The Use of Radio-isotopes in Forensic Science: The Development of the "Isotope Fingerprint" Analysis.

Thesis submitted for the degree of

Doctor of Medicine

at the University of Leicester

by

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January 2004

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ABSTRACT

The Use of Radio-isotopes in Forensic Science: The Development of the "Isotope Fingerprint" Analysis.

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Forensic Pathologists are often requested to examine remains that have been unearthed during building developments. Once it has been established that the remains are indeed human the question of period of interment arises. Despite the extensive literature published upon this subject it remains notoriously difficult to quantify with the majority of cases relying heavily upon the "experience" of the investigating pathologist. Whether such experience yields correct answers is questionable; corroborating evidence is often absent and therefore the pathologist is unable to recognise any errors in judgement.
It is generally accepted that remains should be no more than 75 years old to warrant police interest. Therefore any reliable dating method should distinguish bones from within this interval accurately from those lying outside of it. Although archaeologists have reliable tools for dating material, pathologists have been unable to devise a method that caters for their specific needs. Previous work has focused upon the physiochemical properties of bone or its organic constituents, though the results have failed to produce a workable calibration system.

The first hypothesis of this thesis has proposed the existence of a predictable and measurable relationship between specific radioisotope concentrations in human bone and the post-mortem interval (PMI). It is predicted that the relationship is such that, once a calibration system has been created, it is possible to accurately estimate the PMI in a set of remains of unknown antiquity. Though concentrating upon ^{210}Pb activities, the study also evaluated additional commonly occurring nuclides, both natural and man-made, the latter being subsequent to nuclear experimentation.

The second proposed hypothesis is that the geographical region an individual lived within becomes imprinted within their skeletal system, such that recognisable relationships between isotopes exist, creating a "radioisotope fingerprint". Examination of these relationships allows identification of the country in which a decedent lived.

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ACKNOWLEGMENTS

The author wishes to express his gratitude to Dr Stuart Black, based within the Postgraduate Research Institute of Sedimentology at the University of Reading, for performing the isotope and trace element analyses, and for assisting with the interpretation of the results.

The interest from the International Commission on Missing Persons, notably Dr. Rick Harrington, is also gratefully received and without whom the study would not have progressed or developed to include additional novel techniques such as geolocation assessment. The East Midlands regional police forces have also played a part in the development of the technique, which is continuing to draw interest from outside organisations such as the United Nations.

The basis of the study lies within the suggestion by Professor Ian Lauder as a means of following-up his seminal work with Professor Bernard Knight from thirty years previous and it is through his initial assistance and continued encouragement that the work was completed. The role of mentor passed over to Professor Guy Rutty who equally has been a source of support in completing the work enclosed within.

I would also like to thank my father, Dr. David Swift, who has helped me through the work with encouragement and advice, and has always been able to help with all queries and questions that arose (despite his comments about being a "real doctor"!). My thanks also extend to my mother, for reasons that are obvious!

Finally, my gratitude extends to Dr Anne Swift who has supported me and provided editorial assistance for the duration of the project. This work is dedicated to her and to Harvey A. J. Swift, who entered our lives during the final stages of its production.

Chapter One - The Background to the Study.

1.1 Introduction

Death is inevitable, though when it occurs is unpredictable. Too often deaths are not witnessed, the deceased instead being found at variable intervals after the event depending upon social factors, location and the mode of death itself.[1]

However, "when did they die?" continues to form one of the main questions that require an answer during an inquest into the death of an individual, presided over by Her Majesty's Coroner.[2]

The answer to this question is often evasive and, in the absence of documentary evidence of collapse, forensic investigators must rely upon additional means for estimating when death occurred. Such evidence for the estimation of the postmortem interval (PMI) may be derived from three main sources:

a) Evidence provided by the body itself,
b) Environmental and artefact-based evidence, that being evidence in association with a body, and
c) Anamnestic evidence, based upon the knowledge of the individual's movements and day-to-day activities.

1.2 The Need for a Dating System

The chronological dating of events is of great importance, not only to historians, geologists and archaeologists, but also to forensic investigators. The ability to produce a theoretical timeline that covers the period of history in question, upon which can be plotted specific points of reference such as the construction of buildings or monuments, the invention of specific items of historical interest, or the lifetime of an individual and their social interactions, is one that aids in the deciphering of proceedings past. Many societies documented their history contemporaneously allowing cross-reference with separate cultures to produce a verifiable historical record. This knowledge has been aided through the use of stratigraphic analysis, being the observation of certain geological strata and recognition of the artefacts

[1] Despite Knight's distinction between two definitions of death, (being cellular and somatic death), for the purpose of this thesis death is defined as occurring when circulatory, pulmonary and neurological activity has irreversibly ceased. (Knight, 1996)

[2] 1) Who was the deceased?, 2) Where did they die?, 3) When did they die?, 4) How did they die, and, 5)What were the circumstances surrounding their death?

contained within (Hunter, Roberts and Martin, 1996). Although occasions where stratigraphy may be beneficial often occur, notably the disposal of human remains within clandestine graves, contemporary forensic investigations require additional accurate and reliable methods of documentation.

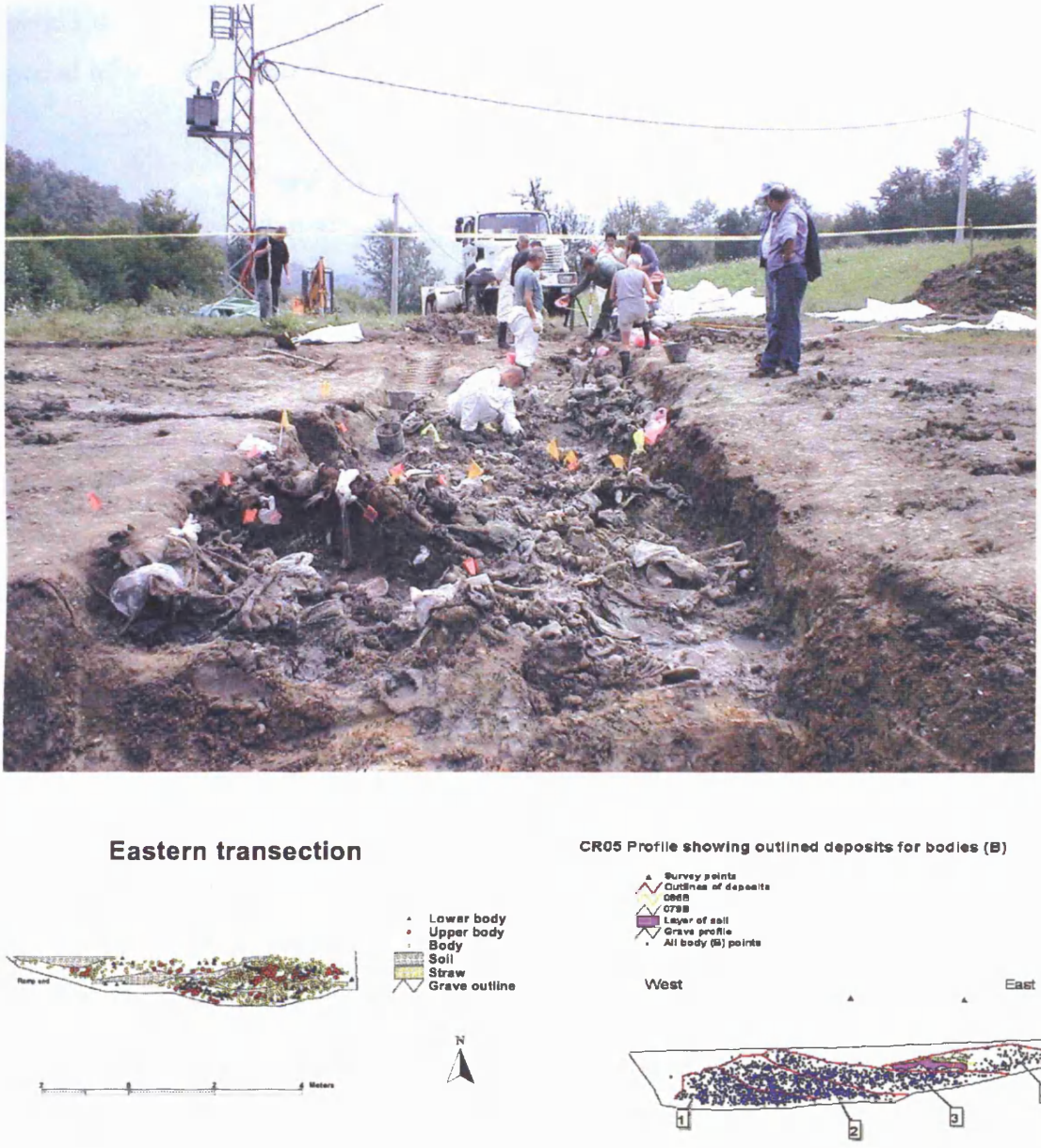


Figure 1.1. A mass grave near Kamanici, Bosnia Herzegovina. Excavation based upon archaeological principles allows the reconstruction of the grave through cross-sectional diagrams. By analysing the data, it is possible to identify stratigraphic layers of human remains, representing separate depositional events. Images courtesy of Dr. Cecily Cropper, International Commission on Missing Persons, BiH.

The search for reliable means for the estimation of the postmortem interval (PMI) has resulted in the production of numerous publications and chapters within forensic textbooks and yet, despite the apparent supernatural abilities of fictional forensic pathologists, there remains no accurate method, especially when examining human skeletal remains. Of the methods published, the majority may be divided into two main categories; those occurring within the early postmortem period, and those during the late. The arbitrarily defined early postmortem period describes the phase in which decomposition of soft tissues occurs, whereas the late period refers to the phase of skeletalisation and subsequent bone-based taphonomic changes.

Chapter Two - The Early Postmortem Interval

The literature on the subject of time since death estimation within the early postmortem interval is extensive, being updated each year with adapted or novel techniques. It is not possible, or practical therefore, to form a comprehensive list of all methods employed and, as such, reference is made to dedicated texts, such as the published work of Henssge, where in-depth information may be found (Henssge *et al.*, 1995).

2.1 Algor mortis

Algor mortis, or postmortem cooling, relies upon the knowledge of a steady state core body temperature during the antemortem period, presumed to be approximately 37°C. Davey first experimented upon temperature measurements from cadavers in 1839, following which numerous methods have subsequently been described (Henssge *et al.*, 1995). The theoretical basis for the test lies within Newton's law of cooling, which states that a body will dissipate its energy (heat) to its surrounding environment at an exponential rate. However, this law is applicable only to regular inorganic spherical objects and as such is inappropriate for complex shapes, such as human bodies. It also fails to account for the effects of clothing, ventilation or physical position of the body.

The principal anatomical sites proposed for temperature measurements are the brain, the skin surface, the nasal cavity, the axilla, the rectum and internal organs (Henssge *et al.*, 1995). Experimentation has revealed that the temperature of bodies fails to conform to the supposed exponential decrease at these sites, producing the lag phase or plateau first described by Professor Rainy, Regius Professor of Forensic Medicine, in 1868 (Rainy, 1868). The result is a sigmoid shaped curve, with a variable lag phase lasting between 0.5-3 hours, thus introducing another unknown into any such assessments. The result was the creation of algorithms and nomograms, required to correct for such anomalies, particular to each anatomical site examined (De Saram, Webster and Kathirgamatamby, 1955;Al-Alousi and Anderson, 1986;Al-Alousi *et al.*, 2001;Al-Alousi *et al.*, 2002;Hensgge, 1988).

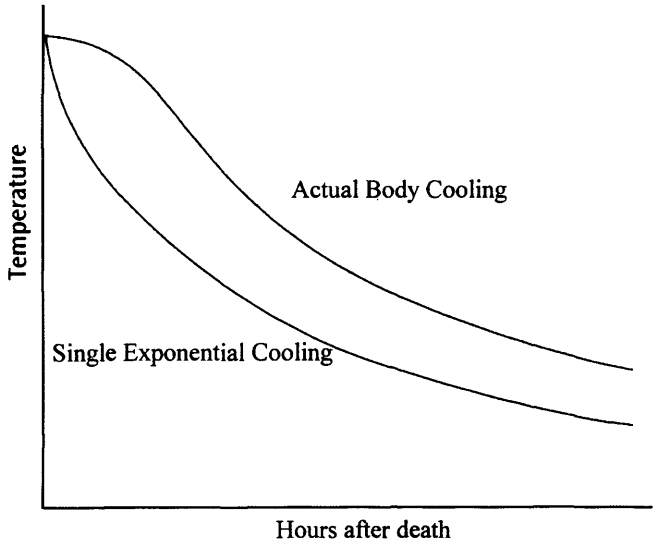


Figure 2.1. The single exponential cooling curve, as represented by Newton's Law of Cooling, compared with the actual cooling curve as recorded in human cadavers. Note the presence of the plateau at the commencement of the latter.

The most frequently quoted nomogram was published by Henssge for use with rectal temperatures. In addition to correcting for the lag phase, the graph also corrects for ambient temperature (Henssge *et al.*, 1995).

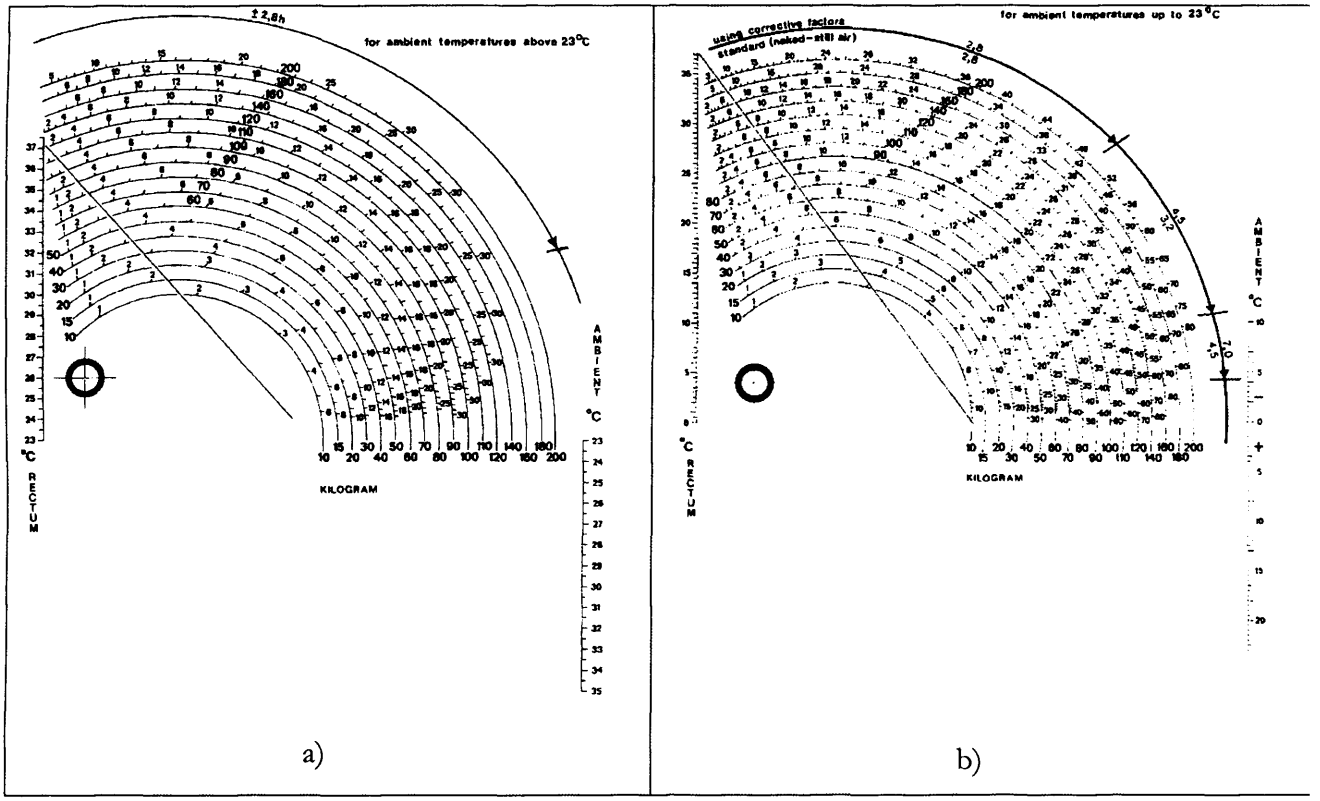


Figure 2.2. The Henssge temperature-time of death relating nomograms for a) ambient temperatures over 23°C and, b), for ambient temperatures under 23°C.

More recent advances have utilised infrared digital thermometers placed within the external auditory canal, the concept being that, in life, the internal carotid artery perfuses the tympanic membrane and thus the temperature is identical to the core body temperature. The results indicate that single temperature measurements recorded from this site can provide accurate methods of estimating the time since death up to 16 hours postmortem, when used in collaboration with a standard algorithm such as Henssge's nomogram for the brain (Rutty, 2001a). It is also noted that, unlike other sites where double or triple exponential cooling curves resultant from lag phases are well documented, there appears to be no lag phase associated with external auditory canal based estimations, resulting in a single exponential curve (Baccino *et al.,* 1996;Rutty, 2001a). External auditory canal based estimations are, however, affected by head position, the presence of wind passing over the cadaver and by natural circadian temperature alterations during the antemortem phase (Rutty, 2001a).

2.2 Rigor mortis

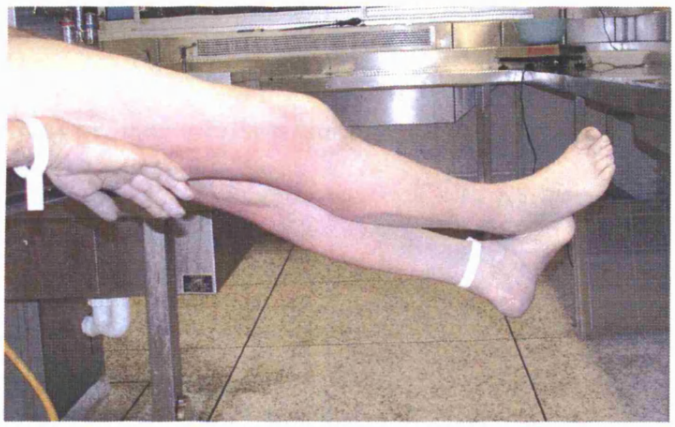


Figure 2.3. Rigor mortis, demonstrated approximately 24 hours after death.

Rigor mortis, or postmortem rigidity, commences after a three to six hour period of muscular flaccidity at death, and lasts up to 36 hours, after which it diminishes (Knight, 1996).
Ultrastructurally, skeletal muscle is composed of two main components; actin and myosin. The interaction between the two proteins produces contraction of individual sarcomeres, resulting in the contraction of the muscles in their entirety. The release of the contraction, and hence the commencement of muscular relaxation, is a process that is dependent upon the concomitant binding of adenosine triphosphate (ATP). The subsequent hydrolysis into adenosine diphosphate (ADP), via an ATPase, releases energy that frees the actin-myosin protein complex (Henssge *et al.,* 1995). The ATP is re-synthesised to allow subsequent

contractions, with energy for this chemical conversion originating from glycogen. As stores are depleted, lactic acid is produced via anaerobic respiration. After death, glycogen resources are rapidly depleted, thus preventing the energy-dependent "breaking" of sarcomere contractions, resulting in rigor mortis. Though previously disputed, true sarcomere shortening similar to antemortem muscle contraction has now been proven to cause rigor mortis (Kobayashi *et al.*, 2001;Liao *et al.*, 2001;Wang *et al.*, 2001). As autolysis commences and the ultrastructural cellular components lose their integrity, the rigor is "released", as is detailed later.

Shapiro previously considered that rigor occurs predictably, first within the temporo-mandibular joint, passing inferiorly to the limbs in a descending and ordered manner (Shapiro, 1954;Gordon and Shapiro, 1975). Though it was assumed that rigor progresses in all muscles simultaneously, the causation of the apparent sequence was suggested to be due to the difference in muscle volume at these sites. However, recent work has disproved this theory, instead explaining the difference through the varying proportion of red and white fibres in individual muscles, the dynamic characteristics of each joint (for example, the elbow being more inherently mobile than the jaw) and the differences in temperature of each muscle (Kobayashi *et al.*, 2001).

By recognising the status of rigor mortis within a cadaver, estimates for time since death may be provided.

Phase of Rigor mortis	Mean with Standard deviations (in hours)	Hours Postmortem				Number of studies
		Limits of 95.5% probability (2sd)		Variations		
		Lower limit	Upper limit	Lower limit	Upper limit	
Delay period	3 ± 2	-	7	<0.5	7	26
Re-establishment possible	Up to 5	-	-	2	8	-
Complete rigidity	8 ± 1	6	10	2	20	28
Persistence	57 ± 14	29	85	24	96	27
Resolution	76 ± 32	12	140	24	192	27

Table 2.1. Time course of cadaveric rigidity, Calculations made from the literature, dating 1811 – 1960. Source: Krompecher in Henssge *et al.*, 1995 after Schleyer, 1975.

Unfortunately, there are many factors affecting the onset of rigor, notably the temperature of the environment, the degree of muscular activity prior to death and the age of the deceased (Knight, 1996). Therefore, despite numerous publications claiming to demonstrate accurate estimation of the postmortem interval based solely upon assessment of rigor mortis, little useful progress has occurred in the field.

2.3 Livor mortis

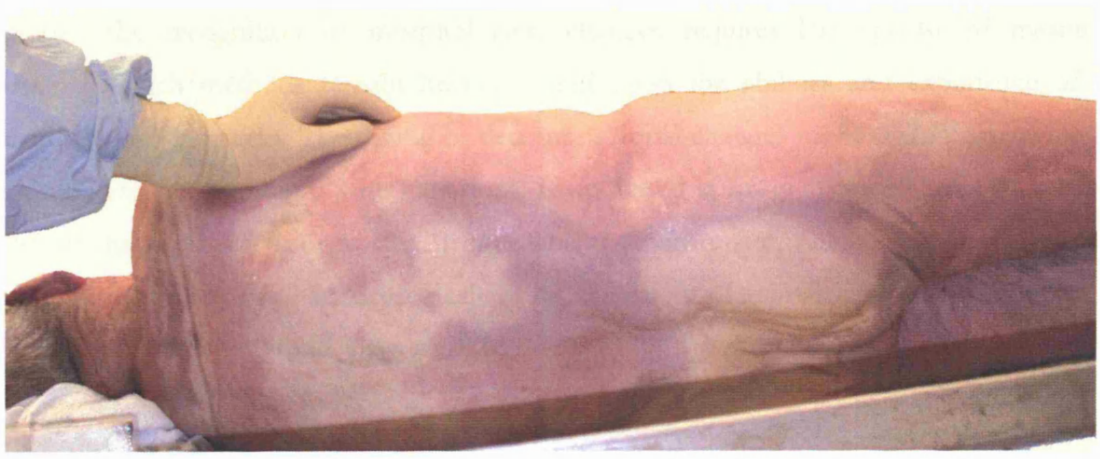


Figure 2.4. Lividity possessing a posterior distribution, suggesting that the deceased had been lying on his back for an appreciable length of time postmortem. The areas of white indicate that pressure from the body against a solid surface has prevented blood from settling within these regions.

Livor mortis, or hypostasis, describes the red-blue hue created in the absence of a cardiovascular circulation, by the gravity-dependent accumulation of blood within small cutaneous or visceral vessels. The spectrum of colouration is great, depending not only on pre-mortem conditions but also on the time since death (Knight, 1996). It is through this recognition that quantitative analysis may assist in PMI estimations; Kaatsch and Bohnert, working independently, examined photometric measurement of livor mortis as a means of assessing PMI, with similar work by Vanezis and Trujillo using a tri-stimulus colorimeter measuring system, though intervals of only up to 48 hours could be estimated with confidence (Kaatsch, Schmidtke and Nietsch, 1994;Bohnert, Weinmann and Pollak, 1999;Vanezis and Trujillo, 1996). Indeed, the rate of occurrence, as well as the distribution and possible re-distribution, is so variable that its use may remain purely within the realm of experimental trials, being unsuitable for the intense scrutiny of medico-legal cases.

Stage	Mean	Standard Deviation	Limits	
			Lower	Upper
Beginning	0.75	0.5	0.25	3
Confluence	2.50	1.0	1.00	4
Maximum	9.50	4.5	3.00	16
Thumb Pressure	5.50	6.0	1.00	20
Complete shifting	3.75	1.0	2.00	6
Incomplete shifting	11.00	4.5	4.00	24

Table 2.2. Hypostasis relative to time since death, as derived from published data (1905-1963), reproduced from Knight, 1996, pg 58.

2.4 Morphological Changes

Although the recognition of morphological changes requires less specialised means of assessment, such methods remain heavily reliant upon the abilities and experience of the individual assessing them. The timing of decompositional changes may assist in narrowing the PMI estimate, but is fraught with variations, many based upon unknown factors such as the health of the deceased prior to death, the ambient environment, (including temperature and humidity), the effects of pharmacological agents, the extent of animal activity and the presence of perimortem trauma (Mann, Bass and Meadows, 1990).

Of these, the environmental conditions in which the deceased laid are the most important and, although animal-based studies continue, little work using human bodies has been performed; published work tends to focus upon the Decay Research Facility in Knoxville, Tennessee. While this small centre remains the nidus of much research, the results cannot be applied internationally, and may even vary within the same locality (Haglund, 2002; Galloway, 1997). Rutty, reflecting upon his experience within the United Kingdom, provides only rough indicators of postmortem changes related to time since death:

Days	Decompositional Changes to the External Surface
1	Green staining of abdomen and flanks.
2-3	Initiation of bloating.
3-4	"Marbling" of the skin.
5-6	Gaseous expansion, skin slippage and "bleb" formation.
14	Marked body swelling.
21	Vesicles burst, eyes expand and tissues soften.
28	Extensive skin liquefaction and blackening.

Table 2.3. Descriptive alteration to bodies in the early postmortem interval.(Source Rutty,2001b)

Caution is advised, however, in giving any definite time since death estimation based solely upon such descriptive findings.

A different morphological method, though only of use for pre-menopausal women, requires the estimation of menstrual cycle based upon the histological changes within the endometrium (Schnabel, Neis and Btratzke, 1997). This is also reliant upon a regular, uninterrupted cycle of known commencement and the absence of autolysis limiting recognition of the uterine phase.

a) b)

Figure 2.5. a)The commemorative statue of Luigi Galvani, Bologna, Italy. b) A close-up of the statue revealing his most famous experiment. Photographs courtesy of Dr. Richard Stitson, Leicester.

Professor Luigi Galvani first established the ability of isolated muscle groups to contract under external electrical stimulation during experimentations with frogs' legs in the 1780s. As the principles became understood the technique was refined, resulting in numerous publications on the subject of postmortem muscular excitability. By subjectively assessing the degree of contraction of specific muscle groups during electrical stimulation, rough estimates of the postmortem interval may be reached (Henssge *et al.*, 1995;Elmas *et al.*, 2001;Querido and Phillips, 2001).

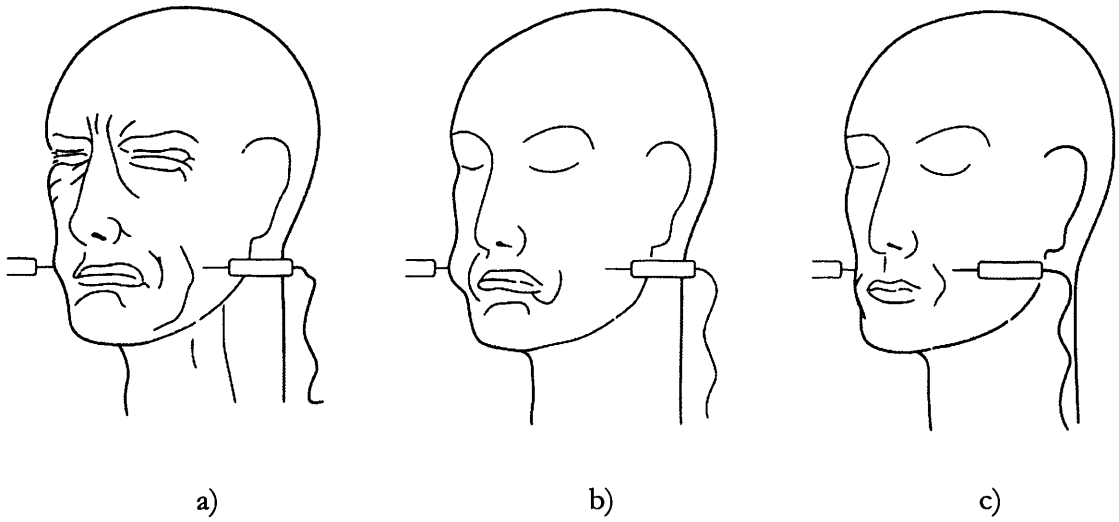


Figure 2.6. Muscular excitability in the postmortem interval: a) Extensive excitability of all facial muscle groups, b) excitability of orbicularis oris muscles only, and, c) fascicular twitching only. (Henssge *et al.*, 1995)

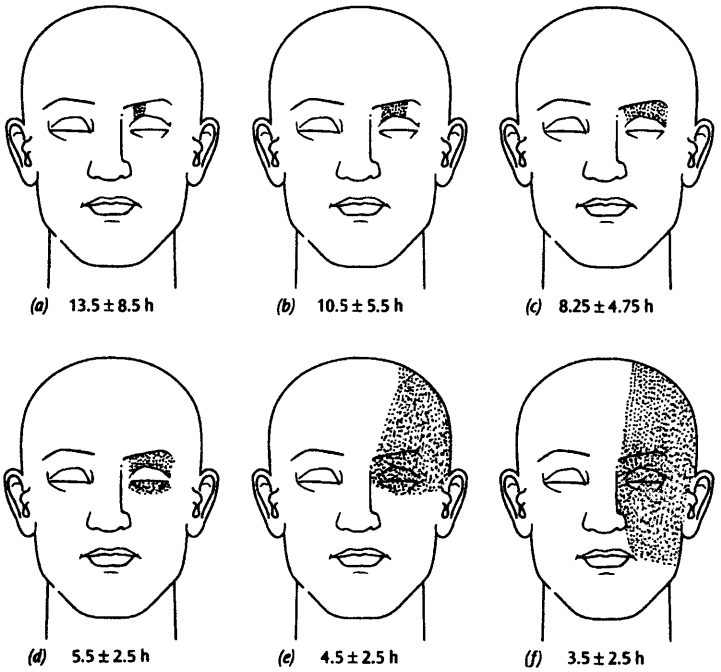


Figure 2.7. Positive stimulation for the orbicularis oculi muscle, with mean values and 95% confidence intervals (in hours) based on Klein and Klein's work, reproduced in Henssge *et al.*, 1995. (Klein and Klein, 1978)

2.6 Gastric Contents Emptying

This method is of limited value, being based upon the assumption that gastric clearing of ingested contents occurs at a predictable rate. Therefore, in the presence of a known time for their last meal, the post-prandial period subsequently elapsed can be estimated providing a time of death. However, these estimates have many confounding variables and are dependent upon not only the type of food ingested, but also the physiological and psychological status of the deceased prior to death.

2.7 Ophthalmological Changes

Methods of analysing the eye have been suggested, ranging from tonometry, (the measurement of intraocular pressure), which may assist only up to 6 hours after the death of the individual, to direct visualisation of the contents of the retinal blood vessels via an ophthalmoscope (Rutty, 2001a). The latter requires the observer's recognition of "trucking", the situation in which the blood separates into distinct units. However, this has been noted within 15 minutes of death, rendering it of limited value (Knight, 1996;Rutty, 2001a).
Reflex contraction of the iris may persist in the early postmortem period in response to the application of an electrical charge, or pharmaceutical stimulation. Localised injection of catecholamine solutions may induce this reflex up to 46 hours following clinical death (Knight, 1996).

Assessment of the chemical constituents of the eye will be considered below.

2.8 Biochemical and Haematological Changes

Postmortem blood samples are notoriously difficult to assess, due to the redistribution of electrolytes and chemicals dissolved within, the loss of cellular integrity and the absence of energy-dependent transmembranous transportation. The extensive animal-based work of Querido has been followed-up in human cadavers by Singh *et al.*, who demonstrated a means of PMI estimation based upon alterations in the serum concentration of potassium and sodium, although external influences continue to affect its accuracy (Querido, 1990a;Querido, 1990b;Querido, 1991;Singh *et al.*, 2002). Later work by the same author also aimed to demonstrate a double logarithmic linear relationship between plasma chloride concentration and time since death, though numerous intrinsic and extrinsic factors appear to alter these findings significantly (Singh *et al.*, 2003).

The blood cells themselves may also assist, with morphological alterations of leukocytes described in the early postmortem period by standard histochemical staining techniques (Dokgoz *et al.*, 2001). Eosinophils, neutrophils and monocytes displayed the earliest recognisable pyknotic degenerative changes (at 6 hours), with lymphocytes becoming altered after 24 hours. The use of this technique appears limited up to 120 hours postmortem. However, these changes are subjective and therefore open to inter-observer variation. The study was also biased in that none of the tests were performed "blind" to the PMI.

The history of analysing vitreous humor chemistry dates back over 40 years, and remains subject to controversy (Henssge *et al.*,1995;Knight,1996;Gong *et al.*,2001;Munoz *et al.*, 2001;Bocaz-Beneventi *et al.*, 2002). The basic principle underlying the test is that the vitreous humor within the eyeball forms a "closed environment" separate to the rest of the body, though is still influenced by ambient temperatures (Knight, 1996). Therefore, the postmortem biochemical changes of the contents may assist, if found to be predictable in their alterations, in estimating the time since death. The most investigated is the potassium concentration, though the hypothesis is somewhat reliant on pre-mortem data, which may be limited given the difficulty with which such biochemical information may be obtained from healthy living patients. Studies have suggested that concentrations increase as potassium leeches out of intraocular cells, creating an exponential increase. There are also suggestions that inter-humoral differences may exist within the eyes of the same individual, though a recent study of 24 individuals found this to occur significantly in only one case (Tagliaro *et al.*, 2001). The possibility of intrahumoral pathology producing spurious results also requires consideration.

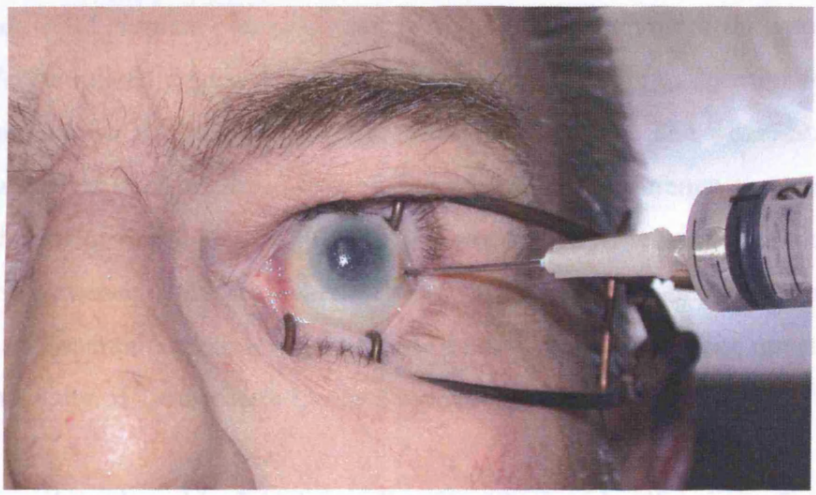


Figure 2.8. The standard postmortem method for sampling the vitreous humor.

The subsequent analytical methods described have become increasingly technical, including the creation of an artificial neural network in collaboration with capillary zone electrophoresis, to assess vitreous chemistry that may improve the predictive value of the test (Bocaz-Beneventi *et al.*, 2002)

Additional studies of vitreous humor have investigated the concentrations of zinc and nickel, though these experiments were within an animal model however and no follow up has confirmed its use in human subjects (Gong *et al.*, 2001).

A similar method has been advocated for both cerebrospinal fluid (CSF) and synovial fluid biochemistry. Like vitreous humor, CSF and synovial fluid reside within closed environments

(Henssge *et al.,* 1995;Madea, Kreuser and Banaschak, 2001). Alterations in potassium, glucose and lactate concentrations are seen to progress in a similar manner (Henssge *et al.,* 1995). A methodology involving synovial fluid may also be of benefit in cases where severe trauma, heat-exposure or decomposition has destroyed the integrity of the globes. However, synovial fluid itself is more viscous, rendering analysis more difficult (Madea, Kreuser and Banaschak, 2001).

Additional studies have analysed bone material for total lipids, proteins, triglycerides and free fatty acids, though only the logarithmic values of protein and triglyceride concentrations correlated to the time since death over a twenty year period (Castellano, Villanueva and von Frenckel, 1984)

Biochemistry has also been used in a novel method of analysis. As early postmortem decomposition progresses, the breakdown products leak from the corpse into the soil, where concentrations of volatile fatty acids and specific cations or anions may be detected (Vass *et al.,* 1992;Vass *et al.,* 2002). Unlike previous studies, Vass' method involves the application of the principles of 'accumulated degree-days', devised by entomologists to account for fluctuations in ambient temperature, to the recorded chemical concentrations. The work, however, fails to address several points such as the effect of altered pH, the difference in soils, exposure to different conditions of burial, or environmental aspects such as temperature alterations, wind exposure or the presence of excess water. Though encouraging results have been gained, the test remains experimental and only applicable to human remains present upon or within the soils of the University of Tennessee's Decay Research Facility, Knoxville.

Additional research within this field is predicted, with the identification of the location of murders possible through the recognition of blood products within soils, even in the absence of the body. Such chemicals have been shown to be present up to 10 years after death. (Hugh Tuller, Personal communication, International Commission on Missing Persons)

2.9 Molecular Techniques

Molecular techniques may also provide information to assist in early PMI estimation. The molecular stability of calmodulin-binding proteins was assessed on rat skeletal muscle through the use of autoradiography (Kang *et al.,* 2003). Lung calmodulin content was also measured by Immunoblot analyses. The results indicated a steady concentration of Ca^{2+}/Calmodulin-dependent kinase II over a 96 hour timeframe, whilst additional binding proteins underwent alteration, possibly indicating future use over short periods of time.

Similarly, cardiac troponin-I, a protein involved in the stimulation of muscle contraction, has been examined through denaturing gel-electrophoresis and Western blot via specific monoclonal antibodies (Sabucedo and Furton, 2003). The results show a correlation between cardiac troponin I degradation and the log of time elapsed since death, which, when assessed against a standard reference material, provides an estimate of PMI, though only over periods up to 5 days.

