P-ST019

The Structure and Elements Analysis of Burnt Bone by ICP-OES,PXRD

Kanit Sawasdee

Advisors: Siwaporn M. Smith/ Montip Tiensuwan/ Atitaya Siripinyanond/ Thamrong Chirachariyavej

Faculty of Science/ Mahidol University

Abstract

This study focuses on the identification of elemental composition and crystalline phase cremated human bones. Seventy-five cremated human bones were collected from two cremators in Khoa Katsapha temple Hua-Hin and Prabahtnamphu temple, Lopburi. This work reports the use of powder X-ray diffraction and inductively coupled plasma optical emission spectrometry (ICP-OES) to study the structure and to quantify the concentration of elements present in the cremated bones, respectively. It was found that all cremated bone specimens are composed of hydroxyapatite. In some specimens, tri-calciumphosphate was found as minor phase. Six elements Ca, Al, Mg, P, K and S are found over the detection limit of ICP-OES. For the statistical analysis, it is found that the amount of calcium and phosphorous are significantly different between the specimens from males and females

Keywords: cremated human bone, elemental analysis, hydroxyapatite

The Structure and Elements Analysis of Burnt Bone by ICP-OES, PXRD

Kanit Sawasdee

Advisors Siwaporn M. Smith, Montip Tiensuwan, Atitaya Siripinyanond, Thamrong Chirachariyavej

Science / Mahidol University

E-mail momo_nid@yahoo.com

Introduction

Cremains and burnt human bones are one of the important evidences for anthropological and forensic investigations. According to one of the most notorious cases, the Tri-State Crematory Incident [1], demonstrated that nonhuman fillers ***i.e.*** cement and concrete powder may be put in the urns to resemble human cremains. It was impossible for family members to notice that the powdered materials in those urns were not their family member's cremains. Previous work reported that human cremains can be distinguished from quarzt, sand and others geological apatite by powder X-ray diffraction method [2]. In addition, Timothy ***et al.*** [3] performed a trace element analysis by using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), and reported the concentration of chemical elements in possible human specimens compared with those in nonhuman material. However, their work reported that, out of 21 examined elements, the concentrations of only seven elements, Sb, B, Li, Mn, Sr, Tl, and V, are higher than the instrumental limit. The concentrations of these seven elements were further used as a classification function to identify the presence of human cremains in the powdered specimens being investigated. From their statistical analysis, there were some ambiguous results possibly due to the small sample sizes of human cremains.

Objective/Research Question

This research aims to present a forensic protocol to identify the elemental composition, crystalline phase and chemical composition of burnt human bones. The data obtained from this work may benefit forensic scientist in distinguishing between cremated human remains and other powdered materials being found in crime scenes.

Research Methods

Burnt bone specimens from 75 individuals (43 males and 32 females), with age at death between 20-70 years old, were collected from two crematoria: Khoa Katsapha temple in Hua-Hin, and Prabahtnamphu temple in Lopburi, Thailand. In detail, burnt bones from 19 individuals (9 males and 10 females) and 56 individuals (34 males and 22 females) were collected from the first and the second crematoria, respectively. Burnt bone fragments of each individual were ball-milled resulting in a fine powder which was kept free from dust and moisture for further study. PXRD patterns of the bone samples were recorded on a Bruker AXS D8 Advance powder diffractometer (Cu Kα1 radiation with PSD detector; 2θ range 5-60°; step size 0.075°; step time 0.5 s). Elemental analyses were carried out on a Spectro CirosCCD (Spectro

Analytical Instruments, Kleve, Germany) ICP-OES system, operating with axial configuration. The ICP-OES operating conditions are shown in Table 1. Burnt bone samples were dissolved in conc. HNO_3 (78%) with the aid of microwave digestion. Clear solutions were obtained by dissolving 250 mg of bone powder in 10 ml of concentrated HNO3 followed by heating (160 °C at 40 psi) in a microwave digester (1200 W) for 20 minutes. Further dilution to 500 ppm of bone solution was carried out with 2% nitric acid solution. All diluted samples were kept at 4 °C prior to use.

