen were hot-plate digested with HNO_3 and H_2O_2. Standard solutions of 21 elements, aluminum (Al), antimony (Sb), arsenic (As), beryllium (Be), boron (B), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), lithium (Li), manganese (Mn), molybdenum (Mo), nickel (Ni), potassium (K), selenium (Se), silver (Ag), strontium (Sr), thallium (Tl), and vanadium (V), were prepared. Samples were calibrated against these standards with ICP-OES. Concentrations obtained from ICP-OES were then used as variables in statistical analysis to determine which elements were most useful in classifying the unknowns as either cremains or concrete.

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INTRODUCTION

Purpose

The purpose of this research was to develop a method that would supplement current methods of analyzing cremains. This method concentrated solely on the ash or powder of the specimen, not the identifiable bone fragments that are the primary focus of most anthropological visual analysis. The necessity of such a thorough analysis had been brought to attention by the recent investigation and subsequent criminal charges involving the Tri-State Crematory in Noble, GA. The operator of the facility did not cremate at least 339 bodies for which he was contracted and instead interred them on the property.[1] Remains returned to families did sometimes contain bone fragments, but were suspected to be filled with other non-human powders such as concrete. In this study, the comparison is made primarily concrete since this is what was the suspected filler-material found in the Tri-State samples.

Cremation

As death is an inevitable part of human existence, what must be done with the deceased is a decision that logically follows. Traditionally, options have consisted of interment or cremation. This practice has existed since pre-historic times when stone-age man cremated his dead in Northern and Eastern Europe.[5] Pyre cremations, or burning bodies atop large piles of wood, were well documented in early civilizations as well, including the Romans and the Greeks.[2] Due to many religious beliefs, cremation lost its following in Western civilization and was only practiced by people who were considered radical free-thinkers. Within the last century however, the practice of cremation has returned as an acceptable method to dispose of the deceased due to practicality, health concerns, and overall cost. Today over 60% of the dead in England are cremated due to the scarcity of land for grave sites.[3] The United States still maintains burial as its primary method of handling the dead, as 2003 statistics indicate that only 28.7% of all deaths were cremated. These numbers are projected to increase in the coming years.[4]

The human body is comprised of bones, carbon-based tissues, and water. Cremation is the direct exposure of a body to direct flame and heat, evaporating all the water, destroying the organic tissues, and reducing it to fragments of bone. Originally, this was done by placing the corpse on a pile of wood and igniting it, often ceremoniously with mourners present.[5] Modern civilization and technology have removed this practice and replaced it with specialized equipment and personnel to perform the task. Typically all jewelry and tokens are removed, before bodies can be cremated. Pacemakers are also removed, as they can explode when exposed to intense heat causing possible damage to equipment or operators. Corpses are placed in combustible containers made out of cardboard, medium density fiberboard, or wood. The container is then placed in a gas-fueled oven or retort for two to three hours at temperatures between 800 and 1100°C. All remains are then swept out of the retort after being allowed to cool. A magnet is then run across the tray of cremains to remove any metal objects left over, including surgical implants and dental work. This leaves the bone fragments which are then ground down to a fine powder before being placed in an urn and returned to loved ones.[4]

In the United States, all handling of deceased individuals is regulated by law on the state and local levels. It is further monitored on the national level by the Federal Trade Commission (FTC).[6] Crematories are also regulated in the United States under the Environmental Protection Agency (EPA) as their operations involve gas emissions.[7] Other accrediting organizations exist to ensure proper procedures and business practices concerning cremation, the largest and oldest among these is the Cremation Association of North America.[5] Despite this amount of organization, unlawful acts of mishandling involving cremation such as the Tri-State case and incidents involving confirmed commingling of cremains have occurred.[8] Due to the large magnitude of the implications of the Tri-State incident, many states have been compelled to reconsider their current regulations on cremations.[9]

Composition of Cremains

The material that actually exits the retort is the concern of this work. The intense heat of the retorts ensures only bone ash remains, as all the organic tissue is destroyed. The powder and small fragments are all remnants of the former skeleton. Knowing what actually composes these bone fragments is the matter of analysis. Bones are composed primarily of calcium phosphates and carbonates.[5] Other trace elements, especially metals, are known to be absorbed by the human body and deposited in the skeleton.[10, 11] The percent composition of these elements in a sample of cremains is also reported from previous research supported by the Cremation Association of North America to be considerably below 0.05% for some metals and over one hundred times lower for some of the trace elements.[12]

What types of environmental exposures cause humans to acquire these particular elements? This is an area of concern because certain concentrations of many substances are considered toxic. The environment in which a particular population lives has a direct influence on the exposure to particular elements. Areas of high industry and emissions with an occurrence of acid rain have water sources with a lowered pH. These lowered pH levels allow for elements like lead, copper, aluminum, and cadmium, that are insoluble in neutral or alkaline conditions, to be leeched from sediments or plumbing materials into drinking water.[13] Arsenic, although an essential element to animals, is also found in drinking water. It was even brought before the U.S. House of Representatives Subcommittee of Environment, Technology, and Standards in 2001 investigating the necessity of placing regulations on its concentrations in water supplies.[14]

Along with drinking water, dietary consumption is another source of elemental intake. Often the elemental uptake in the skeleton is related to the food that was produced on grounds that have elevated levels of each respective element.[12] Some of the elements have properties similar to essential minerals and replace them in anatomical function. Strontium and magnesium are in the same group on the periodic table as calcium and have similar properties. It was found that strontium replacement was much higher in plants than in animals. So it was concluded in certain studies that a person whose diet is largely vegetarian has a higher incidence of strontium.[14] Consumption of specific other elements including vanadium, chromium, manganese, cobalt, and nickel has been determined essential to human diet, and these metals accumulate in the skeleton as well.[15]

Research conducted by the Wittmers *et al* studied the lead concentration in skeletons and used it as means to identify the social status of the remains removed from southern plantation lands. They found that those with higher lead content lived and ate within the main house and kitchen where there was lead based paint. Food in this area was transported in containers of British pewter, which contained lead. Slaves or indentured servants who lived in the fields had lower lead levels in their skeletons.[16] The same principles can be applied to current social positions as poorer, industrialized inner-city areas see a higher incident of heavy metals associated with particular manufacturing processes. Elemental exposure probabilities associated with particular environments are the people inhabiting these areas. Thus, the elemental concentrations found in a person's skeleton and, therefore, their cremains are going to be as varied as the environments and habits of those people.

Analysis of Cremains

Previous analysis of cremains has primarily been the labor of anthropologists with the aid of other specialists ranging from physicians to the analytical chemist. The object of these investigations was to examine whether the contents of the urn are what they are claimed to be or to determine simply if the remains are human or not. This included suspicions of commingling remains. Commingled cremains may have the remains of more than one individual in the sample.[5] Anthropologists rely on what they can visually distinguish from the material present in the whole sample.

This includes all extra materials or contaminants along with the expected fragments of bone. Initial analysis includes noting the overall coloration of the sample and the consistency of it powders or fragment size.[8] Among larger fragments, the anthropologists attempt to distinguish specific structures such as teeth and bones. Clues to commingling are also found in repeated structures, such as the presence of more than one left talus. The particular weight of the sample is also taken into consideration.[17] These methods do not consider of the powdery ash of the sample.

Elemental analysis has been carried out with the use of atomic absorption spectroscopy (AAS) in a study of lead concentrations in skeletal remains. [17] Dietary studies on Eskimo remains in Alaska were conducted by Gorsuch using ICP-OES after incineration.[18] This research was followed later by Radosevich using instrument neutron-activation analysis.[19] The most recent analysis of cremains was performed by Warren et al using proton-induced X-ray emission testing.[20] This particular method, while effective, requires instrumentation that is quite costly and not readily available.

ICP-OES

The inductively-coupled plasma-optical emission spectrometer has two major components: the torch and the spectrometer. These components, along with an efficient sample delivery system and computer operating station, make the ICP-OES well-suited for elemental analysis of the prepared samples of cremains in this study. The principle behind the function of this instrument is spectroscopy, the study of electromagnetic radiation and, in the context of analysis, its interaction with matter.

Specifically, *emission spectroscopy* is the observation of radiation emitted by chemical species. Radiation is emitted from elements as a result of excitation. The added energy causes transitions in electron energy states and as the electrons return to the ground state the energy is emitted as electromagnetic radiation. Measurement of the wave-like properties of this radiation allows for qualitative (identification) and quantitative (how much) analysis of the sample in question.[21]

The inductively-coupled plasma, the torch, is the excitation source for this instrument. A plasma is a partially ionized gas, consisting of electrons, ions, and neutral particles.[22] Argon gas is passed through three concentric quartz tubes that are surrounded by an induction coil. The flowing argon acts as the support gas for the plasma as well as the coolant for the tubes. A Tesla coil sparks the gas to provide the initial electrons and charges inside the induction coil. The induction coil is connected to a radio frequency generator operating at 40.68 MHz. The alternating magnetic fields created by these coils, induces a closed circular current of electrons and ions. The alternating magnetic fields and their induced directions cause further collisions between the argon ions and electrons which further ionize the gas and heat the plasma. This generates a plasma that is sustained at temperatures between 7000 and 8000K. The inert nature of the argon ICP reduces chemical interferences and allows for efficient atomization and excitation of the introduced samples.

The radiation that is emitted from the atoms is detected and measured. The emission consists of multiple photons that are of various wavelengths. A monochromator separates the spectral wavelengths and makes it possible to isolate the line of interest. Light first enters through an entrance slit which selects the source area of radiation. The incoming radiation then is collimated by a curved mirror. After the rays are collimated, they are dispersed by the grating. The positioning of the grating specifies which wavelength is reflected to a mirror that directs the light out the exit slit. Once the radiation is isolated, it is received by a detector that measures its intensity. The strength of the signal detected is directly proportional to the concentration of the source that emitted it. These measurements are then calibrated against prepared standards.[21]

Due partly to the effectiveness of the plasma as an excitation source and the selectivity of the particular monochromator, this instrument has multiple advantages for this study. The high temperatures of the plasma cause high excitation efficiency resulting in measurement of elemental concentrations into the parts per billion (ppb) ranges. Nitrogen gas flows through the monochromator to ensure that no dust or other material does not linger and absorb radiation. The computer interface controls the adjustment of the monochromator and for each measurement and the introduction of samples via a robotic autosampler. It is also responsible for recording the signal. Since this setup is a *sequential* spectrometer, it can only measure one wavelength at a time. The computer assisted operation is indispensable, as analysis of multiple samples for multiple elements can be quite time consuming.

Statistical Analysis

The ICP-OES provides multiple elemental concentrations for each sample. In the case of this research, 21 separate elements were measured and act as variables in the analysis of the cremains. Each of these multiple values must be considered in the analysis of the samples, so simple mean and standard deviation calculations were not sufficient. Therefore, multivariate statistical methods had to be employed. These methods make it possible to isolate the variables that are most important to the analysis as well as to detect structure in the relationship between the variables.