Immunohistochemical staining for thyroglobulin has also been proposed with a positive reaction described up to 13 days postmortem, though this would be dependent upon the type of antibody used, the technical capabilities of the laboratory and the subjective opinion of the observer (Wehner *et al.,* 2000).

2.10 DNA and Ultrastructural Changes

Following cessation of adequate perfusion and the onset of cellular death, the ultrastructural components of cells undergo autolytic alteration. Nuclear deoxyribonucleic acid (DNA) degenerates into discrete fragmentary lengths, their molecular weight altering as time progresses. The theory follows, therefore, that assessment of DNA denaturation, by such means as hybridisation probe analysis, could provide a method of early PMI assessment (Perry, 1988;Boy, Bernitz and von Heerden, 2003;Lin *et al.,* 2000;Cina, 1994;Liu, 2000;Chen and Chang, 2002). Similarly, messenger RNA (mRNA) degradation has been reviewed (Inoue, Kimura and Tuji, 2002).

Flow cytometry may produce an alternative means of quantifying this degradation and has been applied to both human splenic samples and dental pulp tissue, though with somewhat contradictory results (Cina, 1994; Boy, Bernitz and von Heerden, 2003;Di Nunno *et al.,* 2002).

Although the ultrastructural degradation process does not appear to be individual specific, the rate, like other methods, appears to be dependent upon ambient temperature and humidity (Perry *et al.*, 1988).

2.11 Entomological Methods

"But time has set its maggot on their track."
Was There A Time
By Dylan Thomas (1914-1953)

Tzou Sung first described the forensic application of entomology in the thirteenth century (Sung, 1981). Bridging the transition between the early and late postmortem periods, the study of insect life cycles may assist through two main approaches:

1. Recognition of the time-dependent maturation of Blowfly (Calliphoridae spp.) larvae, and
2. The recognition of species succession over an inferred time frame,

(Erzinclioglu, 2003; Anderson and Cervenko, 2002).

Blowflies, such as the commonly encountered green and bluebottle flies, have a predictable lifecycle, passing through larval maturation phases known as instars prior to pupation and subsequent emergence as an adult fly. Likewise the faunal succession by additional species, such as beetles or spiders, which feed upon the primary insect species present, provides additional means of estimation through the knowledge of the average period elapsed for each species to enter the region of the corpse.

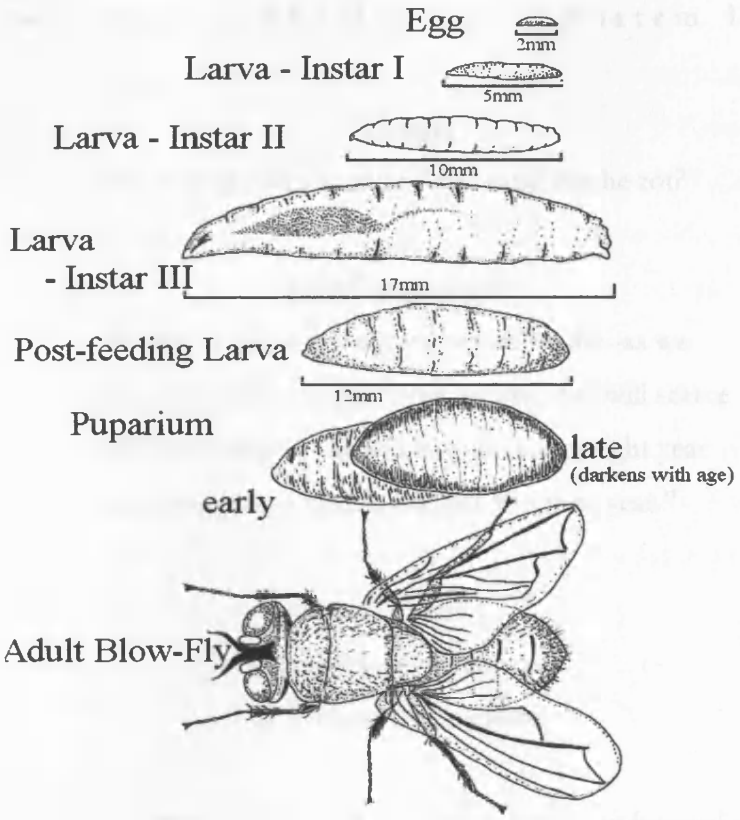


Figure 2.9. An illustration depicting the lifecycle of the blow-fly. The time interval between each stage is dependent upon ambient temperature and is species specific, thus requiring expert entomological opinion in assessing the post-mortem interval.

The periods of time for both of these elements vary depending upon temperature, humidity, manner of death and even the presence or absence of narcotic drugs within the body. The use of 'accumulated degree days' calculations assist by diminishing the effects of the temperature, though expert advice is always recommended to provide accurate PMI estimations. The principle is that the product of the average daily ambient temperature against the number of days exposed will allow evaluation between two different environments. For example;

The maturation of a population of insects after 10 days at 10°C will be the same as a population after 20 days at 5°C.

Chapter Three - The Late Postmortem Interval

"Hamlet
How long will a man lie i' the earth ere he rot?

First Grave-digger
I' faith, if he be not rotten before he die--as we
have many pocky corpses now-a-days, that will scarce
hold the laying in--he will last you some eight year
or nine year: a tanner will last you nine year."

Act V Scene I
'Hamlet, Prince of Denmark',
by William Shakespeare.

When decomposition has advanced, entering the late postmortem interval and resulting in only skeletal elements, the dating of the postmortem interval becomes much more difficult with fewer methods published over the years. These include the use of plant growth and the changes within the remaining skeletal matrix. Those that consider alterations in the bone matrix form the main aspect of this thesis and as such will be considered separately.

3.1 Botanical

"Pile the bodies high at Austerlitz and Waterloo.
Shovel them under and let me work –
I am the grass; I cover all."
Grass
By Carl Sandburg.

Palynology (the study of pollen) and botany can also assist in forensic investigations (Liggett and Swift, 2003); by recognition of pollen species adherent to articles of clothing or parts of the body, seasonal exposure can be indicated, especially within interred remains. Alternatively, rootlet infiltration of remains can similarly aid in estimation through the counting of concentric annual growth rings within perennial plants, similar to that used for dendrochronology. Such methods allow assessment of periods of growth and subsequent dormancy by measuring the cell sizes between each ring, the larger cells indicating a period of

increased growth (Willey and Heilman, 1987). However, growth ring sizes vary between same species plants and irregular growth may introduce eccentric rings producing complications in time assessment. Exposure of rootlets to light, such as may occur following the creation of a so-called 'shallow grave', also inhibits plant growth (Willey and Heilman, 1987). Unlike perennial plants, annual plants do not produce growth rings and, as such, their association with human remains indicates only that the deceased was present before that start of that plant's season of growth.

Although many case studies have been cited where botanical studies have assisted criminal investigations, these methods rarely provide accurate answers; growth rings only indicate the minimal time since death though such techniques may suggest a season or year in which the individual died (Willey and Heilman, 1987).

Chapter Four - The Estimation of the Postmortem Interval in Human Skeletal Remains.

Prior to the main body of this thesis, regarding the development of an "isotope fingerprint" technique within human skeletal remains, a brief review of normal bone histology follows that will provide information of relevance to later discussions.

4.1 Bone Histology

Despite its relatively inert appearance, the skeleton is both dynamic and complex, being constantly remodelled during an individual's lifetime (Bell, Cox and Sealy, 2001). It is composed of an organic phase, being predominantly type I collagen with a smaller non-collagenous protein proportion, and an inorganic phase. The latter constitutes approximately 70% of the total dry weight and is formed from the progressive mineralization of osteoid, a matrix produced by osteoblast cells, resulting in the formation of calcium hydroxyapatite, $(Ca_{10}(PO_4)_6(OH)_2)$, though invariably other ions and elements become deposited within the matrix, such as strontium, lead and barium (Bell, Cox and Sealy, 2001).

The bone is remodelled by three cell types; osteoblasts, osteoclasts and osteocytes.
The osteoblasts are of mesenchymal origin, and are especially prominent under the periosteum and bone trabeculae. These cells secrete a collagen-rich matrix called osteoid, which forms the bone structure.

Osteoclasts are mobile, frequently multinucleate, monocyte-like cells that remodel the osteoid through resorption, ensuring the creation of medullary cavities and allowing biochemical homeostasis of calcium. The resorption of osteoid results in eroded shallows referred to as Howship's lacunae, within which the osteoclasts reside.

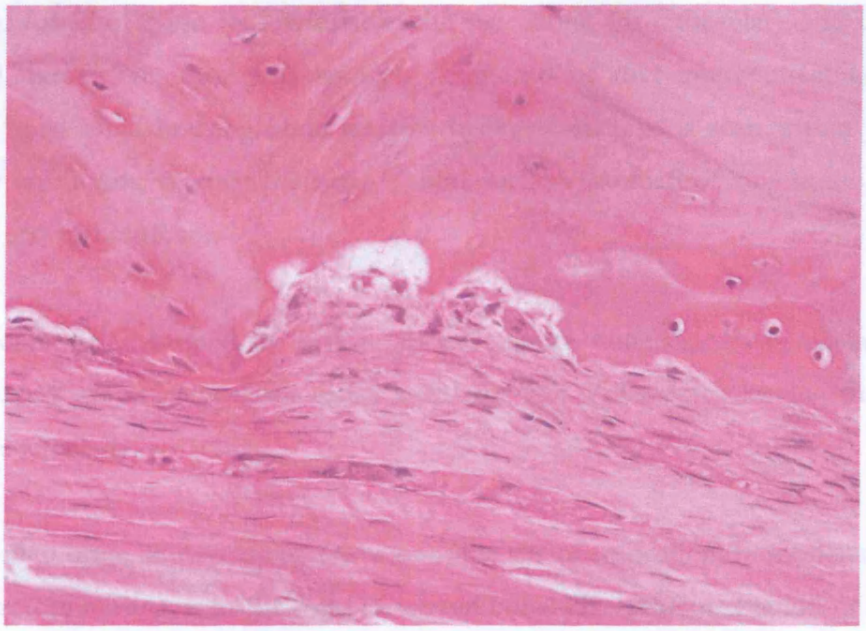


Figure 4.1. The mineralising osteoid (at the top of the image) is being eroded by multinucleate osteoclast cells, located within Howship's lacunae, adjacent to the periosteum.

Osteocytes, it is thought, represent osteoblasts enclosed within matrix. They are identifiable within lacunae and communicate through filapodia, though their function remains uncertain.

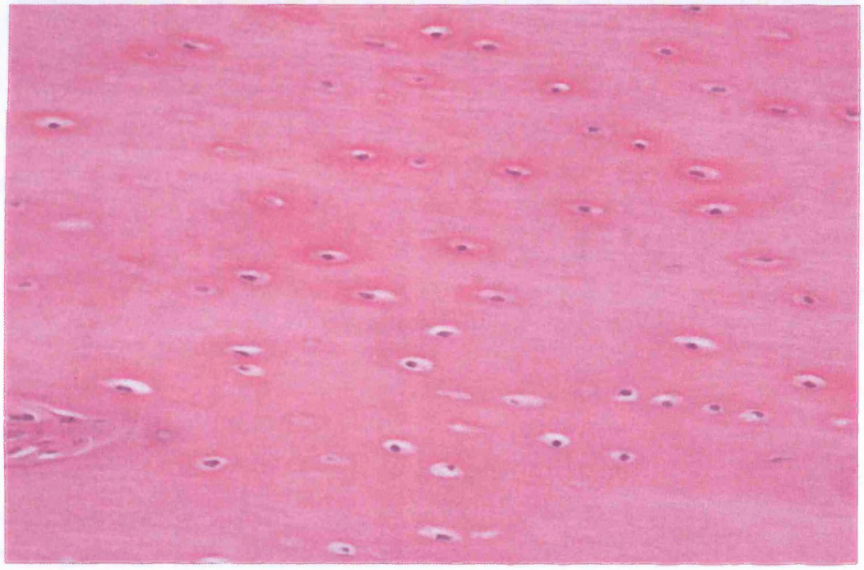


Figure 4.2. Osteocytes are identifiable as individual cells, situated within small lacunae, embedded within the mineral matrix of bone.

The result is a closely controlled dynamic environment created for the purpose of movement, muscle attachment, biochemical homeostasis, soft tissue protection (as with the calvaria), an environment for haemopoiesis and a storage facility for unwanted, or potentially toxic, chemicals such as lead.

Two specific forms of bone are identifiable, the cancellous, (or trabecular), and the compact, (or cortical), bone. The former is spongy, composed of thin interconnecting strands of mineralised bone often housing bone marrow between, such as is seen within the body of vertebrae or within ribs, whereas the latter is solid, such as the shaft of long bones or the bone constituting articular surfaces.

The compact bone is formed by lamellar bone, mature mineralised osteoid laid down in concentric layers around Haversian canals centrally with Volkmann's leaving the Haversian system perpendicularly.

Newly deposited bone, in developing tissue or at the site of resorption/re-modelling such as is seen with fracture healing, is in the form of woven bone. Here, the orientation of the lamellae is more haphazard, being recognisable by viewing histological sections under plane polarised light.

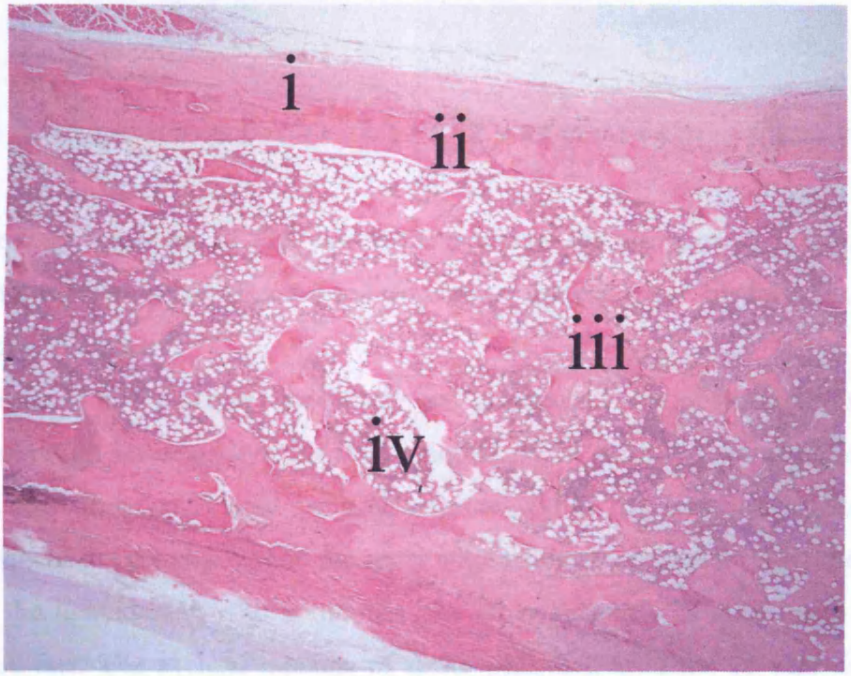


Figure 4.3. A longitudinal section through a long bone revealing i) the periosteum, ii) compact, or cortical, bone, iii) trabecular bone, and iv) haemopoietic bone marrow.

Figure 4.4. Compact bone viewed under plane polarised light, demonstrating a concentric lamellar pattern surrounding central Haversian systems. Lacunae containing osteocytes are also identifiable.

4.2 The Division of the Late Postmortem Interval

To compound the problem of PMI estimations within the late period, it is regarded that human skeletal remains be separated into two distinct groups: those of 'forensic interest' and those of 'historical interest'.

The rather arbitrary difference between these two groupings is the postmortem interval, itself the very point under investigation; for this, a period of 75 years PMI is taken as the cut-off between the two. It is considered by police forces within the United Kingdom that, if a criminal act has been perpetrated and attempts were made to conceal a body, then it remains feasible that the individual(s) who committed the crime may still be alive up to seventy-five years after the fact. The skeletal remains of those who died after the 75-year timeframe are thus considered 'historical' as the chances of convicting anyone diminishes greatly beyond this point. Therefore, for any such PMI dating method to be of forensic use, it must reliably and accurately differentiate between these two categories; herein lies the problem.

The method most commonly employed by pathologists previously has been based purely upon the morphological appearances of the bone. Many authors claim that such an assessment continues to rely heavily upon the experience of the individual examining the bones and yet, unless the individual is identified by additional means such as by DNA extraction or through

the presence of corroboratory evidence interred with the remains, these investigators will fail to learn from their mistakes (Swift, 1998;Swift *et al.*, 2001; Knight, 1996).

4.3 Morphological Appearance

As in the early postmortem interval, during which the soft tissue decays, the appearance of the skeleton alters as time progresses. Within climates such as that of the United Kingdom, skeletalisation is expected within 5-8 years in dry soil conditions, being dependent upon the surrounding environment (Knight, 1996). Following the decomposition of the skin and subcutaneous tissue, muscles that originate from or attach to the underlying bone begin to decompose. The rate is somewhat delayed compared to the former tissues due to the lack of inherent exposure to micro-organisms, unlike the skin or gastro-intestinal tract.

The periosteum and dense connective tissues that are rich in collagen, such as tendons, decompose later and often remain closely adherent for many months or even years after death. The presence of a constant air draught passing over the remains within an enclosed space may, though not invariably, induce mummification thus drying the connective tissue to the bone surfaces.

The presence of animal scavengers within the environment (enclosed or exposed) in which such material remains will also affect the bone. Although rodents may gnaw exposed bone in situ, larger animals, such as canids, may physically remove elements in a predictable sequence, often dragging these elements far from the body (Haglund, Reay and Swindler, 1989). The epiphyseal ends are then chewed open, exposing the metaphysis and medullary cavity, allowing access to the nutrient rich bone marrow within.
The residual soft tissues are removed by fungal, bacterial and insect activity, notably beetles. The bone then undergoes additional changes, though alterations that are recognisable to the naked eye may take decades or even centuries to progress.

The organic components of the bone are then degraded further through continued micro-organism action, with bone itself being destroyed, potentially in as little as 25 years (Pollard, 1996).

4.4 Time-dependent Morphological Alterations to the Bone Matrix.

"First Grave-digger

Here's a skull now; this skull has lain in the earth
three and twenty years."

Act V Scene I,
'Hamlet, Prince of Denmark'
by William Shakespeare.

Initially, bone remains relatively fresh, possibly with blood product evidence still identifiable. Adipose tissue from the medullary cavity leeches out under exposure to warm temperatures, resulting in the "greasy-to-the-touch" feeling to recent remains described by Knight (Knight, 1996). The continued presence of organic components results in a 'heavy' feeling to the bone and, upon exposure to the frictional heat experienced during sectioning of the bone, the smell of burning organic material is appreciable, though admittedly somewhat subjectively so.

As will be described in due course, the organic stromal component is, in the majority, composed of collagen. Like any other protein material, the alpha-helical polymers of collagen are broken down through enzymatic action and heat exposure, with the resultant protein fragments being metabolised by organisms associated with the remains. The result of the continued degradation of collagen is the loss of the organic component of bone, visible on sectioned bone as the concentric loss of the normal appearance on the internal (medulla cavity) and external (subperiosteal) aspects. Organic-poor bone appears 'chalky' and, given that the surfaces may be exposed to environmental degradation, loss of their collagen content tends to occur here first. Therefore, a cross-section of material in which protein degradation of the organic phase of bone had commenced demonstrates a concentric ring of remaining minimally altered bone deep within the compact bone, between organic-depleted matrix (Knight, 1996).

The type of bone itself also affects the rate at which it undergoes postmortem alteration. Large dense compact-rich bones, such as femora, tibia and humeri, are slower to decompose than those that are trabecular bone-rich or possess larger surface area to volume ratios, such as

phalanges, vertebrae and calvarial bones. These issues will be discussed in detail within later chapters.

It should be noted, however, that these morphological alterations are far from diagnostic; in Knight's experience, bones of several millennia antiquity have been noted to retain an excellent morphological appearance, yet the skeletons of those recently deceased which have lain exposed to peat (within which low pHs are experienced) have almost completely decomposed (Knight, 1996).

Despite the fact that the majority of estimates continue to be performed on morphological grounds, the inherent inaccuracies of this method reinforces the requirement for a reliable and accurate method for estimating the time since death in human skeletal material.

4.5 Microscopic Methods

The microscopic alterations in human bone during the late postmortem period have also been assessed.

Within their seminal paper detailing methods of PMI estimation, Berg and Specht concluded that histological changes were evident only after a postmortem interval of ten years, being erosive changes identifiable initially within the Haversian canals and the interstitial lamellae (Berg and Specht, 1958). However, it would appear that quantification of these changes would not allow assessment of the time since death.

Nokes *et al.* analysed five specimens, dating from 11 years to 3000 years PMI (Nokes, Green and Knight, 1987). Sections of compact bone were removed from each specimen, polished flat and enzymatically treated. Following appropriate preparation, the material was examined within a scanning electron microscope. Despite such marked differences in time since death, Nokes *et al.* concluded that the degeneration of the matrix into an appearance of particulate aggregates between 30 and 50nm in diameter was recognisable in all specimens and, thus, the technique was unable to distinguish ancient from modern material. There was no subsequent attempt to identify the constituents of these aggregates, nor was there any evidence that the investigators had excluded the possibility that these changes were actually artefacts introduced by excessive enzyme treatment.

A subsequent publication by Yoshino *et al.* attempted to re-examine the initial findings of Berg and Specht, by investigating the issue of erosive tunnelling (Yoshino *et al.,* 1991). Yoshino *et al.* used compact bone specimens (humeral shaft segments) placed within three different environmental conditions; within soil, within the sea or exposed to the air. The postmortem intervals varied between 0 to 15 years, with all specimens being analysed through microradiography and scanning electron micrography.

Microscopic analysis of air-exposed bones showed alterations only after 15 years, these being the presence of erosive fungal and bacterial tunnels forming characteristic labyrinthine spaces (This topic will be discussed in further detail within later chapters). Prior to this time, few changes were recognisable.

The samples within soil showed similar tunnelling after 2.5 years within both the internal and external circumferential lamellae, being tunnelling recognisable histologically after 5 years when fungi or bacteria infiltrate the mid-zone of the substantia compacta. The result is a loss of both organic and inorganic content, evidenced by the microscopic recognition of erosive changes within both collagen fibrils and the calcified matrices.

Although these results, unlike those published by Nokes *et al.,* demonstrate structural alteration of bone postmortem, they are reliant upon the environmental conditions to which the bone has been exposed and, hence, the subsequent activity of micro-organisms. Like Berg and Specht, no attempt is made to provide a calibration means based upon the quantitative changes recognisable, instead purely stating a minimum time of exposure to soil, air or seawater in which such changes have been observed. In itself, this also fails to take into account the length of the preceding early postmortem interval, as defleshed bone was used within Yoshino's publication. Thus, the conclusion reached with this method fails to address the additional variations that also exist within this timeframe, which in turn are themselves also dependent upon ambient environmental temperature, soil and burial conditions, and the humidity of the surroundings.

Chapter Five - Analytical Methods for PMI Estimation in Skeletal Remains.

As previously mentioned, analytical methods for estimating the postmortem interval are few and often based upon small studies involving numerous techniques. These techniques may themselves be divided into:

i) those measuring physiochemical or serological changes, and

ii) those measuring alterations in radio-isotope concentrations, which will form the remaining part of this thesis.

5.1 Physiochemical and Serological Changes

Berg and Specht, 1958

The first formal investigation into the dating of the postmortem interval in human skeletal remains was published in 1958 by Berg and Specht in which morphological, histological and physiochemical tests were applied to bones of known PMI. Berg later refined this work, by considering a range of techniques from the simple, such as specific gravity, to the complex, including supersonic conductivity (Berg, 1963). However, his conclusions were that the macroscopic examination by an experienced pathologist remained more accurate than any of the tests.

Knight and Lauder, 1967

Despite Berg's findings, Knight and Lauder, in a study of 68 dated samples, aimed to produce a method that was less time-consuming and yet was relatively accurate (Knight and Lauder, 1967;Knight and Lauder, 1969). By employing several predominately physiochemical methods to test bones of known PMI, the research was aimed at identifying which tests may provide the most information regarding the time elapsed since death. Although no one individual test was advocated, a series of separate methods were highlighted to aid forensic investigators in their judgements:

a) Nile Blue and dichloroindophenol staining
b) Reaction with mineral acid
c) Nitrogen content
d) Amino-acid content, including 'free' amino acids.
e) Benzidine reaction
f) Ultra-violet (U.V.) induced fluorescence
g) Anti-human sera immunological reaction, and
h) Fat estimation.

The conclusion of the authors is that many of these techniques failed to discriminate accurately between bones of antiquity and bones of forensic interest. Of the tests described, only four showed a direct correlation with the PMI, being;

i) A loss of nitrogen – retention of greater than 2.5% by weight nitrogen suggests the time of death to be less than 350 years; results above 3.5% suggest less than 50 years.
ii) An overall reduction or loss of particular amino acids from the organic phase of the bone: Fewer than seven remaining amino acid types suggest a PMI of more than 100 years. However, the specific loss of proline, or hydroxyproline, suggests the bone is ancient.
iii) The progressive loss of inherent fluorescence pattern of the cut bone surface. The fluorescence diminishes after 100 years PMI, with concentric loss up to 800 years since death.
iv) Loss of immunological activity or benzidine staining after 5 years and 150 years, respectively.

Given the extent to which the work of Knight and Lauder is referenced in the literature regarding the dating of human skeletal remains, each of these recommended tests will be discussed and critiqued in detail.

5.2 Nitrogen Loss

Amino acids constitute the major nitrogen-containing molecules within the organic phase of bone tissue. Therefore, progressive loss of proteins through decomposition results in a loss of nitrogen content. This may be estimated through assays with concentrated sulphuric acid and subsequent titrations to allow a calculation of original nitrogen content, though automated

methods are used more frequently in modern laboratories. Knight assessed several bone types of known PMI, ranging from "limb bones" to skull fragments, by using a micro-Kjeldhal method, with digestion through a potassium sulphate-copper sulphate-selenium dioxide catalyst system (Knight, 1969).

As suggested, decomposition of the bone results in a steady reduction in nitrogen content, though, as with previously described methods, humidity, exposure to air and both the acidity and the temperature of the ambient environment alter this rate. Despite collagen representing the major bone protein type, some authors consider protein identified within bones of antiquity to originate from the preservation of non-collagen sources (Schoeninger *et al.,* 1988).
The nitrogen content of bone in a live individual, though prone to variation between persons, is regarded as approximately 4.5% by weight (Knight, 1996). Therefore, the difference between time zero and 350 years PMI is, potentially, only 2% by weight nitrogen content, allowing little possibility to improve the accuracy of this estimation.
The surface area to volume ratio also influences these changes and, as would be expected, the protein content of smaller bone fragments experience decompositional alterations earlier than larger, intact bones (von Endt, 1980).
Overall, decompositional changes result in an unpredictable linear decrease in protein content, the rate of which is influenced by numerous external factors. The possibility of fertilisers added to the soils in which remains lie, thus potentially influencing intrinsic nitrogen content, has yet to be examined either. As such, the accuracy and reliability of this method for use within forensic investigations is questionable.

5.3 Amino Acid Content

The amino acid content was extracted by heating bone samples in a hydrochloric acid solution and analysed, in the case of Knight and Lauder, by 2-D chromatography (Knight and Lauder, 1967;Knight and Lauder, 1969). The results of their work suggested that, of the fifteen amino acids naturally present in human bone, proline and hydroxyproline provide the most useful information, despite other amino acids such as glycine and alanine being present in higher concentrations.
Depending upon the storage environment, proline and hydroxyproline are absent in specimens of postmortem intervals greater than 50 years, making them useful in forensic investigations. Other amino acids are later lost over subsequent decades, though glycine may persist for millennia (Knight, 1996).

However, the authors admit that their method was "poor", resulting in technical difficulties identifying the presence, or absence, of particular amino acids. Four of the samples from the 'forensic interval' failed to demonstrate any proline and/or hydroxyproline content (Knight, 1969). Conversely, three of the 16 archaeological bones contained measurable proline/hydroxyproline content.

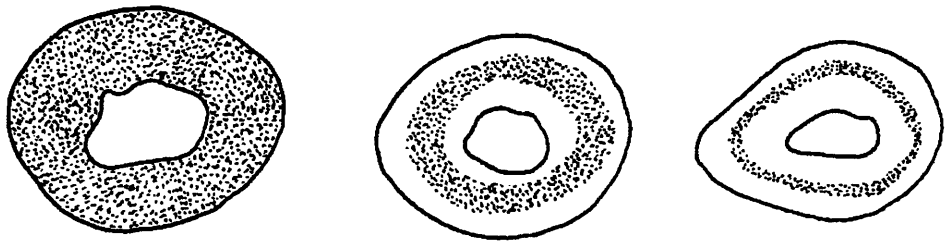
How are these results explainable? Proline and hydroxyproline are water-soluble and therefore vulnerable to the effects of water passing through bone, decreasing the internal concentrations. Also quantities of hydroxyproline are present within ground water itself which, theoretically, may wash into bones producing false results (Mays, 1998). The degree of acid hydrolysis, including the ability to lyse all peptide bonds and thus release all amino acids present, may have also affected the resultant profiles produced by Knight and Lauder.

Additional studies have shown a virtually normal amino acid profile in a 2000-year-old sample, suggesting that it may not be purely the presence of the amino acid types, but the percentage content compared to that within living bone. A total amino acid concentration greater than 10% of that present within modern (time-zero) bone maintains the normal ratio and profile of amino acid types; below this percentage the profile is altered significantly (Hare, 1980).
Zinc concentrations are also said to decrease during this period, presumably as the metal ion binds numerous biological proteins. Thus, the loss of zinc reflects the loss of protein content (Castellano, Villaneuva and von Frenckel, 1984).

Although amino acid content may naturally alter in a somewhat recognisable manner, it is prone to too many unknown variables. As such, it is not recommended as a sole means of estimating the time since death.

5.4 Bone fluorescence

The blue-white fluorescence of the cut diaphyseal bone surface seen under ultra-violet light is also said to alter with time. Knight describes the concentric loss of fluorescence, beginning around the medullary cavity, between 3-80 years PMI, with subsequent loss of the subperiosteal fluorescence. The result is a "sandwich" of residual fluorescence that diminishes over subsequent centuries (Knight, 1996). Japanese publications confirmed this effect, observing fluorescence at 460nm UV, and quantification of the effect suggested that a loss of 20% intensity occurs within fifteen years of death (Yoshino *et al.*, 1991).



a) b) c)

Figure 5.1. Bone fluorescence over the postmortem interval: a) up to several decades PMI the whole cross-section of bone demonstrates a blue fluorescence under ultra-violet light, b) from 3-80 years, the subperiosteal and peri-medullary bone show progressive loss of fluorescence, and, c) the fluorescence diminishes after a PMI of a century, disappearing entirely in the second century. After Knight (Knight, 1996)

Facchini and Pettener (1977) furthered Knight's original research, though using a different specific UV source to Yoshino (366nm). They confirmed the 'sandwich effect' of fluorescence in bone material from Medieval periods and, to a lesser extent, material over 2000 years old. They concluded that fluorescence could be extended up to 350 years, although they attributed the physical attribute to the organic phase of bone, contrary to a previous publication that suggested it was due to the mineral component (McLean and Urist, 1968). If the latter were the case, then diagenetic recrystallisation could affect the fluorescent properties of bone, rendering the test prone to inaccuracies.

5.5 Immunological and benzidine stain reactions

Benzidine reactions detect the presence of blood components, notably haemoglobin, within the bone matrix. The survival of blood proteins over variable periods of interment is unknown, though Knight suggests that it may occasionally be demonstrable through this method up to 150 years after death (Knight, 1969). However, the method is fraught with false-positives. The chemicals are also now considered carcinogenic, with the method being altered to employ aminobenzidine instead (Pollard, 1996). However, negative results have been obtained with bones of forensic interest, being less than 50 years PMI (Knight and Lauder, 1967;Knight and Lauder, 1969).

Knight also used the Anti-Human Serum reaction (AHS), though negative results were produced after only 5 years PMI (Knight, 1969). It was thought that this was highly reliant upon the quality of the antisera available and the concentration used.

Introna *et al.* investigated the use of luminol, an alkaline reagent commonly employed for the identification of bloodstains due to the peroxidase activity of the heme portion of haemoglobin (Introna, Di Vella and Campobasso, 1999). The objective was to identify blood remnants in skeletal remains, but, unlike methods such as those described above, the reaction of luminol creates light, such that it may be photographed for evidential purposes.

Introna *et al.* performed the test upon eighty bones, (postmortem intervals ranging from 1 month to 60 years), the intensity of the bioluminescence being graded both by naked-eye observations and grey-scale image analysis. The inevitable intra- and inter-observer bias variations failed to be addressed, though through the image analysis software the investigators claimed to be able to identify a decrease in percentage activity after 10 years PMI. The test appeared to be ineffective after 50 years. Follow-up studies currently being undertaken aim to reduce the observer bias through the use of high resolution computerised image analysis with initial, unpublished results showing promise.

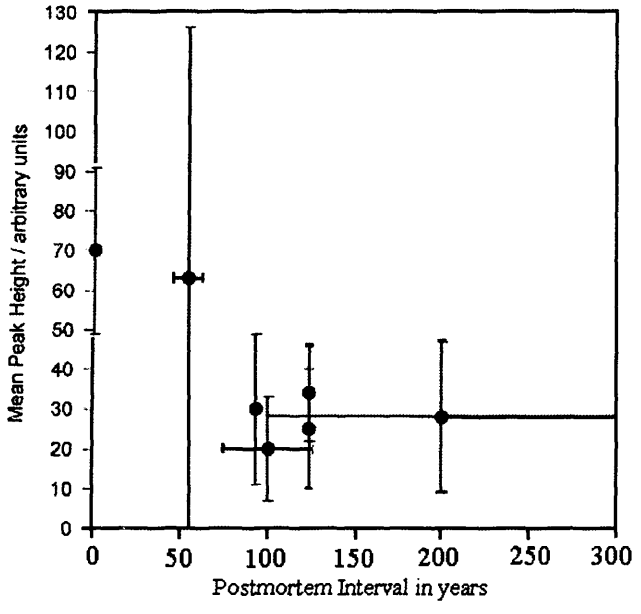


Figure 5.2. The computer-based image analysis results for luminol fluorescence in a collection of human skeletal remains, each being of known PMI. These findings indicate a correlation may exist, though the wide statistical margins may limit these results. Courtesy of Dr. A. Buck, University of Western Australia, Perth.

5.6 Conclusions

Physiochemical testing methods may assist in estimating the postmortem interval, but only when used in conjunction with additional methods. No single reliable method has yet been identified.

These methods are also reliant upon specific environmental conditions; deviation from such conditions alters the results in an unpredictable manner, which cannot be accounted for after the fact. For example, the water table may rise, affecting bone chemistry, but only during specific seasons and, being dependent upon rainfall, may not occur every year of the postmortem interval. The forensic investigator will not usually be aware of these temporary changes in soil environment.

The geographic location also affects rates of decomposition, as alluded to previously. The results from a temperate climate cannot, therefore, be applied to the conditions within a tropical country, or an arid climate. However, it should also be noted that variations might occur within the same locality (Haglund, 1997).

It is therefore considered that the ideal method for estimating the postmortem interval of human skeletal remains is one that is not affected by the surrounding environment to which it lies exposed. Such a means could be seen as resembling a stopwatch, being internal to the bone itself and therefore present in all bone types. The inability to "reset" the stopwatch is of great importance, allowing quantitative assessment and therefore producing an accurate, reliable and reproducible method based upon existing accepted scientific principles.

Chapter Six - Radioisotope-based methods.

Prior to commencing the following section, a brief introduction to isotopes is provided:

6.1 Isotopes

Isotopes are described as elements from the same chemical or elemental family that vary in atomic weight due to the presence of additional neutrons such that, despite similar chemical attributes, they may display different physical attributes. The term is derived from Greek, meaning "equal places", referring to the fact that these elements occupy the same position on the periodic table.

Of the 110 elements recognised, only 21 appear to exist in a natural "pure" single form; varying quantities of isotopes form the remaining. Of the isotopes, many remain unchanged over time. These are the so-called stable isotopes, whereas those that demonstrate spontaneous decay of their nuclei are referred to as radioactive isotopes (also referred to as radioisotopes or radionuclides). During the nuclear decay, radioisotopes emit a variety of particles that could constitute an ionising radiation risk to the environment.
By way of an example, carbon exists as two stable isotopes and a single main radioisotope, as will be described later:

^{12}C, ^{13}C = stable isotopes
^{14}C = radioisotope.[3]

Therefore, the sum of their atomic weights against their prevalence in the environment produces the overall atomic weight of the elements that, in the case of carbon, is 12.0107.

The decay of radioisotopes is predictable, resulting in the production of half-life estimates for each. The half-life is defined as the time taken by a radionuclide to decay to 50% of the original number of atoms present. As they decay the atom changes, resulting in the potential creation of "daughter-isotopes". Ultimately such decay chains terminate as stable isotopes.
The half-life is specific and varies for each radioisotope. For example, Bismuth-210 has a half-life of 5.013 days, whereas Uranium-238 continues to remain abundant in the environment

[3] It should be noted that other radioisotopes of carbon have been described experimentally, though the half-lives are measured in milliseconds or seconds and remain footnote material only.

owing to its half-life of 4.468 billion years. Of the nearly 4000 isotopes currently known to man, most remain confined to the laboratories that artificially created them due to their extremely short half-life.

The manner in which isotopes decay is also of importance biologically. As mentioned above, when isotopes decay they emit specific particles as radiation. These particles denote the type of decay, and hence the form of radiation:

Alpha (α) decay – this type of decay results from the emission of an alpha particle, (a doubly charged helium nucleus (He^{2+})), such as seen with ^{210}Po, ^{239}Pu and ^{241}Am. Alpha particles are relatively large and readily stopped by thin barriers, including the skin surface, thus external radiation risks to humans are minimal. However, internal doses (through ingestion, inhalation or across cutaneous wounds) require monitoring due to the ability of alpha particles to interact with surrounding atoms, through the double-charged nature of the particle, which results in the release of its nuclear energy over small volumes. Therefore, cells exposed to the energy within alpha particles are usually destroyed, such as may occur with haemopoietic cells.

Beta (β) decay – this type of decay commonly occurs in the form of the emission of a singly charged unpaired electron (beta particle) and results in the conversion of a neutron to a proton (β- decay), such as seen with ^{210}Pb, ^{137}Cs and ^{40}K. The depth of penetration by beta particles is an individual function of their energy, thus not only producing similar internal radiation exposure concerns as alpha particles, but also a degree of external radiation exposure risk as the particles are able to penetrate into the "germinal layer" of skin.