Table 1 ICP-OES operating conditions.

Plasma Conditions	
RF generator frequency/MHz	27.2
RF power/W	1,350
Nebulizer gas flow rate/L min^{-1}	0.65
Coolant gas flow rate/L min^{-1}	12.0
Auxiliary gas flow rate/L min^{-1}	1.0
Measurement Parameter	
Number of replicate	3
Sample Introduction	
Sample uptake rate/mL min^{-1}	1

Standard solution was prepared before introducing into ICP-OES. In this study, aluminum (Al), antimony (Sb), arsenic (As), beryllium (Be), boron (B), cadmium (Cd), calcium (Ca), Carbon (C), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), lithium (Li), magnesium (Mg), manganese (Mn), nickel (Ni), potassium (K), selenium (Se), strontium (Sr), sulfur (S), titanium (Ti), vanadium (V) and zinc (Zn) were analyzed. Series of standard solutions were prepared as follows:

- 100, 150, 200, 250 and 300 ppm for Ca
- 25, 50, 75 and 100 ppm for P
- l0, 50, 100, 200, 500 ppb and 1 ppm for Al and C
- 1, 10, 50, and 100 ppm for S
- 0.1, 0.5, 0.9 and 2.5 ppm for K
- 5, 25, 50,100, 200, 500 ppb, 1 and 2 ppm for other elements

Results

Powder X-ray diffraction patterns of six specimens of cremated human bones are shown in Figure 1. All samples are mainly compose of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, denoted as HAp, the principal component found in mammal bones. From our observation, the sample from a 76-year old male gave diffraction peaks at which indicated a coexistence of tri-calciumphosphate as minor phase.

The concentration of chemical elements in cremated bone samples were analyzed by ICP-OES. Over twenty elements were focused i.e. aluminum (Al), antimony (Sb), arsenic (As), beryllium (Be), boron (B), cadmium (Cd), calcium (Ca), carbon (C), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), lithium (Li), magnesium (Mg), manganese (Mn), nickel (Ni), potassium (K), selenium (Se),