When two or three variables are considered, they can be represented graphically in two or three dimensions. Matrix algebra is necessary to understand fully the analysis for this many variables.[23] Computer software is available to overcome this difficulty. Once the data are organized, a cluster analysis identifies for groupings of multiple variables that are spatially close together and can be classified to represent a single component. This method is useful in identifying variables that are related in a non-linear fashion. A dendrogram is a figure that presents the findings of the cluster analysis showing the successive groupings of the variables according to distance or similarity.

Principal component analysis (PCA) is another method used to narrow the scope of the large number of variables being viewed. PCA takes a group of variables that appear to be closely correlated to each other and combines them into a single component. This reduces the number of dimensions in which the variables exist by creating principal components. The first grouping creates a principal component accounting for the largest variance of the data set.[24] The second principal component would then account for a decreased magnitude of variation and so on. Unlike cluster analysis, PCA is not useful for variables that are not correlated.

Discriminant analysis is unlike either PCA or cluster analysis because it requires prior knowledge of the objects and the groups of which they are members. PCA and cluster analyze the known objects and established their components and characteristics. Discriminant analysis takes these known components and creates a rule based on the patterns that make up these components.[25] A mathematical function can be devised that places the unknown objects into the known groups to which they are being compared. Combination of all of these statistical methods is necessary to analyzing the multiple elemental concentrations provided by the ICP-OES analysis.

EXPERIMENTAL

Samples

Eighty-eight samples were analyzed in this study. The samples were initially classified as known human cremains, concrete, and questionable. Table 1 specifies the samples and their sources. The known human cremains (n=54) were attained through cooperation with the Office of the Hamilton County Medical Examiner (HCME). Some samples were acquired by HCME by scientific donation of the bodies that were then cremated by a single crematory. Other known human samples were donated from the urns of local families after reading a newspaper article published concerning this research.[1] These were only accepted as legitimate when accompanied by a *Certificate of Cremation*. Medical school cadavers that had been cremated were also donated by the University of Cincinnati Body donation program, making up the largest portion of the known cremains group (n=33). The other group was the concrete group (n= 10). This group contained all the known, non-human powder material in the study. This included commercial brand concretes, cement, floor underlayments, and grout. All these materials had the appearance of powderized ash and could possibly be used as falsified human cremains.

The questionable group (n=24) was comprised of samples of all types. This includes cremains that were questionable or known to be from the Tri-State Crematory. Also included in this group were two individuals who lived with indwelling firearm wounds, one being from a shotgun and the other a bullet. Also included in this group were a cremated dog (*Canis familiaris*) and wood ashes from a barbecue pit. Mixtures of human and cement (n=13) of known various ratios were prepared to represent more questionable samples. These were made from type I general purpose Portland cement powder and three of the known human cremains. Each was mixed individually in a plastic container and shaken for one minute by hand to insure homogeneity.

Since powderization of the cremated skeleton insures homogeneity of all the remains, sampling of any powder within each container is representative of the entire sample. Large fragments of bone and other outstanding masses were avoided in sampling as they accounted for minor portions of the total sample. These would also be difficult to digest and would be removed by filtration. Since the samples were human, approval was obtained from the UTC Institutional Review Board (IRB Approval #04-128).

TABLE 1: *Sample Details*

Description	**Classification**	**n**
HCME donations	Known	21
University of Cincinnati Body Donations	Known	33
Wood ashes	Questionable	1
Canis familiaris	Questionable	1
Questionable human cremains	Questionable	9
Human:concrete mixtures* 1st individual: 90:10, 75:25, 40:60, 25:75 2nd individual: 75:25, 60:40, 40:60, 10:90 3rd individual: 90:10, 75:25, 60:40, 50:50, 25:75	Questionable	13
Concrete brands	Concrete	10

*****These are three known human donations from HCME and general purpose type 1 Portland cement mixtures

Materials

Nitric Acid Trace Metal Grade, Fisher A509-500

Hydrogen Peroxide 30%, Fisher A325-500

0.22µm nylon membrane filters, Fisher 09-720-3

Spex Certiprep 1000 ppm Metal Standards:

Aluminum, Antimony, Arsenic, Beryllium, Boron, Cadmium, Chromium, Cobalt, Copper, Iron, Lead, Lithium, Manganese, Molybdenum, Nickel, Potassium, Selenium, Silver, Strontium, Thallium, Vanadium

Deionized water from a Barsted® B-Pure UltraPure™ Filtration System

HDPE bottles

Sample Preparation

The glassware used in sample preparation was cleaned thoroughly in a 20% v/v nitric acid bath and then rinsed with distilled, deionized water. For each sample, three 1.0 g sub-samples were taken and placed into 125-mL Erlenmeyer flasks. The sub-samples were digested with trace metal grade nitric acid followed by 30% hydrogen peroxide on a hotplate. Repeated heating, cooling, and addition of acid was required to fully digest the sample so it could be analyzed by the ICP-OES. The procedure is detailed in Table 2.

TABLE 2: *Digestion Procedure*

Step	**Action**	**Time (min.)**	**Acid or Oxidant**	**Volume (mL)**
1	Heated at 100°C	10	HNO_3	10.0
2	Sample allowed to cool			
3	Heated at 100°C	5	HNO_3	5.0
4	Sample allowed to cool			
5	Heated at 200°C	To boil	HNO_3	2.0
6	Sample allowed to cool			
7	Heated at 200°C	10	H_2O_2	2.0
8	Sample allowed to cool			
9	Heated at 200°C	To boil	H_2O_2	2.0
10	Sample allowed to cool			

After the sample was digested, it was quantitatively transferred to a 100-mL volumetric flask and brought to volume with distilled, deionized water. After mixing, this diluted solution was syringe filtered using the 0.22µm membrane filters into a 125-mL HDPE bottle, and stored until analysis.

Analysis

The Jobin-Yvon Ultima ICP-OES was the instrument used for the analytical study of 21 elements in all the samples. This instrument is a sequential monochromator with photomultiplier tube detection. Specifications and operating conditions are listed in Table 3. The instrument used a cyclonic spray chamber with a crossflow nebulizer for sample introduction. The emission wavelengths used for each element and the detection limits are listed in Table 4.

Samples were run against multi-element standards prepared at concentrations of 0.01, 0.10, 1.0, and 10 parts per million (ppm) using 1000 ppm standards. Calibration curves were constructed by plotting emitted light intensity versus concentration. Each sample had three sub-samples, each of which were analyzed by the instrument three times, and their outputs averaged. The mean value of the sub-samples was then used to represent the elemental concentrations (mg/kg) of the entire sample.

The elemental concentrations of the known samples, both the human and concrete groups, were evaluated by variable cluster analysis to identify the trace elements that formed a representative component for each group. A principle component analysis was also performed to further investigate these groupings. Once the elements were assigned to the components of each group, a discriminant function was computed to assign the probability that the samples initially determined questionable were either part of the cremains or concrete group. These statistical procedures were performed by Dr. Stephanie Smullen in the Department of Computer

Science at UTC using SAS 9.1 for Windows (SAS Institute, Cary, NC). This was necessitated due to the extensive knowledge of statistics and computer programming required for the analysis.

TABLE 3: *ICP-OES Specifications and Operating Conditions*

Monochromator	Czerny-Turner, 1.0m
Grating	2400 grooves/mm, holographic
Radio Frequency Generator	1.5 kW at 40.68 MHz
Detector	PMT
Carrier Gas Flow Rate	2.0 mL/min
Plasma Gas Flow Rate	13 L/min
Plasma Observation	Radial
Pump Rate	2.0 mL/min

TABLE 4: *Emission wavelengths and detection limits*

Element	**Wavelength (nm)**	**Detection Limit* (ppm)**
Aluminum	308.215	5.98E-03
Antimony	206.833	3.05E-02
Arsenic	193.695	6.54E-04
Beryllium	313.042	1.35E-04
Boron	249.678	1.08E-02
Cadmium	226.502	5.67E-04
Chromium	276.716	8.49E-04
Cobalt	228.616	2.32E-04
Copper	324.754	3.75E-04
Iron	259.940	5.55E-03
Lead	220.353	3.81E-03
Lithium	670.784	1.01E-03
Manganese	257.610	2.19E-04
Nickel	231.604	8.08E-02
Potassium	766.490	3.67E-02
Selenium	196.026	3.57E-03
Silver	328.068	4.99E-03
Strontium	407.771	6.22E-03
Thallium	190.864	7.57E-03
Vanadium	292.402	6.93E-04

* Detection limits were calculated by ks_{bk}/m, where k is the confidence factor (2), s_{bk} is the standard deviation of the blank measurement, and m is the slope of the calibration curve.

DATA AND CALCULATIONS

Standard Solutions

Using the 1000 ppm Spex Centriprep standard solutions, dilutions were made to 0.01, 0.10, 1.0, and 10.0 ppm. Equation 1 was used to determine the volumes for these dilutions, where M is the concentration in parts per million and V is the volume in micro-liters.

(Eq. 1) $M_1V_1 = M_2V_2$

The dilutions are detailed in Table 5.

TABLE 5: *Standard Solution Dilutions*

Concentration (ppm)	Volume of 1000 ppm Standard Solution (µL)
0.01	1
0.10	10
1.0	100
10	1000

Calibration curves were constructed by plotting the intensity versus the concentration of the standard solutions. The regression equations and correlation coefficients (R^2) are included on each graph. Molybdenum did not generate a linear calibration curve ($R^2 = 0.9876$), and was therefore removed from the study. All other curves showed correlation coefficients very close to or at 1.