Gamma (γ) radiation – Gamma radiation is the release of an electromagnetic pulse from an excited atomic nucleus. Due to its almost insignificant mass, gamma rays are able to penetrate most material, and all human tissues. It is also frequently produced during alpha or beta emission.

For the purposes of wider discussions, radioisotopes may also be broadly divided into two main categories: Artificial (Man-Made) and Naturally Occurring radioisotopes, some examples of which are provided below:

Artificial Radioisotopes	Half Life	Decay Mode and Specific Activity	
Strontium-89 (^{89}Sr)	50.53 days	β-	SpA = 2.905×10^4 Ci/g
Strontium-90 (^{90}Sr)	28.79 years	β-	SpA = 136.4 Ci/g
Yttrium-90 (^{90}Y)	64.00 hours	β-	SpA = 5.44×10^5 Ci/g
Caesium-135 (^{135}Cs)	2.3×10^6 years	β-	SpA = 1.151×10^{-3} Ci/g
Caesium-137 (^{137}Cs)	30.07 years	β-	SpA = 86.98 Ci/g
Barium-140 (^{140}Ba)	12.752 days	β- γ	SpA = 7.317×10^4 Ci/g

Table 6.1. Examples of artificial (man-made) radioisotopes, with their half-lives, method of decay and specific activity.

Naturally Occurring Radioisotopes	Half Life	Decay Mode and Specific Activity	
Potassium-40 (^{40}K)	1.277×10^9 years	β- β+ γ	SpA= 5.65×10^{-6} Ci/g
Radium-228 (^{228}Ra)	5.75 years	β- γ	SpA = 272.7 Ci/g
Actinium-228 (^{228}Ac)	6.15 hours	β-	SpA = 2.24×10^6 Ci/g
Actinium-227 (^{227}Ac)	21.773 years	β-	SpA=72.34 Ci/g
Lead-210 (^{210}Pb)	22.3 years	β-	SpA = 76.3 Ci/g
Radium-226 (^{226}Ra)	1600 years	α γ	SpA = 0.989 Ci/g
Polonium-210 (^{210}Po)	138.376 days	α	SpA = 4.493×10^3 Ci/g

Table 6.2. Examples of naturally occurring radioisotopes, with their half-lives, method of decay and specific activity.

However, it should be noted that several radioisotopes could be considered to be both naturally occurring and produced in significant quantities through the activities of man, the most notable example being radiocarbon (carbon-14,^{14}C). Considering the broad ranging applications of radiocarbon in historical studies, this particular isotope will be discussed first in isolation to these main category headings.

Radiocarbon (^{14}C)

In 1949, the dating systems employed by archaeological studies were altered, through the publication of an article by Willard Libby (Libby, Anderson and Arnold, 1949). Within this article, the principles of radiocarbon (^{14}C) dating were introduced: an analytical process that would revolutionise the study of events past.

Radiocarbon is the naturally occurring radioisotope of carbon that decays, through the beta emission of an electron (average energy = 160keV), to form stable nitrogen:

Carbon	Protons	Neutrons	Proportion (%)	Half-life (t½)
^{12}C	6	6	98.89%	Stable
^{13}C	6	7	1.11%	Stable
^{14}C	6	8	0.0000000001%	5730 years

Table 6.3. Atomic differences and prevalences of the main isotopes of carbon.

Radiocarbon is rapidly oxidised within the atmosphere to form $^{14}CO_2$, which enters the oceans via atmospheric exchange, forming dissolved carbonates, and also into plants and phytoplankton through photosynthesis. Hence, radiocarbon may enter animals via the progression of the isotope through the subsequent food chains, where it is incorporated into organic biochemicals. Plants and animals may therefore exist during their lifetime in equilibrium with atmospheric ^{14}C. At death, metabolic function ceases resulting in a steady decay of the radiocarbon content present, and the loss of this equilibrium.

6.2 The Development of Radiocarbon Dating Systems

The most significant outcome from the development of a new isotope-based system was the recognition that 'civilised societies' had become established many years prior to our understanding at that time. However, by the latter half of the twentieth century, it became apparent that there was an error in the principles of radiocarbon dating. Cross-calibration with dendrochronology indicated that the altering rate of atmospheric radiocarbon production over the recent centuries required an addition of several centuries to the first millennia BC, and earlier periods; these were times occupied by civilisations that had not documented their period contemporaneously. The result was that these societies were recognised as older than had been originally considered, requiring previously assumed social or technological connections with other cultures to be radically re-thought (Renfrew, 1973).

Early analytic methods required the monitoring of radiocarbon decay, during which it converts back to stable nitrogen. Given the very small percentage of ^{14}C in total carbon, (see Table 6.3), the process was slow and expensive. The introduction of more sensitive and faster analytical methods, such as the creation of Accelerator Mass Spectrometry (AMS) improved the science, by specifically analysing only the heavier ^{14}C, compared to normal ^{12}C atoms, and requiring smaller quantities (milligrams) of material to be destroyed. Therefore, the new technique allowed historical artefacts to be dated with minimal damage. By not relying on the recognition of beta decay, the absolute values of ^{14}C and ^{12}C could be identified by AMS.

The result of this technology was an expansion in our knowledge, with many dates for historical cultures and objects now given only in terms of radiocarbon activity. There has been a general acceptance of the technique in all historical disciplines and several additional radioisotope-based methods have since been identified, such as uranium series dating. However, ^{14}C testing is somewhat limited in the archaeological timeframe (up to 50,000 years) and provides wide age estimates which are acceptable in archaeology, but may be too broad for forensic application (Pollard, 1996)(Swift, 1998).

6.3 The use of radiocarbon dating in forensic science.

As alluded to earlier, Willard Libby made the incorrect assumption that environmental radiocarbon has remained constant over millennia, such that modern values may be correlated to initial concentrations in historical material. This is not the case. The magnetic fields of the Sun and the Earth result in global variations in atmospheric production, and the global fractions vary, being the quantities of radiocarbon within the atmosphere, the biosphere and the oceans.

Also, when Libby created his system, the half-life of radiocarbon was considered to be 5568 years, again incorrect ($t_{½}$=5730 years). These errors have resulted in the recognition that any material produced between 1650 AD and modern day will produce inaccurate carbon-based dating results (Pollard, 1996). Given that the system had in-built errors, it required the development of a correction calibration system to counteract the errors.

By accurately analysing samples from the annual growth rings in trees of known age, it was possible to establish atmospheric ^{14}C concentrations through knowledge of the isotope's true half-life. In such a manner, a correction curve was created, represented as calendar years against radiocarbon dated years.[4] By calculating the radiocarbon date from material of unknown origin, using Libby's incorrect assumptions, cross-reference with the established correction curve allows easy estimation of the calendar year value (given as CalBP, CalAD or CalBC, to indicate the fact that the date has been corrected.).

Since the industrial revolution in the late 1800s, the use of fossil fuels as an energy source has resulted in the release of large quantities of carbon, predominately as carbon dioxide, into the atmosphere over a relatively short period of time. Given the age of the fuel source, (being millions of years in creation), the ^{14}C content of these materials has decayed to virtually zero. The result was a large increase in the overall carbon content of the atmosphere, but this being radiocarbon depleted. Thus, up until the mid-1940s, the radiocarbon signal became diluted by up to 2%. For material created between 1890 and 1950, the estimated ages based on radiocarbon dating were inaccurate. A standard reference material was identified to assist in the calibration of such analyses, this being wood from 1890 AD.

[4] It should be noted that the latter is always expressed as BP (Before Present), which is taken as 1950.

Following 1945, atmospheric nuclear weapon detonations have rapidly increased the radiocarbon burden. This is represented as a sudden peak, often referred to as the "Bomb Peak", when large quantities of artificial carbon isotopes were created through nuclear fission. The result has been a doubling of the ^{14}C activity in terrestrial organisms (Taylor, 1987). The geographic distribution of this peak also varied, being higher in the Northern Hemisphere where the majority of nuclear weapons testing occurred. Since the signing of the atmospheric nuclear test ban treaty by the majority of nations in 1963, this radiocarbon has rapidly redistributed into the biosphere (Wild *et al.*, 2000).

Several publications have used this recognisable increase in atmospheric radiocarbon content when attempting to differentiate bones of forensic interest from bones of historical interest. Taylor *et al.* detailed several case studies in which the ^{14}C content was analysed and remains were placed in one of three categories:

i) Non-modern period (prior to 1650 AD) and of no forensic interest,
ii) Pre-modern period (1650 AD to 1950 AD), being of possible forensic interest, and
iii) Modern period (1950 AD to the present), being of definite forensic interest.

(Taylor *et al.*, 1989)

Despite the apparent success of this methodology in these cases, the limitations are obvious, with the second category covering three centuries that includes cases that may warrant police interest. Delineation between these cases and ancient material is not possible using the method described.

Researchers have recently focused upon the bomb peak in forensic casework. Wild *et al.* uses the peak as a calibration curve based around the recorded atmospheric ^{14}C values to allow assessment of different human biological material to produce accurate radiocarbon-based dates (Wild *et al.*, 2000;Geyh, 2001). Ubelaker also recognises the use of the peak, though the estimate produced is wide ranging, and potentially of little practical use when compared to macroscopic examination based estimations (the results indicated that a "95% probability of death occurring between 1670AD and 1955AD") (Ubelaker, 2001).

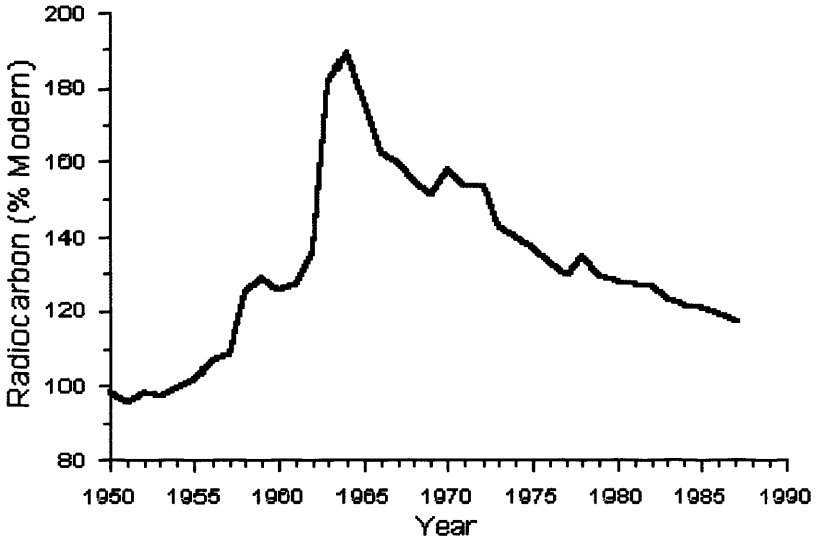
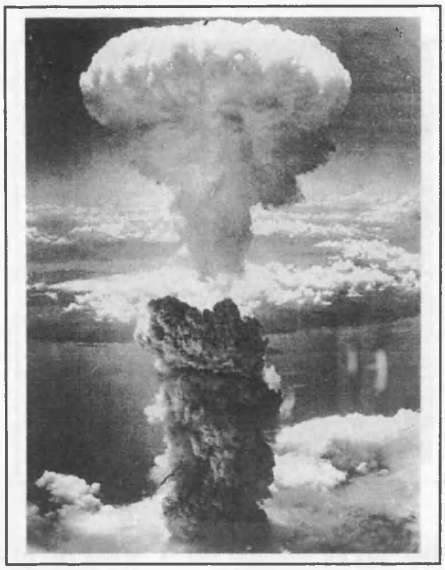


Figure 6.1. A graphic illustration of the bomb peak: as radiocarbon concentrations against calendar years, as registered in the Northern hemisphere. Recognition of the sudden increase in 14C concentrations may assist in the estimation of the PMI within the forensic interval.

One of the potential problems raised by Wild *et al.* is the biological half-life of the carbon content of human tissue (Wild *et al.*, 2000). Radiocarbon, biologically identical to ^{12}C, is incorporated into the organic phase of bone matrix. Therefore, the majority resides within collagen. Estimates based upon previous work suggests that collagen turnover may occur every 15 to 30 years, depending upon the nutritional status and the chronological age of the individual during life (Wild *et al.*, 2000;Katzenberg, 1993). Therefore, in an individual who is 50 years old and who died in the early 1990s, the collagen phase of their bone potentially retains the radiocarbon profile from when the protein was created in the early 1960s, when atmospheric radiocarbon was high. The result is an inaccurate estimate of time since death. This may be corrected for through the testing of several tissue types in combination, such as bone marrow or hair, which have faster production rates and hence quicker turnover rates. However, in the event of the discovery of human skeletal remains devoid of soft tissue, the applications of the technique may be limited.

6.4 Artificial, or Man-Made, Radioisotopes



"If the radiance of a thousand suns
Were to burst at once into the sky
That would be like the splendour of the Mighty one --
I am become Death,
The shatterer of Worlds."

Bhagavad Gita, Hindu Spiritualist.

An interesting approach was suggested within the concluding remarks of Knight and Lauder's preliminary publication (Knight and Lauder, 1969). It was considered that the measurement of man-man (artificial) isotopes might provide information of value. Unlike the methods that depend upon chemical changes, radioisotopes are less affected by changes within the physical environment to which bones have lain exposed. As will be discussed, many of these isotopes are also known to accumulate within the calcified matrix of bones.

6.5 Strontium-90 and the time since death

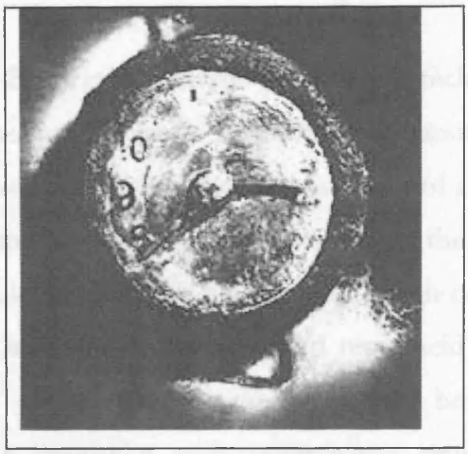


Figure 6.2. A wristwatch stopped by the atomic bomb dropped on Hiroshima by the Enola Gay, a Boeing B-29 bomber, at 08:15am on the morning of August 6th 1945.

Exposure of the population to radiation has increased in the last century through fallout from nuclear weapons testing. The isotopes of the alkaline earth metals, for example strontium and barium, are similar in their biochemical properties to calcium; though having no apparent metabolic function, they are absorbed across the intestinal mucosa at absorption levels of between 20 and 40% and preferentially incorporated in to the matrix of the skeletal system (MacLaughlin-Black *et al.,* 1992). Therefore, man-made fission products that entered the biosphere following atmospheric nuclear weapons detonations should be present in the bone matrices of those who died after 1945 and remain absent in those who died before that date.

MacLaughlin-Black *et al.* tested this hypothesis by measuring the ^{90}Sr concentrations in contemporary femora (from postmortem examinations) and compared them with concentrations in medieval femora (MacLaughlin-Black *et al.,* 1992). Though initial results seemed encouraging, the archaeological samples possessed significant concentrations of radio-strontium. The authors of the study explained the process through diagenesis. This is the process whereby radionuclides, in solution with groundwater, percolates through the soil, ultimately being passively absorbed and adsorbed by the bones buried within it. Obviously this applies to both bones of antiquity and the bones of forensic interest, with isotopes being exchanged between the hydroxyapatite matrix and the soil. MacLaughlin-Black *et al.* noted this potential postmortem variation, however, soil samples were unavailable for comparison. To determine the postmortem interval, it was suggested that future investigations into the viability of using ^{90}Sr-dating must relate the concentrations of radionuclide in the samples to the

location and condition of the burial with respect to the process of diagenesis. It would also be essential to map the concentrations of isotopes present against the timetable of atmospheric nuclear detonations, which peaked in the 1960s, though isotope concentrations may vary nationwide.

For their study, MacLaughlin-Black *et al.* followed an analytical technique from a publication in 1965 (Parker *et al.,* 1965). The method was a form of precipitation extraction, requiring long periods of time for the in-growth of ^{90}Y and the establishment of an equilibrium, which would be unacceptable for forensic investigations. This occurred after the extraction of the strontium nitrate precipitate from the calcium matrix. The method, though not described by the authors within the published article, was altered; concentrated nitric acid (70%) was substituted for fuming nitric acid (95-96% w/w). Prior to the in-growth, however, purification of the precipitate is essential; many impurities and contaminants remain present which, if not completely removed, may produce false positive results. Due to the time constraints, the MacLaughlin-Black pilot study may have inadequately purified the resultant precipitates (Joanna Norris, personal communication). It is therefore likely that the beta-activity detected in the specimens tested was not purely ^{90}Sr, but a mixture of radiostrontium and contaminate beta-emitters. These include not only man-made isotopes, but potentially also naturally occurring radioisotopes, such as ^{226}Ra and ^{40}K, both of which possess long half-lives. It is the probable presence of these isotopes that may account for the beta-activity, detected by liquid scintillation counting, measurable within the ancient bone material rather than the incorrectly assumed diagenetic in-growth of ^{90}Sr.

Neis *et al.* (1999) continued the work using ^{90}Sr, claiming it to be an ideal means of estimating the postmortem interval. The results of the study revealed the expected lower concentrations of ^{90}Sr in older bones, though no significant activity was demonstrated in bones from the 1930s. These claims appear to contradict the findings of MacLaughlin-Black *et al.,* possibly confirming the flawed analytical method employed by the latter. The use of occipital bone as a standard, however, also prevents accurate comparison with other studies. Conversely, should any diagenetic in-growth have occurred, it would be expected to be greater in bone, such as calvarial material, which possesses a high trabecular content (see later discussion).

A possible limitation inherent to the methods measuring man-made radioisotopes is the irregular creation of such nuclides. Between 1945 and 1998 over 2040 nuclear weapons tests were performed at irregular intervals around the world. Each produced different isotope ratios, dependent not only upon the warhead design used, but also on how it was detonated. For

example atmospheric detonations resulted in rapid cycling of isotopes into the biosphere and food chains compared with subterranean detonations. Ultimately, these isotopes became distributed globally. The relative determinations of isotope concentrations must be plotted against the intermittent creation of new concentrations, each "topping-up" the existing quantities present within the environment. Also, nuclear accidents such as the Chernobyl disaster add to the worldwide isotope burden. It may, therefore, be that bones of greatly differing postmortem intervals will in fact have similar concentrations of fall-out isotopes as the decay versus creation times alter over the decades.

Country of Origin	Number of detonations	Programme History Information
United States of America	1054 tests	Conducted first test, "Trinity," July 16, 1945, at Alamogordo, New Mexico. The last was "Divider" on Sept. 23, 1992.
Former Soviet Union	969 devices	Conducted its first test, "Joe-1," Aug. 29, 1949. Last test on Oct. 24, 1990
United Kingdom	45 tests	Conducted its first test, "Hurricane," on Oct. 3, 1952. Last test, "Bristol," on Nov. 21, 1991.
China	43 tests	Conducted its first test, fusion U-235, on Oct. 16, 1964. Final test was conducted on July 27, 1996.
France	210 tests	Conducted its first test on Feb. 13, 1960. Last test conducted on Jan. 28, 1996.
India	11 tests	Conducted its tests between May 11 and 13th 1974, and in May 1998.
Pakistan	6 tests	Conducted six tests between 28th and 30th May 1998
Israel	No testing to date.	Widely assumed to have nuclear capabilities.
South Africa	No testing to date.	Announced in 1993 that it had secretly created six devices, which were subsequently dismantled.
Iraq	No testing to date	Launched an ambitious nuclear weapons programmed, but was ordered by the UN to be dismantled following the Gulf War.
North Korea	No testing to date	Announced in 2003 the re-starting of its nuclear programme. Continues to deny UN inspection of facilities.

Table 6.4. The international nuclear weapons programmes and device testing, from 1945 to 1998.

6.6 Naturally Occurring Radioisotopes

Every population is exposed to significant concentrations of radiation through the inhalation of naturally occurring radioisotopes, such as radon and ^{210}Pb, and through ingestion of isotopes present within food and water supplies. These primordial elements are unrelated to nuclear explosions and their uptake should remain constant throughout an individual's life. Naturally occurring radioisotopes have a regular and predictable background concentration, one that has remained relatively unchanged over millennia (Smith *et al.,* 2001). Though localised increases have been observed around certain industrial facilities, these have not been shown to produce identifiable increases of ingested concentrations (Flues, Moraes and Mazzolli, 2002;Vukovíc and Mandíc, 1996). Because of these reasons, this thesis therefore proposes to focus primarily upon the potential viability of using naturally occurring nuclides as a means of estimating the postmortem interval.

6.7 Study Design

A study was designed, firstly to produce a pilot study using bones of known PMI to confirm or refute the existence of a correlation between specific isotope activities and the year of death. Additional work would then be performed based around any initial findings.
To fulfil the remit of an isotope-based dating system, the isotope selected must comply with the three criteria described by Swift *et al.*:

i) to have some biological function, so as to be incorporated into the human bone,
ii) to have a half-life commensurate with the time scale of investigation required, and
iii) to be abundant enough to be detected readily through conventional analytical techniques.

(Swift *et al.,* 2001)

Prior to the discussion and selection of possible isotopes that may be of use within the forensic interval, the chosen bone sample is described.

6.8 Standardisation of anatomical site for dating time since death.

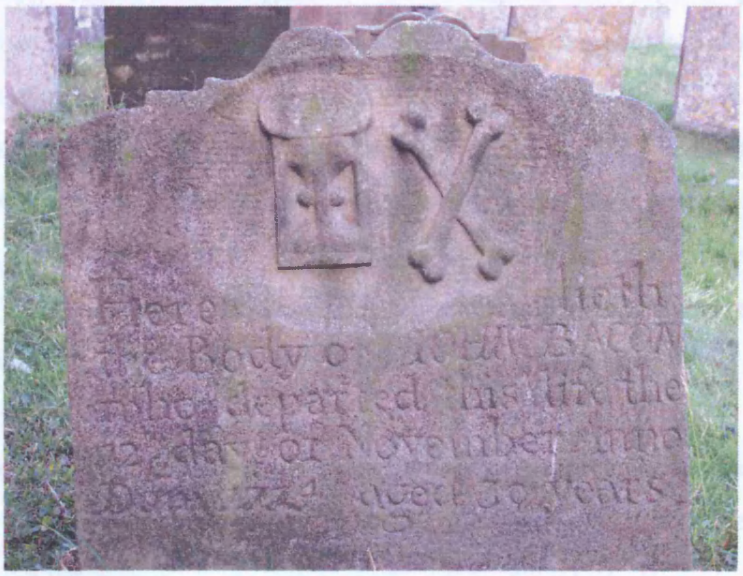


Figure 6.3. A gravestone, in All Saints & St. Margarets Parish Churchyard, Pakefield, Suffolk, from 1724 depicting an hourglass timepiece with crossed femora.

Previously published studies regarding isotope concentrations in human skeletal material have described differences existing between bone sites. The authors of these studies highlight the differences between the bone structure, notably the proportion of compact to trabecular bone. As described previously, the compact bone is solid, forming the functional aspect of the skeleton. Resorption and deposition of new material is limited to the subperiosteal aspects. Trabecular bone produces little in the way of structural integrity, instead forming a framework for haemopoiesis and an available source of calcium and phosphate to correct biochemical fluctuations in blood concentrations. The obvious difference that may account for the altered concentrations within these bone types is the dissimilar surface area-to-volume ratios. Holtzman states that analyses based on trabecular-rich bones, such as ribs, may not be representative of the total body burden and may instead indicate an upper limit of isotope concentrations (Holtzman, 1963). By recognising the potential for different results between bone types, it becomes essential to identify a standardised anatomical site from which all measurements must be taken. These differences may, in taphonomic studies, result from two principles:

a) altered resorption/deposition activity, and
b) increased diagenetic activity.

Classically long bones such as femora are used within analytical studies (MacLaughlin-Black *et al.,* 1992;Bradley, 1993; Henshaw, Hatzialekou and Randle, 1988). Being proportionally high in compact bone femora are slow with regards to both resorption and, ultimately, decomposition. It is also, therefore, low in trabecular bone ensuring little diagenetic inflow or outflow of elements into the compact bone being analysed.
Practically, femora are also recognisable as human in origin and are more likely to be recovered from clandestine graves or surface depositions, even in the presence of canid scavenging (Haglund, 1997). Additional forensic studies may also be performed upon femoral diaphyseal sections, such as DNA identification. Therefore, no additional specialist procedures need to be undertaken at postmortem examination to retrieve an appropriate sample.

It is therefore proposed that any naturally occurring radioisotope based method be performed using the human femoral diaphyseal cortex as the standardised anatomical site.

6.9 Radioisotope Selection

Through the criteria set for a naturally occurring isotope study to be of forensic potential, an isotope with a half-life well within the forensic timeframe is required. Being set at 75 years, a half-life shorter than this period is essential so that any analysis performed will register a fluctuation from biological "normal" concentrations that exist within the living population being assessed, allowing extrapolation and estimation of the PMI. Therefore, a half-life of less than 40 years would be ideal.

The isotope should also be incorporated into the skeletal system naturally, whether it possesses a biological function or not, in recognisable concentrations and at a steady rate throughout the lifetime of the individual, allowing measurement through existing analytical means. The International Committee of the Red Cross (ICRC) has recently compiled a Declaration, within which it suggests similar requirements to this third criteria as defined by Swift (International Committee of the Red Cross, 2003)(Swift *et al.,* 2001).[5]

Following a review of all the potential isotopes, it was decided that lead-210 (^{210}Pb) and polonium-210 (^{210}Po) were the most promising candidates based upon these criteria.

[5] "... (the) framework shall include: defined protocols for exhumation, antemortem data collection, autopsies and identification based on **reliable and scientifically valid methods and technologies**." (International Committee of the Red Cross, 2003)

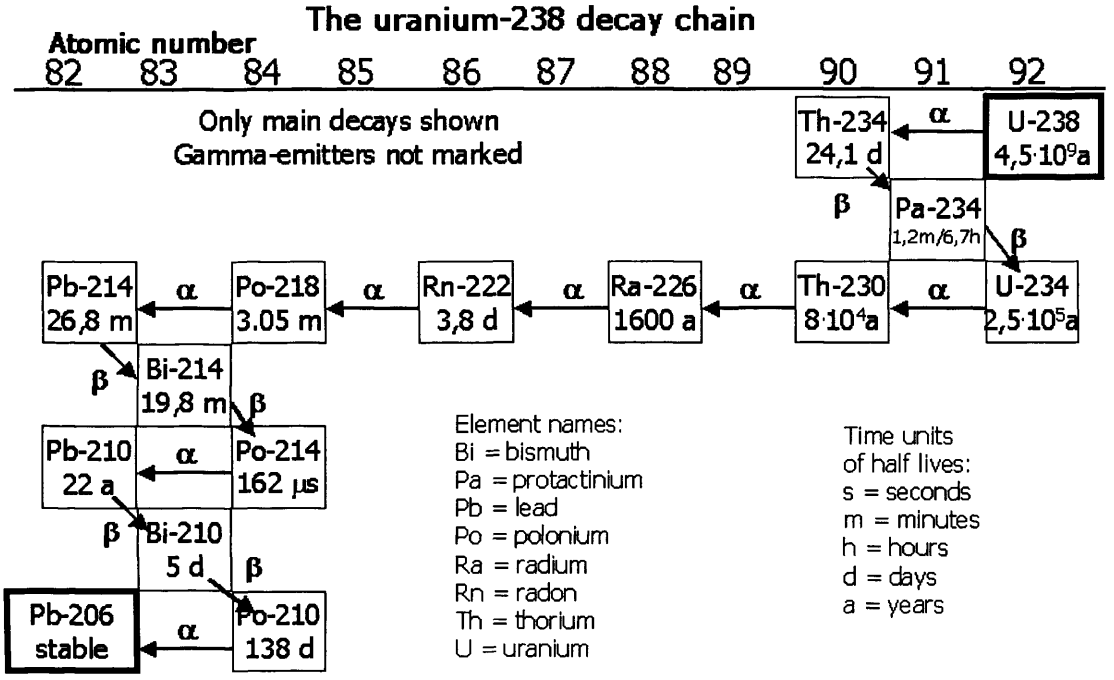


Figure 6.4. The Uranium-238 (^{238}U) decay series, of which ^{210}Pb and ^{210}Po constitute the latter parts of the chain.

^{210}Pb forms part of the Uranium-238 (^{238}U) decay chain series, being derived naturally through subsequent decays of parent isotopes. It possesses a half-life of 22.3 years, being ideal for the purposes of forensic estimation, and naturally occurring within human tissues during an individuals lifetime. The isotope is also preferentially deposited within the calcium hydroxyapatite matrix of bone, as will be described later, ensuring measurable quantities by standard analytical means. Also, being present within the inorganic phase of bone, it is unaffected by postmortem decompositional changes, as is experienced by ^{14}C.

As stated by Blanchard there are, in fact, similarities between ^{210}Pb and fission products, such as ^{90}Sr, with respect to their behaviour in the atmosphere and to their rate and mode of deposition (Blanchard, 1966). Exposure to ^{210}Pb occurs through both inhalation and ingestion of the element, with average annual dose equivalents of approximately 41µSv for members of the UK population (Smith-Briggs, Bradley and Potter, 1986). Once ^{210}Pb has been absorbed into the bloodstream, either across the alveolar air spaces or across the small intestinal mucosa, it is preferentially incorporated into bone, replacing calcium within the matrix in a manner similar to the alkaline earth metals. However, unlike ^{90}Sr, the uptake of ^{210}Pb is slow, with ^{210}Pb circulating within erythrocytes for a period of time (Leggett, 1993). It has been estimated that 55% of the total radio-lead absorbed into the body is ultimately retained within the mineral bone matrix, of which 10-15% may occur within a few hours of exposure (ICRP, 1979;ICRP,

1980;Heard and Chamberlain, 1984). Ultimately, the adult human skeleton constitutes the main reservoir for approximately 90% of all lead within the human body (Gross *et al.*, 1975).

The International Commission on Radiological Protection's (ICRP) model of isotope metabolism was modified by Leggett for specific use with lead, the result being a biokinetic model that illustrates the passage of lead from the intake to the final tissue distribution, or its ultimate excretion (Leggett, 1993;ICRP, 1979;ICRP, 1980). Through this model it is assumed that the quantity of lead reaching the bone is exchanged at the bone surface, which may be divided into compact and trabecular surfaces, where further exchange allows ^{210}Pb to enter the compact or trabecular volumes respectively. Leggett further divides these volumes into deep and shallow, where the former provides a non-exchangeable "pool" of ^{210}Pb, unless displaced by the process of bone resorption. With a half-life of 22.3 years, ^{210}Pb decays within the matrix to ^{210}Bi (half-life = 5.013 days) of which virtually 100% decays further to ^{210}Po (the remaining 0.0001% forms an insignificant concentration of ^{206}Tl).

Individual bone types have shown variations in isotope concentrations. In the study described by Fisenne, concentrations of ^{210}Pb were found to be highest within rib material, followed by vertebrae (Fisenne, 1994). Fisenne also states that the concentrations in trabecular bone may be higher when compared with compact bone. This result would be in agreement with the analytical findings of Holtzman (Holtzman, 1963) and could support the finding of highest levels being within ribs and vertebrae, where a higher proportion of the bone volume is trabecular.

^{210}Po differs from ^{210}Pb in the respect of its short half-life (138.4 days), resulting in the decay of ^{210}Po to stable ^{206}Pb. After a given time, an equilibrium ultimately develops in life between ^{210}Po and ^{210}Pb.

Although not generally a large part of the diet, Rollo *et al.* indicate that ^{210}Pb concentrations are relatively high in seafood (Rollo *et al.*, 1993). It is therefore feasible that those living in coastal or fishing regions may have higher initial concentration of radio-lead than, say, individuals living inland. Smoking has also been suggested to raise ^{210}Pb concentrations (Spencer *et al.*, 1977). It is obvious that knowledge of an individual's habits will not be available immediately upon recovery of skeletal remains.

The equilibrium between ^{210}Pb and ^{210}Po may be offset in areas of high radon concentrations, though it will re-establish at individually higher concentrations. Several publications, however,

have recognised that both ^{226}Ra and ^{222}Rn will contribute to the ^{210}Pb and ^{210}Po levels by their chain of decay, but direct ingestion of these isotopes are considered the most important under normal circumstances (Kawamura *et al.*, 1991; UNSCEAR, 1982; Henshaw, Hatzialekou and Randle, 1988).

The ICRP model of ^{210}Po postulates that over 99% of the total ^{210}Po in bone arises from the decay of ^{210}Pb, though this assumes a continuous annual intake at the average level for any country in question. The findings of Bradley confirm this hypothesis, describing levels of over 97% (Bradley, 1993). Unfortunately, it has been shown that, biologically, ^{210}Po does not possess the same degree of affinity for the hydroxyapatite matrix as ^{210}Pb, being instead relatively mobile. Though it is theoretically possible for the nuclide to ultimately redistribute into other tissues, it is unlikely that this would provide a serious complication when utilising the activities of these isotopes for the purpose of PMI estimation (Henshaw, Hatzialekou and Randle, 1988;Harrison and Haines, 1996). In fact, no independent movement of ^{210}Po has been observed and, despite lacking the osteotropic behaviour of its parent isotope, it remains, apparently "locked" into the crystal bone lattice (Salmon *et al.*, 1998).

It is therefore proposed that, by measuring concentrations of ^{210}Pb within a set of discovered human remains, it would be possible to extrapolate backwards to the average initial concentration for a given population, calculated through in vivo studies, therefore providing an estimate for the time elapsed since death. Within this section of the thesis it is suggested that by analysing ^{210}Pb, and also ^{210}Po, concentrations it may be possible to accurately and reliably estimate the time since death. ^{210}Pb has been used successfully in the past to date sediment depositions over the last four centuries, and has proved useful in the dating, and hence the verification of the authenticity of works of art (Gelen *et al.*, 2003; Aitken, 1990;Keisch, 1968). The half-life of ^{210}Pb also fulfils the criteria required for investigations within the forensic interval, and is present within the natural environment in measurable concentrations when using currently employed analytical methodologies. Radon contributes little to the overall concentration of ^{210}Pb within human bone, therefore little variation is expected within the adult population of a single country, though each country examined would require its own individual calibration system.

Independently, both Swift and Pollard recognised this potential at similar times, both suggesting a study to verify the theory using modern material and bones of antiquity to create a calibration system for future isotope based estimations (Swift, 1998; Pollard, 1996).

In concluding this section, it is recommended that a pilot study of human bone material be commenced which may provide a new tool in estimating the date of death within the late postmortem period. In order to validate this, a study of skeletal material of known postmortem intervals covering the last century should be undertaken, preferably with the different decades represented to include the recently deceased. The unknown variables that would require further investigating may include the effects of diet and diagenesis, though soil samples and multiple isotope analyses, where possible, may minimise the latter.

6.11 Predicted Limitations

The effect of diagenesis upon bone isotope profiles remains unknown at present. Lambert *et al.* examined the diagenetic movement of elements within human femora (Lambert *et al.*, 1982; Lambert *et al.*, 1983). Electron microprobe analysis was employed to visualise elemental distributions. Their results indicate that lead possesses a homogenous distribution across the cross-section of excavated femora, suggesting no inflow or outflow of this element, being therefore "free from diagenetic effects". Therefore, the hypothesis that the lead content of femora would be relatively resistant to the effects of diagenesis appears borne out.

Shinomiya *et al.* described the work of Lambert, yet highlighted the fact that only in-flow measurements had been taken (Shinomiya *et al.*, 1998). The possible exchanges between bone and the soil within which it was embedded was investigated over a two-year period. Unfortunately, the bone chosen as the experimental standard was a vertebra which, being high in trabecular bone content and therefore different to compact bone, would produce inherently different outcomes to femora. Nor did they measure lead concentrations either. Shinomiya's article mentions only that the bone was embedded within "reference soil"; no mention as to whether the soils were then exposed to the natural environment was made. Therefore, the co-action of rain and other effects of weathering failed to be monitored.
The results were in agreement with those of previous workers. They identified three groups of elements;

a) Group I, the in-flow group, in which elements increased in the soils, notably iron, aluminium and barium,
b) Group II, the balanced decrease between both soil and bone, including sulphur, magnesium and zinc, and
c) Group III, in which there is out-flow from the soil into the bone, including calcium and phosphate.

(Shinomiya *et al.*, 1998)

The concluding remarks of the investigators suggested that the analysis of such elements may allow estimation of the time bones have spent exposed to soil. However, in the absence of data for weathering effects and no standard duration for the soft-tissue decomposition in human remains, the results remain relatively unworkable.

To test the hypothesis that naturally occurring isotopes, notably ^{210}Pb and ^{210}Po, could provide a means of estimating the postmortem interval in human skeletal remains, a small pilot study was undertaken. As suggested, this study utilised bone samples of known PMI, covering the decades of the last century, to see if such a relationship existed.

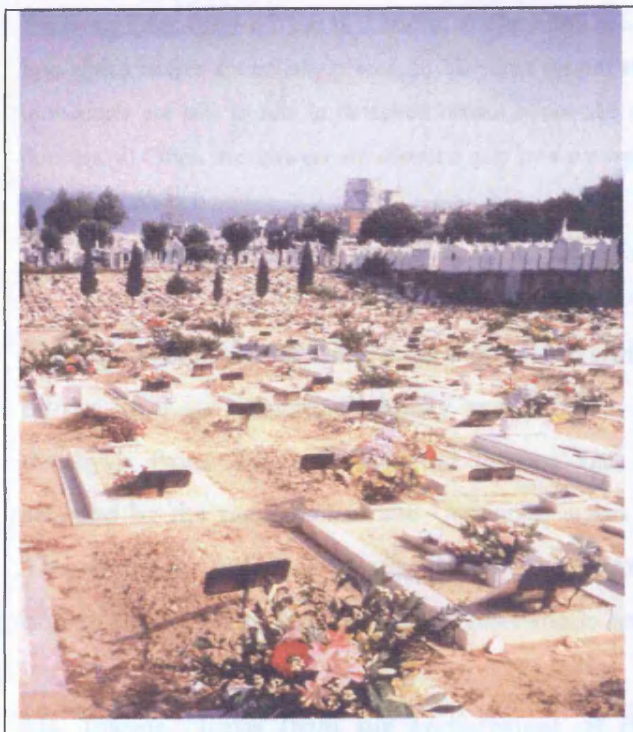
The study would form the basis of a collaboration between two research centres, the University of Leicester and the University of Reading, each providing relevant expertise in their fields. The methodology would be based upon existing, and therefore reliable and scientifically valid, analytical practices to assess concentrations of large numbers of isotopes, especially radio-isotopes, and trace elements within human skeletal material.