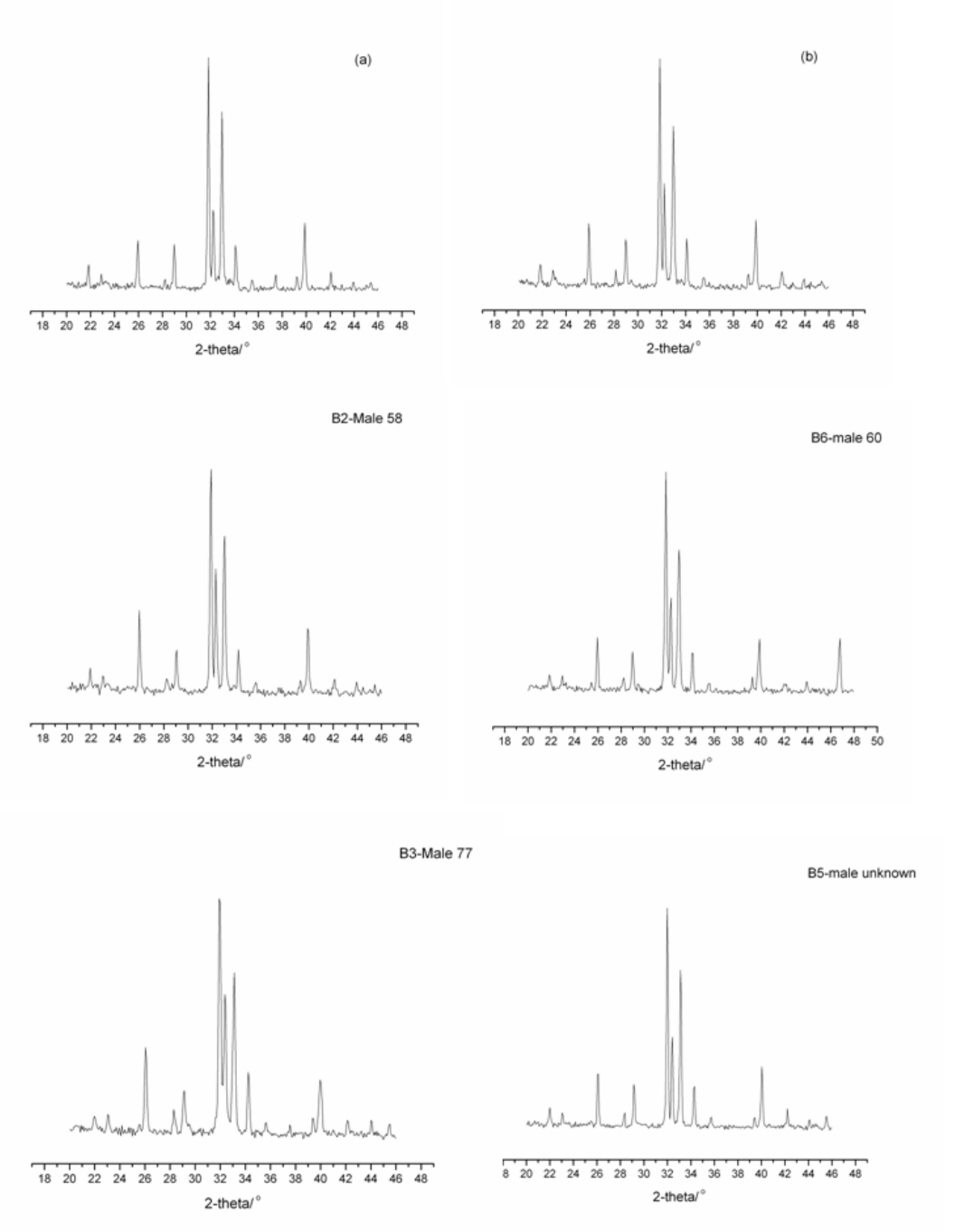

Figure 1 Powder X-ray diffraction patterns of six cremated bone specimens from six individuals.

strontium (Sr), sulfur (S), titanium (Ti), vanadium (V) and zinc (Zn). However, only the following elements, Ca, P, Mg, S, K and Al will be further focused as their quantities are above the instrumental detection limit (IDL), the lowest amount of analyte that can be detected. The instrumental detection limit of all elements studied has been shown in Table 2. For triplicate measurements, ICP-OES results indicated that only six elements, Al, Ca, K, Mg, P, and S were present above the instrumental detection limit (7.0 ppb, 11.7 ppb, 1.2 ppb, 8.8 ppb, 3.9 ppb, and 3.6 ppb for Al, Ca, K, Mg, P, and S, respectively) in the bone specimens. Note that the detection limit is calculated as $(3)SD_{bk}/m$, where SD_{bk} is the standard deviation of the blank measurement, and m is the slope of the calibration curve.

Table 2 Instrumental detection limit (IDL) of samples from ICP-OES measurement.

Observed elements	Wavelength (nm)	Detection limit (ppm/250mg)	Observed elements	Wavelength (nm)	Detection limit (ppm/250mg)
Al	167.078	0.0007	Li	460.289	0.793
Al	308.215	0.0103	Li	670.784	0.792
As	189.042	0.4538	Mg	279.079	0.0088
As	193.759	0.9523	Mn	257.61	0.1096
Be	313.042	0.0011	Mn	260.569	0.1941
C	193.091	0.0637	Ni	221.648	0.0151
Ca	317.933	0.0117	Ni	231.604	0.0138
Ca	422.673	0.0138	P	177.495	0.0039
Cd	214.438	0.031	Pb	220.351	0.0033
Cd	226.502	0.0313	Pb	261.418	0.0175
Cd	228.802	0.0501	S	180.731	0.0036
Co	228.615	0.0109	Sb	206.833	0.813
Co	230.786	0.0188	Se	196.09	1.031
Cr	267.716	0.084	Se	203.985	1.286
Cr	284.325	0.011	Sr	407.771	0.0209
Cu	224.7	0.015	Ti	190.646	0.0022
Cu	324.754	0.025	Ti	334.188	0.0067

Fe	238.204	0.0125	V	290.882	0.0204
Fe	239.562	0.0011	V	292.402	0.0113
Fe	259.94	0.0033	Zn	202.548	0.0016
K	766.49	0.0012	Zn	206.191	0.0022

Preliminary data analysis was carried out to obtain descriptive statistics such as mean ($\overline{X}$), standard deviation (SD) and $\overline{X} + 2\text{SD}$. The results for each descriptive analysis are reported in Table 3.