Figure 1: Calibration Curve of Al

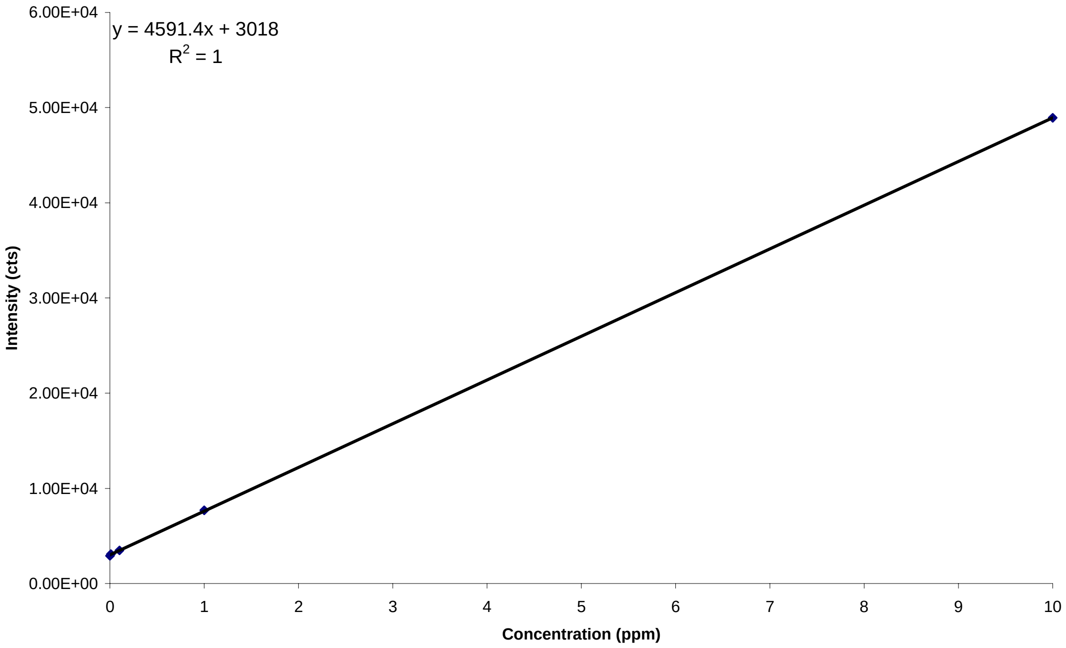


Figure 2: Calibration for Sb

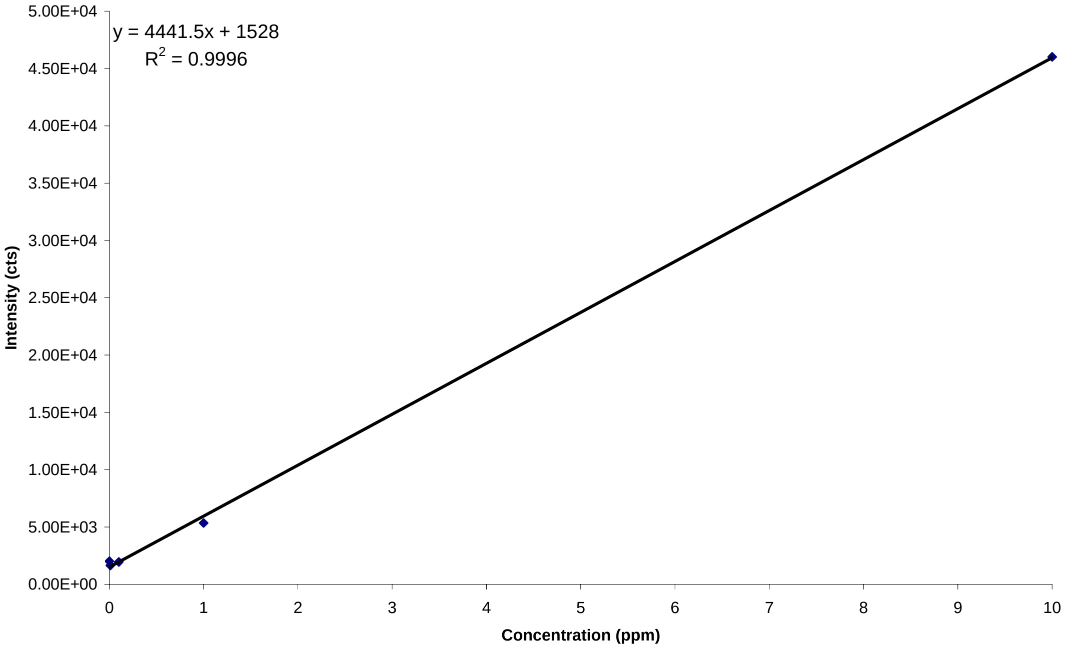


Figure 3: Calibration Curve for As

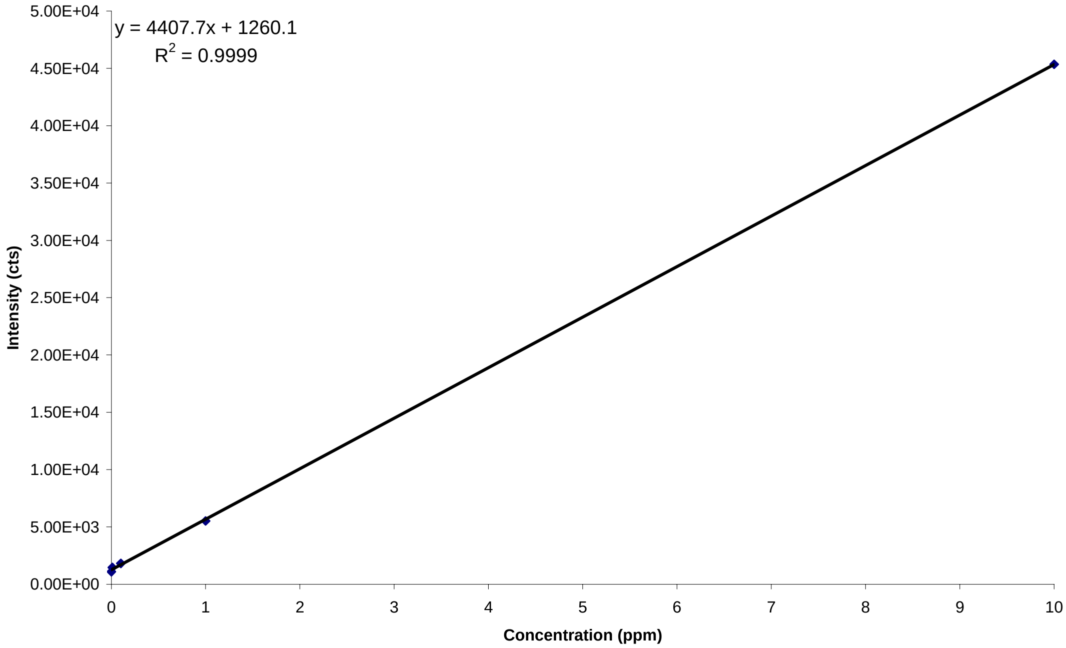


Figure 4: Calibration Curve for Be

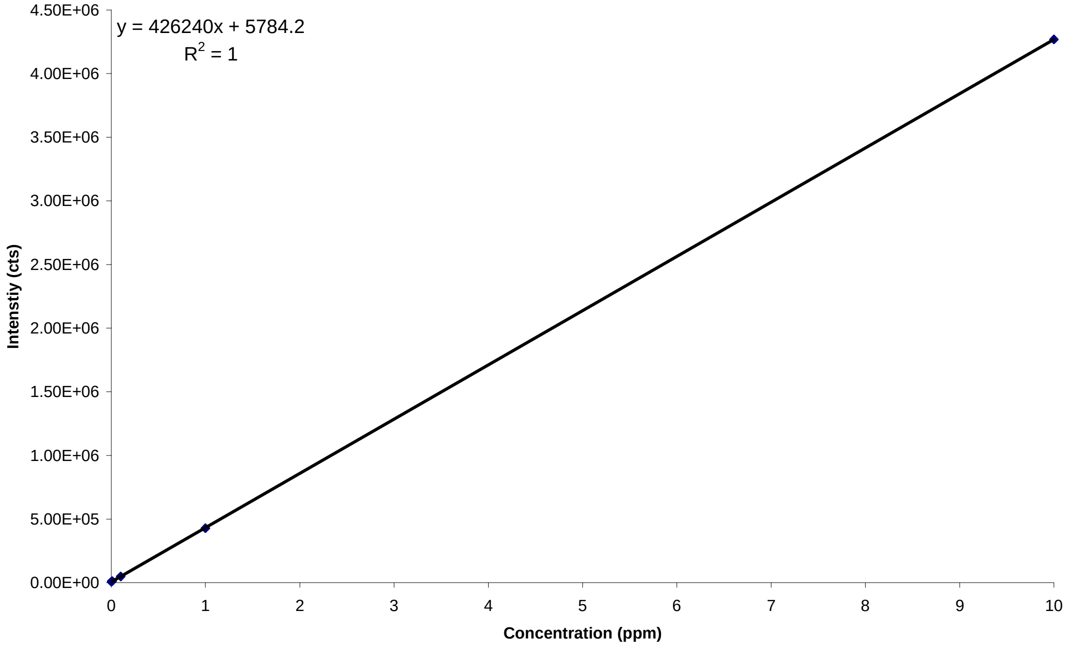


Figure 5: Calibration Curve for B

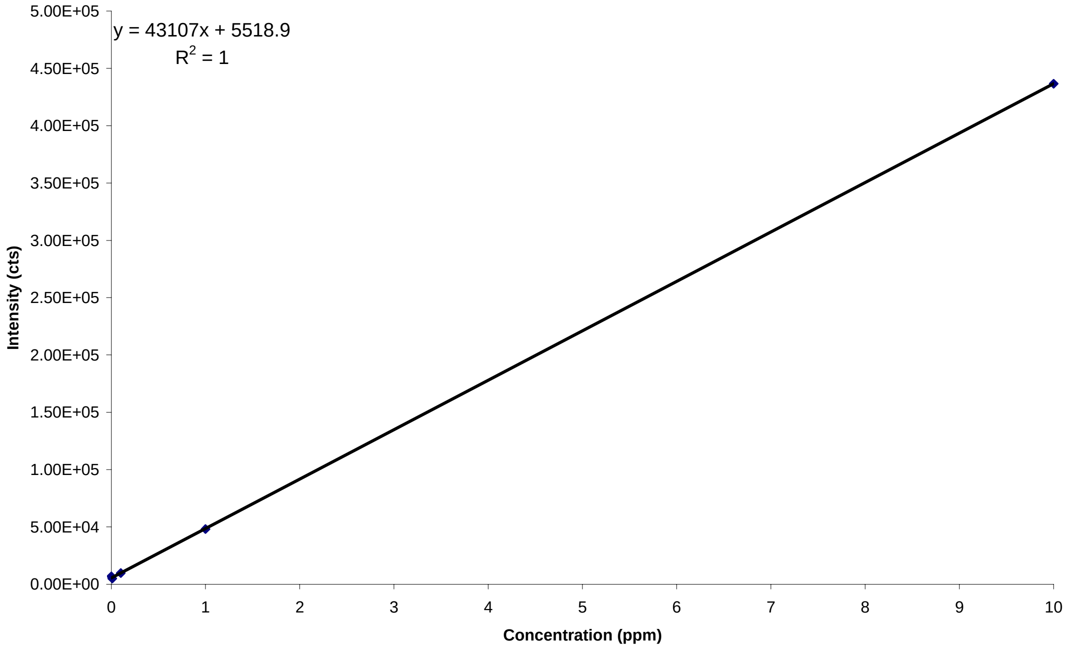