Chapter Seven - The Pilot Study: Portuguese Specimens

7.1 Background Information

Despite a close ancestral history, funereal practices vary between European countries, the most commonly employed practices currently performed within the United Kingdom being cremation and permanent burial. Additional methods, such as burial-at-sea, overall form a smaller percentage. Portugal, however, varies from these practices in that relatives of deceased commonly request, after temporary burial within the natural soil environment of the Alto De S. Jōao cemetery for a period of five years or more, that the skeletalised remains be exhumed and re-coffined. The remains are then re-housed within a marble-faced drawer, being adorned with a nameplate inscribed with the individuals' details; the large numbers of drawers form rows that ultimately contain numerous individual sets of human remains at any one time. The bones continue to be housed in such a surrounding until the formal lease agreement expires or no surviving relatives exist to honour the annual payments. At this point the final funereal stage is initiated, being the subsequent removal to an ossuary, or re-burial of the remains within a mass grave situated within the cemetery grounds. Much of the bone was also sold to a commercial company, to be ground and used as a fertilizer. Cycling of the occupants of the drawers' results in the re-burial of over one thousand coffins each year.

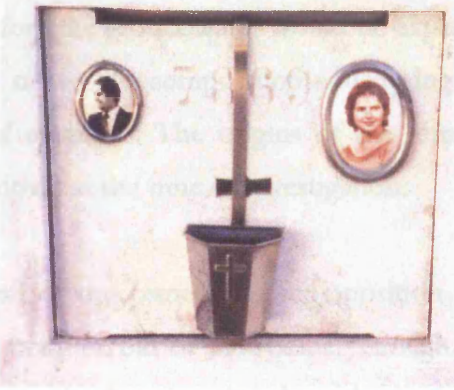
During the early 1990's, the Museu Bocage in Lisbon was granted permission by the Portuguese government to retain skeletons being removed from the drawers for the purposes of research.



a)

b)

c)



d)

Previous Page Figure 7.1.a, b, c and d. a) The Alto De S. Jōao cemetery, Lisbon. The photo reveals the graves into which bodies are initially placed, b) The class system still remains apparent, even in death; the more affluent individuals are laid to rest in detached ornate house-like properties, c) whereas the poor lay in rows of small drawers, d) Often the drawers are adorned only by a nameplate or photo of the occupant. (Photographs courtesy of Dr Sue Black.)

During the summer of 1992, members of the University of Aberdeen were granted permission by the local government authority and museum for the retention of sections of left femoral diaphysis from 228 individuals of known age, sex and time since death, the latter being selected by the members of the University team to cover a timeframe from 1920 until 1983. Therefore, the selected material adequately provided specimens from individuals who died before and after the introduction of man-made radioisotopes into the environment.

Ms. Joanna Norris from the Department of Forensic Anthropology at the University of Aberdeen donated these samples, of known postmortem interval from the single cemetery in Lisbon, to the University of Leicester in 1997. It is the analysis of these that forms the pilot study arm of this thesis.

The local cemetery environment itself introduced uncertainties into the assessment of the results, being located on a downward gradient. Therefore the groundwater would be expected to accumulate at the lower levels, producing altered rates of decomposition and during the burial phase increased rates of diagenetic transfer of elements. The origins of the remains relative to areas within the cemetery grounds was unknown at the time of investigation.

Despite the short period of interment within the soil of the cemetery, decomposition had advanced to the late stages in all cases; the remains were devoid of soft-tissue. Though the limited period of soil burial decreased the risk of diagenetic alteration, it is hypothesized that the conditions within the marble drawers increased the risk of microorganism tunneling and bone matrix resorption. (see later discussion)

7.2 Methods

From the complete collection, fifteen sections of human femora (13 female, 2 male) were selected covering post mortem intervals, at the time of analysis, from 15 to 77 years. These samples, therefore, included two different individuals that had died during each decade from the 1920's until the 1980's, with an extra specimen from a person who had died immediately following the 1945 test detonations, allowing comparison of man-made radioisotopes concentrations.

Pilot Study Code	Internal Sample Code 1	Internal Sample Code 2	Gender	Year of death	Age at time of death	Interval between death and analysis (years)
1	33695	340	Female	1980	"Retired"	18
2	13175	734	Female	1946	76	52
3	6328	4890	Female	1935	76	63
4	8832	2991	Female	1927	76	71
5	6758	3570	Female	1921	77	77
6	10454	193	Male	1939	76	59
7	15987	4823	Female	1949	72	49
8	29460	3615	Female	1965	"Old"	33
9	10277	881	Female	1959	77	39
10	6592	276	Male	1978	"Retired"	20
11	38094	3442	Female	1983	"Retired"	15
12	26014	395	Female	1967	"Retired"	31
13	16743	1808	Female	1954	72	44
14	7032	639	Female	1976	"Retired"	32
15	11186	4561	Female	1943	78	55

Table 7.1. Details of bone samples analysed during this pilot study. All information is as accurate as the records allow.

In an attempt to minimise variations due to gender or age-related differences, the majority of the samples were of the same gender and within a similar age range.

Each selected bone section was dried and any adherent soil was dislodged from the bone surface by brushing with a coarse-haired paintbrush. The sample was then washed in de-ionised water. Care was taken to ensure that the trabecular component of each bone was removed prior to analysis of the sections, ensuring only compact bone tissue was analysed. Any adherent adipocere, haemopoietic marrow or fat still residing within the medullary cavity was removed with a scalpel and forceps.

A sub-sample of prepared bone was then ashed to concentrate the sample, eliminating the water and organic components of the bone, though specific 'volatile' isotopes may be lost during this process depending on the physical properties of the element in question. Bone may be ashed at temperatures above 450°C resulting in the required loss of the organic component, though potentially decreasing the content of ^{210}Pb and ^{210}Po (Martin and Blanchard, 1969). This may be the result of either excessive heat or excess oxygen intake into the furnace used, resulting in localised areas of the sample reaching significantly higher temperatures than surrounding material. Though it should be noted that such changes are commonly experienced in soft tissue specimens, it remains less likely in sections of compact bone tissue. However, this risk was minimised by ensuring that the samples were thoroughly air-dried prior to the ashing process within a cold muffle furnace, which digitally controlled internal temperatures.
The bone was ashed at a temperature of 600°C, which was maintained for 12 hours or until no further carbon remained. The ashes were ground into a homogenous powder in an electronic mill, the resultant material forming the analytical source for all determinations, except ^{210}Po and ^{137}Cs which were analysed on unashed fractions due to the potential to volatise both elements. (Martin and Blanchard, 1969)

All samples were analysed for ^{210}Po, ^{226}Ra, ^{234}U, ^{238}Pu, ^{238}U, ^{239}Pu and ^{240}Pu concentrations by alpha spectrometry, and for ^{40}K, ^{137}Cs and ^{228}Ac (^{228}Ra) by gamma spectrometry.

Each analysis was performed 'blind' to the postmortem interval at the Postgraduate Research Institute of Sedimentology, University of Reading under the direct supervision of Dr Stuart Black, being coded internally by two different numbers generated at random.

(see Appendix 2)

7.3 RESULTS

Data collected on the bone samples are presented in Tables 7.2 – 7.4.

Man-made Radioisotopes

The measured ^{239}Pu and ^{240}Pu concentrations range from 6-67mBq kg^{-1} (mean = 34 ± 22 (1sd), n=15) and are similar to previously measured levels in human skeletal material (Popplewell, 1986; O'Donnell *et al.*, 1997; Burkinshaw, Bayhreyni-Toosi and Spiers, 1987; Popplewell *et al.*, 1985; Popplewell *et al.*, 1998; O'Donnell, 1993).

In two samples ^{238}Pu was also detectable, (12mBq kg^{-1}). Although the 2σ counting errors were high for these samples, it was still identifiable above the detection limit.

^{137}Cs was only recordable in samples 1, 10 and 11, comprising the specimens with the shortest postmortem interval. Of these, two also possessed detectable concentrations of ^{238}Pu, (Samples 1 and 11, both of whom died during the 1980s), and would be consistent with the recent deposition of man-made nuclides originating from nuclear weapons testing.

Naturally-Occurring Radioisotopes

^{238}U values ranged from 0.04-0.9Bq kg^{-1}, (mean = 0.43 ± 0.28 (1sd), n=15). These values correspond to a total uranium range of between 0.3 and 7.2 μg kg^{-1} and are consistent with the published data on 40-60 year old males from Milwaukee, USA (0.014-0.125Bq kg^{-1}; Harley and Fisenne, 1990). Interestingly Harley and Fisenne's data concerning the measurable (^{234}U/^{238}U) quotients is in the range 1.3-2.6, similar to data reported within these samples (0.9-3.5). Despite being the dominant radioisotope form of uranium within the environment, these findings suggest ^{234}U values are similar, if not greater, than ^{238}U.

^{226}Ra analyses range from 0.21-0.94Bq kg^{-1} (mean = 0.54 ± 0.3 (1sd)), being similar to analyses conducted involving large sample numbers (n=140-865). (Neis *et al.*, 1999; Harley and Fisenne, 1990; Fisenne, Keller, and Harley, 1981; Walton, Kologrivov and Kulp, 1959; Muth and Globel, 1983). ^{228}Ra concentrations, however, are all much higher at 0.5-7.6Bq kg^{-1} (mean = 3.6 ± 2.5 (1sd)) and may represent slightly higher levels of parent isotope (^{232}Th) concentrations in the bone material.

Pilot Sample	^{238}Pu	^{239}Pu + ^{240}Pu	^{137}Cs	^{238}U	^{234}U	^{226}Ra	^{210}Po	^{228}Ra	^{40}K
	Alpha	Alpha	Gamma	Alpha	Alpha	Alpha	Alpha	Gamma	Gamma
	(mBq kg^{-1})	(mBq kg^{-1})	(Bq kg^{-1})	(Bq kg^{-1})	(Bq kg^{-1})	(Bq kg^{-1})	(Bq kg^{-1})	(Bq kg^{-1})	(Bq kg^{-1})
1	12 ± 31	67 ± 15	0.75 ± 0.61	0.787 ± 0.015	0.725 ± 0.012	< LLD	2.78 ± 0.03	< LLD	59.6 ± 4.1
2	< LLD	5 ± 11	< LLD	0.319 ± 0.013	0.631 ± 0.011	0.23 ± 0.1	0.33 ± 0.02	5.5 ± 0.9	64.2 ± 5.8
3	< LLD	< LLD	< LLD	0.307 ± 0.011	0.625 ± 0.010	< LLD	0.24 ± 0.01	0.5 ± 0.3	37.4 ± 2.9
4	< LLD	< LLD	< LLD	0.042 ± 0.005	0.113 ± 0.009	0.21 ± 0.1	0.21 ± 0.01	1.0 ± 0.3	38.9 ± 3.8
5	< LLD	< LLD	< LLD	0.073 ± 0.009	0.253 ± 0.010	< LLD	0.06 ± 0.01	< LLD	64.1 ± 5.3
6	< LLD	4 ± 6	< LLD	0.276 ± 0.010	0.521 ± 0.026	< LLD	0.20 ± 0.01	< LLD	74.8 ± 5.5
7	< LLD	< LLD	< LLD	0.209 ± 0.011	0.562 ± 0.036	0.21 ± 0.1	0.21 ± 0.01	< LLD	104.9 ± 9.1
8	< LLD	43 ± 12	< LLD	0.701 ± 0.032	0.709 ± 0.045	0.83 ± 0.3	1.03 ± 0.02	< LLD	59.9 ± 4.3
9	< LLD	52 ± 28	< LLD	0.528 ± 0.031	0.605 ± 0.033	< LLD	0.96 ± 0.02	2.4 ± 0.4	62.8 ± 4.8
10	< LLD	61 ± 22	0.97 ± 0.71	0.774 ± 0.035	0.705 ± 0.045	0.22 ± 0.1	3.86 ± 0.06	5.6 ± 0.8	58.9 ± 3.0
11	12 ± 14	8 ± 6	1.11 ± 0.68	0.899 ± 0.055	0.800 ± 0.064	0.92 ± 0.4	3.48 ± 0.08	< LLD	47.2 ± 2.7
12	< LLD	< LLD	< LLD	0.691 ± 0.037	0.669 ± 0.035	0.74 ± 0.3	1.32 ± 0.02	< LLD	64.6 ± 4.9
13	< LLD	45 ± 23	< LLD	0.402 ± 0.055	0.943 ± 0.062	< LLD	0.88 ± 0.02	2.7 ± 0.4	55.3 ± 2.5
14	< LLD	7 ± 6	< LLD	0.285 ± 0.020	0.348 ± 0.022	0.94 ± 0.4	1.54 ± 0.02	7.6 ± 0.5	9.4 ± 0.9
15	< LLD	6 ± 7	< LLD	0.083 ± 0.009	0.122 ± 0.012	0.53 ± 0.3	1.03 ± 0.01	< LLD	40.1 ± 2.2
IAEA 134	3.1 ± 0.3**	14.8 ± 0.9**	N.A.	4.1 ± 0.1	3.8 ± 0.2	2.9 ± 0.1	7.3 ± 0.3	3.7 ± 0.2	220 ± 60
IAEA 134 reported	3.1 ± 0.4**	15.0 ± 1.2**	N.A.	4.0 ± 0.7	3.7 ± 0.7	2.8 ± 0.5	7.5 ± 2.0	3.6 ± 0.8	212 ± 40

Table 7.2. Radioisotope activities for the Portuguese study bone samples. The IAEA reference material (control sample) is also provided, together with reported values for comparison. The associated errors are 2σ from counting statistics. Reproducibility based on five analyses of a standard is better than 1.5 % for all nuclide abundances.

**Results are in Bq kg^{-1} dry mass. < LLD = below lowest limit of detection.

	Ca	As	^{239}Pu	^{238}U	^{234}U	^{226}Ra	^{210}Po	^{40}K	Ce	La	Pb	Rb	Sr	Zn	Zr	Nd	Year	U	^{210}Po DC
As	-0.15																		
^{239}Pu	0.44	0.62																	
^{238}U	0.42	0.26	0.59																
^{234}U	0.26	-0.03	0.45	**0.73**															
^{226}Ra	0.12	-0.53	-0.36	0.50	0.27														
^{210}Po	**0.70**	0.17	0.41	**0.80**	0.46	0.30													
^{40}K	-0.38	0.52	0.32	0.07	0.23	-0.50	-0.19												
Ce	-0.16	0.14	0.62	0.21	0.41	-0.20	-0.01	0.60											
La	-0.05	-0.13	0.39	-0.07	0.14	-0.46	-0.14	0.38	**0.91**										
Pb	-0.21	-0.16	-0.66	**-0.80**	**-0.81**	-0.57	-0.55	-0.01	-0.25	0.32									
Rb	0.12	-0.13	0.35	0.21	0.04	0.19	0.09	-0.23	0.13	0.20	-0.28								
Sr	-0.09	0.04	-0.45	-0.49	**-0.72**	-0.30	-0.38	0.03	-0.59	-0.50	0.67	-0.30							
Zn	0.32	-0.09	0.12	-0.22	-0.43	-0.11	0.03	-0.48	-0.20	-0.08	0.18	0.00	0.23						
Zr	-0.40	0.11	-0.35	-0.15	-0.09	-0.28	-0.23	0.24	-0.18	-0.43	0.02	-0.02	0.49	-0.12					
Nd	-0.17	0.35	**0.70**	0.22	0.37	-0.31	-0.01	0.69	**0.95**	**0.71**	-0.28	0.07	-0.43	-0.14	-0.01				
Year	-0.47	-0.12	-0.52	**-0.90**	-0.64	-0.61	**0.86**	0.06	-0.15	0.05	**0.75**	-0.29	0.53	0.15	0.22	-0.11			
U	0.41	0.26	0.58	**0.99**	0.73	0.50	**0.80**	0.07	0.22	-0.06	**-0.80**	0.21	-0.50	-0.23	-0.15	0.23	**-0.90**		
^{210}Po Unsup.	**0.71**	0.30	0.56	**0.75**	0.47	0.06	**0.95**	-0.08	0.09	-0.07	-0.52	0.08	-0.36	0.11	-0.17	0.14	**-0.77**	**0.75**	
^{210}Po DC	**0.72**	0.19	0.61	0.64	0.56	0.07	**0.85**	-0.16	0.10	-0.08	-0.57	0.18	-0.45	0.08	-0.13	0.13	-0.68	0.64	**0.93**

Table 7.3. A correlation matrix between trace elements, radioisotopes and the time since death (^{210}Po DC = decay corrected ^{210}Po). Values greater than 0.7 (as depicted by bold print) are suggestive of a potential underlying relationship. Some may be explained through the similar biochemical affinities or presence within the same radioisotope decay chain. Others represent previously undescribed associations, notably the apparent time-related uranium content or ^{210}Po activity with the time since death.

Pilot Sample	Ca (wt.%)	Ba (μg/g)	Ce (μg/g)	La (μg/g)	Pb (μg/g)	Rb (μg/g)	Sr (μg/g)	Zn (μg/g)	Zr (μg/g)	Nd (μg/g)	As (μg/g)	U (μg/g)
1	40.96	<LLD	18	7	25	4	<LLD	210	21	11	0.19	0.063
2	39.05	<LLD	13	5	43	4	10	196	23	9	0.11	0.026
3	38.97	<LLD	10	3	43	4	1	150	23	8	0.13	0.025
4	41.12	<LLD	4	<LLD	114	4	211	207	22	6	0.13	0.003
5	40.01	<LLD	14	5	71	3	85	202	21	10	0.16	0.006
6	38.18	<LLD	9	<LLD	47	<LLD	158	157	961*	8	0.17	0.022
7	38.34	<LLD	15	6	68	3	63	120	16	10	0.22	0.017
8	38.97	<LLD	11	3	19	4	36	150	18	8	0.22	0.056
9	40.40	4	10	3	20	5	30	175	21	8	0.25	0.042
10	42.55	<LLD	11	3	25	4	14	165	21	9	0.30	0.062
11	42.23	8	7	2	23	3	53	158	20	7	0.08	0.072
12	40.01	3	16	7	38	5	5	125	18	10	0.05	0.056
13	42.47	<LLD	13	5	28	4	38	171	21	9	0.01	0.032
14	40.56	14	6	2	37	5	<LLD	202	21	6	0.03	0.023
15	39.93	2	10	5	70	4	13	164	21	7	0.03	0.007
Mean value	40.25	1.38	11	4	45	4	55	170	21	8	0.14	0.034
H-9 analysis	0.230	N/A	N/A	N/A	0.16	7.9	3.1	28.4	N/A	N/A	0.90	N/A
H-9 reported	0.231	N/A	N/A	N/A	0.16	8.0	3.0	27.5	N/A	N/A	0.88	N/A

Table 7.4. Trace element concentrations for bone samples. * = ashed in a zircon crucible, introducing contaminant Zr.

Reproducibility based on measured triplicates is better than 2% for all element abundances. <LLD = below lowest limit detection. H-9 represents the analytical standard material..

^{40}K analyses ranged widely from 9.4Bq kg^{-1} (0.04-0.41μg g^{-1}) indicating variable postmortem contents in our samples. Potassium is considered a bone calcium moderator, however, the large calcium range in our samples may indicate potassium mobilisation relative to individuals' dietary habits.

Trace Elements

Trace element contents were calculated using the ashed samples, performed by X-ray fluorescence analysis and atomic absorption spectroscopy.

Calcium values range from 38.1-42.6% of weight. These results are consistent with published studies (Manea-Kricheten *et al.,* 1991;Harley and Fisenne, 1990). The large range of calcium values may be due to pathological variations in individual bone matrices. Due to the selection of samples, the resultant age-at-death range for the samples examined is relatively large. Therefore the very nature of the specimens, being from elderly individuals, increases the likelihood of osteoporosis or metabolic bone diseases which could account for such differences.

Barium concentrations range from 3.4-13.6μg g^{-1} and are similar to values reported elsewhere (2.8-14.6μg g^{-1}) (Manea-Krichten *et al.,* 1991). Other trace elements known to possess a physiochemical affinity for the calcium hydroxyapatite structure of bone, namely zinc and strontium, are all in the range previously reported (Baranowska, Czernicki, and Aleksandrowicz, 1995). The quotients of strontium to calcium ($1.2x10^{-4}$ - $3.08x10^{-6}$) and barium to calcium ($1.9x10^{-5}$- $4.5x10^{-6}$) are within the reported range for human skeletal remains (Manea-Krichten *et al.,* 1991).

One of the main elements of interest in the samples is lead, which shows relatively elevated concentrations (19-114μg g^{-1}, mean 45 ± 25 (1sd)) and is consistent with data reported previously from environments exposed to elevated lead levels;

Authors of the Study	Total Lead (Pb) concentrations
This thesis (Portuguese Specimens)	19-114μg g^{-1} mean 45 ± 25 (1sd)
Baranowska, Czernicki, and Aleksandrowicz.(1995)	3-205μg g^{-1}, mean 58 ± 49 (1sd)
Samuels *et al.* (1989)	1.4-82.0 μg g^{-1}, mean 12-15μg g^{-1}
Jaworowski, Barbalat and Blain. (1985)	5-35μg g^{-1} , mean 16.9 ± 10 (1sd)

Table 7.5. Published lead concentrations in human bone compared to the sample results from the Portuguese material.

Though the possibility of lead contamination should be raised, I am confident that this is not the case; all instruments used during the sample preparation and subsequent analyses were lead-free. Similarly the crucibles used during the ashing process were free of detectable lead. The lead concentrations from our samples are, however, elevated above older human skeletal remains that have been analysed, ranging from 1200-3000 BC (0.1-7μg g^{-1}) (Drasch, 1982) and for samples from 4000 BC-1100 AD (0.65-5.11μg g^{-1}) (Ivanovich and Harmon, 1992). The lead/calcium ratios are also elevated above those reported by Manea-Krichten *et al.* (Manea-Krichten *et al.*, 1991).

One sample had been ashed within a zirconium crucible and has clearly become contaminated with Zr (sample number 6). However, all other elements, including those for the Zr-contaminated sample, remain unaffected by the ashing.

7.4 Discussion

Examination of the results reveals a number of interesting correlations between elements. The following discussion will concentrate on the correlations that exist in this database.

Radionuclides

A correlation exists between the $^{234}U/^{238}U$ quotient, plutonium, uranium and the postmortem interval (Figs.7.2 – 7.4).

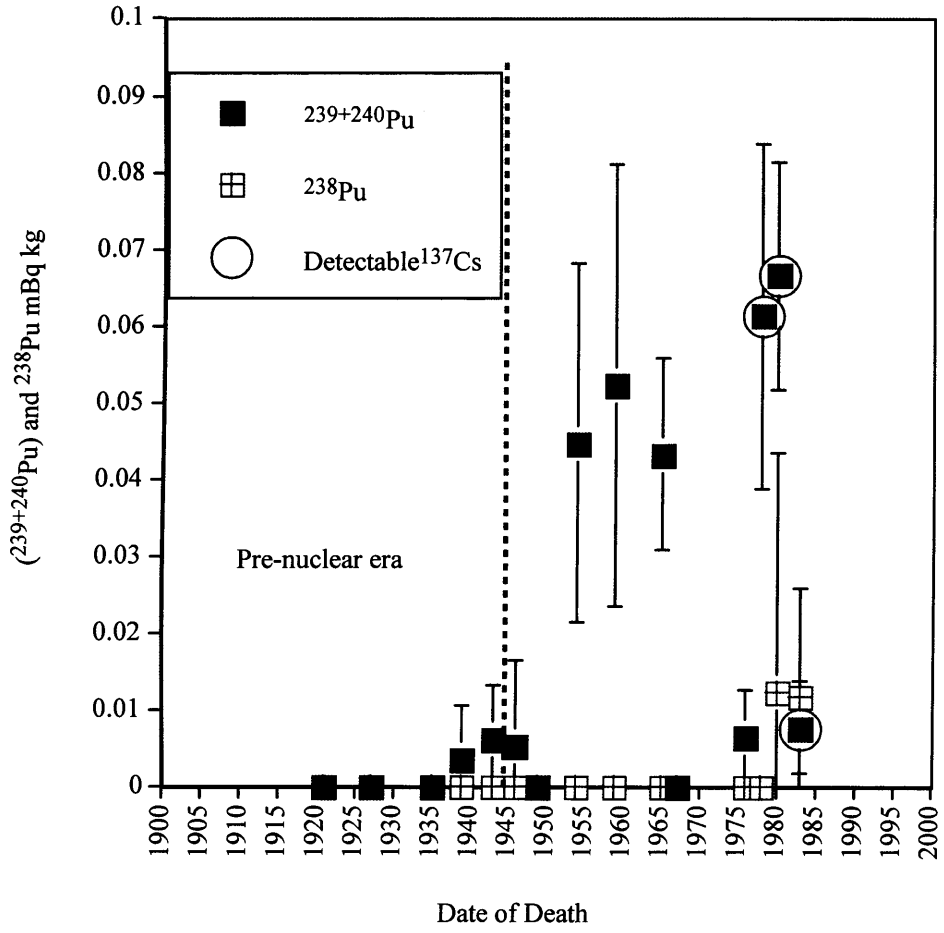


Figure 7.2. Pre- and Post-nuclear activities of Plutonium-238, 239 and 240. The 95% confidence interval error bars are produced through knowledge of the Poisson distribution in radioactivity decay analyses (see later discussion regarding ^{210}Pb analyses.)

Plutonium concentrations are relatively low, but, as would be predicted, only those samples from individuals who had died within the last 50 years have detectable Pu, (i.e. were alive within the nuclear era). Similarly, measurable concentrations of ^{137}Cs are present in the bones of only those alive after 1945. Conversely, concentrations of both plutonium and caesium are undetectable in the individuals that had died prior to the nuclear age. Not only is this

consistent with the hypothesis that the presence of man-made isotopes in human bone may be used as a means of delineating those who died prior to 1945 from those who died after that time, but it also indicates that the bones have not undergone any postmortem uptake of these nuclides. This finding would be consistent with the conditions in which the specimens had been buried, i.e. a short period of interment within soil followed by a variable period stored out of the ground.

Uranium concentrations decrease from around 0.07μg g^{-1} in samples which have experienced small postmortem intervals, to almost zero after 80 years. Given the long half-life it would suggest that uranium might have been mobilised from the bone material, although the time scale for this mobilisation is short. This result appears contrary to the knowledge of fossil bone material contents, where elevated levels of uranium (up to 1000μg g^{-1}) have been recorded, due to the absorption of uranium from groundwater (Ivanovich and Harmon, 1992). During the process of diagenetic recrystallisation, uranium enters the hydroxyapatite lattice, tending to concentrate in the medullary cavity surfaces. As a precaution against this potential uptake of radionuclides, we have analysed samples of compact bone that were free from recrystallisation.

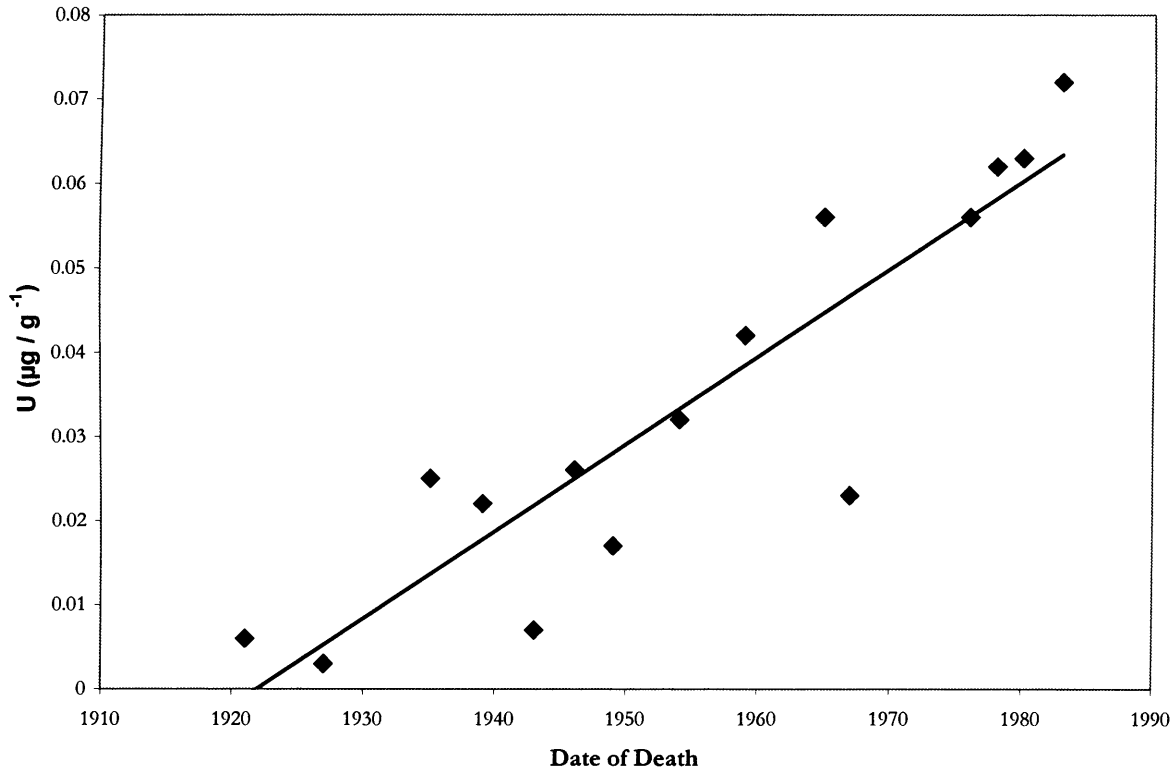


Figure 7.3. Total uranium concentrations show a relatively linear decrease with time over the last century.

Our observations of decreasing uranium content with increasing age are also consistent with those made by Szabo who suggested that the time-dependent decay of the organic phase of

bone matrix causes localised uranium reduction, resulting in a form of precipitation (Szabo, 1979). We have tried to minimise the amount of enrichment/depletion of uranium from our samples by analysing only compact bone material, which previous publications have recommended as the ideal dating material (Rae and Ivanovich, 1986). Uranium deficiency may occur adjacent to the Haversian canals, which act as channels allowing inflow and outflow of elements within groundwater or soil. However, given the short PMI (less than 100 years) it is felt that this possibility can be considered negligible. Thus, it may be considered that the uranium depletion documented within this study is related to hydrolysis during soft tissue decay.

The rapid increase in $^{234}U/^{238}U$ ratios with age suggests that, if uranium *is* being removed from the bone matrix as has been observed, then the depletion of ^{238}U is not being accompanied by concomitant ^{234}U loss. In the natural environment $^{234}U/^{238}U$ ratios in water and rock systems tend to be nearly always greater than 1. This is also true for the basic human diet, which has been shown to contain $^{234}U/^{238}U$ ratios greater than 1 (Shraishi *et al.*, 1992). In addition, previous data on uranium concentrations within human bone samples indicates that recent remains may have $^{234}U/^{238}U$ quotients greater than 1 due to increased ^{234}U intake (Hamilton, 1971;Harley and Fisenne, 1990;Fisenne, Keller and Harley, 1981). Thus the alteration in uranium concentrations may also reflect changes in diet over the last 100 years in addition to the subsequent removal of uranium from the skeletal remains.

Fig 7.4. $^{234}U/^{238}U$ activity against the date of death. There appears to be a positive relationship between time since death and total activity. Each individual isotope activity also shows a similar pattern.

The correlation between ^{210}Po and time since death is illustrated in Figure 7.5. The samples that have experienced the shortest postmortem interval have the highest concentrations of ^{210}Po, (being greater than 3.5Bq kg^{-1}); these concentrations decrease with increasing time since death producing a statistical significance ($p<0.0001$ at the 95.5% interval, Mann-Whitney test[6]). A model decay curve fitted to the data may explain this correlation by decay of unsupported ^{210}Pb with time (assuming that the concentrations of ^{210}Po equal those of ^{210}Pb). However, given the 22.3-year half-life of ^{210}Pb and the potential age range of the samples under investigation, the radioactive decay of ^{210}Pb since death must be accounted for.

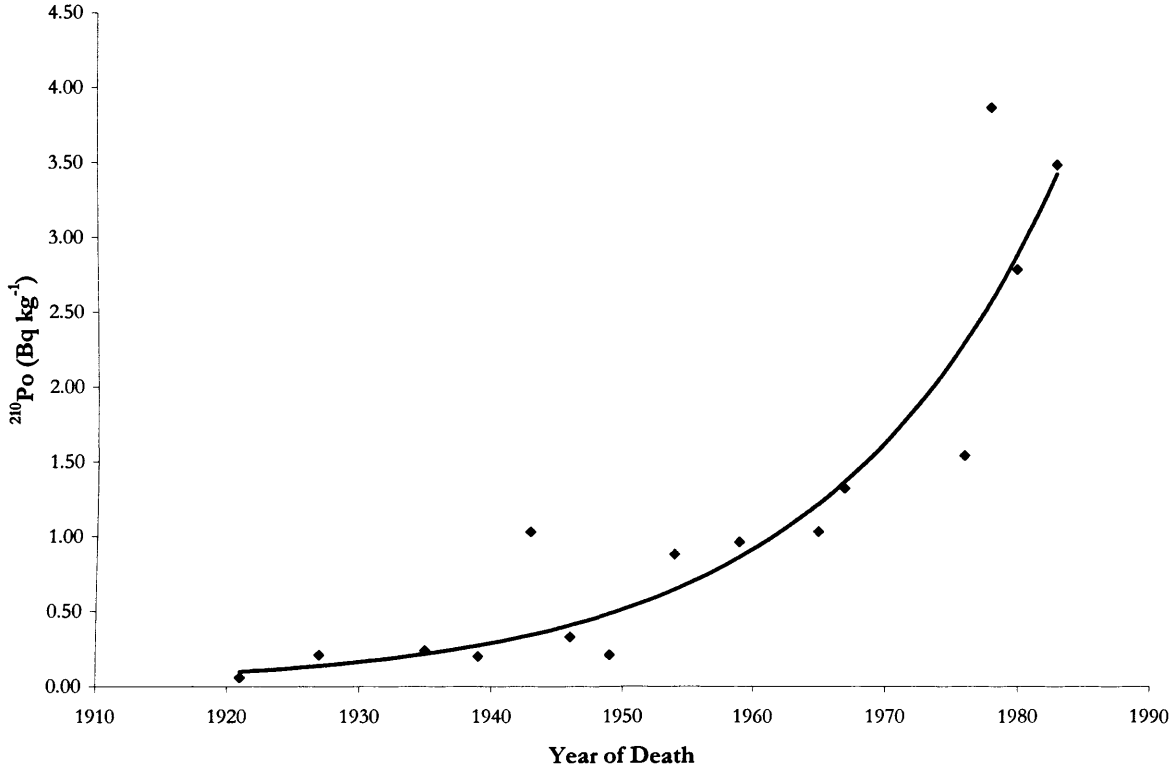


Figure 7.5. ^{210}Po (and hence ^{210}Pb, assuming equilibrium) activity against time since death, based upon ashed samples.

Additional samples from the same Portuguese collection was subsequently analysed using an alternative technique (described in Appendix 3) that did not require an ashing phase (see Table 7.6). The result was a limitation of the volatisation of certain isotopes; specifically caesium, polonium and lead content (see Figure 7.6). The reanalyses recorded a higher activity than previously identified within cases of similar PMI's, but confirms the exponential decay curve noted ($p<0.0001$ at the 95.5% interval, Mann-Whitney test[7]). These results are consistent with

[6] Nonparametric statistical significance (Mann-Whitney test) calculated using Minitab Version 13, Minitab Inc, USA.
[7] Nonparametric statistical significance (Mann-Whitney test) calculated using Minitab Version 13, Minitab Inc, USA.

the data presented in Table 7.2, where samples with high ^{210}Pb, for example sample 10 (3.9Bq kg^{-1}) has only 0.2Bq kg^{-1} of ^{226}Ra, indicating that the ^{210}Pb component found within this bone material is unsupported by radioactive decay of radium.

WHOLE BONE Sample Number	Date of Death	Years since death	^{210}Pb (Bq kg^{-1})	^{226}Ra (Bq kg^{-1})	^{137}Cs (Bq kg^{-1})
R3380	1931	69	0.65	0.15	0
R3319	1963	37	7.78	2.16	3
R5191	1951	49	3.62	1.44	0.57
R2402	1964	36	7.64	1.56	2.95
R1810	1943	57	1.57	0.43	0
R1611	1957	43	4.77	1.03	0.78
R263	1955	45	4.83	1.52	1.06
R915	1959	41	5.23	1.34	1.99
R2550	1937	63	1.47	0.65	0
R191	1982	18	29.03	5.02	3.32
R3177	1950	50	3.23	1.42	2.34
R1255	1966	34	8.93	2.55	3.45
R1753	1969	31	10.93	2.44	4.53
R3939	1945	55	2.65	1.27	0.01
X	1975	25	17.23	3.42	3.23
Y	1980	20	25.46	2.13	2.34
D8832	1927	73	0.68	0.34	0
D6758	1921	79	0.257	0.12	0

Table 7.6 The results of whole bone analyses from specimens within the Portuguese collection, housed within the University of Leicester. The methodology employed did not require an ashing phase, thus limited the loss of isotope activity through volatisation (the years since death relate to the year of analysis, being 2000).

These results are impressive, despite the small sample size for this pilot study, and confirm, as would be predicted scientifically, the presence of an exponential decay correlation between time since death and the concentrations of ^{210}Pb. The results from the unashed specimens also produce a closer-fitting exponential trendline, suggesting that some of the variation noted in the ashed specimens may indeed have resulted from volatisation (Figure 7.6).