Table 3 Mean, SD, p-value and $\overline{X} \pm 2SD$ of Ca, P, Mg, S, K and Al in cremated bones for males (M) and females (F).

Element	**Gender**	**n**	**Mean(mg/g)**	**SD(mg/g)**	**p-value**	$\overline{X} \pm 2SD$ **(mg/g)**	
						Lower	**Upper**
Ca	M	129	382.45	22.52	0.00	337.41	427.49
	F	96	412.18	25.47		361.24	463.12
Al	M	129	0.04	0.02	0.45	0.0044	0.0724
	F	96	0.05	0.04		-0.03	0.14
Mg	M	129	6.83	0.68	0.00	5.46	8.19
	F	96	7.15	0.55		6.05	8.24
P	M	129	175.25	4.56	0.18	166.13	184.37
	F	96	174.50	6.78		160.94	188.06
K	M	129	2.24	0.79	0.07	0.65	3.83
	F	96	2.04	0.68		0.70	3.40
S	M	129	4.41	0.92	0.44	2.57	6.24
	F	96	4.39	1.00		2.38	6.40

n = number of samples
mean = average value
standard deviation = dispersion of a statistical population
p-value = statistical hypothesis testing

Discussion and Conclusion

Surprisingly, the elemental species found in cremated human bones in this work are totally different from those reported by Timothy's work [3]. It should be emphasized that some specimens in the previous reported work are questionable whether they were nonhuman materials. Thus, the purity of those cremains specimens is also questionable. On the other hand, in our work, the elemental analysis was carried out for the cremated human bones, which are carefully collected from crematoriums. In addition, the presence of Ca, P, Mg, S, K and Al in the cremated bones is of high possibility as those elements are commonly found in biological system. From Table 3, it was found that the concentration of Ca and Mg are

significantly different between males and females (p-value < 0.05). Although bones mainly consist of inorganic mineral calcium phosphate, ion substitution could occur during recrystallisation due to dissolution and reformation of bone crystal [4] resulted in the co-presence of several other inorganic components such as calcium carbonate, calcium fluoride, calcium hydroxide, citrate as well as other trace elements. From fetal period to mature and post-mature stages, both calcification and dissolution processes resulting changes in the size and shape of bones, and perhaps the chemical composition in bones.

Suggestions

It is highly possible that the concentrations of elements and the elemental fingerprints in burnt bones have some relations with the individual's lifestyle and dietary. To obtain such relations the collected chemical composition data should be statistically analyzed in variable form, representing different values from each individual and each burnt bone specimen.

References

1. D.A. Markiewicz, Few takers for fake cremains; GBI seeks 'closure in crematory scandal; families conflicted, The Atlanta Journal-Constitution Metro News, August 10, 2005 1B
2. Bergslien, E.T., Bush, M., Bush, P.J., (2008). Identification of cremains using X-ray diffraction spectroscopy and a comparison to trace element analysis, Journal of Forensic Science, 175, 218-226.
3 Timothy, R.B., Tom, E.B., Gretchen, E.P., and Stephanie, S., (2006). Elemental analysis of human cremains using ICP-OES to classify legitimate and contaminated cremains, *J Forensic Sci*, *Vol.51*, No.5., 967-973
4 E.L. Smith, R.L. Hill, I.R. Lehman, R.J. Lefkowitz, P. Handler, A. White, Principles of Biochemistry: Mammalian Biochemistry. seventh ed. McGraw-Hill, New York, 1983.