Figure 6: Calibration Curve for Cd

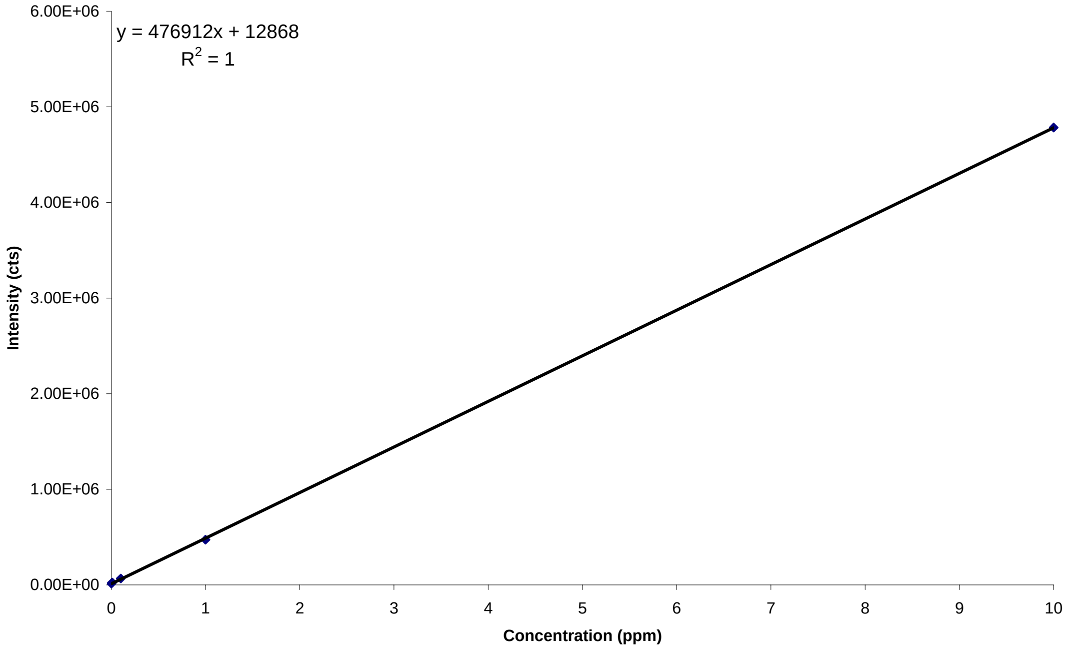


Figure 7: Calibration Curve for Cr

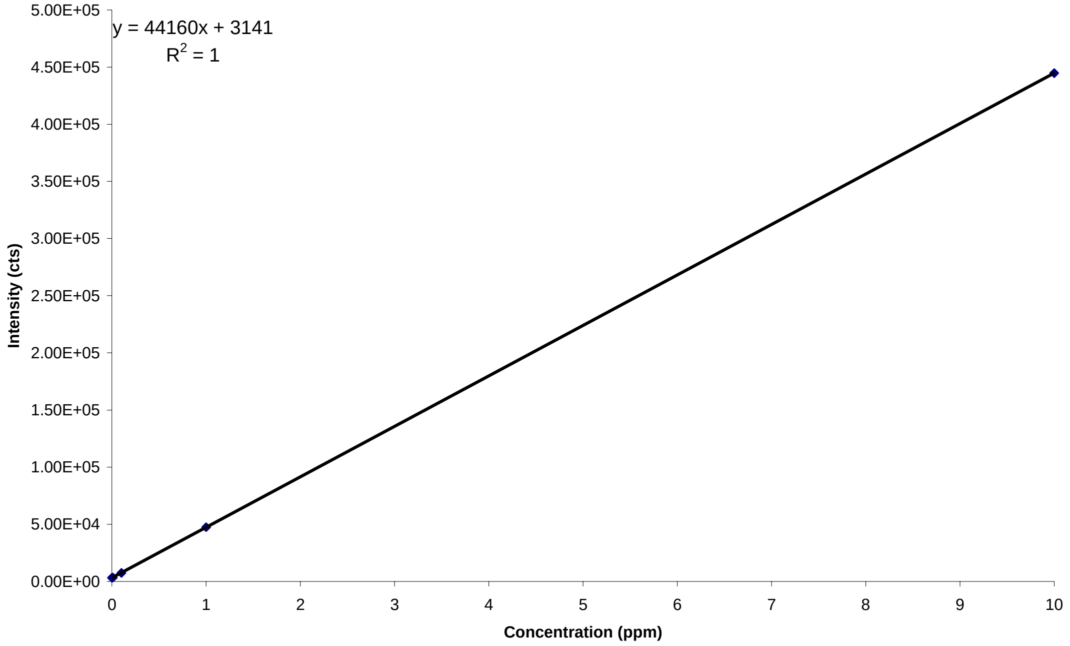


Figure 8: Calibration Curve for Co

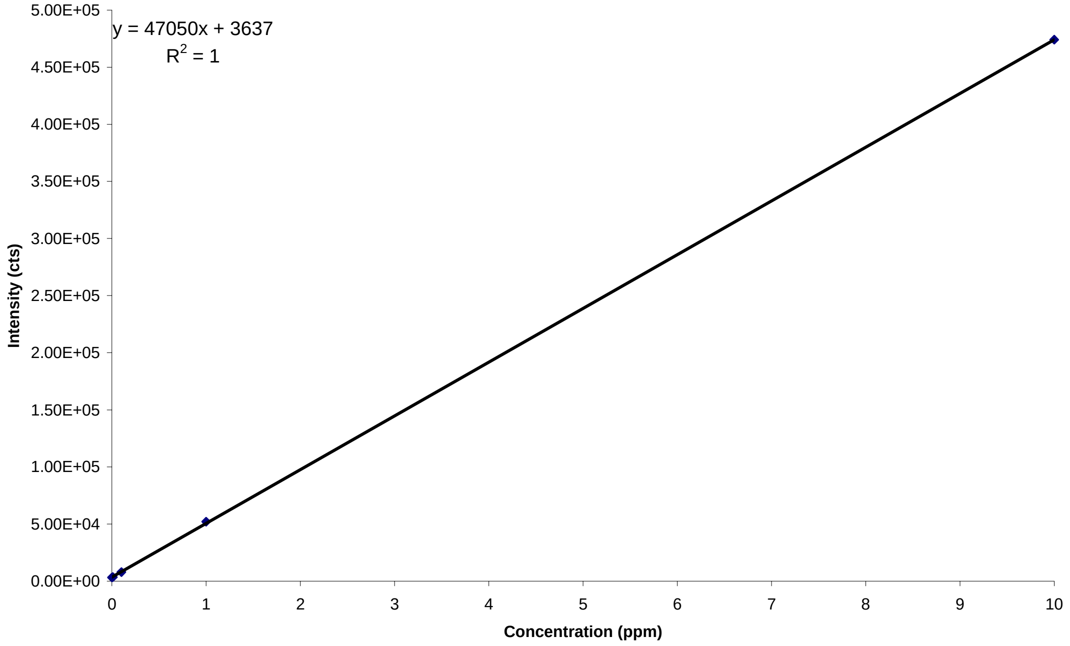


Figure 9: Calibration Curve for Cu

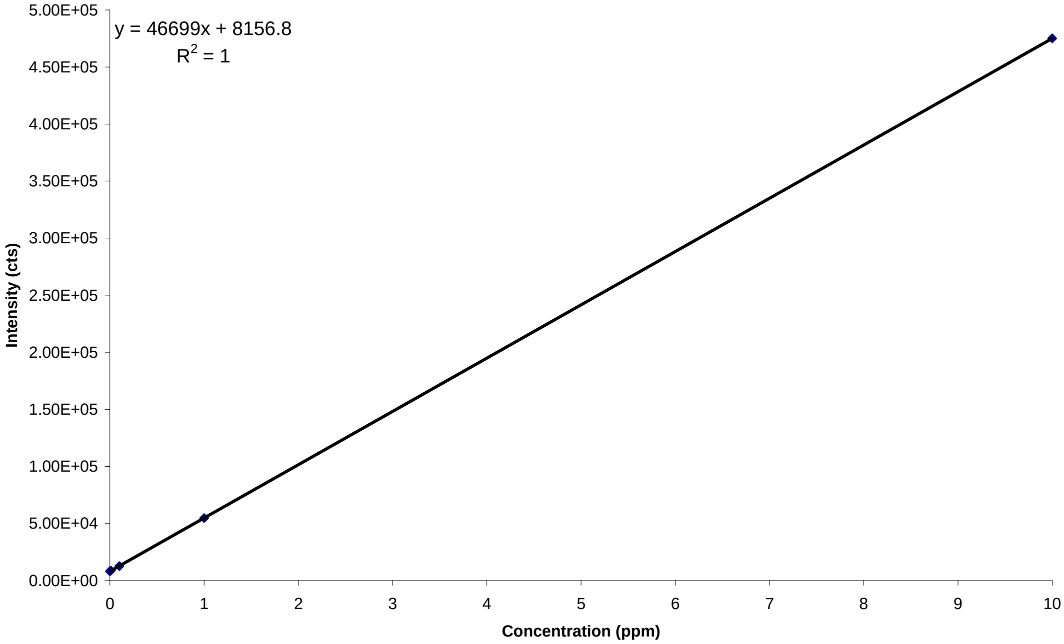


Figure 10: Calibration Curve for Fe

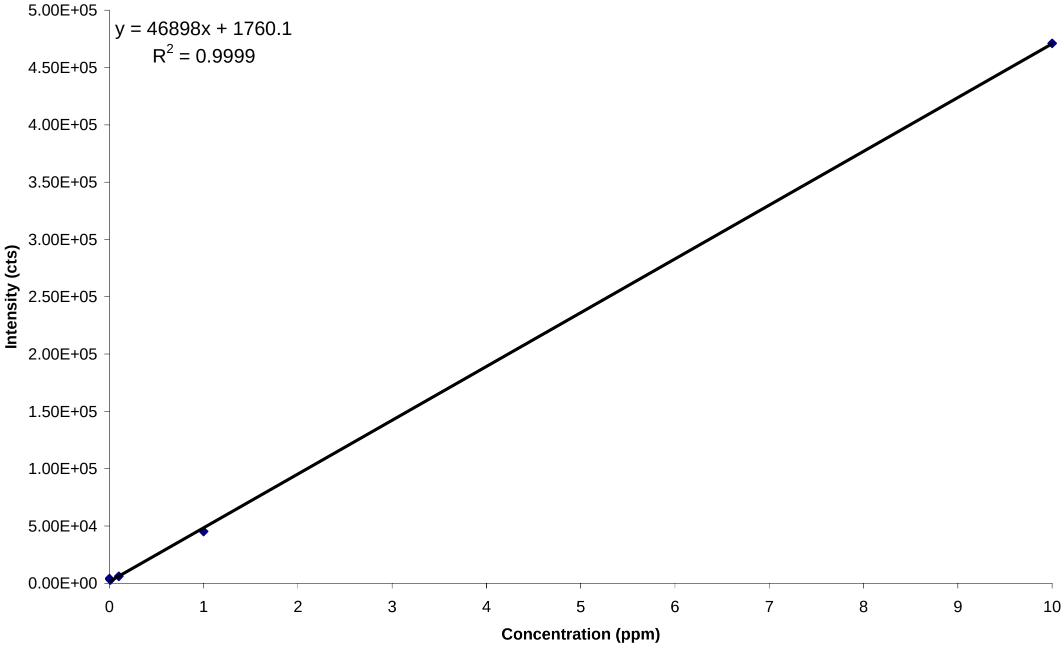