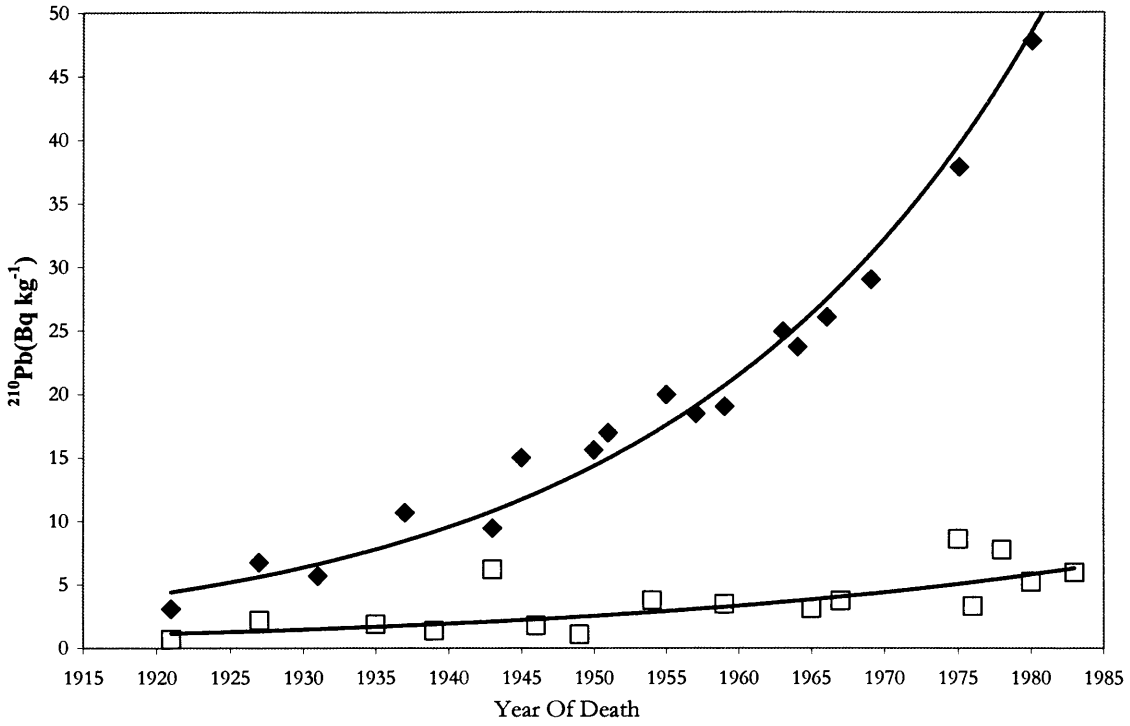


Figure 7.6. The decay corrected ^{210}Pb activities (indicated by black diamonds) gained through additional analyses of unashed material from the same Portuguese collection reveals a more readily identifiable exponential decay correlation when plotted against time, compared with ashed specimens (white squares) that form the basis of the pilot study.

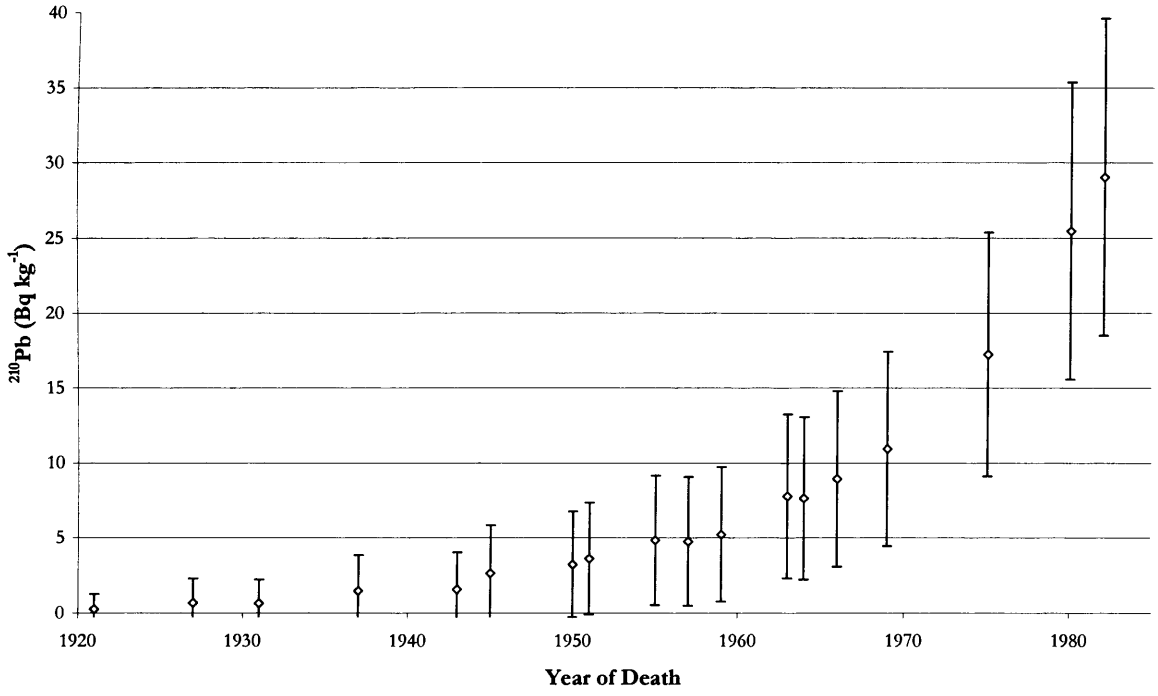


Figure 7.7 The measured ^{210}Pb activities with 95% confidence intervals from the unashed Portuguese specimens.[8]

[8] The radioactive decay of isotope atoms in unpredictable, thus any counting is subject to sampling error. The resultant activity counts follow a Poisson distribution and relate to the intrinsic variability of radioactive decay. Calculating the counting errors,

The ^{137}Cs activity is also corrected by the use of a nonashed technique, confirming the presence of man-made isotopes only after 1945, though the increase is not regular owing to the irregular creation of the isotope and its decay rate (half life = 30.3 years) (see Figure 7.8).

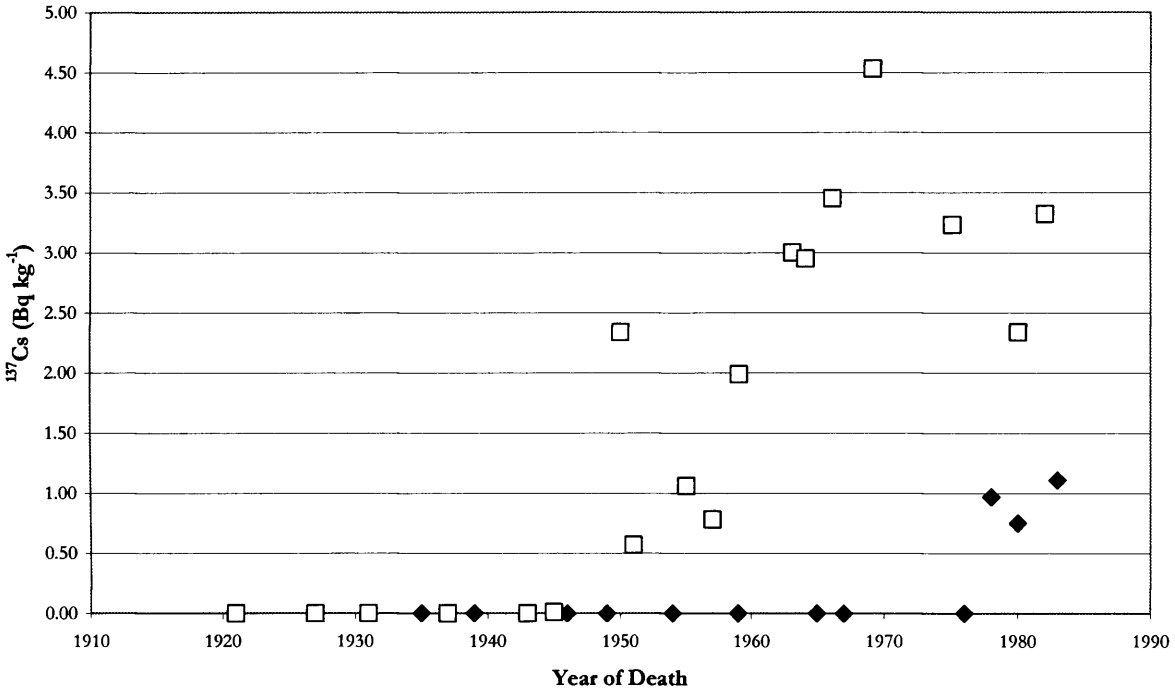


Figure 7.8 The activity of ^{137}Cs within the two samples of Portuguese remains. The specimens which have been analysed in the absence of an ashing phase (white squares) reveal higher activities compared to those that have been ashed (black diamonds) though only in sets of remains after 1945, the isotope was first created.

Trace elements

Interestingly, total stable lead concentrations increase with the postmortem interval, from around 20μg g^{-1} within the modern samples, to 115μg g^{-1} in those samples greater than 60 years old (see Figure 7.9). This increase of lead concentration is unusual, especially considering the certainty that none of the samples analysed were stored for any length of time (postmortem) in any lead-rich container. Previous studies have shown a trend of increasing lead concentrations in bone with increasing time since death, during the last two centuries (Manea-Krichten *et al.*, 1991;Samuels *et al.*, 1989;Jaworowski, Barbalat and Blain, 1985;Drasch, 1982). These findings are consistent with our results and must be attributed to lead contamination from the environment. This may reflect increased prevalence of lead water-piping in past years, or other such environmental exposures.

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and hence the 95% confidence interval, for the radioactive decay of an isotope against time is produced using the following equation:
95% Confidence interval for a specimen = $(n-1.96\sqrt{n})$ to $(n+1.96\sqrt{n})$,
where n = counting value (in Bq kg^{-1}).

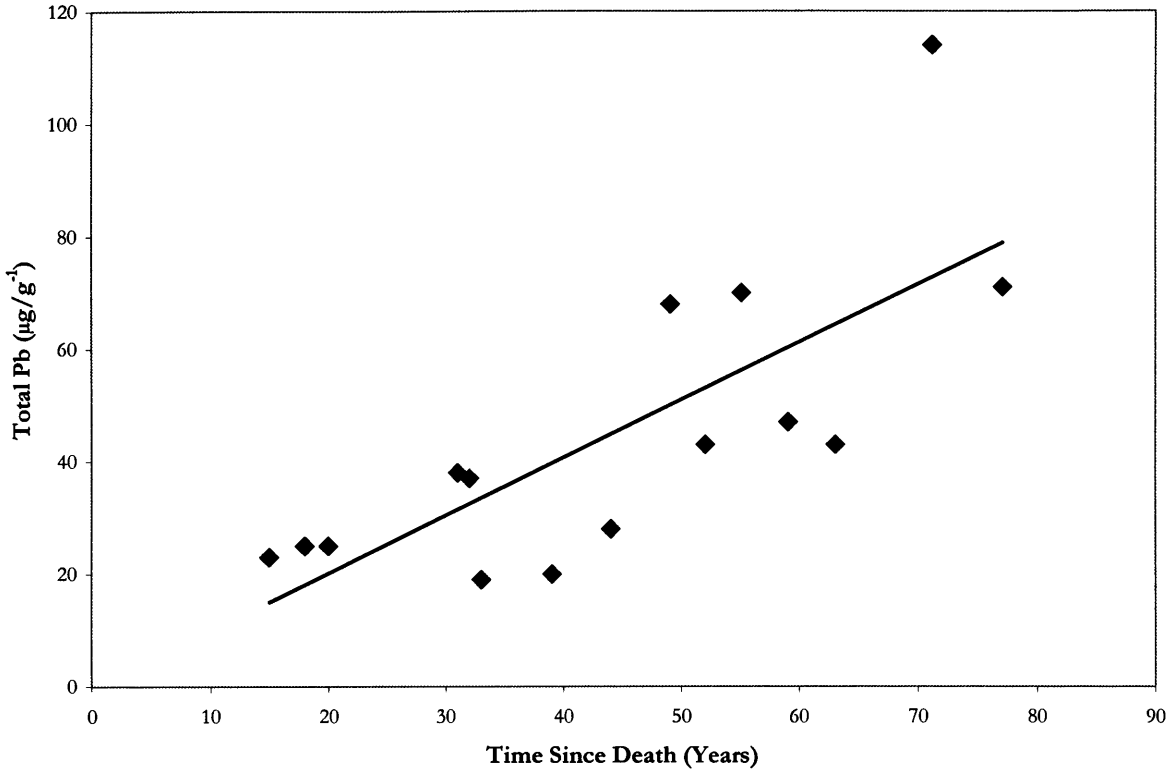


Figure 7.9. Total lead concentration of bone samples against time since death.

Other correlations between trace elements can be seen, notably the correlations with potassium and the light rare earth elements (lanthanum, cerium and neodymium), and the inter-correlations that exist amongst them (See Table 7.3).

7.5 Possible Routes of Contamination.

Physical infiltration of skeletal remains, as mentioned previously, may provide a route of contamination. Studies have shown fungal hyphae, plant rootlets and carbonized material may invade bone matrix, even dense compact tissue, by entering the vascular channels and along the Haversian canals (Marchiafava, Bonucci and Ascenzi, 1974;Hackett, 1981;Ascenzi and Silvestrini, 1984;Bell, Skinner and Jones, 1996). Active destruction, or 'osteoclastic' tunnelling, may also alter the elemental composition of bone. Therefore trace elements present within the soil may be actively carried into, or take up by, the matrix of the bone affecting concentration analyses.

Contaminants may also be introduced as solid grains, (especially silica rich grains), which infiltrate voids and fractures within the decomposing remains. Within these remains we cannot, therefore, rule out contamination occurring during the interment period, (either soil or during dry storage), but in the future the use of Scanning Electron Microscopy (SEM) fitted with an Energy Dispersive X-Ray Counter (EDX) will enable the pinpointing of uncontaminated sections much more accurately.

7.6 Comparisons with Different Countries.

Though these initial results from the pilot study are encouraging, it must be stressed that the sample size was small and that these samples originated from a single country. This may affect the accuracy of such a method for estimating the postmortem interval when applied to human skeletal remains found within different regions of the world. For example, it has been suggested that seafood provides a relatively high source of ^{210}Po (Aarkrog *et al.*, 1997). Therefore, countries with a higher than average annual intake may be exposed to a higher internal radiation dose (UNSCEAR, 1993). It is known that Portugal consumes three times the annual intake per capita of seafood compared with the United Kingdom, (60.1 kg y^{-1} per capita and 20.3 kg y^{-1} per capita, respectively; Fisheries of the United States, 2002) Nevertheless, with the improved detection of these nuclides, it is predicted that similar postmortem findings will be identified within the UK population.

In order to further assess these results, the findings of additional studies were examined. Carvalho (1995) calculated the average intake of ^{210}Po and ^{210}Pb for the population living around Lisbon, close to the cemetery from which the samples studied were recovered. Samples of the surface air, household water, major food groups and three most popular brands of cigarettes were analysed. The results suggested that water and beverages provide the lowest

^{210}Po and ^{210}Pb concentrations, though certain villages supplied by spring water filtering through granitic rocks, rich in uranium series radionuclides, may have an increased intake compared to other regions of Lisbon. However, Carvalho suggests that, even in these cases, the concentrations ingested are relatively low compared to the concentrations ingested within food.

Inhalation concentrations from atmospheric concentrations were found to be lower than UNSCEAR estimated concentrations for the Northern Hemisphere. However, by comparison, cigarette smoking was shown to expose an individual to almost 50 times the daily atmospheric concentration of ^{210}Po. Therefore it is unlikely that any increased radionuclide exposure would be resultant from the effect of local industry, rather it instead relating to personal habits.

For both ^{210}Po and ^{210}Pb, it was shown the majority of exposure was through food ingestion (95% and 83%, respectively.). Interestingly, 70% of the dietary ^{210}Po was ingested in the form of seafood (Carvalho, 1995). However, Torvik *et al.* showed that ingested ^{210}Po does not produce significant concentrations within the human skeleton (Torvik *et al.,* 1974), thus it is unlikely that seafood would produce an appreciably increased polonium burden within bone. Furthermore almost 80% of the ^{210}Pb exposure provided by food originates from meat, vegetables and cereals, and not seafood (Bunzl, Kracke and Kreuzer, 1979), (Khandekar, 1977), (Smith-Briggs and Bradley, 1984), (Smith-Briggs, Bradley and Potter, 1986).

The study by Carvalho concluded that Portuguese adults have a much higher total body lead burden than estimates from a reference population, but the author believes a proportion of this difference can be attributed to the use of fresh food samples by Carvalho compared to other studies.

Although further studies may be required before it is possible to allow comparisons between the Portuguese samples and remains unearthed within different countries, the findings of the pilot study suggest that this method may provide a new tool in estimating the date of death in skeletal remains within the forensic interval.

7.7 Conclusions for the Pilot Study.

i) The pilot study has demonstrated for the first time that, in recent human skeletal remains covering postmortem intervals (at the time of the analyses) from 15-77 years, there exists a correlation between certain radionuclide content and the time since death.

ii) The apparently useful radionuclides in question are ^{238}U, ^{234}U, ^{210}Po and ^{210}Pb from the U-series chain and artificial radionuclides such as ^{238}Pu, $^{239+240}Pu$ and ^{137}Cs.

iii) Trace element analysis of the same material revealed inter-correlations as well as correlations with time since death, (for example lead). However, further work needs to be carried out to see if such element concentrations are present in additional populations.

iv) The possibility remains that these radionuclides and trace element concentrations can be used to give some meaningful, quantitative estimation regarding the postmortem interment period for human skeletal remains.

Bosnia – Herzegovina and the International Commission on Missing Persons.

8.1 A Brief History of the Former Yugoslavia

Yugoslavia was formed in the wake of the First World War, a discohesive collection of six territories: Slovenia, Croatia, Bosnia and Herzegovina, Montenegro, Macedonia and Serbia, the latter including the regions of Kosovo and Vojvodina. Three main "ethnic groups" populated these countries: the Serbs (Orthodox), the Croats (Catholic) and the Muslims, the last being the least well defined culturally. The result was the creation of a forced united nationalism, described by Agnew and Corbridge (1995) as the idealized attempt to coalesce apparently incongruent populations into a territorially defined region. It was this strategy that President Tito employed relatively successfully within Bosnia and Herzegovina as a means of redressing the imbalance created by lingering Croatian and Serbian patriotism.

Figure 8.1. A Map of the Former Yugoslavia.

Following the dissolution of the Soviet Socialist Republic in 1991, regions of Yugoslavia declared themselves independent from each other. In October 1991 Bosnia followed suit by pronouncing sovereignty, with a referendum apparently affirming independence in March 1992, despite the protestations of Bosnian Serbs. This affirmation was formally recognised by the European Union, the result being the beginning of a four-year civil war initiated by an armed attack by Bosnian Serb Nationalist Members upon a demonstration within the Bosnian capital, Sarajevo (Gjelten, 1995).

The rise to prominence of Nationalist leaders, such as Slobodan Milosevic in Serbia, in the years following the death of President Tito in 1980 fuelled the ethnic animosity and ensured a long and violent conflict. The result was distrust and hatred between people who had lived for years as neighbours, often marrying between families. The hatred, fuelled by propaganda, culminated in the destruction of towns and the initiation of "ethnic cleansing", a process of religious and cultural elimination used by Bosnian Serbs to enable majority and power over the now fractured territories.

The population of Bosnia and Herzegovina was (and remains) composed largely of secular "Muslims", their history dating back to the Ottoman occupation when Bosnia was a self-governing region during the fifteenth to the nineteenth centuries (CIA World Factbook, 2003;Robinson, Engelstoft and Pobric, 2001). Over the centuries, these individuals continued to retain this label, more for reasons of convenience and for both economical and political gain rather than their strict adherence to the teaching of Islam. The term has now been superseded by the name "Bosniak", or "Bosnjak", to distinguish them from Serbians or Croatians (Wood, 2001;Gellner, 1983). By belonging to such a collective identity, the Bosniaks aimed to gain strength in number and, by seeking out a religion "lost" during Communist leadership, sought to claim a territorial ownership that would justify their occupancy (Gellner, 1983). It is this loss of an inherited "motherland", combined with the belief frequently held by separate nationalists that the Muslims were considered "relics of a former colonial period", that hindered their plight during the conflict and which to their oppressors used to justify their criminal actions (Robinson, Engelstoft and Pobric, 2001).

The Croatians and Bosnian Serbs originated (historically) from the territories of Croatia and Serbia, respectively. Typically Croats are followers of Catholicism whilst Serbs are Eastern Orthodox in religion, though such stereotyping does not always apply leading to difficult cultural divides that are beyond the scope of this thesis. The Serbs were aided by the population of the neighbouring Serbia and Montenegro in an attempt to further divide the region along religious lines, the ultimate goal being the creation of a single large nation: Greater Serbia. To this end, the Serbian forces practiced genocide on an almost industrial scale and aimed to "cleanse areas", allowing their ultimate occupation. Over 50% of the population was displaced during the conflict, either internally or having fled the country entirely. (Bisogno and Chang, 2002) Torture, rape and murder were utilised as weapons of warfare against Bosniaks. Civilians were also detained within concentration camps established in Omarska,

and Trnoplje during 1992, allowing further segregation from the Serb population in a manner previously employed by the Nazis during the Holocaust (Wood, 2001).

The creation of the Bosniak/Croat Federation of Bosnia and Herzegovina, through the union of the Bosniaks and Croats in March 1994, reduced the civil war to a conflict of two entities, blurring the lines of war further still. With the diplomatic assistance of international countries, the warring sides finally reached an agreement and, on the 14th December 1995, the Dayton Peace Accord was signed in Paris, France, though the conflict officially ended the following year. The result of the Peace Accord was the cessation of war and the creation of two areas within Bosnia, with joint government foundations and democratic elections. The areas were named the Federation of Bosnia and Herzegovina, (BiH), and the Republika Srpska, (RS), being separated by an internal inter-entity boundary line.

In the four years of fighting, over 250,000 people were killed. To date, over 40,000 remain missing, presumed buried within mass graves scattered throughout the landscape of the territory (ICMP website, 2003).

In May 1993, the United Nations Security Council accepted documentation of the humanitarian law violations, resulting in the establishment of the International Criminal Tribunal for the Former Yugoslavia (ICTY). The effect has been the on-going criminal trials of prominent members of the warring sides.

8.2 The International Commission on Missing Persons for the Former Yugoslavia (ICMP)

The ICMP was created at the G-7 summit in 1996, ratified by a single fax from President Bill Clinton, with the purpose of assisting families, regardless of their ethnic or religious origin, and in determining the fate of individuals lost during the armed conflicts in the Yugoslav region (ICMP website, 2003). This is achieved through working relations with governmental organisations, through civil society initiatives and, finally, through establishment of forensic sciences. The latter constitutes a massive DNA blood sample programme, aiming at identifying as many of the 40,000 dead through DNA from surviving relatives and postmortem DNA from bone material. It also requires large-scale forensic archaeological excavations of mass graves, resulting in over 12,000 sets of human remains being removed to storage facilities to date.

In February 2002, Dr Rick Harrington, Head of Exhumations and Examinations within the ICMP, invited me to visit the Sarajevo Headquarters to discuss the potential application of the PMI estimation methods within Bosnia and Herzegovina:

From : Rick Harrington <rick.harrington@ic-mp.org>
To : "swift_ben@hotmail.com" <swift_ben@hotmail.com>
Subject : Consultation
Date : Fri, 18 Jan 2002 10:43:21 +0100

From: Dr. Richard J. Harrington (Forensic Anthropologist)
Head of Exhumations and Examinations
ICMP
Sarajevo, Bosnia-Herzegovina
rick.harrington@ic-mp.org mobile phone: 387-66-102 921

Dear Dr. Swift,
I would like to discuss with you the possibility of arranging a consultation with you regarding a forensic science issue we are faced with here in the former Yugoslavia. First, some background: Currently, the International Commission on Missing Persons for the Former Yugoslavia (ICMP) is engaged in a truly ambitious attempt to identify thousands of individuals who were killed in the varous conflicts in the former Yugoslavia in the 1990s. We expect our success to be largely contigent on advances in DNA technology in recent years, but successful integration of traditional approaches (mostly from forensic anthropology, given the largely skeletal nature of the remains) with the DNA program is vital as well. (You may wish to check our website at www.ic-mp.org; I must confess, however, that as of this writing, we have yet to add the page for Recovery and Identifications.) To the extent that the entire process can be science-driven, we do expect enormous success, but as you can well imagine, there are numerous political, cultural, and financial issues that must be factored in as well. In looking to enhance our success on both scientific and nonscientific fronts, my colleagues and I will be considering various feasibility studies, pilot projects, and perhaps even large-scale projects. One issue that does occasionally arise, and is often extremely contentious, is that of assessing time-since-death. A representative of one of the "former warring parties" (Serb, Croat, or Bosniak (ie, Muslim)) may claim that certain interred remains are from individuals killed during the 1990s conflict, and should therefore be exhumed, examined, and repatriated; another party may dispute this, claiming that the remains are from individuals who died in, say, the 1960s, and therefore should not be disturbed. Given that burials have often been disturbed (perhaps even repeatedly), traditional approaches to assessing time-since-death (archaeological/forensic context, soil profile, skeletal state-of-preservation) are of diminished value. To augment these traditional approaches, I would like to assess the feasibility of conducting radionuclide and/or trace element analysis and soil chemistry analysis (eg, as per your article: B Swift et al (2001) Forensic Science International 117:73-87). I realize, of course, there are limitations and obstacles (obtaining local permission to exhume enough remains for analysis; establishing baselines for a large geographical region; costs and time limits; sensitivity and specificity of results, etc). I feel, however, that the potential benefits of even a feasibility study could be substantial. By the way, I will be attending the upcoming AAFS meetings in Atlanta (along with some of my DNA colleagues), so if you or some of your colleagues are there, that would give us an opportunity for an extended chat. Otherwise, I do hope that you might have some valuable feedback for me--positive or negative--in terms of recommendations and in terms of your willingness to consider providing a formal consultation by you or a recommended colleague.

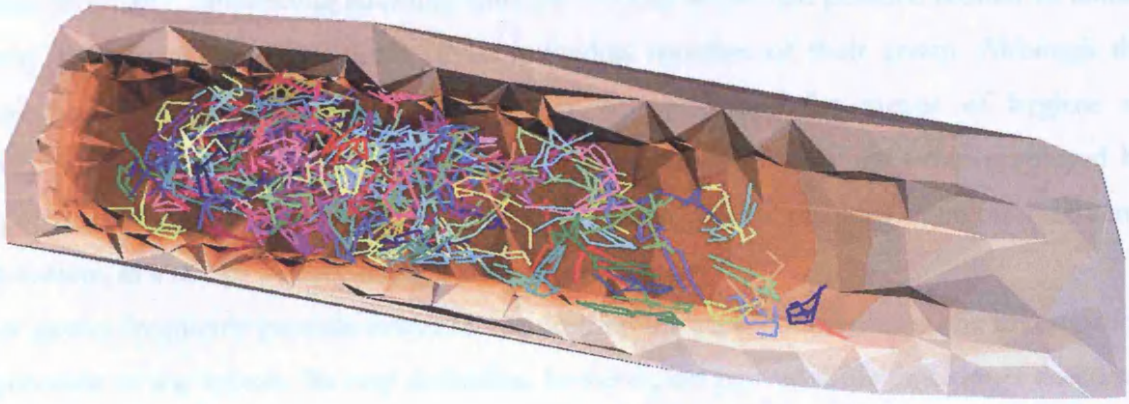
Best Wishes,
Rick Harrington

The majority of scenarios dealt with by ICMP involve mass graves. Prior to continuing this section, the mass grave will be briefly discussed.

8.3 The Mass Grave.

a)

b)



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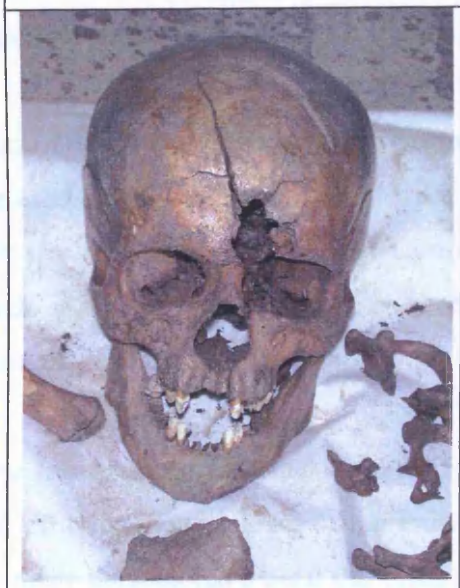

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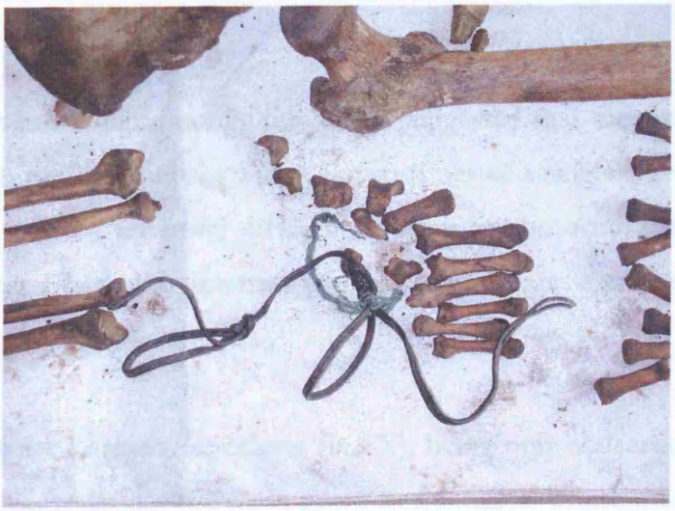


e)

Figure 8.2. (Previous Page) a) A Mass Grave in BiH being excavated according to approved archaeological standards. This grave is estimated to hold approximately 150 individuals, the disordered nature of the bodies results from their removal from the primary site of burial by earth-moving equipment at least a year after death. The subsequent interment, constituting a secondary burial, results in disruption of the skeletal elements. b) Careful excavation allows recognition of the original tyre-marks left by the truck disposing of the human remains 7 years previously, c) The archaeological excavation, together with the use of theodolite-based measurement taking, allows for 3-D reconstruction of the grave and its contents, from which the tyre-marks are evident to the right of the image, producing a sloped pit into which the remains were unloaded. d)The cause of death of many individuals is apparent, being gun shot wounds, e) however, artefacts present within the remains, such as these bindings found around the wrists, indicate that the deaths did not occur during combat. These people were instead executed, representing war crimes. Image c) courtesy of Dr. Cecily Cropper, ICMP, BiH. Photographs d) and e) are courtesy of Professor G.N. Rutty, University of Leicester.

Within 'normally' functioning societies, burial is a social or cultural practice created to confer dignity and respect into the death of an individual member of their group. Although the simultaneous burial of many individuals may be performed for means of hygiene or humanitarian purposes, such as in the time of mass disasters, they are often employed by perpetrators of widespread war crimes, or crimes against specific religious or cultural elements of a society, as a means of concealing the acts.

Mass graves frequently provide evidence required by the prosecution during the investigation of genocide or war crimes. Its very definition, however, has proved difficult. Skinner described it in terms of the minimum number of bodies contained, being more than five (Skinner, 1987). Later work by the same author describes the relative organisation of the occupants, indicating that the bodies of victims of genocide are frequently treated disrespectfully (Skinner, York and Connor, 2002).

Mant (1987), drawing upon his background in taphonomic processes, suggested that the term be used when two or more bodies are in physical contact within the confines of a single grave, yet this definition does not take into account temporal differences between the occupants, such as would be present where a spouse has been interred within the same grave as their partner, who died years previously.

The International Criminal Tribunal for the Former Yugoslavia (ICTY), being only concerned with the manner of death of the individuals, defines the mass grave as a location where three or more persons have been the victims of extra-judicial, summary or arbitrary executions, and not having died during armed confrontation or combat (United Nations, 1997).

However, a definition is required that reflects upon both the cultural or social context in which the individuals died, and the circumstances in which they died. By way of addressing this need, Schmitt provides the following working definition:

"a criminal mass grave contain(s) the remains of a group of individuals who share some common trait that justified their assassination in the eyes of the perpetrators" (Schmitt, 2002).

8.4 The Creation of a Bosnian Study

During the period from February 2002 until March 2003, I visited Sarajevo, BiH, on three separate occasions, to discuss a collaboration between the British centres and ICMP. This resulted in a formal study proposal and the first specimens were received from Bosnia in mid-March 2002.[9]

ICMP routinely remove a section from the femur of each body recovered. This is divided into two, the first forming the primary source for their DNA based identification technique, and the second forming "back-up" material should the primary source fail. The study would therefore utilise the back-up material, being of sufficient size and quality (compact bone and free from contamination), therefore ensuring that no additional material would be retained. The samples are typically 3 to 5cm in length and 15-25g in weight. A barcode system has been implemented by ICMP such that all analyses were performed blind to the postmortem interval and any identifying information.

The cutting and preparation of the sections follow established Standard Operating Procedures at ICMP to ensure no contamination between samples.

a) b)

Figure 8.3. a) The Missing Persons Institute near Tuzla, BiH. This single facility currently houses over 4500 sets of remains, b) Sections of femora are removed and given barcode identities. These are then stored within the ICMP facility in Tuzla prior to DNA analysis.

[9] All specimens entering the United Kingdom were declared to H.M. Inspector of Anatomy in accordance with the Draft Code of Practice, dated July 2002.

It was decided to examine a limited number of remains from mass graves separated both geographically within BiH and also temporally, to allow calibration of the time since death estimation technique. The date of death of all the individuals was known through witness testimony and the anthropological examination had produced estimated ages at death although, at the time of testing, the individuals remained unidentified.

Mr. Gordon Bacon, ICMP Chief of Staff, and the Bosnia-Herzegovina Ministry of Health granted permission to proceed with the study. Mr. Adnan Rizvic, Head of ICMP's Identification Coordination Centre (ICC) in Tuzla, BiH, selected a total of 20 case numbers from two mass graves, controlled for the place of residence, the place of death, the place of burial, the date of death, the grave type (i.e. large mass graves), gender, age range (25 to 45), the absence of skeletal pathologies, and the same ethnic identity (all Bosniak).

The two mass graves selected for this purpose were as follows:

Mass Grave One: Kevljani, near Prijedor.

On the 30th April 1992, the community of Prijedor experienced a takeover by Serb forces. All non-Serbs living within the region became unemployed, with children banned from their own schools. Less than a month later, fearing a rebellion by local Muslims and Croats, the Serb forces created the detention camps at Omarska, Keraterm and Trnopolje. The camps remained in operation for over 3 months during 1992, until international media and governments became aware of their existence, during which time the prisoners experienced widespread poverty, torture, rape and murder. Witnesses described piles of corpses accumulating on a regular basis and removed by lorry. The rate at which inhabitants were murdered appeared to accelerate in the latter part of the camps' existence, as fears over international exposure grew. The true number of individuals that died within these detention camps is unknown.

The individuals being tested died during June or July 1992. Their remains were excavated in July 1999.

Mass Grave Two: Lazete, near Srebrenica.

In 1993, over 40000 Muslims sought refuge in Srebrenica, a town located in eastern Bosnia. Serbian forces progressed towards the region, resulting in the refugees seeking assistance from a United Nations (UN) enclave sited within an abandoned battery factory at Potocari, close to Srebrenica. Despite a UN mandate declaring the factory and surrounding area as a "Safe Area", hence protected under International Law by the right to respond to hostilities with air and ground forces, the Serbs proceeded in July of 1995, under the command of General Mladic, to enter the facility. Despite the presence of Dutch UN soldiers, armed Serbs removed over 20,000 individuals in lorries (Wood, 2001). It was later learnt, through survivor testimony, that the men and boys were separated from the females and transported away. Those 7063 humans were never seen again. (Wood, 2001)

The individuals being tested died in July 1995. Their remains were excavated in August 1996.

From these two mass graves, the following individuals were selected to form the basis of the study:

Code Number	Sex	Estimated Age
002	M	45-55
003	M	35-45
004	M	45-60
008	M	30-40
010	M	35-45
011	M	45-55
016	M	20-99
017	M	28-35
018	M	45-55
019	M	30-40

Table 8.1. Details regarding the specimens chosen from Mass Grave One, Kevljani, used to establish the method of dating the postmortem interval. All individuals died in June or July 1992, with the remains recovered in July 1999.

Code Number	Sex	Estimated Age
001	M	42
005	M	52
006	M	50
007	M	58
009	M	16
012	M	54
013	M	53
014	M	45
015	M	60
020	M	44

Table 8.2. Details regarding the specimens chosen from Mass Grave Two, Lazete, used to establish the method of dating the postmortem interval. All individuals died in July 1995, with the remains recovered in August 1996.

8.5 Methods

Due to the advances in technology, and the availability of more accurate analytical techniques, the methodology employed differed to that for the pilot study, though these techniques are equally accepted scientifically (See Appendix 3).

8.6 Results

ICMP No.	Al (ppb)	Ba (ppb)	Cd (ppb)	Co (ppb)	Cr (ppb)	Cu (ppb)	Fe (ppb)	K (ppb)	Mg (ppb)
001	$1.49x10^4$	$1.68x10^3$	0.0	$3.49x10^2$	$1.06x10^3$	$4.45x10^3$	$1.82x10^4$	$7.03x10^5$	$2.23x10^6$
002	$1.49x10^4$	$3.18x10^3$	0.0	$1.18x10^2$	$1.33x10^3$	$4.29x10^3$	$6.42x10^4$	$4.05x10^4$	$2.54x10^6$
003	$1.47x10^4$	$1.43x10^3$	$2.49x10^1$	$6.05x10^2$	$6.01x10^2$	$2.92x10^3$	$8.92x10^4$	$1.66x10^5$	$2.23x10^6$
004	$1.40x10^4$	$1.02x10^3$	0.0	$8.12x10^2$	$1.65x10^2$	$2.54x10^3$	$1.15x10^4$	$1.55x10^5$	$2.45x10^6$
005	$1.80x10^4$	$1.70x10^3$	0.0	$3.85x10^2$	$6.61x10^2$	$2.80x10^3$	$1.64x10^4$	$3.16x10^5$	$2.14x10^6$
006	$1.43x10^4$	$2.14x10^3$	$4.76x10^1$	$3.58x10^2$	$4.35x10^2$	$2.67x10^3$	$4.98x10^3$	$1.28x10^5$	$2.28x10^6$
007	1.8410^4	$8.39x10^3$	0.0	$2.23x10^1$	$1.07x10^3$	$3.26x10^3$	$9.12x10^4$	$2.42x10^5$	$2.71x10^6$
008	$1.42x10^4$	$4.93x10^3$	$1.62x10^1$	$2.36x10^2$	$8.15x10^2$	$2.81x10^3$	$2.70x10^4$	$1.01x10^5$	$3.05x10^6$
009	$1.64x10^4$	$6.82x10^3$	5.30	$3.21x10^2$	$1.98x10^2$	$2.96x10^3$	$1.06x10^4$	$4.25x10^5$	$2.44x10^6$
010	$1.67x10^4$	$5.38x10^3$	0.0	$2.02x10^2$	$6.92x10^2$	$2.82x10^3$	$1.62x10^4$	$9.47x10^4$	$2.40x10^6$
011	$1.32x10^4$	$1.84x10^3$	$1.77x10^1$	0.0	$1.10x10^3$	$2.83x10^3$	$8.29x10^3$	$1.60x10^5$	$1.99x10^6$
012	$1.43x10^4$	$1.68x10^3$	0.0	0.0	$2.93x10^2$	$2.70x10^3$	$1.10x10^4$	$7.12x10^5$	$2.11x10^6$
013	$1.61x10^4$	$2.51x10^3$	0.0	$2.85x10^2$	$1.83x10^3$	$2.83x10^3$	$2.26x10^4$	$9.05x10^5$	$2.03x10^6$
014	$2.12x10^4$	$2.49x10^3$	0.0	$9.74x10^2$	$9.10x10^2$	$4.34x10^3$	$1.07x10^5$	$2.75x10^5$	$2.11x10^6$
015	$1.62x10^4$	$2.73x10^3$	$6.89x10^1$	$3.17x10^2$	$3.99x10^2$	$2.82x10^3$	$1.50x10^4$	$1.24x10^5$	$2.15x10^6$
016	$1.49x10^4$	$2.16x10^3$	0.0	$4.17x10^2$	$3.38x10^2$	$2.62x10^3$	$2.14x10^5$	$2.48x10^4$	$2.21x10^6$
017	$1.83x10^4$	$2.33x10^3$	0.0	0.0	$5.91x10^2$	$2.65x10^3$	$2.53x10^4$	$1.75x10^5$	$2.46x10^6$
018	$1.56x10^4$	$2.45x10^3$	0.0	$7.07x10^2$	$2.88x10^2$	$2.74x10^3$	$1.89x10^4$	$7.12x10^4$	$2.50x10^6$
019	$1.62x10^4$	$3.72x10^3$	0.0	$2.87x10^1$	$6.43x10^2$	$2.62x10^3$	$8.62x10^4$	$7.28x10^4$	$3.12x10^6$
020	$1.06x10^5$	$2.79x10^3$	0.0	$2.25x10^2$	$3.15x10^2$	$2.86x10^3$	$9.28x10^4$	$3.63x10^5$	$2.14x10^6$

Table 8.3. The aluminium, barium, cadmium, cobalt, chromium, copper, iron, potassium and magnesium content of the Bosnian samples. The methods employed for analyses are detailed within Appendix 3.