Figure 11: Calibration Curve for Pb

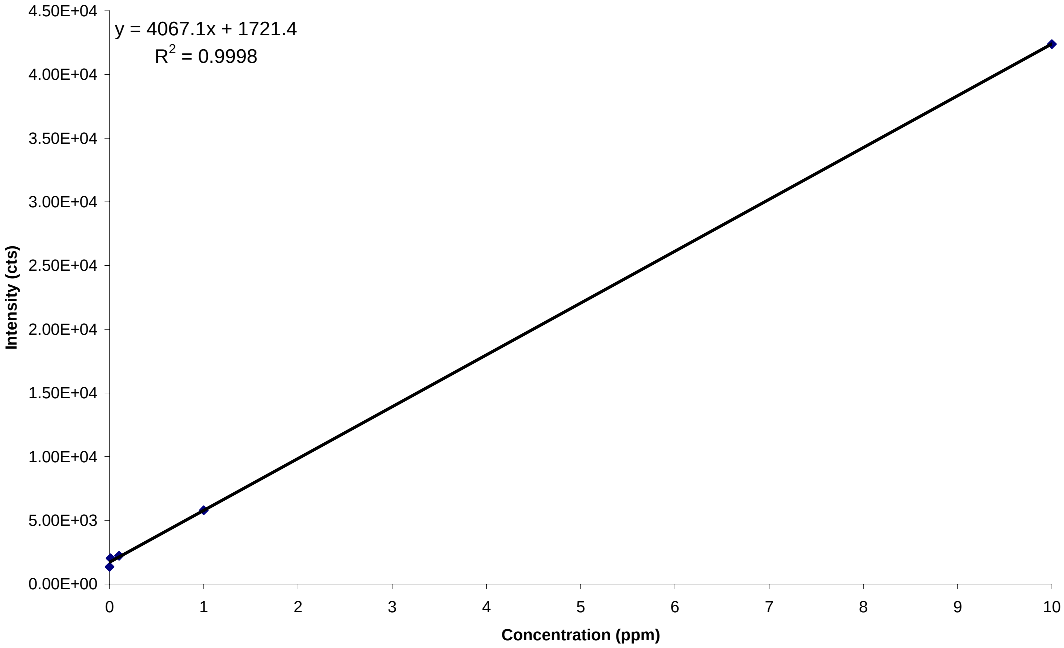


Figure 12: Calibration Curve for Li

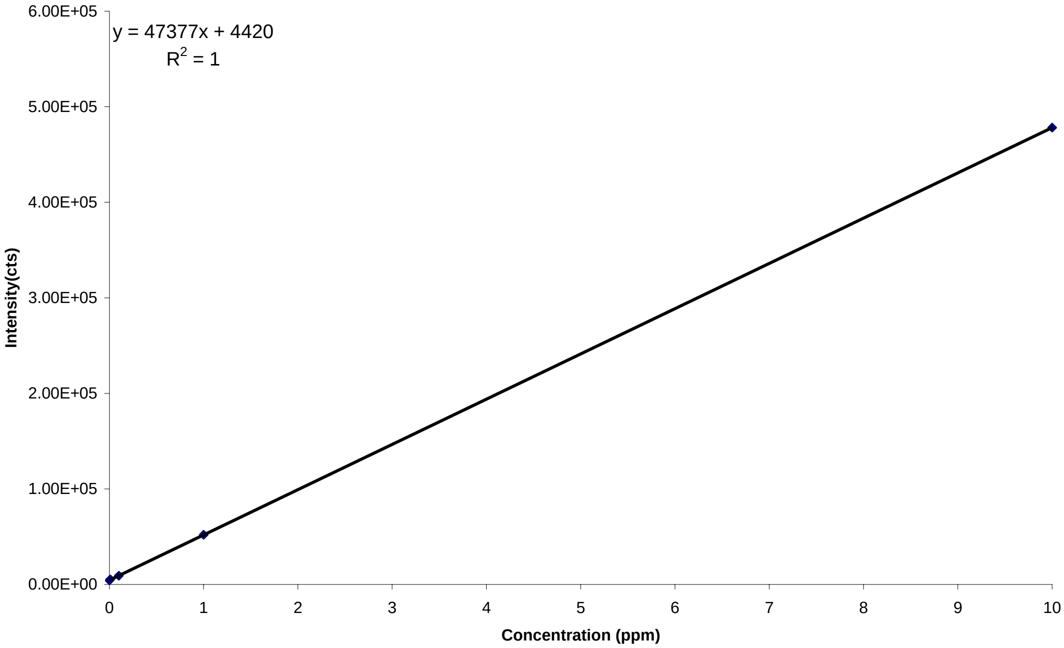


Figure 13: Calibration Curve for Mn

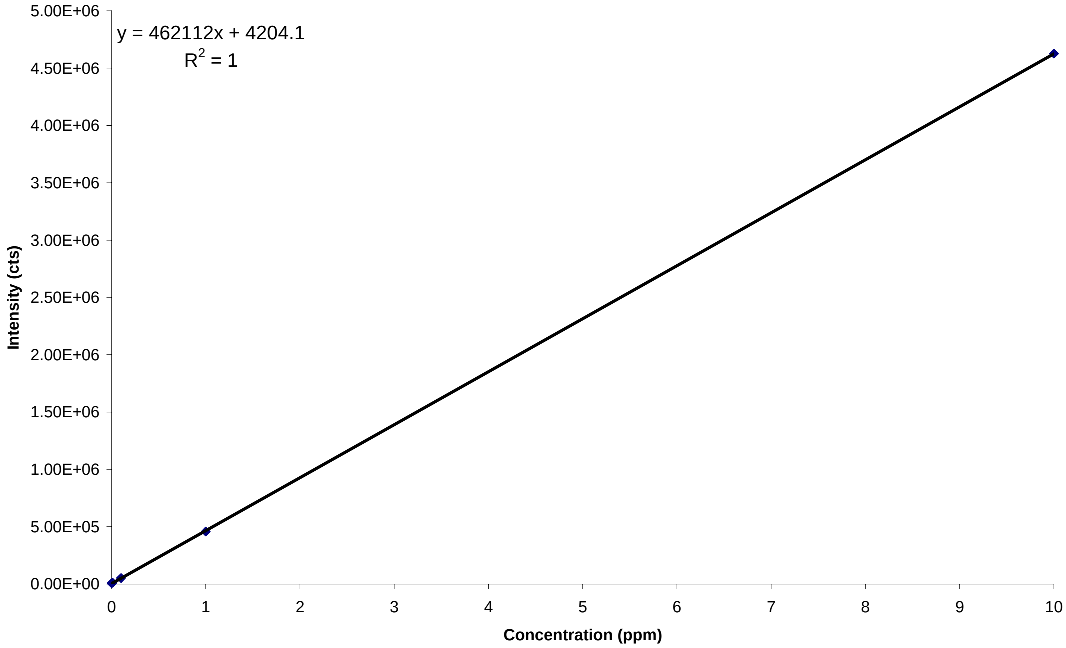


Figure 14: Calibration Curve for Ni

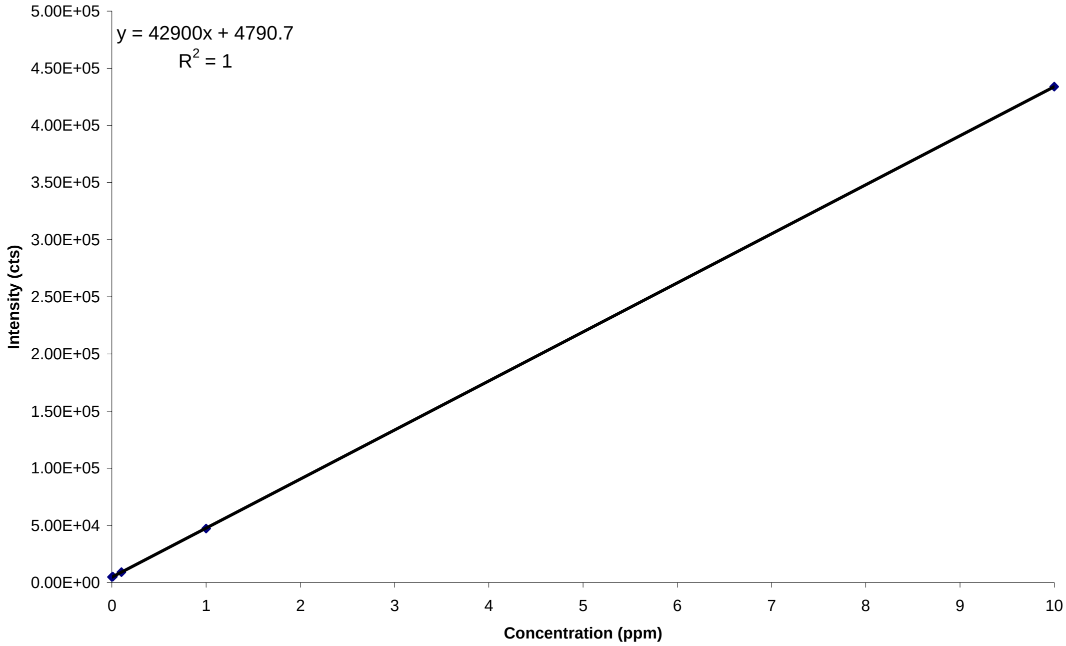


Figure 15: Calibration Curve for K

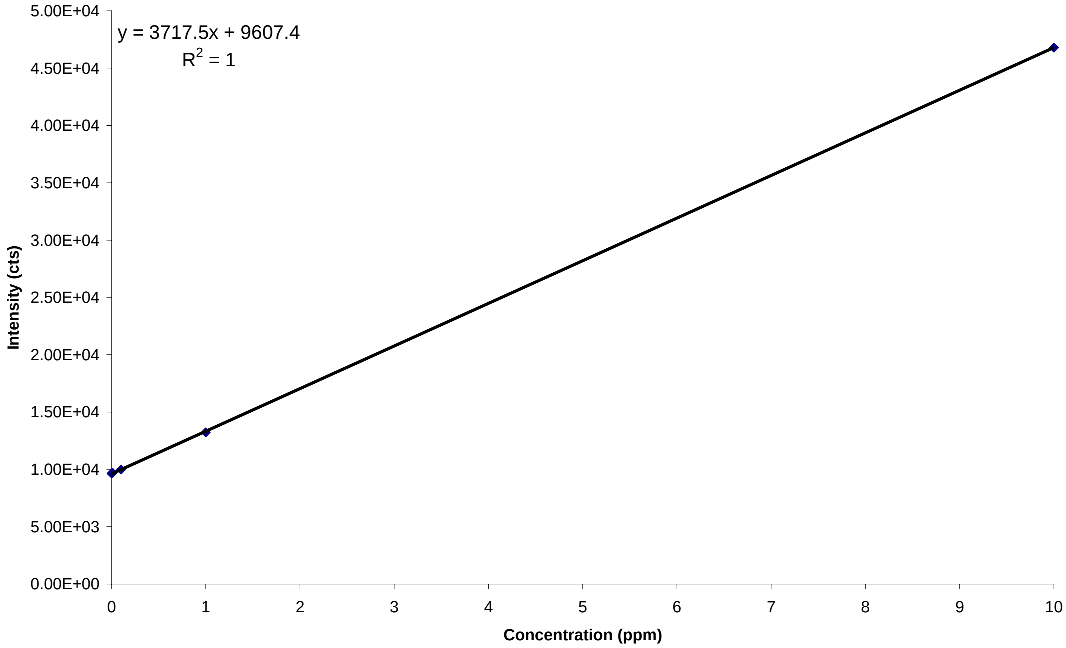


Figure 16: Calibration Curve for Se

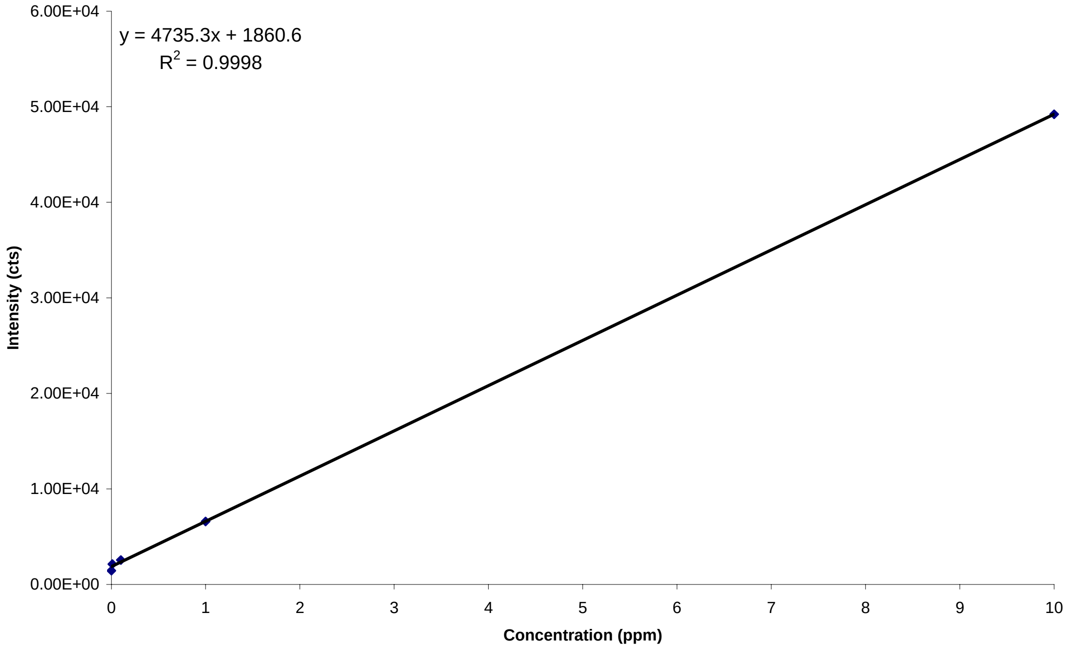


Figure 17: Calibration Curve for Ag

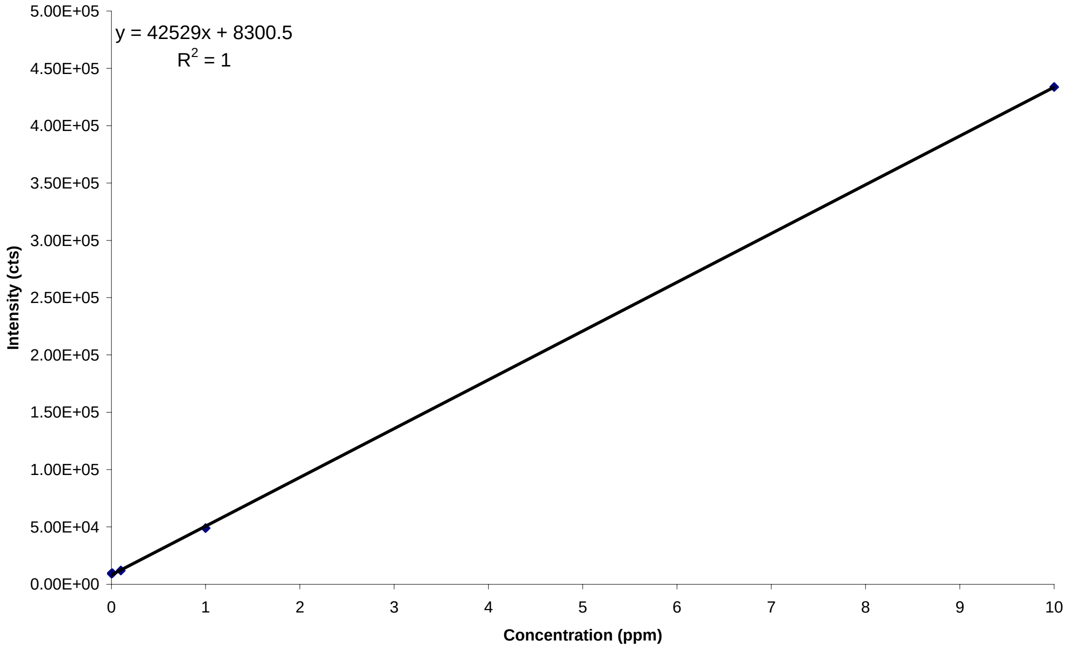


Figure 18: Calibration Curve for Sr

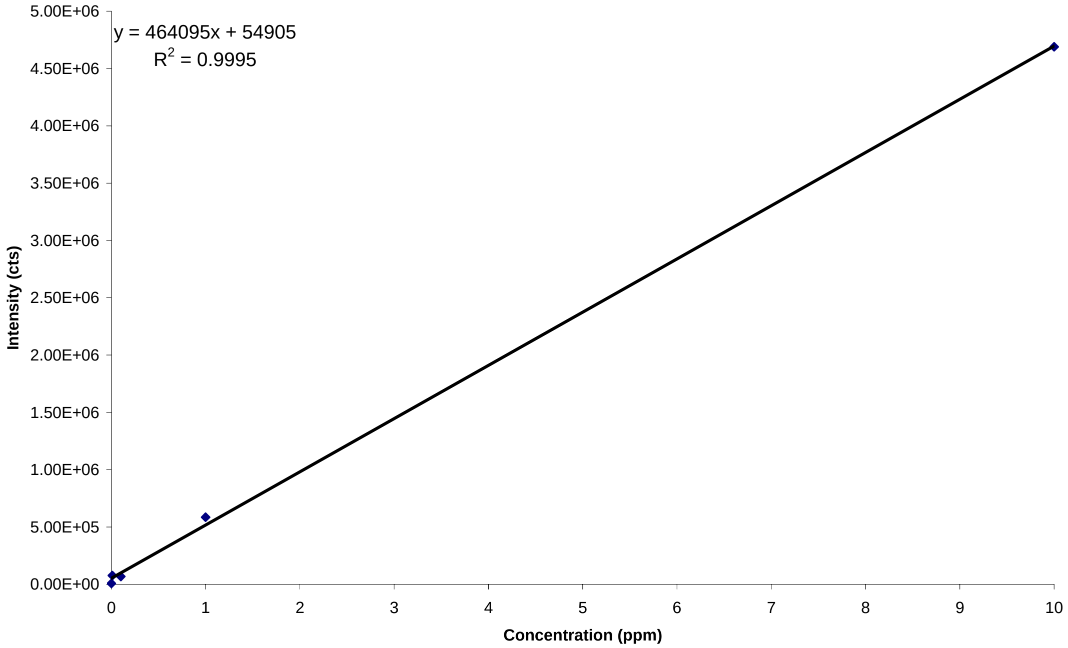


Figure 19: Calibration Curve for Tl

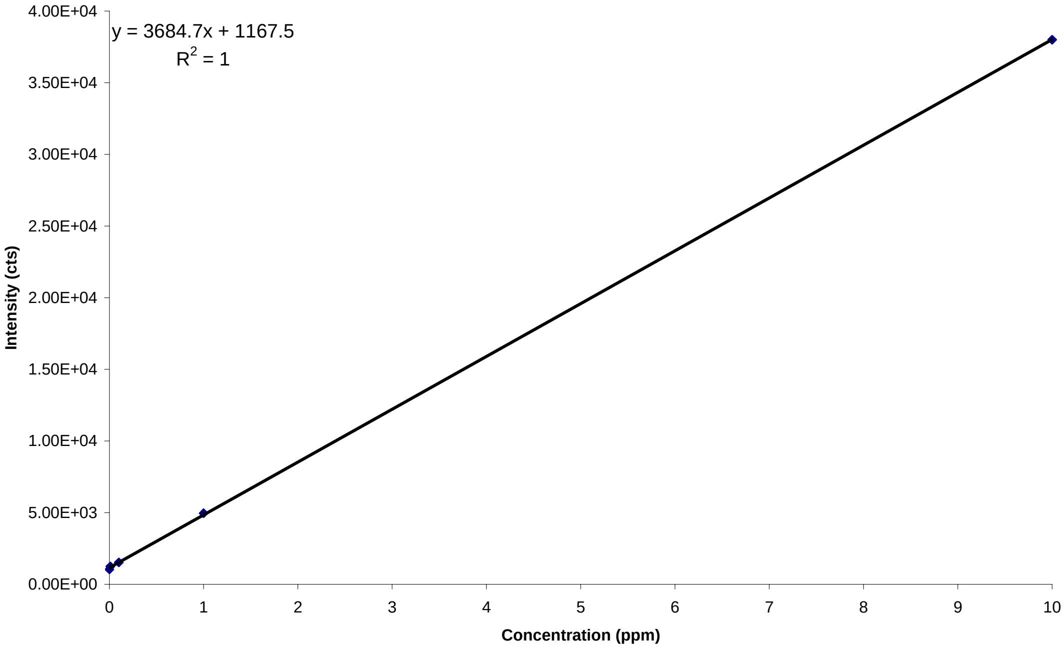
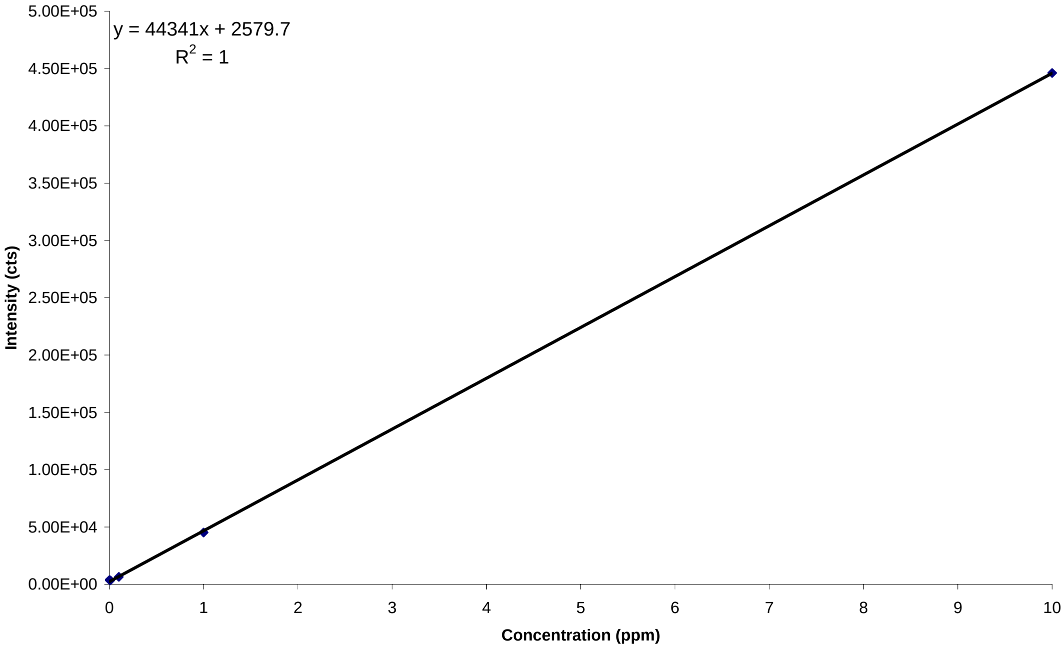