ICMP No.	Mn (ppb)	Na (ppb)	Ni (ppb)	Pb (ppb)	S (ppb)	Si (ppb)	Sr (ppb)	Ti (ppb)	Zn (ppb)
001	$4.29x10^2$	$5.09x10^6$	$2.49x10^3$	$3.56x10^3$	$2.24x10^6$	6.1610^3	$9.49.10^4$	$2.31x10^2$	$9.50x10^4$
002	$3.11x10^4$	$4.38x10^6$	$1.65x10^3$	$2.22x10^3$	$2.07x10^6$	$6.36x10^3$	$5.75x10^4$	$4.19x10^2$	$1.01x10^5$
003	$1.53x10^4$	$4.75x10^6$	$6.49x10^2$	$4.27x10^3$	$2.02x10^6$	$6.88x10^2$	$5.32x10^4$	$1.76x10^2$	$7.43x10^4$
004	$2.00x10^3$	$4.32x10^6$	$3.82x10^2$	$3.42x10^3$	$1.99x10^6$	$5.91x10^2$	$4.72x10^4$	$1.99x10^2$	$8.99x10^4$
005	$3.17x10^3$	$4.69x10^6$	$4.79x10^2$	$2.58x10^3$	$2.06x10^6$	$5.43x10^3$	$8.66x10^4$	$3.76x10^2$	$6.21x10^4$
006	$1.31x10^2$	$4.26x10^6$	$1.44x10^2$	$8.54x10^3$	$1.88x10^6$	$1.36x10^3$	$6.91x10^4$	$2.12x10^2$	$7.94x10^4$
007	$9.75x10^3$	$5.00x10^6$	$6.64x10^2$	$3.12x10^3$	$2.09x10^6$	$5.98x10^3$	$6.76x10^4$	$2.01x10^2$	$8.07x10^4$
008	$9.37x10^3$	$4.21x10^6$	$5.97x10^2$	$1.71x10^3$	$1.89x10^6$	$1.61x10^2$	$3.85x10^4$	$9.46x10^1$	$7.83x10^4$
009	$1.08x10^3$	$4.50x10^6$	$5.38x10^2$	$2.29x10^3$	$2.00x10^6$	$7.09x10^3$	$8.02x10^4$	$1.93x10^2$	$7.47x10^4$
010	$9.49x10^2$	$4.55x10^6$	$1.06x10^3$	$5.26x10^3$	$1.99x10^6$	$6.81x10^2$	$4.94x10^4$	$3.19x10^2$	$8.46x10^4$
011	$1.44x10^2$	$4.46x10^6$	$5.59x10^2$	$3.22x10^2$	$1.86x10^6$	0.0	$3.88x10^4$	$3.12x10^2$	$8.93x10^4$
012	$5.06x10^2$	$5.11x10^6$	$8.53x10^1$	$2.90x10^3$	$1.95x10^6$	$4.58x10^3$	$7.60x10^4$	$2.73x10^2$	$8.16x10^4$
013	$3.33x10^3$	$4.86x10^6$	$9.98x10^2$	$3.54x10^3$	$1.70x10^6$	$7.83x10^3$	$4.99x10^4$	$4.40x10^2$	$6.85x10^4$
014	$3.80x10^3$	$4.51x10^6$	$3.51x10^2$	$1.84x10^3$	$1.89x10^6$	$1.62x10^3$	$9.32x10^4$	$6.14x10^2$	$7.70x10^4$
015	$2.72x10^3$	$4.45x10^6$	$3.61x10^2$	$1.21x10^3$	$2.04x10^6$	$3.67x10^3$	$8.30x10^4$	$3.51x10^2$	$9.72x10^4$
016	$3.63x10^4$	$3.89x10^6$	$3.38x10^2$	$5.11x10^3$	$1.87x10^6$	$5.71x10^3$	$4.95x10^4$	$2.04x10^2$	$7.24x10^4$
017	$2.43x10^3$	$4.49x10^6$	$2.41x10^2$	$2.61x10^3$	$1.84x10^6$	$9.90x10^2$	$3.18x10^4$	$2.47x10^2$	$8.34x10^4$
018	$5.99x10^2$	$4.72x10^6$	$5.27x10^2$	$4.18x10^3$	$2.02x10^6$	0.0	$4.85x10^4$	$2.47x10^2$	$8.48x10^4$
019	$9.85x10^3$	$4.45x10^6$	$7.20x10^2$	$2.90x10^3$	$1.92x10^6$	$3.62x10^3$	$4.70x10^4$	$2.09x10^2$	$7.82x10^4$
020	$6.16x10^3$	$4.62x10^6$	$1.07x10^2$	$3.03x10^3$	$1.93x10^6$	$4.53x10^4$	$9.97x10^4$	$5.06x10^3$	$8.91x10^4$

Table 8.4. The manganese, sodium, nickel, lead, sulphur, silicon, strontium, titanium and zinc content of the Bosnian samples. The methods employed for analyses are detailed within Appendix 3.

ICMP no.	Na_2O Wt%	MgO Wt%	P_2O_5 Wt%	CaO Wt%	^{210}Pb (Bq kg^{-1})	^{226}Ra (Bq kg^{-1})	^{40}K (Bq kg^{-1})	^{137}Cs (Bq kg^{-1})	^{241}Am (Bq kg^{-1})	^{90}Sr (Bq kg^{-1})	$^{239+240}Pu$ (Bq kg^{-1})	^{238}Pu (Bq kg^{-1})
001	0.63	0.36	27.47	39.13	30.77	11.31	283.23	0.22	< LLD	< LLD	< LLD	< LLD
002	0.51	0.37	26.58	39.03	21.94	25.63	78.54	0.19	< LLD	< LLD	< LLD	< LLD
003	0.59	0.42	26.52	29.03	20.32	9.54	124.31	0.21	< LLD	< LLD	< LLD	< LLD
004	0.61	0.48	28.45	39.04	21.14	6.77	102.35	0.12	< LLD	< LLD	< LLD	< LLD
005	0.59	0.35	27.59	39.09	32.32	10.43	173.54	0.13	< LLD	< LLD	< LLD	< LLD
006	0.51	0.33	26.73	28.99	35.54	13.55	100.01	0.46	0.10	0.002	< LLD	< LLD
007	0.61	0.48	27.11	39.03	33.99	58.77	169.43	0.19	< LLD	< LLD	< LLD	< LLD
008	0.54	0.88	26.92	28.98	19.34	36.31	83.41	0.26	< LLD	< LLD	< LLD	< LLD
009	0.58	0.43	26.08	29.05	25.65	53.43	225.42	0.18	< LLD	< LLD	< LLD	< LLD
010	0.58	0.37	27.6	38.98	20.31	42.43	90.42	0.40	0.077	0.001	< LLD	< LLD
011	0.6	0.32	27.29	39.01	21.43	14.68	111.44	0.21	< LLD	< LLD	< LLD	< LLD
012	0.66	0.34	27.31	39.10	32.23	13.23	300.21	0.22	< LLD	< LLD	< LLD	< LLD
013	0.41	0.17	21.54	19.06	33.34	16.54	362.40	0.18	< LLD	< LLD	< LLD	< LLD
014	0.67	0.43	28.74	39.05	29.45	16.45	180.21	0.17	< LLD	< LLD	< LLD	< LLD
015	0.54	0.32	27.4	39.02	34.24	18.43	89.57	0.56	< LLD	< LLD	< LLD	< LLD
016	0.55	0.39	27.88	39.05	18.89	12.10	66.74	0.11	< LLD	< LLD	< LLD	< LLD
017	0.61	0.52	27.6	39.01	19.32	15.54	135.42	0.21	< LLD	< LLD	< LLD	< LLD
018	0.48	0.53	25.81	29.00	21.32	12.34	78.65	0.20	< LLD	< LLD	< LLD	< LLD
019	0.49	0.87	26.11	29.02	20.00	31.23	72.34	0.12	< LLD	< LLD	< LLD	< LLD
020	0.74	0.45	28.79	39.51	30.43	20.31	201.21	0.42	0.09	0.002	< LLD	< LLD

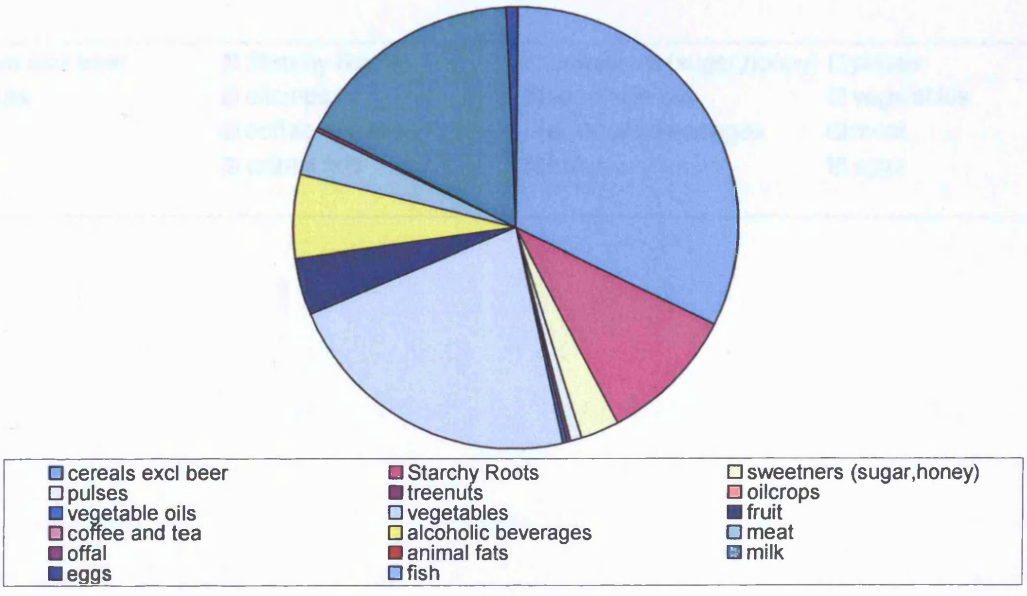
Table 8.5. Chemical and isotope analysis results for the Bosnian Mass Grave Specimens, as analysed at the Postgraduate Institute for Sedimentology, University of Reading. The methodology for these analyses is detailed within Appendix 3. (<LLD = below the lowest level of detection)

Diet Related Isotope Activity

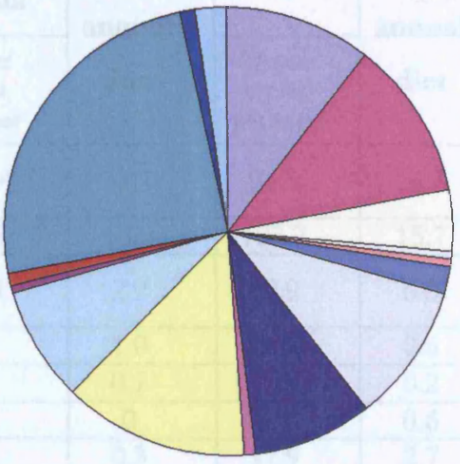
Firstly, the most apparent difference between the Portuguese dataset and the Bosnian is that the concentrations of ^{226}Ra are much higher in the latter set. A similar relationship exists with ^{40}K, again being higher than was measured in the Portuguese remains. This may be, in part, diet-related, ^{226}Ra being present in proportionally higher concentrations in cereals, (which formed up to 32.1% of their diet during the conflict), and hence bread, beverages and vegetables (which accounted for a further 22.2%) (Pietrzak-Flis *et al.*, 2001).

Personal experience and published studies of immigrant populations originating from BiH have indeed shown that the Bosnian diet consists of bread as the staple food, with meat, dairy products (especially butter and buttermilk, often being home prepared) and some vegetables (Noah and Truswell, 2001;Jonsson *et al.*, 2002). The flour used is essentially white wheat and much of the meat (mostly beef) is cured or smoked. Unlike other countries bordering the Mediterranean Sea little olive oil is consumed, though some small areas of the region grow this commodity. One of the commonest beverages consumed is strong Turkish-style coffee (referred to locally as Bosnian coffee). All of these would be predicted to increase the ^{226}Ra burden internally. It should be noted, however, that many of these commodities would have been available in only limited quantities during the conflict, when poor dietary habits were reflected in the health of the population (Ivanković *et al.*, 2003;di Giovanni, 1995).

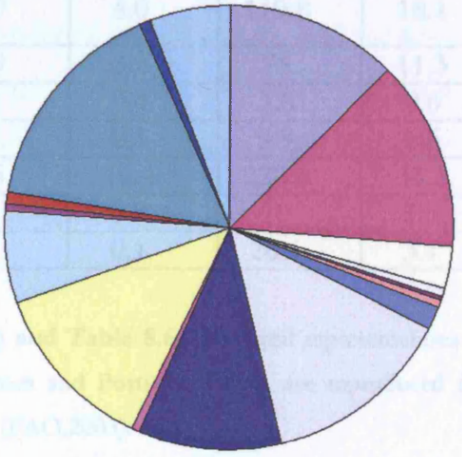
Bosnian Food Consumption, 1992.



United Kingdom Food Consumption, 1992.



Portuguese Food Consumption, 1992.



cereals excl beer	Starchy Roots	sweetners (sugar,honey)	pulses
treenuts	oilcrops	vegetable oils	vegetables
fruit	coffee and tea	alcoholic beverages	meat
offal	animal fats	milk	eggs
fish			

	Bosnia	%age annual diet	UK	%age annual diet	Portugal	%age annual diet
	kg per capita per year		kg per capita per year		kg per capita per year	
Cereals (excl beer)	184.4	32.1	94.5	14.3	125.9	12.7
Starchy Roots	57.5	10.0	103.7	15.7	136.1	13.7
Sweetners (sugar,honey,etc)	16.4	2.9	39.9	6.0	31.6	3.2
Pulses	5.7	1.0	3.8	0.6	5.6	0.6
Treenuts	0.7	0.1	1.5	0.2	3.9	0.4
Oilcrops	0	0	3.7	0.6	3.7	0.4
Vegetable oils	1.6	0.3	17.9	2.7	18.5	1.9
Vegetables	127.4	22.2	89.8	13.6	134.2	13.5
Fruit	24	4.2	77.1	11.7	103.5	10.5
Coffee and tea	0.7	0.1	8.1	1.2	4.9	0.5
Alcoholic beverages	34.7	6.0	119.8	18.1	124.9	12.6
Meat	18.9	3.3	75	11.3	66.4	6.7
Offal	2.1	0.4	3.8	0.6	5.2	0.5
Animal fats	0.5	0.1	9.9	1.5	9	0.9
Milk	94.5	16.4	220.6	33.3	156.9	15.8
Eggs	4.8	0.8	10	1.5	8.8	0.9
Fish	0.7	0.1	20.6	3.1	58.2	5.9

Figures 8.4 – 8.6. (previous pages) **and Table 8.6.** Graphical representations of the annual dietary intake for Bosnia-Herzegovina, United Kingdom and Portugal. Values are reproduced from the Food and Agricultural Organisation of the United Nations (FAO,2003).

The radionuclide concentrations are also relatively high compared with UK based studies (especially the ^{226}Ra and ^{40}K concentrations), though this may reflect the nature of the methodology employed; for the majority of analyses within this study unashed bone (dry bone mass) was used, being tested in solid, powder and liquid forms.

The seafood-rich diet of Portugal, as described before, could also account for some differences, notably the ^{210}Pb and ^{210}Po concentrations. Despite the country being 51,129 sq km in size, the Bosnian population is relatively land-locked, possessing only 20km of coastline (CIA World Factbook). As such, seafood consumption for the Bosnian population is currently estimated at only 1.6kg per year per capita, compared with the Portuguese who consume up to 60.1kg per year per capita (FAO, 1993). The low ^{210}Pb activity was reflected in the additional counting times required.

Inhalation Exposure

Bosnia once possessed one of the worst air qualities in Europe due to the large numbers of industries present throughout the country. The conflict resulted in many of these industries collapsing. Rather ironically therefore the air quality has improved since the early 1990s. Personal experience, however, suggests that the air pollution still exceeds that in Western European cities. The risk through inhalation of radioisotopes may therefore be higher than that experienced within a reference population. Some cities, such as Tuzla in North-East Bosnia, have been built around a coal-burning power station, combusting large quantities of lignite-rich fuel. To date, no air quality data exists for any region of BiH.

Smoking is also prominent in Bosnia, forming a highly significant air pollutant indoors. Current estimates suggest that up to 80% of the adult population smokes, risking higher ^{210}Po intakes. The local brand cigarette (Drina™, DIN, Serbia) was the most smoked tobacco product during the conflict and, because of this, this variety of cigarette was purchased in Sarajevo and analysed for isotope concentrations. The results were compared with those for a major UK brand.

Sample	^{210}Pb (Bq kg^{-1})	±%	^{226}Ra (Bq kg^{-1})	±%	^{40}K (Bq kg^{-1})	±%
BiH Cigarette tobacco	240.04	5.32	69.04	7.9	1139.51	10.32
BiH Cigarette filter (unused)	121.6	6.43	0.27	9.47	671.34	7.64
BiH Cigarette filter (used)	186.43	6.98	2.81	6.44	839.43	8.64
BiH Cigarette ash	467.44	4.64	10.78	4.57	5512.34	9.64
UK Cigarette tobacco	247.93	8.65	243.14	7.9	1178.23	9.69
UK Cigarette filter (unused)	-	-	-	-	-	-
UK Cigarette filter (used)	157.94	5.54	18.12	4.63	491.34	9.54
UK Cigarette ash	197.06	7.37	11.24	7.54	8575	7.9

Table 8.7. Comparison of the radioisotope content of Bosnian and British cigarette brands. The intervening columns provide uncertainty values for each isotope.

The results show that the inhalation risk for the Bosnian population is comparable with the UK population. Indeed, it is apparent that the UK brand tobacco has a higher ^{226}Ra content. Smoking the cigarette results in the concentration of some isotopes, such as is seen for the concentrations of ^{210}Pb between the unused and ashed samples, though volatisation may deplete other isotope concentrations, such as seen with ^{226}Ra.

Given the percentage of the population that smokes, however, the risk of passive smoking would also increase the overall inhalation exposure.

Postmortem Interval Correlations

One thing is apparent from the results: ^{210}Pb concentrations do not appear to correlate well with ^{226}Ra, as would usually be predicted, being part of the same decay chain series (Figure 8.7).

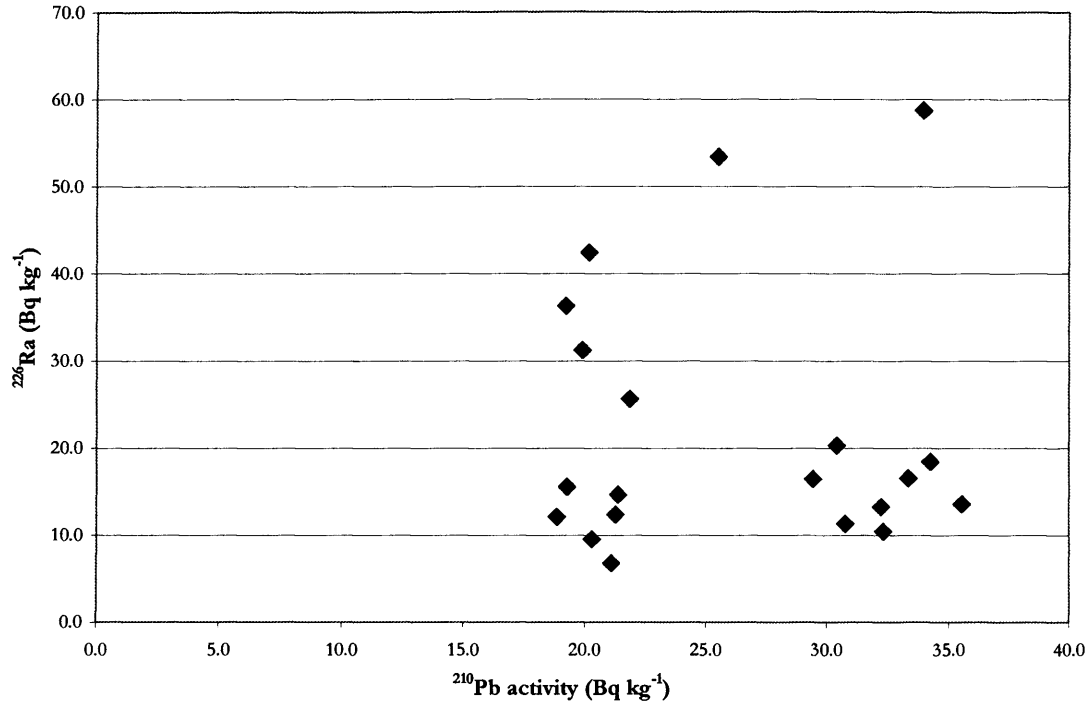


Figure 8.7. The expected correlation between ^{226}Ra and ^{210}Pb, members of the same decay chain, appears inhibited.

This may indicate either:

1) The hypothesised dating system does not work, or
2) ^{226}Ra and ^{210}Pb have de-coupled from the decay series within this population.

These possibilities will be discussed further.

^{226}Ra appears to correlate with other alkali metals, such as barium (Figure 8.8) and potassium, indicating it is incorporated into the bone under similar physiochemical conditions.

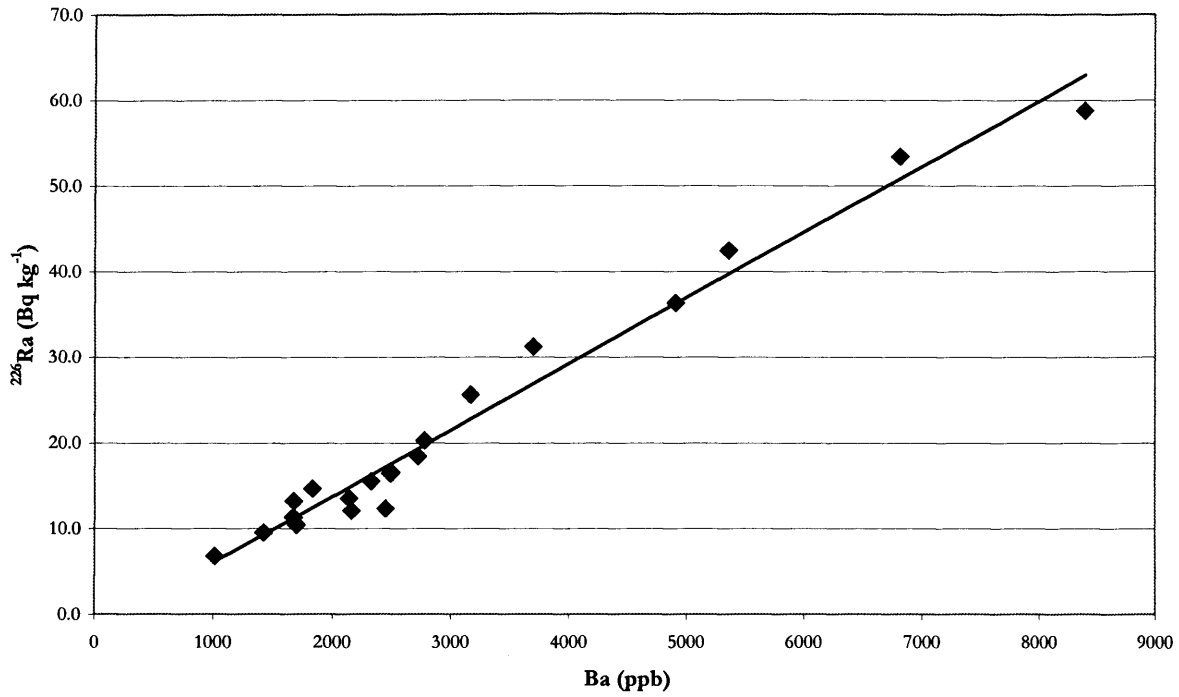


Figure 8.8. The correlation between ^{226}Ra activity and Ba concentration forms a linear relationship, as would be expected given their reaction-based similarities.

The measured concentrations of ^{210}Pb correlate well with strontium, lead and ^{212}Pb (from the ^{232}Th and Thoron chain), indicating accumulation has proceeded in a manner that would be expected. The loss of the correlation between concentrations is therefore assumed to be due to an unsteady deposition. Despite this, ^{210}Pb still correlates well with time since death within each of the two groups, indicating that a time-based decay function is occurring and that, therefore, the first possibility raised of an unworkable dating technique appear incorrect. In fact, as shown in Figure 8.9, the activity of ^{210}Pb is appreciably different over an interval of only 3 years. It could even be suggested that a PMI less than this may still be distinguished with confidence.

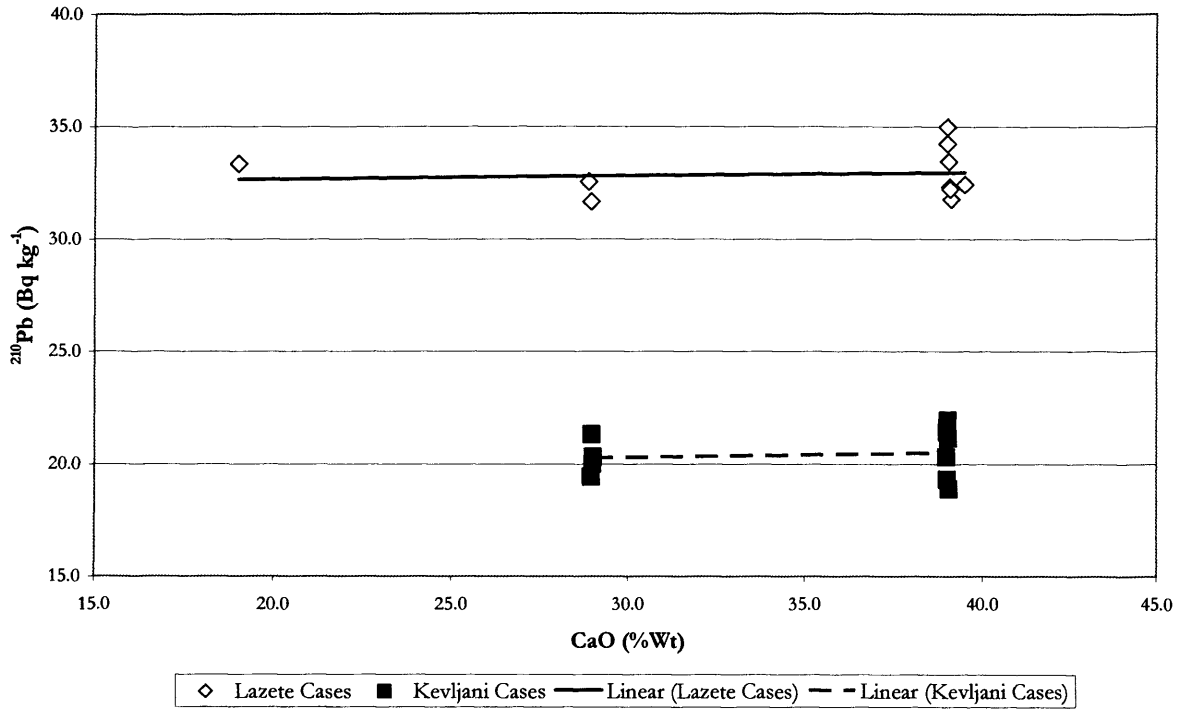


Figure 8.9. The ^{210}Pb activity measured within the two mass grave specimens. These cases from Kevljani (year of death 1992) and from Lazete (year of death 1995) reveal an appreciable difference in activity, confirming the presence of a time since death decay correlation.

So, what could explain this apparent de-coupling of the ^{226}Ra and ^{210}Pb decay? One important correlation that appears to exist is between ^{210}Pb concentration and the chronological age of the individual at the time of death (Figure 8.10). This therefore suggests that ^{210}Pb increases with age (as does cadmium, amongst other elements) and it is this age-related increase, producing unsteady isotope deposition over time that is in effect masking the apparent relationship with ^{226}Ra. This is certainly true of total lead concentrations in bone, as noted by Wittmers *et al* (2002) (Figure 8.11).

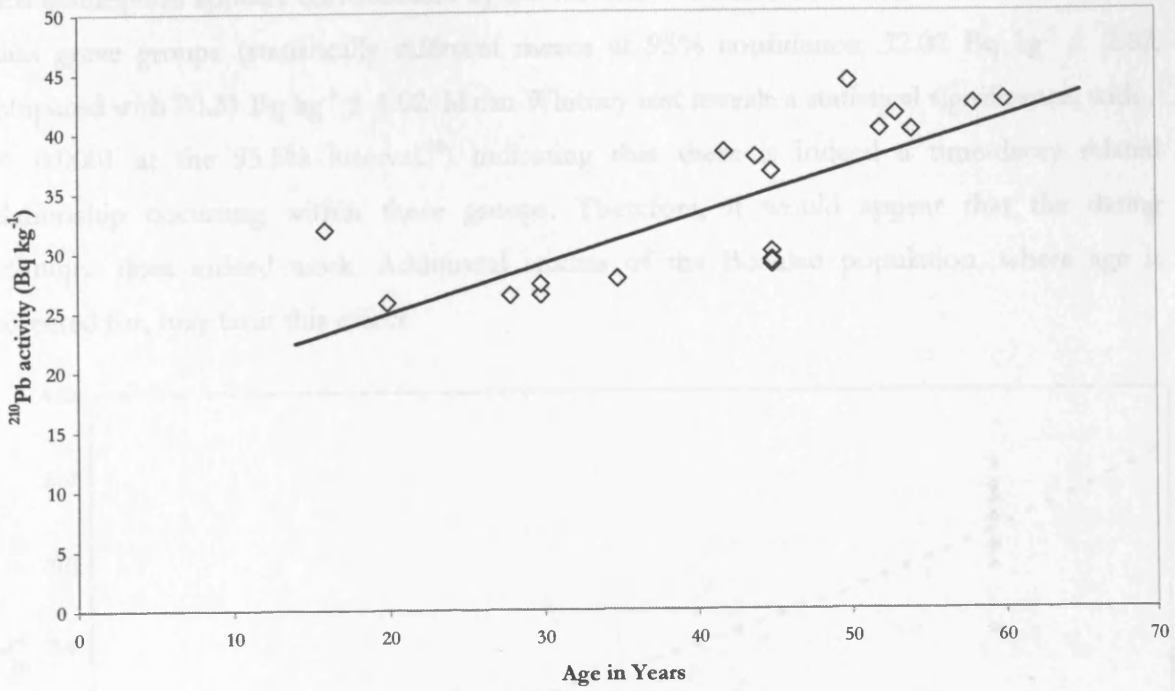


Figure 8.10 Decay correcting the results from both Bosnian mass graves to the year in which the analyses were performed confirms an apparent age-related 210Pb activity in human skeletal material.

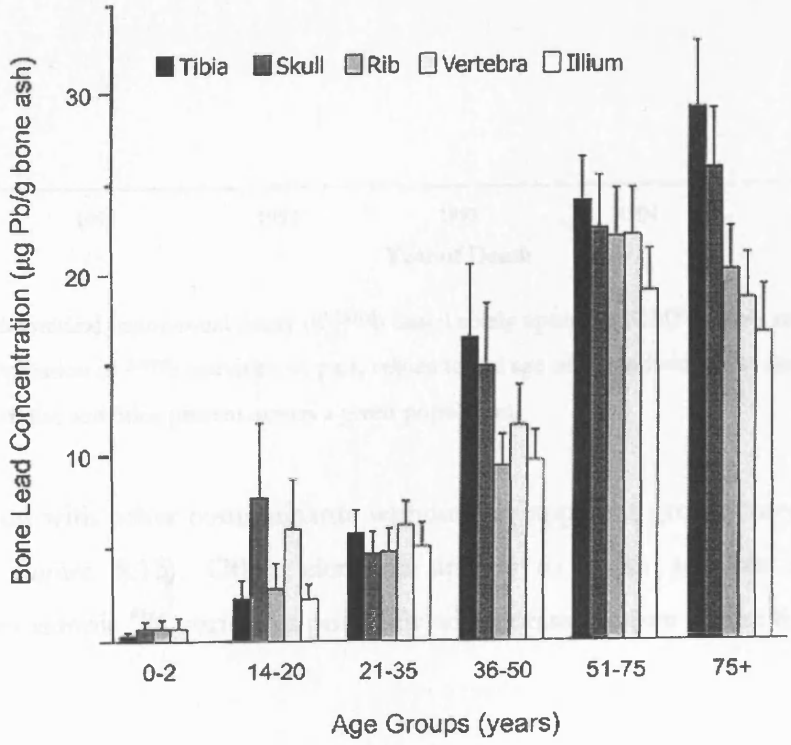


Figure 8.11. Lead distribution in modern human skeletons. (Bars indicate one standard deviation) A general trend to increasing lead content is seen, especially within compact bone rich bones such as tibia. Reproduced from Wittmers *et al.*, 2002.

This assumption appears corroborated by the fact that ^{210}Pb also correlates well within the two mass grave groups (statistically different means at 95% confidence; 32.02 Bq kg^{-1} ± 2.82, compared with 20.31 Bq kg^{-1} ± 1.02: Mann-Whitney test reveals a statistical significance, with $p < 0.0001$ at the 95.5% interval.[10]) indicating that there is indeed a time-decay related relationship occurring within these groups. Therefore, it would appear that the dating technique does indeed work. Additional studies of the Bosnian population, where age is corrected for, may limit this effect.

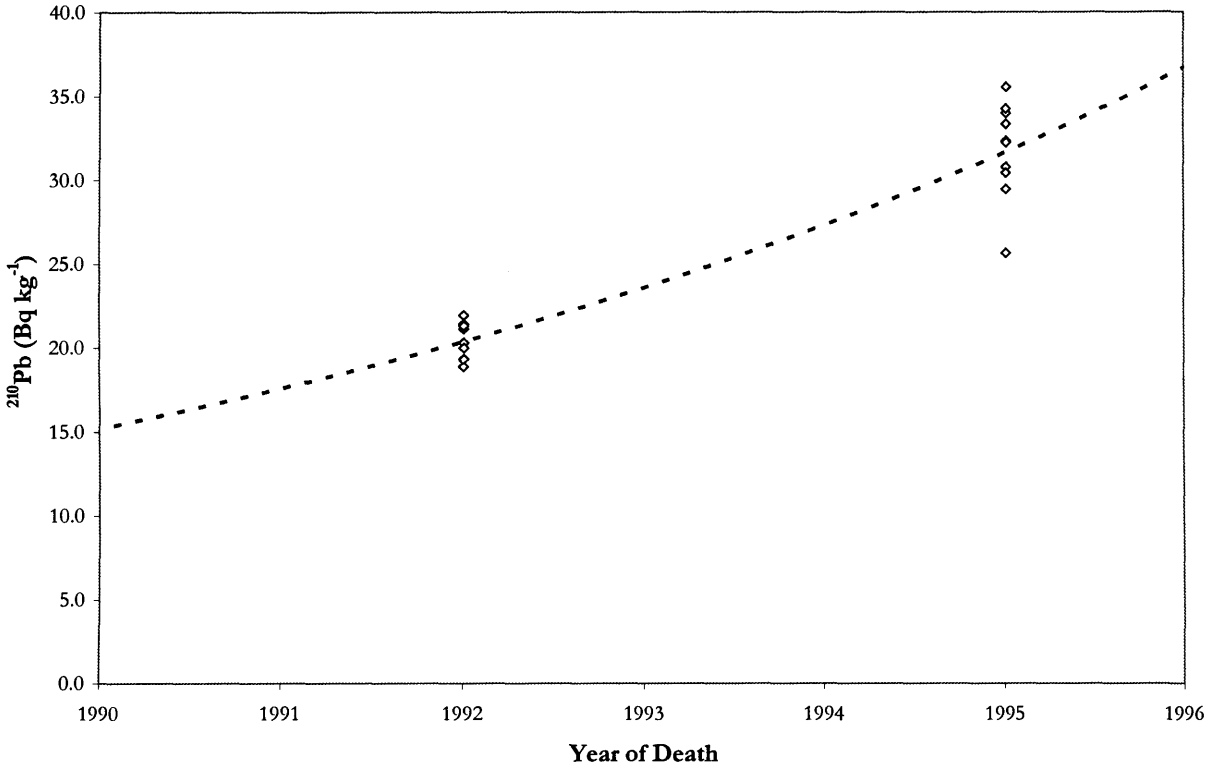


Figure 8.12. The theoretical exponential decay of ^{210}Pb based solely upon the ICMP sample results from both Mass Graves. The variation in ^{210}Pb activities, in part, relates to the age of the individuals at death, but may include a variation in the activities present across a given population.

^{137}Cs is correlated with other contaminants without any apparent group correlations, such as cadmium (see Figure 8.13). Other elements appear to make sensible and predictable correlations, for example ^{40}K correlates positively with potassium (see Figure 8.14).