Figure 20: Calibration Curve for V



Concentration Data

All samples were analyzed for the 21 elements. Of the 21 elements, only 16 elements provided significant concentration values (Sb, As, B, Cd, Cr, Co, Cu, Pb, Li, Mn, Ni, Se, Ag, Sr, Tl, and V). Three elements (Al, Fe, and K) had concentrations greater than the10.0 ppm range of the calibration curves. Molybdenum did not have a linear calibration curve and beryllium had values below the detection limit of the instrument. Further investigation into the volatility of six elements (Sb, As, B, Cr, Mn, and Se) resulted in As and Se being removed from the study. These two elements are volatile at the temperatures of the retort (800 to 1100°C) and their concentrations following the cremation would be unreliable.[26] The mean concentrations of the remaining 14 elements are listed in Tables 6 through 8.

The elemental concentration of the cremain samples in mg/kg was determined by considering the measurement by the instrument of the digested solution and the mass of the sample before digestion.

(Eq. 2)

$$\frac{Concentration\left(\frac{mg}{L}\right) \times Volume(L) \times \frac{1000g}{1kg}}{SampleWeight(g)} = \left(\frac{mg}{kg}\right)$$

The elemental concentrations for the questionable human remains, those that were originally claimed to legitimate or from Tri-State, were included in Table 9. The wood ash and canine samples are represented in Table 10, and the human:concrete mixtures are detailed in Tables 11 through 13.

TABLE 6: *Mean values and standard deviations of each element in the Cremains Group*

Element	**Mean (mg/kg)**	**Standard Deviation**
Sb	17.4	7.6
B	138.2	179.9
Cd	4.2	7.0
Co	4.0	9.9
Cr	18.2	31.7
Cu	92.2	187.2
Pb	43.0	99.7
Li	9.8	11.6
Mn	69.5	195.1
Ni	10.9	16.0
Ag	8.5	9.8
Sr	127.2	91.9
Tl	13.5	5.1
V	5.1	5.3

TABLE 7: *Mean values and standard deviations of each element in the Concrete Group*

Element	**Mean (mg/kg)**	**Standard Deviation**
Sb	24.7	22.9
B	14.4	9.9
Cd	4.8	7.3
Co	1.6	2.2
Cr	27.3	41.4
Cu	10.6	18.2
Pb	22.8	10.7
Li	35.3	83.3
Mn	192.0	235.7
Ni	6.6	11.8
Ag	4.2	5.6
Sr	265.9	213.2
Tl	33.5	24.8
V	22.7	27.8

TABLE 8: *Mean values and standard deviations of each element in the Questionable Group*

Element	**Mean (mg/kg)**	**Standard Deviation**
Sb	29.2	9.5
B	60.0	38.0
Cd	2.5	0.9
Co	4.1	3.1
Cr	22.6	21.4
Cu	59.7	79.3
Pb	181.2	515.5
Li	7.4	5.1
Mn	306.8	927.3
Ni	11.9	10.2
Ag	8.6	6.2
Sr	306.6	255.8
Tl	28.7	11.3
V	22.1	18.2

TABLE 9: *Elemental concentration (mg/kg) of Questionable Human Remains, samples Q1-Q9*

	Q1	**Q2**	**Q3**	**Q4**	**Q5**	**Q6**	**Q7**	**Q8**†	**Q9**‡
Sb	26.6	40.2	28.4	16.3	24.4	29.1	16.2	29.4	19.2
B	95.3	116.4	104.2	125.3	39.1	5.0	59.5	96.6	79.9
Cd	1.2	2.4	2.1	2.0	2.8	0.6	1.4	2.5	1.0
Co	10.2	1.3	3.8	1.7	3.2	7.5	1.4	5.3	1.4
Cr	46.9	110.2	19.2	12.1	18.7	2.4	9.2	8.6	14.3
Cu	135.7	215.9	125.1	55.4	60.6	4.8	18.8	18.4	353.3
Pb	17.7	174.6	24.7	13.1	25.7	18.8	11.7	1555.1	2106.2
Li	10.6	7.8	6.8	4.1	3.3	1.2	3.2	2.8	3.0
Mn	91.3	247.1	239.8	78.4	180.1	34.3	9.2	44.4	520.4
Ni	6.4	25.1	7.8	14.1	13.7	1.4	0.0	3.1	8.0
Ag	6.8	23.3	9.9	16.1	7.8	1.1	17.5	3.2	1.9
Sr	163.2	91.6	93.1	103.8	196.6	27.9	73.0	88.8	113.2
Tl	19.5	28.9	24.8	18.9	24.1	25.4	13.5	16.7	25.1
V	7.3	11.0	10.3	4.6	7.3	4.2	3.5	7.1	8.6

† Individual with indwelling shotgun pellets.

‡ Individual with indwelling copper-jacketed bullet.

TABLE 10: *Elemental concentration (mg/kg) of wood ash and canine samples*

	Wood Ash	**Canine**
Sb	21.7	9.9
B	129.2	2.1
Cd	2.3	1.7
Co	2.9	2.2
Cr	10.1	9.8
Cu	74.5	50.2
Pb	29.6	51.0
Li	5.0	4.3
Mn	4618.7	34.6
Ni	51.5	4.3
Ag	2.2	1.4
Sr	1040.6	109.8
Tl	41.3	17.3
V	8.9	5.5

TABLE 11: *Elemental concentration (mg/kg) of human:concrete mixtures prepared with human sample 1.*

	10:90	**40:60**	**60:40**	**75:25**
Sb	47.4	40.1	30.0	27.0
B	17.3	25.7	27.3	33.2
Cd	4.0	3.4	2.7	2.2
Co	5.1	5.3	3.5	3.2
Cr	25.2	23.3	18.6	17.7
Cu	13.2	16.2	16.7	20.8
Pb	33.1	28.4	20.4	15.0
Li	11.8	10.2	7.2	6.6
Mn	106.0	82.6	56.5	48.6
Ni	15.0	12.4	10.7	9.7
Ag	4.9	6.5	7.3	9.5
Sr	737.4	537.3	346.6	266.5
Tl	52.6	41.9	29.8	20.8
V	59.0	44.5	29.7	22.1

TABLE 12: *Elemental concentration (mg/kg) of human:concrete mixtures prepared with human sample 2.*

	25:75	**40:60**	**75:25**	**90:10**
Sb	42.2	42.2	29.9	23.0
B	23.4	33.0	45.0	49.2
Cd	3.7	3.5	3.0	2.6
Co	4.1	14.9	2.8	2.1
Cr	22.0	20.2	14.4	12.3
Cu	14.8	18.5	25.5	26.0
Pb	31.4	31.0	29.8	27.3
Li	10.2	9.4	6.2	4.6
Mn	91.3	80.7	46.2	30.3
Ni	13.3	14.3	10.1	6.5
Ag	7.5	8.3	24.4	12.4
Sr	620.9	532.8	276.9	170.3
Tl	45.4	41.3	27.2	20.1
V	51.4	45.7	23.4	12.8

TABLE 13: *Elemental concentration (mg/kg) of human:concrete mixtures prepared with human sample 3.*

	25:75	**50:50**	**60:40**	**75:25**	**90:10**
Sb	42.7	31.6	31.5	29.2	23.0
B	34.2	60.7	68.7	79.9	88.5
Cd	3.7	3.4	2.7	2.9	2.0
Co	4.3	3.8	3.4	2.9	2.2
Cr	23.5	49.2	19.6	18.8	16.0
Cu	19.2	51.0	27.6	36.2	34.5
Pb	30.6	25.1	16.6	21.2	10.7
Li	10.6	26.8	8.3	7.6	5.4
Mn	95.9	447.7	70.4	62.9	44.5
Ni	14.8	20.5	9.9	8.0	5.4
Ag	5.5	4.0	9.2	7.6	7.4
Sr	622.8	371.6	355.2	270.6	147.0
Tl	46.4	41.6	27.0	26.9	13.5
V	50.8	47.1	30.6	22.9	11.4

Statistical Data

Statistical analysis was performed using the concentrations obtained for the samples. Variable cluster analysis identified elements useful in classification of the questionable samples. The details of the clusters found in the computer output of SAS 9.1 for Windows are given in Table 14. Further analysis to determine the useful elements for classification of the questionable samples was done by principal component analysis. This produced five components with eigenvalues greater than one which accounts for 73% of the variability. The factors of these components are described in Table 15. From the seven elements identified in Cluster 1 in Table 14, linear discriminant functions were computed for cremains group and the concrete group. The coefficients for the discriminant functions of each group are listed in Table 16.

The function was used to classify the questionable samples into either the cremains group or the concrete group. Table 17 displays the probability of the questionable samples belonging to each group. The samples were then classified into a group if there was 0.90 probability of membership. Any sample below 0.90 remained classified as questionable.

TABLE 14: *Correlation coefficient values of elements to specific clusters*

	Element	**R-squared Value**
Cluster 1	Tl	0.84
	V	0.74
	Li	0.66
	Sr	0.21
	B	0.05
	Mn	0.33
	Sb	0.69
Cluster 2	Cd	0.66
	Pb	0.82
	Co	0.04
	Cu	0.11
	Ni	0.27
	Ag	0.00
	Cr	0.74

TABLE 15: *Factor pattern for principal component analysis of cremains and concrete groups*

	Component 1	**Component 2**	**Component 3**	**Component 4**	**Component 5**
Sb	0.82	-0.16	0.03	0.06	0.23
B	-0.22	-0.04	-0.25	0.50	0.58
Cd	0.28	0.78	-0.23	-0.15	-0.10
Co	0.08	0.20	0.79	0.36	-0.07
Cr	0.71	0.57	-0.23	-0.05	0.06
Cu	0.01	0.42	0.30	0.21	-0.06
Pb	0.32	0.85	-0.26	-0.08	-0.02
Li	0.80	-0.18	-0.09	0.28	0.21
Mn	0.51	-0.31	0.17	-0.36	0.08
Ni	0.30	0.41	0.69	0.05	0.08
Ag	-0.02	-0.02	0.34	-0.70	0.45
Sr	0.39	-0.30	0.01	0.00	-0.60
Tl	0.86	-0.28	-0.08	0.07	-0.02
V	0.79	-0.33	0.03	-0.01	-0.06
% Variance	28%	18%	11%	8%	7%

TABLE 16: *Linear discriminant functions for cremains and concrete groups with Sb, B, Li, Mn, Sr, Tl, and V*

	Concrete	**Cremains**
Constant	-12.09	-4.78
Sb	0.17	0.28
B	0.01	0.01
Li	-0.13	-0.10
Mn	0.00	0.00
Sr	0.02	0.01
Tl	0.56	0.24
V	-0.09	-0.14

TABLE 17: *Posterior probability of group membership using linear discriminant functions from TABLE 16*

	Cremains	**Concrete**	**Group**
10:90-2*	0.00	1.00	Concrete
25:75-1	0.00	1.00	Concrete
25:75-3	0.00	1.00	Concrete
40:60-1	0.00	1.00	Concrete
40:60-2	0.00	1.00	Concrete
50:50-3	0.01	0.99	Concrete
60:40-2	0.14	0.86	Questionable
60:40-3	0.35	0.65	Questionable
75:25-1	0.44	0.56	Questionable
75:25-2	0.82	0.18	Questionable
75:25-3	0.51	0.49	Questionable
90:10-1	0.91	0.09	Cremains
90:10-3	0.99	0.01	Cremains
Wood Ash	0.00	1.00	Concrete
Canine	0.91	0.09	Cremains
Q1	0.98	0.02	Cremains
Q2	0.93	0.07	Cremains
Q3	0.92	0.08	Cremains
Q4	0.96	0.04	Cremains
Q5	0.80	0.20	Questionable
Q6	0.91	0.09	Cremains
Q7	0.99	0.01	Cremains
Q8[†]	0.99	0.01	Cremains
Q9[‡]	0.78	0.22	Questionable

* This represents human:concrete by mass. Number indicates the source of the cremains.

[†] Individual with indwelling shotgun pellets.

[‡] Individual with indwelling copper-jacketed bullet.

DISCUSSION

All samples were analyzed for the 21 elements. Four elements (Al, Be, Fe, and K) fell outside the calibration curve and one (Mo) failed to produce a linear curve, so they were excluded. The investigation that caused As and Se to be removed from the study also questioned the volatility of Sb, B, Cr, and Mn. These elements are volatile as halides and halogens, especially at the high temperatures of the retort (800 to 1100°C). However, reduced forms of these elements are unlikely in the oxygenated environment of the skeleton. The oxides of these elements have boiling points above the retort temperatures.[26] Thus, 14 elements were used for the statistical analysis of cremains.

The data presented in Table 6 for the concentrations of the known cremains group shows substantially high standard deviation values. None of the 14 elements have standard deviation values within acceptable limits. The same holds true for the values of the concrete group concentrations detailed in Table 7. This is partly due to the relatively small sample size of the group. A larger number of samples could possibly alleviate this. Also, this represents the fact that elemental concentrations for human cremains are spread out over a broad range from one individual to another. This fact makes distinguishing human cremains as legitimate based on expected individual element concentrations an unlikely method. Abnormalities of specific elements within bodies can occur, further deviating a legitimate sample from what would be considered normal if the respective element is a determining factor. Evidence of this is displayed in Table 9, as samples Q8 and Q9 are known to be human remains, but lived with indwelling projectiles left in their person. Lead concentration within both these individuals was elevated, and the person containing the bullet had elevated copper concentration. These values are far beyond the limits of the means of the cremains group, as well as the values of the concrete group. Further examination of Table 9 showed samples that have outliers for one or two elements but are within the mean range for other element concentrations.

Cremain and concrete mixtures were created to increase the size of the questionable group. The known ratios of these mixtures provided a way to compare the concentrations for concrete to cremains. Concrete was the same in each of these mixtures and three different human samples were used. For the mixtures with all three individuals, displayed in Tables 11 through 13, the trends in concentrations for each element as the ratios proceeded from more human to concrete were consistent. Even more encouraging is that the concentrations for each element among the three different tables were very analogous at the similar ratios. The differences in concentration at the same ratios were accounted for by the differences in concentrations contributed by the human part of the mixture. There was suspected error in Table 13, as values for Cr, Cu, Li, Mn, and Ni at the 50:50 ratio were outside the expected trend. Mn had a concentration of 447.7 mg/kg that was well outside the expected outcome. Since the standard deviation for this particular sample was within limits, it is possible there was some sort of contamination of the sample prior to analysis.

The high standard deviation values for elements in each of the known groups as well as the discrepancies that occur as a result of having multiple elements for analysis necessitated the use of multivariate statistical methods. In using these methods the questionable samples were classified as either concrete or cremains. All the elements were not equally useful to this goal. Since the sample sizes of the groups were small, statistics that were less dependent on normality were used. Variable clustering was initially used to discover which elements proved most useful in classification of the questionable samples. In Table 14, two clusters are detailed showing the correlation of each element to the respective cluster. Cluster 1 was more tightly grouped, especially with Tl, V, Li, and Sb having R-squared values above 0.60. The element making up Cluster 2 consisted of the seven other elements that did not comprise Cluster 1, and were less closely correlated.

Principal component analysis was used to further determine which elements were useful for classification. The five components detailed in Table 15 show the factor loadings for each element by component. Component 1's largest loadings were on Sb, Cr, Li, Mn, Tl, and V. Except for Cr, these were also the elements found in Cluster 1 from the variable cluster analysis. Since Component 1 accounts for 28% of the variability this verifies the results found in the cluster analysis. The discriminant functions computed for each group, whose variables are detailed in Table 16, were done using the seven elements (Sb, B, Li, Mn, Sr, Tl, and V) identified in Cluster 1.

The probability of membership to one of the known groups, either concrete or cremains, is detailed in Table 17. For the purpose of reliability, 0.90 probability was used for classification into a group. As expected, wood ash was classified as concrete. The canine sample had 0.91 probability of being cremains, identifying the possibility of distinguishing a mammalian corpse from the concrete.

Of the questionable cremain samples, only samples Q5 and Q9 did not classify. Sample Q1 was classified as questionable prior to analysis despite coming from a reputable source due to an unusual color upon visual inspection. It had 0.98 probability of being cremains, showing that color might not be a reliable factor in considering legitimacy. Five of the Tri-State cremains (Q2, Q3, Q4, Q6, and Q7) were classified as cremains. Sample Q5 (a Tri-State sample) had been stored in a shed exposed to rain and standing water prior to analysis. This presented the possibility of elemental contamination by way of rainwater after cremation.

Samples Q8 and Q9 were both known cremains, but had abnormalities resulting from indwelling projectiles. Sample Q8 had 0.99 probability of being cremains, as the only abnormal value in this sample was with respect to Pb content from the indwelling shotgun pellets. Since Pb was not one of the variables of the discriminant function, it was not a factor in its classification. Sample Q9 had a copper jacketed bullet, giving it abnormal values for both Cu, Pb, and Mn as well. This sample had 0.78 probability of being cremains because Mn, unlike the Cu or Pb, was a variable in classification, and the high concentration of Mn within this sample removed it from the cremains group. The classification for this sample remains questionable, although it does have a higher probability of being cremains.

The human:concrete ratios produced more predictable results. Samples that were 90% cremains classified as cremains. All the mixed samples that were less than 50% human classified as concrete. The samples that were between 50 to 90% human all classified as questionable. Probabilities of being cremains decreased for each of the mixtures as the percentage concrete increased. Once the percentage of cremains in the mixture was below 50%, there was almost no probability that they were cremains. Again the variations seen in the probability of the mixtures being cremains at similar ratios is due to the differences in element concentrations between the different human samples that comprise the mixture.

CONCLUSION

The goal of this project was to create a method of analyzing cremains that focused on the elemental concentrations of the ashes. This came from a need to supplement the current methods of analysis performed by forensic anthropologists. From the data gathered in this study, it is possible to ascertain the legitimacy of cremains using elemental analysis by ICP-OES in conjunction with multivariate statistical analysis.

With the knowledge of what happened at the Tri-State Crematory, it was expected that the samples that came from there would be highly contaminated and classified as concrete by this research. This method classified all the Tri-State samples in this study as cremains with the exception of sample Q5, which was likely contaminated post-cremation. This indicates that the crematory did in fact cremate some bodies to which it was entrusted.[*] More Tri-State samples included in this study could indicate that the cremains were actually concrete.

The data obtained for the ratios of human:concrete bring the issue of what defines *legitimate* cremains. The samples that were 90% cremains were classified as cremains, but were known to be contaminated with 10% concrete. Despite the fact that this sample was contaminated it was still classified as legitimate. This raises the question of contamination for the samples that had similar probability ratios among the questionable samples, but were classified as cremains. So, at what percentage of

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[*] Though, it does not mean the cremains were from the correct individual. Identification of cremains is practically impossible due to the absence of genetic material.

impurity should the cremains be considered illegitimate? Outside of contamination whose source is filler material like concrete added to the urn is the contamination that is from the retort itself. This contamination was accepted in the study and represents natural conditions that were not lab controlled. The methods used in this research will detect large amounts of replacement material, those being greater than 50% by mass, instead of human ash.

Elemental abnormalities that occur among the known human cremains creates a difficulty for this method. For samples Q8 and Q9 these abnormalities were explained by the indwelling projectiles, however, for other samples there was no clear explanation of why element concentrations were outside the normal range. These are a possible result of the environments in which the persons lived. Knowing the environments to which the person in the study were exposed or having knowledge of their medical history would be beneficial to the study. This is especially important if the abnormal element concentrations within the individual is one that is considered in the discriminant functions.

Increased numbers of known cremain and concrete samples is needed for consideration in future research to improve the comparative analysis. Further study into the elements used for classification is also needed to understand their relationship to one another. It would also be useful to include elements that were excluded from this study for availability purposes, but have the possibility of surviving the cremation process and being found in cremains.

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