[10] Nonparametric statistical significance (Mann-Whitney test) calculated using Minitab Version 13, Minitab Inc, USA.

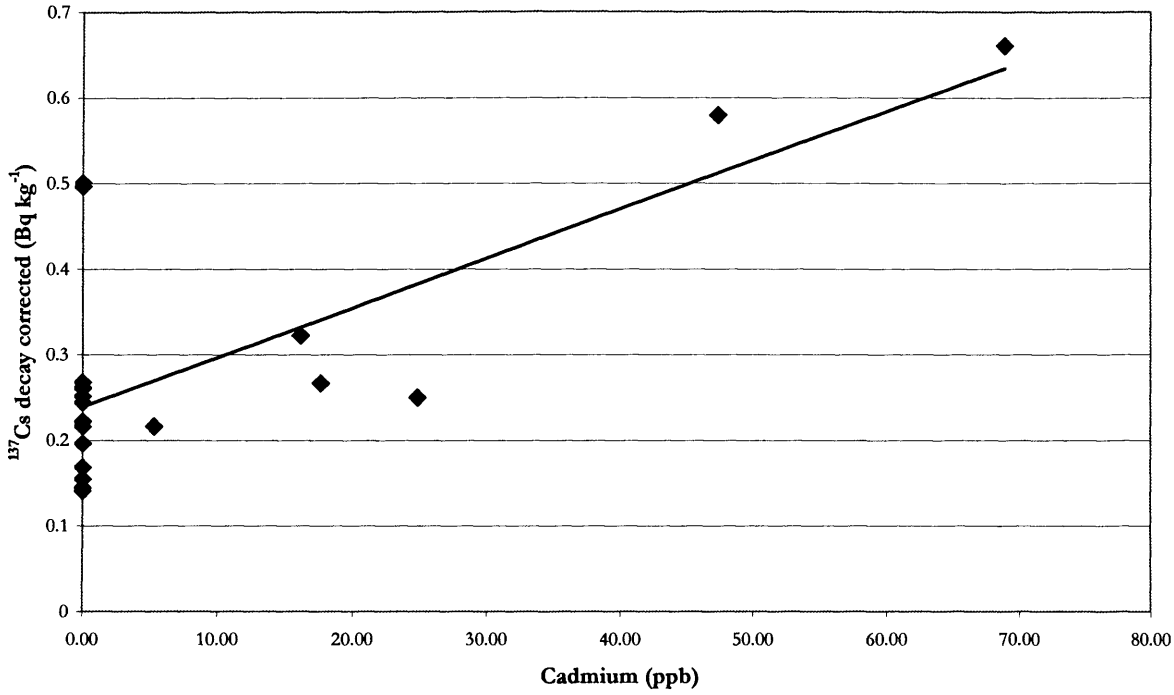


Figure 8.13 The decay corrected 137Cs activity shows a correlation with cadmium, a contaminant heavy metal, though there appears to be no site specific relationship between the two graves examined.

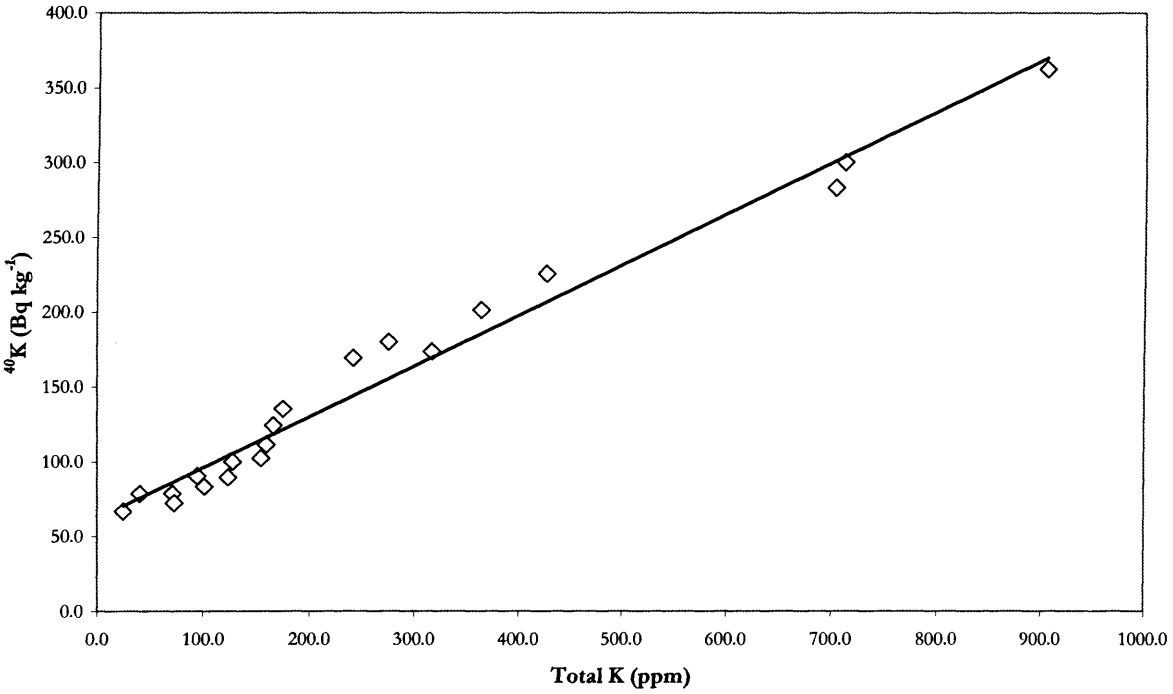


Figure 8.14 The correlation between 40K and total potassium, as would be predicted given their similar physiochemical reactive properties.

The data from the Bosnian samples produce a correlation in keeping with the results from the Portuguese pilot study, though the sample numbers are low. There is a definite need to create a Bosnian calibration curve which may allow more accurate results in future investigations.

8.8 Future Work

One of the things we have seen in the ICMP data set is that ^{210}Pb concentrations increase with the chronological age of the individual, suggesting that uptake and deposition may not be as steady during life as was previously considered. The original Portuguese study (Swift *et al.*, 2001) did not note this effect as the individuals were standardised to within one age range, in an attempt to limit such variables. To produce accurate results in the future, we may need to counter this effect, possibly by bone density fractionation to remove the oldest bone deposits from the younger (see later discussions). The results are very encouraging and appear to corroborate both the theoretical model and the pilot study findings.

9.1 Lav Cemetery, Sarajevo, BiH.

Following the end of the conflict both warring sides in the Former Yugoslavia began the process of exhuming potential mass graves, with a view to collecting evidence for the occurrence of war crimes during this time.

In 2000, excavations began within the grounds of the oldest cemetery in Sarajevo, known as Lav (Bosnian for 'Lion') cemetery, following allegations of war crimes against Serbs, alleged to have been committed by Bosniaks during the siege of Sarajevo (1992-1995). It had been suggested by the Republika Srpska Commission, following preliminary unwitnessed excavations, that three individuals had been murdered, and their bodies hidden under legitimate burials within the cemetery, a claim denied by the Federal Commission that stated the remains were of those who had died before the Second World War.

a) b)

Figure 9.1. a)The Lion sculpture, from which the cemetery derives its name, still guards the entrance to the cemetery despite the damage caused by artillery rounds, and b) Modern Bosniak gravestones within the cemetery.

The allegations centred upon a specific grave, that of "Andja Bosnjak". ICMP representatives performed an exhumation of the remains from the legitimate burial in November 2001 with permission from both Commissions and deeper excavations revealed the presence of three more sets of human remains.

The bodies were intact, articulated but fully skeletalised, lying side-by-side in an ordered fashion, two with their heads next to each other, the third lying in the opposite direction. The depth was approximately 1.5m, however, due to the grave being opened partially previously in 2000, stratigraphic evidence was limited. The bodies themselves had not been disturbed, indicating that this was a primary burial occurring at similar times. Beneath the skeletons was a layer of wood and nails.

A Serbian pathologist examining the three skeletons claimed that appearances suggested a PMI of no more than 8 years (rather conveniently dating them back no earlier than the start of the conflict.). This was based purely upon opinion and appearances.

ICMP-based anthropological examination suggested that the individuals were all male, with similar age ranges.

ICMP Code	Gender	Estimated Age Range
NN-1	Male	40-60
NN-2	Male	35-45
NN-3	Male	40-60

Table 9.1. The age and gender results from the three skeletalised remains from Lav cemetery, Sarajevo, based upon their anthropological appearances.

During the summer of 2001, the political situation over these cases of alleged war crimes threatened to re-ignite already rather difficult post-war negotiations. It was because of this that, in July 2002, ICMP was granted permission by both the Republika Srpska and Federation Commissions to analyse the Lav cases.

Method

Three sections of human femoral shaft were received from ICMP, each labelled with the appropriate case numbers.

Figure 9.2. ICMP specimens from Lav cemetery, as received by the Division of Forensic Pathology, University of Leicester.

The bone was analysed at the Postgraduate Research Institute of Sedimentology, University of Reading, using the analytical methods described within Appendix 2, though it was found that additional counting time was again required.

Results

The results from the isotope analysis were as follows:

ICMP code	U (ppb)	^{238}U / ^{235}U Bq kg^{-1}	^{210}Pb Bq kg^{-1}	^{226}Ra Bq kg^{-1}	^{40}K Bq kg^{-1}	^{137}Cs Bq kg^{-1}	^{241}Am Bq kg^{-1}	^{90}Sr Bq kg^{-1}
icmp-av	1334.7	138.45	26.42	21.73	152.0	0.25	0.09	0.63
NN-1	33.2	137.45	2.41	9.49	49.32	<LLD	<LLD	<LLD
NN-2	47.9	137.45	2.23	11.51	93.91	<LLD	<LLD	<LLD
NN-3	39.5	137.45	3.44	10.44	77.65	<LLD	<LLD	<LLD

Table 9.2. The results from the Lav cases, compared with the ICMP average (icmp-av) from the study of two mass graves described previously. <LLD = no activity at the lowest level detected.

Internal controls were assessed and found to be within similar ranges to those described earlier within this text.

The results of the analysis have shown, by several means, that the individuals were not alive during the 1990s conflict. These are as follows:

1) The absence of man-made isotopes within these remains (being ^{137}Cs, ^{241}Am and ^{90}Sr). It can therefore be inferred that these individuals were not alive after 1945.

2) It is also noted that ^{210}Pb and ^{226}Ra are in disequilibria; this indicates that an excess still exists within the bones. Equilibrium is expected after at least 100 years have passed, suggesting that these individuals are younger, PMI wise, than this.

3) The ^{210}Pb concentrations are significantly lower than the average observed within the ICMP database, indicating extensive decay from the level during life. This point will be discussed further.

To create a PMI estimation, a time-zero starting point is required. Knowing that ^{210}Pb concentrations are age-dependent, (see Figure 8.10), at least within this population if not all

populations, from the small Bosnian dataset only the individuals that were within the age ranges for each case (based upon anthropological estimations) were used in the calculations. Taking the ^{210}Pb concentration of individuals of known PMI that fell within each age range, an upper and lower estimate was produced. For this, the following equation was required to assess concentrations:

Equation One - $$N_t = N_0 e^{(-\lambda * t)}$$

where the decay constant $\lambda = \ln 2 / T½$

$= 0.693 / T½.$

The activity at time zero (N_0) is inferred through the studies of material of known PMI. The concentrations of the radioisotope in the material analysed constitutes the second value, (N_t), and the time that has elapsed between the time zero and the time of analysis (t) remains the unknown. This equation is therefore dependent upon the time units used for calculating the decay constant: if the half-life is measured in days, then the value for t will also be in days. The equation can then be re-arranged as follows:

Equation Two - $$t = -((T½)/\ln 2)(\ln(N_t/N_0))$$

In order to proceed with time since death estimations, a few calculations are required.
The time zero value represents the ^{210}Pb activity within the living population. This true value remains, at present, an unknown as no data based on either living people or autopsy tissue has been published. To calculate a theoretical activity an average activity of ^{210}Pb is produced based upon our mass grave results. Knowing that diet varies over the region, and that the availability of food was limited during the conflict, it was decided to concentrate upon the individuals within the mass grave closest to Sarajevo, being Lazete, making the assumption that food sources would have been similar in these areas.

To calculate the activity within the living population, it is necessary to decay correct the results obtained through the analysis of the Lazete cases. By this, we are aiming to produce a value for ^{210}Pb at death for each case, knowing that they died 7 years prior to analysis (1995). Using equation one, where N_0 is the measured activity and t = -7 (as it is correcting for seven years in the past rather than in the future) and N_t is the unknown, the following results are obtained:

Lazete Case Number	Age at Death	^{210}Pb Activity Measured	Decay Corrected ^{210}Pb Activity
001	42	30.80	38.30
005	52	32.32	40.18
006	50	35.47	44.10
007	58	33.99	42.25
009	16	25.65	31.89
012	54	32.23	40.07
013	53	33.34	41.45
014	45	29.45	36.61
015	60	34.24	42.57
020	44	30.43	37.83

Table 9.3. ^{210}Pb activities for the Lazete cases, decay corrected for the time of death.

Knowing from our previous work that ^{210}Pb activity is related to the chronological age of the individual at death, it was decided to only use those individuals from Lazete that form the two ends of the anthropology-based age estimations for the Lav cases. Though not ideal, the absence of any large scale population based activities required the use of the only available data. The results would therefore be rough estimations only, based upon the data from the two mass grave sites examined previously, though the main goal would be to differentiate recent "forensic interval" cases from remains of no forensic interest.

Case NN-1 was estimated to be between 40 and 60 years of age at the time of their death. By examining the Lazete cases, three cases fall close to the lower age limit of 40 years (being cases 001, 014 and 020). Taking an average of these Lazete cases ^{210}Pb activities produces the following time-zero value:

$$(38.30 + 36.61 + 37.83) / 3 = \underline{37.6}\ \text{Bq kg}^{-1}\ \underline{^{210}\text{Pb}}$$

Two cases from Lazete fall close to the upper age-at-death estimation (being cases 007 and 015). An average of these ^{210}Pb activities produced the following time-zero value;

$$(42.25 + 42.57) / 2 = \underline{42.4}\ \text{Bq kg}^{-1}\ \underline{^{210}\text{Pb}}$$

By entering these upper and lower ranges into Equation Two produces the following PMI estimations for Lav case NN-1:

Lower PMI Limit	Upper PMI Limit
t = -(22.3 years / ln2) x (ln (2.413 / 37.6) t = -(22.3 years / 0.69315) x (ln 0.0642) t = -(32.172) x (-2.7456) t = 88.3 years	t = -(22.3 years / ln2) x (ln (2.413 / 42.4) t = -(22.3 years / 0.69315) x (ln 0.0569) t = -(32.172) x (-2.8664) t = 92.2 years

Therefore, the results would suggest that the individual, NN-1, died between 1909 and 1913 AD.

Through these estimations, based upon the averages for the age-specific results gained previously from Lazete and using identical equations, the following PMIs are suggested for each individual:

LAV Case Number	**Estimated PMI**	**Calendar Years**
NN-1	Between 88 and 93 years	1909-1914 AD
NN-2	At least 91 years	1911 AD, at the latest
NN-3	Between 77 and 81 years.	1921-1925 AD

Discussion

Though the limitations for these PMI estimations are apparent, being based upon a small study, it would reflect the benefits of this test, giving results that are reproducible and verifiable, and would appear to be internally consistent across several isotope types. By increasing the Bosnian database in the future, through analyses of bones of known PMI, the accuracy of such tests could be improved dramatically.

The time since death estimations would also appear to show that, of the three individuals, two have similar PMIs (those being NN-1 and NN-2), whereas the third appears to have died more recently. This finding would also be in keeping with the funereal positions in which the bodies were recovered; NN-1 and NN-2 lying parallel, in the same direction head-to-toe, whereas NN-3 was found facing in the opposite direction. The organised and respectful nature of the burials would also be in keeping with Skinner's suggestion that these were not criminal acts (Skinner, York and Connor, 2002).

In concluding, the claims made by the Republika Srpska Commission appear refuted, the isotope results appearing to confirm the suggestions of the Federation Commission. These results were made available to ICMP for distribution to the appropriate authorities.

icmp International Commission on Missing Persons

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From: Richard Harrington, PhD
International Commission on Missing Persons
Sarajevo, BiH

Subject: Update on Case Status: *ICMP Forensic Science Official Report on Postmortem Interval Testing of the Undocumented Remains Recovered from beneath the "Andja Bosnjak" Gravesite, Lav Cemetery, Sarajevo, BiH*, (a.k.a. *Lav Report*), dated 30 October 2002

Date: 7 August 2003

Update of Case Status

Based on initial verbal responses from the chairmen of each commission, it appeared that both commissions were willing to accept the ICMP/Leicester findings as sufficient to conclude that the three individuals died prior to 1992-1995, and therefore were "not of forensic interest" to organizations focused solely on recovering missing persons from the recent conflicts.

However, at least one representative of the RS commission has reportedly stated an interest in obtaining a "second opinion," i.e., submitting bone samples to another laboratory for physiochemical testing. As stated, ICMP has no vested interest in this case, and has no objections to additional testing. Regardless of the outcome of this case, the service provided by Dr. Swift's team has had an extremely positive impact on ICMP's scientific operations and its standings with the commissions. The ICMP Forensic Sciences Program promotes scientifically valid and reliable methodology, practiced to the highest standards possible. Integrating new forensic techniques—notably DNA and postmortem interval testing—with the traditional techniques has vastly improved our ability to assist the people of the former Yugoslavia in identifying the thousands of missing persons and putting forth new ways to resolve disputes that inevitably arise among former warring parties.

It is also possible that additional cases from Lav Cemetery (among others) will be scrutinized further, possibly to include requests for additional postmortem interval testing. It is currently ICMP's hope that Dr. Swift's group would be in a position to assist in such matters, given the confidence we have in the system being developed at Leicester.

Richard J. Harrington, PhD
Forensic Anthropologist and *Lav Report* author

A duplicated copy of the official document ('*The Lav Report*') submitted by the International Commission on Missing Persons to the Federal Commission and the Republika Srpska Commission is provided as Appendix 4.

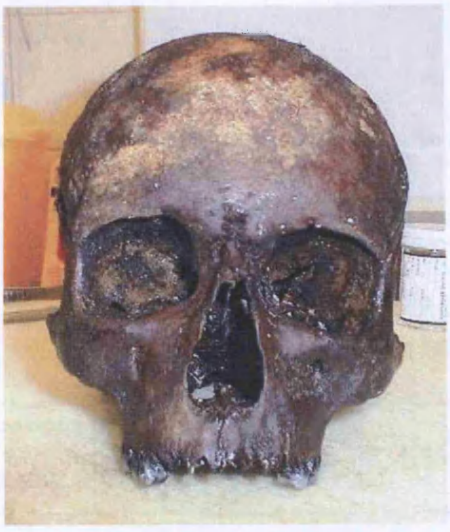
9.2 East Midlands, United Kingdom

The following is a case report from work that has been performed by the Division of Forensic Pathology for a UK police force:

An 18 year old female was murdered by two blunt-force blows to the head with a hammer. Her body was defleshed and eviscerated with a knife, then dismembered with a standard hacksaw. The body parts were disposed of in bags, which were thrown into a local river. The river was searched by professional police divers who, during one pass of the area, recovered a human skull which had been embedded within the silt on the river bottom. The dentition did not match any missing individual from the region. Given the possibility of a further police investigation, PMI estimation was requested by the senior investigating officer.

Examination of the skull revealed it to possess prominent masculine-type features. There was no residual soft tissue or hair associated with the skull. The bones and teeth were discoloured and, subjectively, had an appreciable increase in their weight compared to recent deaths, as is often seen following a prolonged post-mortem interval. The molar teeth were eroded flat, as is recognised in studies of previous historical periods due to the presence of small fragments of stone within ground flour. There was no evidence of dental reconstruction.
A large defect was present superiorly within the calvarium, though the nature of this was uncertain. There was no evidence of bone re-growth, indicating that the damage was either peri-mortem or post-mortem in nature.

Due to the increased risk of diagenetic alteration with trabecular-rich bone, especially within a fluvial environment, a molar tooth was extracted and analysed. The isotope content of the whole tooth would assist in PMI estimations.



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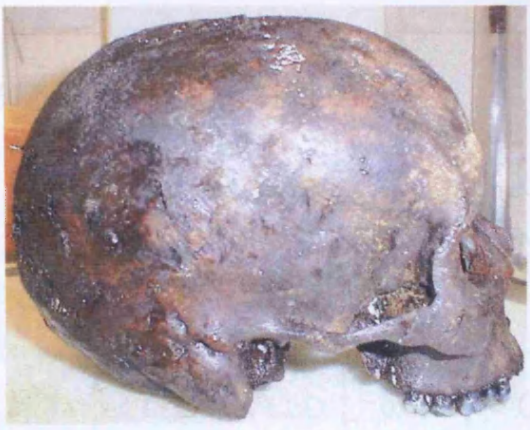

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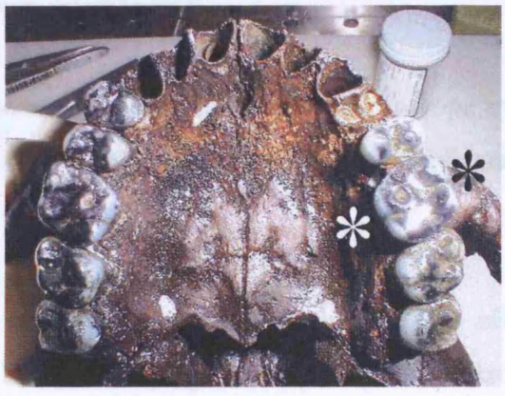


d)

Figure 9.3. a and b) Photographs of the skull of an unknown male retrieved from the River Witham, Lincolnshire. c) Note the unusual fracture to the parieto-occipital region superiorly. d) The teeth are particularly worn-down, as is frequently seen in skulls of ancient origin. The tooth extracted for isotope analysis is indicated in the last image.

Results

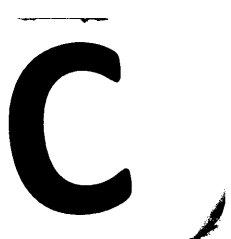
The results of the isotope analyses for the molar tooth extracted are as follows:

Tooth	$^{238}U/^{235}U$ Bq kg^{-1}	^{210}Pb Bq kg^{-1}	^{226}Ra Bq kg^{-1}	^{40}K Bq kg^{-1}	^{137}Cs Bq kg^{-1}	^{241}Am Bq kg^{-1}	^{90}Sr Bq kg^{-1}	$^{239+240}Pu$ Bq kg^{-1}
Measured activity	137.55	1.02	1.33	69.4	< LLD	< LLD	0.01	< LLD

Table 9.4. Radioisotope activities measured within the tooth extracted from the River Witham Skull (<LLD = activity below the lowest detection limit).

These results indicate that the individual had no appreciable man-made radioisotope exposure (^{137}Cs, ^{241}Am and $^{239+240}Pu$). As such, his childhood (when the enamel was deposited) must have occurred prior to 1945.

The presence of ^{90}Sr is interpreted as being a contaminant, probably through the effects of fluvial-based diagenetic dynamics. As such, it has probably 'grown-in' from the surrounding environment, or through the action of bacterial decay. It may even be hypothesised that the measurable activity results from the effects of faunal scavenging altering the calcified matrix (See Figure 9.4).



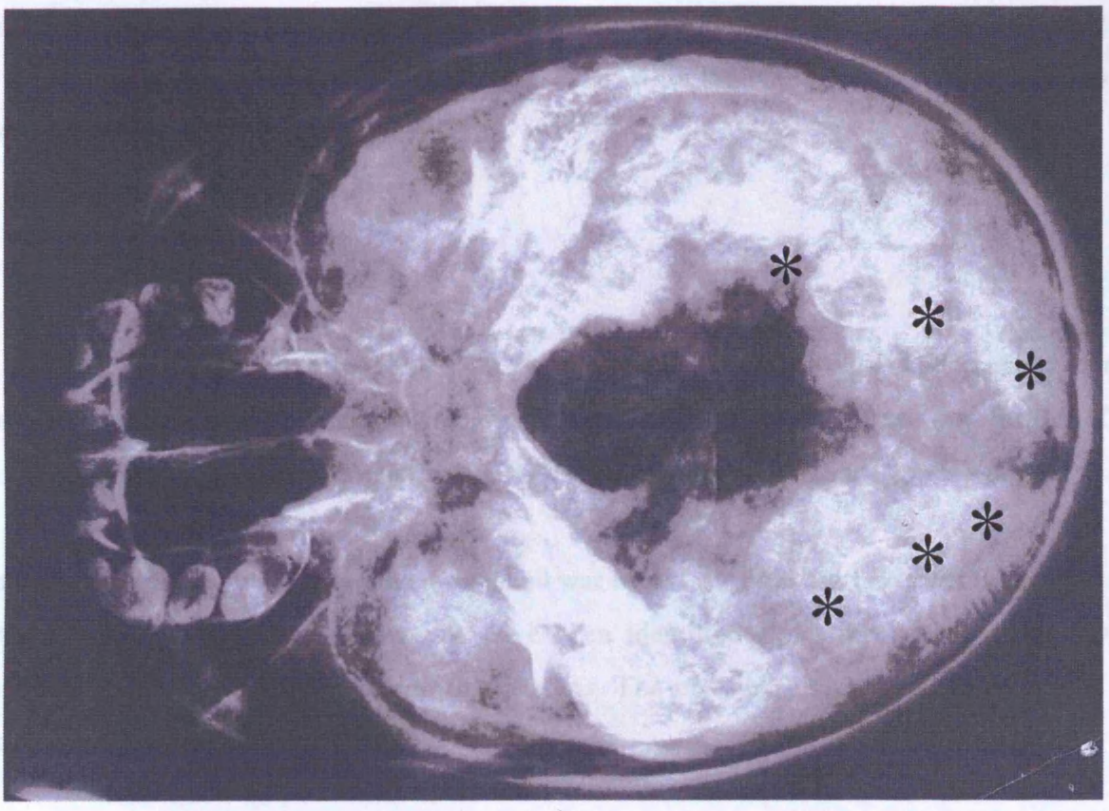


Figure 9.4. Radiological examination of the River Witham Skull prior to osteological examination revealed the presence of a colony of freshwater bivalve molluscs within the cranial vault. The calcified outlines of their shells are visible as ovoid structures (between the asterixes) and, though the skull provided shelter from the water currents, the mussels may theoretically have altered the chemical contents of the bone or teeth through erosive feeding activities.

The ^{226}Ra and ^{210}Pb activities are in equilibrium, referring to the similarity in activities between the two isotopes together with the apparent low ^{226}Ra activity, indicating that the PMI must be greater than 100 years; thus each being at lower quantities than would be expected for a recent death. A study published by Henshaw *et al.* measured the activity of ^{226}Ra in children's teeth (Henshaw, *et al.*, 1994). Through this study, children over the age of 10, when the permanent teeth enamel had been deposited, have an average level of 7.00 Bq/kg (± 0.15) ^{226}Ra. Using the same equation from above, a PMI estimate may be produced from the ^{226}Ra activity present within our sample, where the half-life of ^{226}Ra is 1600 years:

$$t = -((T½)/\ln 2)(\ln(N_t/N_0))$$

$$t = -((1600\text{years})/0.69315)(\ln(1.33/(7.00+/-\ 0.15)))$$

$$t = -(2308.31)(-1.664)$$

$$t = \underline{3790.4 - 3889.4 \text{ years.}}$$

As the analysis was performed in 2002, it is estimated that the deceased's childhood occurred at the earliest between 1788 – 1887 BC, thus falling within the era of UK history referred to as the Bronze Age.

Discussion

It was therefore concluded that the skull was from an individual who died outside of the interval of forensic interest. As such the police decided not to investigate any further. The outcome therefore enabled valuable police funds and manpower be allocated elsewhere.

Interestingly, the region within which the skull was found is of known historical interest. Both Bronze Age and Iron Age settlements have been identified along the course of the river, including the recovery of partial human skeletons. The possibility that the skull was from an individual from one of these periods was raised.

The diets of Bronze Age humans were considered to be high in meat content and high in cereals, a fact reflected in the wear to the molar teeth relating to the presence of ground millstone within their meals (as noted in this case also). Compared with modern societies this would have resulted in a higher ^{226}Ra intake and potentially a higher radioactivity burden within the enamel of children of this era. As such the estimation of time since death provided for this case was given as an "earliest" point; if a higher activity did exist in children from the Prehistoric era, a longer PMI would be produced in this scenario.

The benefit of additional radiocarbon dating to confirm our PMI estimation was suggested. This decision was left with the police force, which declined to investigate further.

Chapter 10. Geographical Variations and Geolocation

Geological and anthropological studies routinely employ isotope analyses to identify geographical origins of materials. Such methods measure both radioisotopes and stable isotopes and, to date, have been employed for both biological and non-biological materials, from butterfly migratory patterns to the origins of precious stones (Wassenaar and Hobson, 1998; Guiliani *et al.,* 2000).

10.1 Oxygen, Carbon and Nitrogen Isotopes

The isotopic profile of regions differ across the continents, and commonly within a single country. The variation reflects the underlying geological strata, often established with the creation of the rocks themselves. The stable isotopes of oxygen, nitrogen and carbon are frequently used, being expressed as a delta (δ) value. Delta values measure the deviation of a ratio from a recognised standard. The value is expressed using the units of parts per thousand, given the symbol ‰, and may be calculated as follows:

$$\delta 13C = \left[\frac{[13C/12C]sample}{[13C/12C]standard} - 1 \right] x1000$$

where, for example, PD Belemnite (PDB) represents the ($^{13}C/^{12}C$)standard carbon standard.

The delta value varies relative to physical processes experienced by the geology, such as evaporation, condensation, diffusion or biological processes, such as photosynthesis or metabolism of the plants or animals that live upon these soils and rocks (Mays, 1998). The outcome is that an isotope content is observed to vary along food chains, from soil to plants and on to animals.

Forensic studies have used these values in identifying the geographical origin of narcotics and confirming the origin of environmental pollutants, though additional anthropological based research has suggested the diets and routes of human migration over the millennia, and the subsequent movement of materials across countries. (Ehleringer *et al.,* 1999;Tommasini, Davi and Elliott, 2000;Ehleringer *et al.,* 2000;Horita and Vass, 2003;Åberg *et al.,* 2001;Budd, *et al.,* 2000;Silva, *et al.,* 2002;Kutschera and Müller, 2003;Åberg, Fosse and Stray, 1998;Gulson, Jameson and Gillings, 1997;Schweissing and Grupe, 2000;Beard and Johnson, 2000;Hoogewerff *et al.,* 2001).

As mentioned, the paleodietary habits may be indicated through the isotope profiles, though this technology is now being furthered commercially; food manufacturers have created a new niche market based upon claims of certain brands or products being superior to others (Angerosa *et al.,* 1999;Ogrinc *et al.,* 2001;Pillonel *et al.,* 2003). Therefore companies selling high quality products such as Basmati rice or Champagne can only legally market such items if they were produced within their eponymous geographical regions. To confirm their origins, testing centres such as that established within the University of East Anglia, analyse foodstuffs for their stable isotope content to confirm or refute advertised claims. This testing can be of benefit during trade-embargos. During the BSE and Foot-and-Mouth crises, the exportation of British meat was banned by certain countries. If, as was claimed, meat was still being removed and sold abroad, stable isotope analysis may assist in criminal prosecutions. This technology may therefore be of benefit in future legal proceedings.

10.2 Strontium, Neodymium and Lead

By recognising the ratios present, it should be possible to suggest the geographical origin of certain non-biological materials, such as through the lead isotope analysis of environmental contaminants or even projectiles (Chiaradia, Gallay and Todt, 2003; Henshaw, Keithch and James, 1995; Buttigieg, *et al.,* 2003). The geolocation of biological material may also be assessed; unlike the isotopes of carbon, oxygen and nitrogen, the ratios of the stable isotopes of strontium, neodymium and lead have shown no fractionation between different positions in a food chain. Thus the isotope signature deposited within hard tissues is said to reflect accurately the soil and bedrock upon which an individual has resided in life (Kutshera and Müller, 2003). Certain strata, such as granites, basalts and limestones have recognisable isotope ratios. Such is the ratio of ^{87}Sr, formed by the decay of ^{87}Rb, and ^{86}Sr. As such, plants growing on these soils will incorporate these isotopes at an identical ratio, which is then subsequently inherited by herbivore animals, and latterly carnivores.

The Sr/Nd/Pb isotope values within bone should reflect the dietary intake during the ossification of the individual bones or the production of enamel. Should a person subsequently move to a different region, the isotope value within cortical bone will gradually reconfigure to reflect the new geo-location, as bone is resorbed and remodelled. Enamel, however, does not undergo remodelling, forming a static indicator of childhood domicile.

By applying this principle to forensic cases it is hypothesised that it would be possible to identify where someone had lived during their lifetime based upon the isotope ratios within their skeleton. The isotopic signature, or "fingerprint", within bone should reflect the geology of the area in which they lived. The benefits of such a system would be that, given the limited annual budgets, such a tool could assist police investigations by dramatically narrowing search parameters when trying to establish the identity of an unknown individual.

Chapter Eleven – Isotope-Based Geolocation Case Studies:

11.1 Bosnian Case Results.

To test this hypothesis, the strontium isotope results from the Bosnian mass grave material described above was examined, assuming that the individuals' within the graves had lived within, or close to, the area in which they later died. Due to the construction of the initial study, such geographical diversity was in-built into the selection of the graves.

(The method employed was as described in Appendix 3)

Results

Mass Grave One, Kevljani.		Mass Grave Two, Lazete.	
ICMP No.	$^{87}Sr/^{86}Sr$	ICMP No.	$^{87}Sr/^{86}Sr$
002	0.7089266	001	0.711245
003	0.7086026	005	0.710382
004	0.7096946	006	0.710548
008	0.7093318	007	0.708655
010	0.709543	009	0.710452
011	0.7093598	012	0.710374
016	0.708109	013	0.709227
017	0.7091319	014	0.7102812
018	0.709095	015	0.7105561
019	0.7085971	020	0.710271
Average = 0.709040		Average = 0.710199	
Mann-Whitney Test : $p < 0.0036$ at 95.5% interval.[11]			

Table 11.1. Strontium isotope ratio results produced during the Bosnian mass grave study.

[11] Non-parametric statistical significance (the Mann-Whitney test) calculated using Minitab Version 13, Minitab Inc., USA.

Discussion

The results appear to show a definite sub-division within the two groups. The inherent $^{87}Sr/^{86}Sr$ ratio, as demonstrated in Figure 11.1, appears to confirm the existence of a geo-location dependent factor within the bones, being statistically significant.

Two individuals from Lazete (ICMP Cases 007 and 013) do not appear to conform to the overall pattern. The life histories of these individuals, at present, remain unknown. Future DNA identification and next-of-kin interviews may reveal that these men did not live within the region that they were found, having fled their homes during the conflicts, or that they had lived elsewhere for an appreciable length of time.

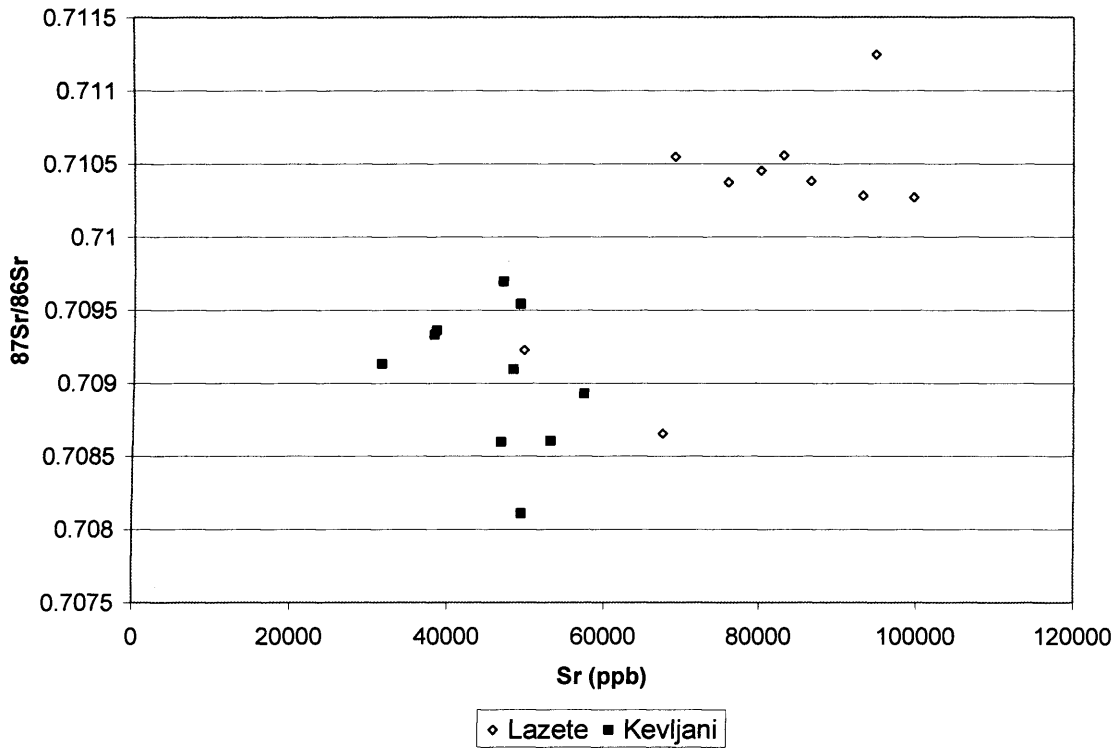


Figure 11.1. The ratio of $^{87}Sr/^{86}Sr$ against total Sr content reveals a division between the two groupings, a relationship that appears to be only contested by two individuals from the Lazete graves. The possible reasons for these cases are described below.

In considering the application of the method, one should remember the physiology of the skeleton. The entire calcified matrix of the skeleton is resorbed and redeposited over each 10-15 year period of life (Bell, Cox and Sealy, 2001). Therefore, for a skeleton to take on the "fingerprint" profile of the environment within which a person lives, that individual must reside there for such a length of time. In fact, should a person move to a different region, then

the profile of the isotopes and elements will gradually shift over time, passing through transient phases between the two. This may have occurred in these instances.
To understand the principles requires a knowledge of the background soil and geology for each region. Although the chemical and isotope composition of many of the world's countries' geology is known, Bosnia remains relatively unknown, or at least not published internationally. Geological maps were donated by the University of Tuzla, BiH, and the nature of the bedrock was assessed.

Large areas of Bosnia are composed of dolomite and limestone outcrops, formed by precipitation from Cretaceous and Jurassic seawater, and ophiolites, regarded as remnants of ancient oceanic crusts formed at spreading centres that thrust upwards.
The Kevljani graves have an average value of 0.71012, in keeping with that expressed by gneisses and granites, whereas the Lazete graves have a value of 0.70904, which is suggestive of pure carbonate sediments, such as limestone. Though attempts have been made to verify this through contacts within Bosnia, no accurate geological survey maps have been identified to confirm this issue. (No widespread isotope profile assessments have been performed either.)

Overall these results would suggest that the use of strontium isotopes in forensic science could supplement established means of investigation. As mentioned previously, it could assist in the investigation of unidentified decedents through the suggestion of geographical domicile. It could also be supplemented with analysis of dental enamel to produce a timeline of habitation across their lifetime, possibly with other established techniques such as pollen analysis deposited upon nasal sinus structures.

11.2 East Midlands Case: The unidentified female.

'Desperate' woman dies from leaked drugs

"Police have issued an artist's impression of a mystery woman who was found dead beside a motorway. The woman, aged about 60, had swallowed 10 small packages of drugs, which are believed to have leaked and killed her. Officers said they believe the woman could have been smuggling the drugs into the country when she died.

The woman's body was discovered face down in the grass by a workman at the side of the westbound carriageway of the M45 in Northamptonshire on Wednesday morning.

Detective Chief Superintendent Chris Cross said police still did not know where the woman was from, where she was going or how she had arrived at the side of the motorway. It is believed the woman died between 1800 BST on 8 April and 0800 BST on 9 April, and was found more than a day later beside the M45, about three miles west of junction 17 of the M1, near Kilsby.

Mr Cross said: "Although we know where she was found, we can't be certain that she met her death there. We don't know where she has travelled from or where she was travelling to."

The woman was described as black, 5ft 3ins, with black hair, which had a gingery tint and was braided at the back and greying at the front. She was wearing distinctive clothing, including a bright orange flimsy floral top, a layered black and pink skirt and black leather moccasin shoes."

BBC News, United Kingdom (15th April 2002)

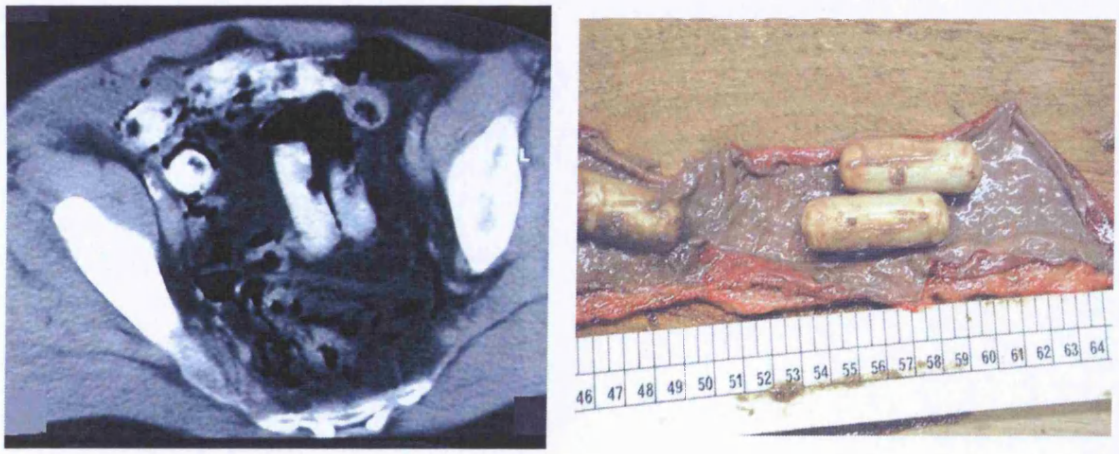
Case History

The body of an Afro-Caribbean female was discovered by the side of a motorway in Warwickshire. The clothing associated with the body was expensive 'designer' brand names, though she did not possess any forms of identification about her. The body was removed for autopsy examination, following post-mortem CT scanning within the Leicester Royal Infirmary.

a) b)

Figure 11.1. The location of the body: a) M45, Warwickshire, b) the body lying to the side of the hard-shoulder of the motorway.

The CT scan revealed the presence of packages within the small intestine. Subsequent toxicological analysis confirmed the contents as cocaine.



a) b)

Figure 11.2. The nature of the deceased's presence within the United Kingdom: a) Post-mortem CT scan of the pelvis reveals small discrete radio-opaque masses within the small intestine. Each appears to possess a small air-bubble associated with it, b) Post-mortem examination confirms the presence of cocaine packets within the ileum.

Therefore, the scenario forming was that the woman had been a drug trafficker, or "mule", who had entered the United Kingdom with a view to passing the narcotics to a contact who would deal the cocaine. The cause of death was ultimately ascribed to a myocardial infarction, though the individuals who collected her from the airport probably assumed incorrectly that her collapse was due to a drug overdose from leaking packages. They would probably have left her by the side of the motorway to die, rather than risk arrest by taking her to a hospital.

Thus it was in the polices' interest to identify the individual, with a view to investigating this trafficking route.

Method

At post-mortem, and with full consent granted by Her Majesty's Coroner, a 6cm section of the left femur was removed and submitted whole for isotope and trace element analysis.

(The method employed was as described in Appendix 3)

Results

Al ppb	**As** ppb	**Ba** ppb	**Cd** ppb	**Co** ppb	**Cr** ppb	**Cu** ppb	**Fe** ppb	**K** ppb
16115.7	<LLD	2312.71	44.85	618.03	289.02	2635.4	13097	562148
Mg ppb	**Mn** ppb	**Na** ppb	**Ni** ppb	**Pb** ppb	**S** ppb	**Si** ppb	**Sr** ppb	**Ti** ppb
2084072	100.74	5437931	459.5	7039.06	1907703	4582.65	67589.9	300.7
U ppb	**Zn** ppb	**$^{87}Sr/^{86}Sr$**	**$^{238}U/^{235}U$**	**^{210}Pb** Bq kg^{-1}	**^{226}Ra** Bq kg^{-1}	**^{40}K** Bq kg^{-1}		
114.76	83180.3	0.70857	137.68	61.23	43.67	171.37		

Table 11.2. Trace element and isotope values for the unidentified females' femoral sample.

Discussion

The results show that the individual has a $^{87}Sr/^{86}Sr$ ratio of 0.70857, within the range described for unaltered pure carbonate sediments ($^{87}Sr/^{86}Sr$ = 0.706 – 0.709), indicating the geology of her home area derived from precipitated seawater (Kutschera and Müller, 2003). Comparing the value to international published examples of strontium ratios reveals a close similarity to those within Caribbean dolomitic limestones.

Geographical Region	$^{87}Sr/^{86}Sr$	
	Min.	Max.
Ghana	0.701	0.701
Canary Islands	0.70209	0.70332
Mid-Atlantic Ridge	0.70212	0.7041
Hawaii	0.702642	0.703827
Caribbean Plateau Mantle	0.702977	0.704976
Hawaii	0.703	0.705
DR Congo	0.703	0.703
Ethiopia	0.7031	0.7039
Caribbean Plateau Lava	0.7031	0.7041
Puerto Rico Lava	0.7033	0.704
Ethiopia	0.70362	0.70373
Mexico	0.7037	0.70469
Trinidad	0.70377	0.70421
Hawaii	0.7038	0.7048
Namibia	0.70425	0.70465
West Angola	0.70448	0.70752
Lesser Antilles	0.7045	0.7045
New Jersey	0.7052	0.7052
California	0.7054	0.7054
Mexico	0.70586	0.70465
Mexico	0.70674	0.70682
Falkland Plateau	0.706789	0.707428
SE Brazil	0.707	0.71
Mississippi	0.7073	0.7075
Canada	**0.7077**	**0.709**
Belize	0.707745	0.707872
NE Brazil	**0.7083**	**0.719**
Caribbean Dolomites	**0.7085**	**0.7092**
St Croix	0.70885	0.70887
Dutch Antilles dolomite	0.708866	0.708915
Caymans	0.7089	0.70922
Barbados	0.70912	0.70921
Bahamas	0.709179	0.709179
Mid-Irish Dolomite	0.70921	0.710873
Salzburg, Germany	0.70927	0.71403
Spain	0.70961	0.71059
Cuba	0.7102	0.7117
Barbados	0.710259	0.710259
Merseyside, NW England	0.71049	0.7112
St Lucia	0.712	0.713
N. Germany	0.7124	0.7142
South Africa	0.7125	0.7125
Dartmoor, SW England	0.71814	0.71968
Gulf of Mexico	0.72	0.7317
Ivory Coast	0.7221	0.7257
Asian Continent	0.724	0.726

Table 11.4. A collection of $^{87}Sr/^{86}Sr$ ratios from international publications. Those ratios including the femoral specimen ratio result (= 0.70857) are reproduced in bold.

Although other countries, such as Nigeria, Congo, The Ivory Coast or the United States where suggested as possible geographical origins of the deceased by the police, as shown in Table 11.4, the strontium isotope ratios appear to refute these suggestions

Sample	$^{87}Sr/^{86}Sr$
Drug Mule Case	0.70857
Caribbean Dolomites*	0.7085 - 0.7092

Table 11.5. Comparison of bone strontium isotope ratios to Caribbean rock samples. *data published by Hans Machel (Machel, 2000) for dolomite samples obtained from Barbados, Grand Cayman, St. Croix and Jamaica.

From Table 11.4 it is also possible to see that other possible countries of origin include Canada and Brazil, though the latter possess quite a variable ratio range, as noted by the numerous results. Of these, it is regarded that the Caribbean forms a major trafficking route to the United Kingdom. Therefore, the focus was placed upon the Caribbean region.

As noted within the table, the regions of the Caribbean have varied results due to their geological composition, be it volcanic or carbonate in origin.

Hope Gate is a region in the northern aspect of the island, formed by Miocene chalk and dolomite. Published data for rocks from this region indicate that an $^{87}Sr/^{86}Sr$ ratio falls within the appropriate range:

Origin of material tested	$^{87}Sr/^{86}Sr$ ratio	
	Min.	Max.
Hope Gate, N. Jamaica - chalk	0.70834	0.70883
Hope Gate, N. Jamaica – dolomite	0.70884	0.70906
Hope Gate, N. Jamaica - limestone	0.70881	0.70889
Unidentified Female - femur	0.70857	

Table 11.6. $^{87}Sr/^{86}Sr$ ratio results for the geology of Hope Gate, North Jamaica, based upon published results, compared to the femoral shaft sample analysed (Land, 1991).

Again, these findings appear to corroborate the initial suggestion of Jamaica as home of the individual over the last 10 years or so.

The trace element analysis allows closer localisation: it is noted that the deceased had high levels of cadmium within her bone (44.85 ppb, compared with the ICMP average, in which cadmium was present below the lowest level of detection.). Cadmium is acutely toxic and, in chronic exposure, can induce renal failure. The soil of Jamaica is well recognised to possess higher levels of cadmium than those worldwide, (Jamaica range 0.3-94 mg kg^{-1}, world range 0.01-2 mg kg^{-1}), though these concentrations are not reflected in the stream or surface waters (Lalor *et al.*, 1998; Knight *et al.*, 1997). The source of the high cadmium concentrations remains uncertain and does not appear to relate to any established industry on the island. However, its presence within the bone sample would appear to corroborate the strontium ratio suggestion of a Jamaican origin.

One of Jamaica's major industries is bauxite (aluminium) mining. Large areas of the island continue to be mined for the metal and, if the individual lived close to these areas, it would be suggested that the aluminium concentrations should be high. However, the concentrations recorded in this postmortem sample are relatively low (16115.67 ppb, compared with the ICMP Bosnian average of 19649.24 ppb) suggesting that, if she did indeed originate from Jamaica, that she lived away from the mined areas. Hope Gate is, itself, close to the Northern Bauxite resource. As such, the likelihood of this area as a possibility appears to be diminished. Aluminium uptake by bone is diet-dependent, thus if she was not eating food grown within the region, then no excessive aluminium would be expected.

Interestingly, a published ratio result for a region of Jamaica indicates a $^{87}Sr/^{86}Sr$ value of 0.7084, similar to that within the femoral sample. This sample was from the Above Rocks region within St. Catherine's district of Jamaica, west of Kingston. It was therefore feasible that the individual may have lived in and around this region of Jamaica during her lifetime.

Additional information was made available through police investigations which appeared to corroborate these suggestions. Intelligence gathered during interviews with known drug-traffickers revealed that the deceased was recognised as a person who had lived in the St. Catherine's region of Jamaica and had contacts with people in the United Kingdom. Despite this information the individual remains a "Jane Doe".

New leads over mystery body

New leads are being followed up by police trying to identify a suspected drugs smuggler found dead on the M45 in Northamptonshire. The woman's body, which was found in April 2002, contained 10 bags of cocaine.

Detectives say they are now convinced the mystery woman was from the St Catherine's district of Jamaica. It is thought she may have been known by the nickname of "Modern Girl." Police are now making appeals through Jamaican radio and newspapers to try to confirm her identity. They are also using a family history website.

The woman's body was found on a grass bank beside the M45 near Barby last April. She was later buried in Daventry. Senior officers and detectives from Northamptonshire Police attended the funeral at Welton Road Cemetery.

A postmortem examination indicated that she may have died from a heart attack.

The woman was black and aged about 60. She had black braided hair which had a gingery tint and was slightly greying at the front.

BBC News, United Kingdom
11th April 2003.

Chapter Twelve - Conclusions.

The developments in isotope analyses, as described within this thesis, have resulted in the creation of new technologies that may assist in forensic investigations. The outcome of this work forms the beginning of a "forensic isotope fingerprint" analysis that can, from an unidentified body, produce estimations of the postmortem interval and the geographical origin of a person throughout their lifetime.

This study has also shown that ^{210}Pb, a viable isotope for use in PMI estimations, accumulates throughout the lifetime of a person, producing an age-dependent activity in bone. This may, in itself, be of use to anthropologists and pathologists alike; after the age of 45, little variation occurs to the skeletal system, making osteological based estimates of age-at-death inaccurate. Knowing that ^{210}Pb accumulates in such a manner may also provide a means of estimating the deceased's age at death. The only problem arises when also considering the PMI. Both techniques measure the same isotope and if measured to produce a time since death estimate, it is dependent upon knowing the age of the individual. But, if the age is also unknown, this produces an analytical dilemma. Such problems could be resolved through the use of emerging technologies such as Bone Density Fractionation, an analytical technique used in archaeology (Bell, Cox and Sealy, 2001). As osteoid is deposited and mineralised, the density of the bone units increases. By grinding the sample to a homogenous powder of known particle diameter, it is possible to selectively fractionate out the particles based upon their density. This produces a time-dependent separation means, such that the more recently deposited bone (of lower density) can be removed from the older material. Knowing that the more recently deposited bone occurs closest to death, the ^{210}Pb content is within this fractionate, in effect, representing the starting of the postmortem stopwatch. Thus, newly deposited bone could form the basis for the PMI estimations, whereas the overall ^{210}Pb activity for the whole bone sample could provide information upon the age-at-death. Future studies into this combination of analyses is therefore strongly recommended.

By combining analytical methods with isotope ratios, the "fingerprint" may be produced, providing such information upon the PMI, age-at-death and geographical domicile of that person. It would readily benefit police forces possessing limited budgets and resources, being relatively inexpensive (approximately £400 per sample) with initial results often available within 7 working days. This compares favourably with radiocarbon dating, with a similar cost per sample though the current estimate for turnaround time for the Oxford Radiocarbon Accelerator Unit at the time of writing is 7.4 months (ORAU Website).

Though the limited nature of this study is recognised, being based upon relatively small numbers of specimens from few countries worldwide, the principles are based upon firm scientific foundations, the techniques being accepted methods which are reproducible and verifiable fulfilling the criteria for both the ICRC recommendations described above, and Swift *et al.* (Swift *et al.*, 2001). The accuracy of the estimations may be questioned, especially when calculating to within a year of death, and the absence of a "gold standard" against which to test the new methodology renders critical analyses difficult (though it maybe argued that this was the purpose of the study; to try and produce a usable method). The true uncertainty in PMI estimation appears to lie within the inherent variability of ^{210}Pb activities within a given population and, without a large scale study to assess this variation, may require the use of biokinetic models to estimate this.

Our case work has reflected the potential benefits of these technologies and has helped in the establishment of information for cases of both forensic and political importance. With global transportation ever increasing, the legal, or otherwise, movement of people, the need to assist in identifying personal traits of an unknown individual is essential in modern forensic practice, helping to establish who the person was and where they lived, two of the main questions that Her Majesty's Coroner is required to answer during forensic investigations.

Appendix 1

Information regarding the provenance of the Portuguese specimens

supplied by Dr. Sue Black OBE BSc PhD DSc, Consultant Forensic Anthropologist.

Dr Sue Black OBE BSc PhD
Consultant Forensic Anthropologist

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27th August 2003

To Whom It May Concern

Skeletal Material from Lisbon – Dr Ben Swift

With regards to the provenance of the above material I can offer the following information and in due course should be able to furnish you with copies of the original communications between myself and Professor Almaca of the Museu Bocage, Lisbon and Dr Luis Lopez of the same.

In the summer of 1989 I travelled to Lisbon with a colleague and an MSc student to study the skeletal remains that are housed in the Museu Bocage in Lisbon. This material originated from the cemeteries around Lisbon and the Cemetery San Juan in particular. In Lisbon at that time the tradition re burial was as follows:

The deceased was laid to rest by the family in the cemetery and a 5 year 'rent' was paid. At the end of the second year, the bones were exhumed from the ground and placed into a wooden box. This box was transferred to a wall of drawers within the cemetery and remained there for a further 3 years. This allowed continual use of the ground for burials as there is a land shortage in Lisbon. At the end of the 5 years, the family had to option to continue to pay the 'rent' or let it lapse. If they either chose not to continue payment or they did not respond to the request from the cemetery, then the remains were eventually removed from the wooden box in the wall and transported to a commercial company where they were ground down and use essentially as fertiliser (I believe). This was only done when room was required within the burial wall and so much of the material dated from the 1950's and 1960's. Dr Luis Lopez was a PhD student in Lisbon at the time and he felt that the material could be more appropriately be conserved as a skeletal collection that would serve scientific research. In 1989 he had already catalogued and stored over 400 individuals but had a further 1200 still to go. He was unable to keep apace with the material that was passing to him and so he had to call a halt to any further 'deliveries'. The material waiting to be catalogued was stored in a variety of outhouses in the vicinity of the botanical gardens in Lisbon.

In 1992, I took on a PhD student, Ms Joanna Norris and her research was to look at the levels of strontium 90 in bones to determine if it would prove to be useful in the

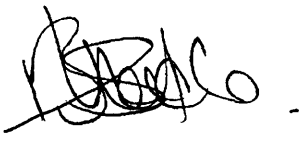
identification of the time-death interval. I obtained permission from both Professor Almaca and Dr Luis Lopez for Joanna to go to Lisbon and remove samples of femoral shaft for her research. These letters are in my storage facility at present and it will take me some time to find them – as it was over 10 years ago. They sought approval from the Portuguese government and we obtained permission to transport them into the UK. The research that we proposed to undertake would necessitate ashing the samples and so there was no requirement or request to return the material at a later date.

Unfortunately the PhD met with several problems and Ms Norris did not complete her project and I moved onto another position. As far as I can remember, as of 1994 the specimens were still within the Anatomy Department at Aberdeen University. At some point, and I cannot remember the specific date, I wrote to Professor Bernard Knight informing him of the material that we possessed and as I knew he was interested in dating techniques I asked him if the samples would be of any benefit to him. The samples then passed into his care and then (I believe) to Dr Swift who undertook research on the material much along the lines of the original design for which the material was obtained.

It is certain that these samples were obtained with full permissions from the Museu Bocage who undertook the responsibility to ensure that approval was obtained from the Portuguese Government and the cemeteries authorities. It was not possible to contact relatives as these represented remains unclaimed by families or the families had made it clear that they did not wish to maintain responsibility for their relatives. When we contacted the UK government, at that time their only comment was that providing the airline would transport the material, then they had no objections. Our flight was with British Airways and they had no objection to carrying the material. Within the UK we spoke with Custom's and Excise and because of the letters of authorisation we carried, they offered no objections to the material coming into the country.

I will endeavour to locate the necessary letters but they are now over 10 years old and may take some time to uncover. I do hope that this will not delay or impede Dr Swift's continuing research.

Yours sincerely



Dr Sue Black OBE BSc PhD DSc

Appendix 2

Radiochemical analyses by alpha spectrometry

^{210}Po and ^{137}Cs method:

All unashed samples were spiked with a ^{209}Po yield tracer. By adding a known quantity of a separate isotope prior to analysis, an estimate or yield value (expressed as a percent) can be calculated. This result will indicate the potential quantity of isotope lost during the test, enabling correction for the concentrations of isotopes under investigation, thus providing estimates for the true original values existing within the material. Similar tracer methods will be employed for subsequent isotope analyses.

The samples were completely dissolved in concentrated (15M) nitric acid, the resulting solution then evaporated to produce a residue that was taken up in 6M hydrochloric acid (HCl), evaporated and re-dissolved in similar concentrations of HCl. Hydroxylammonium chloride, sodium citrate, bismuth nitrate and ascorbic acid were then added to the solution, ensuring the correct oxidation of polonium, and the pH was adjusted to 2. Under such physiochemical conditions polonium spontaneously deposits onto standard analytical silver discs, allowing assay via low background EG&G 'Ultra'® detectors (PerkinElmer, USA). Sample counting was commenced for a minimum of $1x10^6$ seconds. Detection limits were in the order of 100μBq. Recoveries, assessed through the ^{209}Po yield tracer, varied between 87-96%.

The resultant solution from the plating media, now being polonium-depleted, was evaporated to a dry residue and analysed for ^{137}Cs by a high purity gamma detector, as described later, using the 662keV peak.

Separate aliquots of ashed bone tissue were weighed and similarly spiked with ^{242}Pu and ^{232}U-^{228}Th-^{224}Ra yield tracers. After chemical separation and purification using conventional techniques (nitric acid and hydrochloric acid digestions, followed by anion exchange resin separation on 10M HCl/ 0.1M NH_4I and 7.2M HNO_3 columns), samples were electrodeposited on stainless steel discs (Black, 1994;Black, Macdonald, R. and Kelly,

1997;Black *et al.* 1998).

For uranium analyses the ^{232}U yield tracer was used, resulting in typical tracer yields of between 70 and 97%. Radium was analysed following the chemical separation method, by using the ^{228}Th-^{224}Ra fraction of the yield monitor. This Th-Ra fraction was analysed rapidly (less than 48 hours) to prevent extensive loss of ^{224}Ra yield monitor once extracted from the thorium component; the yield range was between 80 and 98% for radium. A decay and in-growth correction was applied for all the yield monitors used.

Blank and background determinations were carried out frequently, forming negative controls and ensuring no environmental exposure. These analyses averaged less than 10 counts in 10,000 under the resultant peaks for the isotopes being determined, and less than 25 counts in 10,000 under the ^{232}U-^{228}Ra yield monitor peaks (Black, Macdonald, and Kelly, 1997; Black *et al.*, 1998). Detection limits were in the order of 1, 4 and 3mBq for Pu, Ra and U, respectively.

The reproducibility of Pu, U and Ra analyses was checked against multiple analyses of a standard reference material, (IAEA 134, Cockle flesh from the Irish Sea), forming a positive control method. All such analyses were within 4% of the reported mean values published by 120 UK laboratories for all nuclides and were well within the 2σ statistical uncertainty count (Popplewell, 1986).

The analyses of the standard material were based on five separate aliquots, resulting in an internal reproducibility better than 1.5% for all nuclides examined. A similar reproducibility was obtained for the bone samples of known PMI. We are, therefore, confident that our analyses are scientifically accurate and reproducible.

Gamma spectrometry analyses

^{228}Ra and ^{40}K were measured using gamma spectrometry of ^{228}Ac at 911 keV and 1460 keV, respectively for each. All samples were sealed in airtight plastic containers with a capacity of $32cm^3$ and counted on a Gamma X-ray high purity germanium coaxial photon detector. To keep self-absorption differences negligible, the plastic containers were calibrated for a range of densities and masses by spiking with a mixed gamma solution obtained from The National Physical Laboratory over the range of masses encountered. The accuracies for the ^{228}Ra and ^{40}K concentrations were assessed equally by comparing against a sample of known

concentrations and similar matrix components (IAEA 134, Cockle flesh and shell from the Irish Sea).
The results of all analyses were within 4% of reported values (Popplewell, 1986).

X-ray Fluorescence analyses

Samples of bone tissue, ashed by the above method, were ground using an agate pestle and mortar. The resulting powder was then pressed into pellets and analysed by X-ray fluorescence on a Phillips PW1400 X-ray fluorescence detector. The instrument was calibrated with human bone material 'loaded' with known quantities of additional chemical constituents (e.g. $PbNO_3$, added gravimetrically) following homogenisation to a similar powder form. All the calibration curves correlated well against the expected and corrected for interferences by elements and background concentrations. The reproducibility of the analyses was compared to the IAEA standard H-9 (mixed human diet) in addition to unknown samples.
All the elements analysed were within 3% of previously reported values from 120 laboratories (Popplewell, 1986).

Atomic Absorption Spectroscopy (AAS)

Calcium and arsenic concentrations were analysed on separate ashed sub-samples following digestion with nitric acid (15M). Calcium was analysed in the flame mode, while arsenic was analysed in a graphite cell using a Perkin-Elmer 2280 AAS with a HGA 400 controller and AS40 autosampler. For arsenic analyses an magnesium/palladium modifier was employed in a volume ratio of 2:1 in addition to the bone sample. This was performed to increase the atomisation efficiency of the arsenic content.
Following the possibility of interference from whitlockite, $(Ca_3(PO_4)_2)$, an apatite phase potentially present within biological bone, arsenic analyses were conducted at temperatures above 2300°C using a $5x10^{-2}$M $Ca_3(PO_4)_2$ solution as reference material, and for the purpose of plotting calibration curves. Analysis of the standard IAEA H-9 provided results of 0.9μg g^{-1} using this calibration for As, being very close to the reported mean value (0.88μg g^{-1}) and a value of 0.23% Ca, being identical to the reported value (Popplewell, 1986).

Appendix 3

Mass Spectroscopy - Isotopes

The outer 1-2mm of the bone samples were removed, minimising surface contamination, by twice washing with acetone, then with ultra-high quality (UHQ) water. This was followed by washing with 5mls of 0.05M (0.25%) nitric acid until all surface discolourations were removed. (This solution was also retained for isotope analysis.) After rinsing, each sample was oven dried at 70°C overnight prior to analysis. Known sample masses (250-750mg) were digested in Teflon beakers on a hotplate at 130°C for 5-15 minutes using ultra-high grade concentrated (69%) nitric acid (distilled Aristar-grade HNO3; BDH, UK). Each sample was subsequently homogenised to ensure complete dissolution. Negative controls were run alongside samples to quantify the contamination from the reagents and during both the processing and analysis of samples. These blanks registered as less than 0.1 $\mu g\ l^{-1}$ during the entire course of this study.
A standard reference material (see below) was also used, acting as a positive control, to further quantify the extent to which the lead isotope ratios could be reproduced. Separation of lead was achieved using a small-scale anionic ion-exchange resin technique similar to that of Mahnes, Minster and Allègre, (1978).

Isotope ratios were determined using a Perkin-Elmer single collector ELAN SCIEX 6000 ICP-MS (ElmerPerkin, USA). For isotope ratio measurements, the instrument was used in the electrical scan (isotope ratio) mode. The magnet was set at up to 15% below the highest scanned mass (208) by altering the acceleration voltage. Thus, the resulting fast peak hopping reduced short-term noise by collecting data over a small time window. Typical scan times were of the order of 10-100 ms/isotope. The instrument was carefully tuned for peak shape and mass bias (<0.05 %/amu) to ensure accurate and reproducible isotope ratio measurements. The external mass bias correction of the data was performed with the NIST SRM981 common lead isotopic reference material. The samples were introduced in a double spray chamber set-up to improve precision and were run in a "two standard samples - sample - two standard samples" analysis bracket routine with the detector dead time monitored at 175Lu. In order to reduce dead time errors the standards were run in closely matched concentrations to the unknown samples (1ng g^{-1} ± 20 %). Samples were rejected if the four bracketing standard analyses relative standard errors (RSE) exceeded 0.1% precision level. Approximately 7 % of samples were rejected; nevertheless, all the resulting re-analyses were within 0.23% of the original values. The "double standard-sample-double standard" set was replicated 20 times for

each individual sample (at low scan rates) and a mean taken of each. The data for differing scans and rinses shows that the data has a low variance from the mean with a standard deviation of (< 0.002) on each measurement. Aliquots of the NIST SRM981 were also run as unknowns giving $^{208}Pb/^{206}Pb$ ratios of 2.1688 ± 0.0012, $^{207}Pb/^{206}Pb$ ratios of 0.91460 ± 0.00051, very close to reported values of 2.1681 ± 0.0008, and 0.91464 ± 0.00033, respectively.

Mass Spectroscopy - Trace elements

Trace elements were also run on separate 250 mg samples after dissolution using the same ICP-MS and methods as described above. Internal (10 $\mu g\ l^{-1}$) Rhenium and Rhodium standards were used to check the quality of the data, which was found to be within 0.25% of expected.

Gamma Spectroscopy

A small quantity (0.5-4.4g) of bone was dissolved in ultra high-grade concentrated (69%) nitric acid (distilled Aristar-grade). The ^{210}Pb was analysed on a large (90mm^2) Harwell Instruments Broad Energy Germanium Detector (Canberra-Harwell Ltd, USA) by direct detection using the low abundance (4.05%) low energy peak at 46 keV. ^{137}Cs (661 keV) and ^{241}Am (59 keV) were also analysed in this manner. This was possible due to the extremely low background in this region (in the photopeaks that ranged from less than 0.003 to less than 0.011 counts per second from 63 - 1000 keV, respectively) and the very high absolute efficiency of the detector at the low energy end of the gamma spectrum (being the efficiency of the detector in comparison to certified solutions greater than 50 %).

Alpha Spectroscopy

$^{239+240}Pu$ and ^{238}Pu were separated from the bone using the methods of McLaughlin *et al.* (Mclaughlin *et al.*, 2003) and were analysed on Canberra passive ion-implanted planar silicon (PIPS) detectors, mounted on two separate four-channel alpha spectrometers (Quad Alpha, Canberra Model 7404; Canberra-Harwell, USA). The spectra were recorded on 450 mm^2 detectors at a source to detector distance of 4 mm. Polonium assays were performed in a similar manner, using the PIPS detectors.

Appendix 4

The Official "Lav Report"

as authored by Dr. Rick Harrington PhD,

Head of Exhumations and Examinations,

International Commission on Missing Persons, Sarajevo, BiH,

October 2002.

icmp International Commission on Missing Persons

ICMP Forensic Science Official Report on Postmortem Interval Testing of the Undocumented Human Remains Recovered from beneath the "Andja Bosnjak" Gravesite, Lav Cemetery, Sarajevo, BiH

30 October 2002

icmp International Commission on Missing Persons

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ICMP Forensic Science Official Report on Postmortem Interval Testing of the Undocumented Human Remains Recovered from beneath the "Andja Bosnjak" Gravesite, Lav Cemetery, Sarajevo, BiH

30 October 2002

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Contributing Forensic Experts:

Dr. Ana Boza Arlotti, ICMP Senior Forensic Anthropologist, on results of archaeological/forensic context and anthropological findings.

Dr. Richard J. Harrington, ICMP Head of Exhumations and Examinations Program and Forensic Anthropologist, on results of archaeological/forensic context, anthropological, and odontological findings.

Mr. Ed Huffine, ICMP Director of Forensic Sciences Program and DNA expert, on results of DNA analysis.

Dr. Benjamin Swift, Forensic Pathologist and Lead Investigator for physiochemical testing, Division of Forensic Pathology, Leicester Royal Infirmary, University of Leicester, United Kingdom, on results of physiochemical testing for postmortem interval.

Purpose of Report:

This report constitutes the official findings of a scientific inquiry conducted by ICMP and University of Leicester forensic scientists. This inquiry was conducted at the joint request of Mr. Amor Masovic, Co-Chairman, Federal Commission on Tracing Missing Persons (herein "Federal Commission"), and Mr. Nedeljko Mitrovic, Chairman, Commission on Tracing Detained and Missing Persons of the Republic of Srpska (herein "RS Commission").

Purpose of Inquiry:

The inquiry was conducted to assess postmortem interval ("time-since-death") for three individuals whose mortal remains were recovered from Lav Cemetery, Sarajevo, BiH; more specifically, to render a scientific opinion on whether the three individuals did or did not die in the period of regional conflict during the 1990s.

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I. Case Background

The three individuals in question are herein referred to as Lav Cases NN1, NN2, and NN3. The following case designations were also duly noted as being in official use: Sa-103/01 for NN1; Sa-104/01 for NN2; and Sa-105/01 for NN3. For analytical purposes (e.g., use of barcodes in the ICMP centralized database system), other designations have also been used.

The mortal remains of these three individuals were reportedly buried together in a position underneath the marked grave of one "Andja Bosnjak" in Lav Cemetery, Sarajevo, BiH. The remains are designated "undocumented" in this report to reflect the fact that ICMP has not been provided with reliable documentation regarding possible personal identities or other relevant background information.

On 23 November 2000, the aforementioned mortal remains were uncovered and examined *in situ* by an RS Commission-appointed forensic pathologist while in the presence of Dr. Brenda Kennedy, erstwhile Director of the ICMP Forensic Sciences Program.

On 27 November 2001, the remains were exhumed and transported to the RS mortuary facility at Lukavica. Dr. Ana Boza Arlotti, ICMP Senior Forensic Anthropologist, was present at the exhumation and later for postmortem examinations conducted at the facility. On 1 August 2002, Dr. Richard Harrington, ICMP Head of Exhumations and Examinations Program, examined the remains at the Lukavica facility.

On 25 April 2002, ICMP representatives recommended to the RS Commission Chairman and the Federal Commission Co-Chairman that bone samples suitable for DNA testing be removed from the three individuals and submitted to ICMP for both DNA testing and postmortem interval testing.

On 8 July 2002, bone samples that had been cut from both femora of each individual were submitted by the RS Commission to the ICMP Forensic Sciences Program for the purpose of analytical testing. Two types of testing were authorized: (1) DNA profiling and matching to facilitate personal identification, and (2) physiochemical testing to assess postmortem interval.

II. Summary of ICMP and University of Leicester Forensic Expert Findings

1. Physiochemical Test Findings:

One bone sample from each case was submitted to Dr. Benjamin Swift of the Department of Pathology, Leicester Royal Infirmary, University of Leicester, England, for physiochemical testing. "Gamma detector" technology was used to measure radioactive decay from the bone samples and thereby determine levels of selected radionuclides that have been demonstrated by Dr. Swift's team to be sound in theory and in practice as estimators of postmortem interval.

Results of the physiochemical testing indicate with high confidence that the individuals died prior to 1945. The primary evidence for this conclusion is the absence of man-made radionuclides, notably Caesium-137, Americium-241, Strontium-90, Plutonium-239, and Plutonium-240. The testing of (and military use of) nuclear weapons in 1945 introduced for the first time man-made radionuclides into the atmosphere and the biosphere (living systems), and therefore measurable levels of some man-made radionuclides would be expected to be found in the systems of all individuals alive after 1945. Additional evidence for postmortem interval exceeding several decades for all three individuals is obtained from results of Lead-210 analysis. It is the expert opinion of Dr. Swift and colleagues that postmortem

environmental factors affecting levels of these radionuclides in a manner that mimics prolonged postmortem interval cannot reasonably account for these findings.

Therefore, the findings are, in and of themselves:

Strongly indicative of postmortem interval of over 57 years, i.e., all three individuals died before 1945

2. Archaeological/Forensic Context Findings:

Evidence such as soil stratigraphy and soil composition reportedly had not been taken into account by the initial investigators for assessing postmortem interval during first exposure of remains on 23 November 2000. Even if such evidence had been documented, the results may be equivocal with respect to distinguishing between moderate and prolonged postmortem intervals, that is, these lines of evidence will not necessarily distinguish postmortem interval of several years from postmortem interval of more than a decade. Artifacts retrieved from the general vicinity of the mortal remains (specifically, a "Yugoslav Army" jacket and rusted nails) are judged by ICMP forensic experts to be of questionable relevance for time-since-death determination in these cases. The jacket was not found in direct association with the remains, and therefore cannot be proven to belong to any of the decedents. The presence of nails which could have been used in coffin construction are irrelevant to determination of time-since-death.

Therefore, the findings are:

Inconclusive in and of themselves, with no evidence to contradict physiochemical test findings

3. Anthropological Findings:

The remains are completely skeletonized, with no discernible odor, indicating advanced organic degradation. Inorganic degradation appears to be minimal, i.e., there is minimal gross evidence for weathering or appreciable loss of mechanical integrity of the osseous remains. State-of-preservation is judged by ICMP forensic experts to be consistent with moderate to prolonged postmortem interval, i.e., postmortem interval of several years to several decades.

Therefore, the findings are:

Inconclusive in and of themselves, but consistent with physiochemical test findings

4. Odontological Findings:

A complete upper denture of what appears to be synthetic resin composition with metallic attachment was associated with the NN1 individual. Dentures of this type apparently have been manufactured since about 1935.

The NN2 individual has metallic restorations, possibly of amalgam composition, of two teeth. Restorations of this general type have been in use since the 19th century.

Therefore, the findings are:

Inconclusive in and of themselves, but consistent with physiochemical test findings

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5. DNA Test Findings:

One bone sample from each case was submitted to the ICMP DNA Laboratory Operations for the purpose of DNA extraction and profiling. Preliminary results of the DNA test were inconclusive due to relatively poor preservation of the DNA. The pattern of biodegradation of the DNA is consistent with both prolonged postmortem interval under normal environmental conditions and short to moderate postmortem interval with exposure to harsh environmental conditions.

Therefore, the findings are:

Inconclusive in and of themselves, but consistent with physiochemical test findings

III. Interpretation of Findings

In conclusion, it is my professional opinion that the sum of scientific evidence weighs in favor of the interpretation that the individuals referred to herein as Lav Case NN1, Lav Case NN2, and Lav Case NN3 died prior to the period of regional conflict during the 1990s. This opinion is based on my belief that the physiochemical tests have provided reliable guidance in postmortem interval estimation; that all other findings cited in this document, although inconclusive when considered in and of themselves, are compatible with this interpretation; and that no submitted evidence has been omitted from consideration that could be construed as contradictory to this interpretation.

Signed,

Richard J. Harrington, Ph.D.
Head of Exhumations and Examinations, ICMP
30 October 2002

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