

**Biogenic lead levels in human skeletal remains from the cemetery of the  
British Royal Navy hospital (1793-1822CE) at English Harbour, Antigua,  
West Indies**

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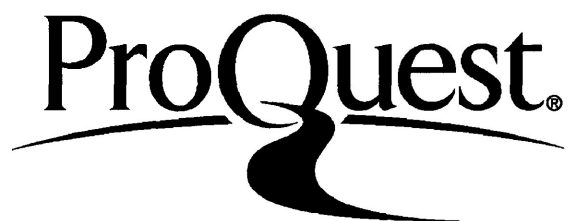
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## **Abstract**

Though lead has been known to be a cause of human poisoning since ancient times, it was a widely used metal. The relationship between ancestry or age and lead exposure in the Colonial period of Antigua, West Indies was explored by comparing the lead levels in bone samples from individuals of two different ancestries, African and European, from a cemetery associated with a British Royal Navy hospital (1793-1822CE) at English Harbour in Antigua, West Indies. The non-segregated nature of this cemetery is believed to have been unique for the period, and allowed for more direct comparisons between ancestral groups in this study. Cortical samples from the fibular diaphysis of 24 male individuals were analyzed for lead by ICP-MS. The mean lead levels were found to be  $74.3 \pm 51.8$  ppm for the African population, and  $87.7 \pm 78.6$  ppm for the European population. No significant difference was found between the mean lead levels of the two ancestries. Furthermore, no discernible pattern in lead levels was found in relation to the individuals' ages. The biogenic origin of the lead was confirmed by scanning the bone samples using synchrotron radiation X-ray fluorescence imaging (SR-XFI). Visible evidence of incorporation of lead into the microstructures of bone indicated that lead uptake likely occurred during the individuals' lifetimes. These results are in contrast to previously published studies comparing lead levels of individuals from similar and contemporaneous populations. The outcomes of this research suggest naval personnel of both African and European ancestry at English Harbour in Antigua, West Indies had very similar experiences with regards to lead exposure. Their exposure to the toxic metal was not consistent over time, however, as steady exposure would likely have resulted in a positive correlation of lead level with individual age. This study assists in addressing historical questions regarding both the prevalence of lead poisoning in the British Royal Navy during the Colonial period and lead's potential involvement in the deaths of naval personnel.

## **Lay Summary**

The use of lead during the Colonial period was common, even though it was well-known to be toxic. Due to symptoms described in medical reports from the period it is believed that naval personnel frequently suffered from lead poisoning. Exposure to lead could have occurred through a variety of sources, including drinking water and rum. Lead circulating in the blood can take the place of calcium and other divalent cations and activate or inhibit many of the enzymes in the human body by altering their structure, resulting in widespread effects to our systems and significant changes to normal body functions. After lead has entered the body, most of it becomes incorporated into bone, replacing calcium. Bone lead can be an important indicator of health status related to lead exposure as the lead can be released from bone back into the blood.

This thesis analyses lead detected in the bones of individuals who were a part of the British Royal Navy during the Colonial period, either as regular personnel or as enslaved labourers. These individuals ranged in age from their early teens to late adulthood and were of both African and European ancestries. They were excavated from the cemetery associated with the Royal Naval Hospital at Nelson's Dockyard in English Harbour, Antigua, which operated from 1793-1822CE. This cemetery is believed to be the only unsegregated cemetery from that time period in the Caribbean. The analysis of the lead levels detected through the course of this research indicates that a significant portion of this population of individuals would have experienced effects of lead poisoning. Contrary to expectations based on previous publications, there was no significant relationship between ancestry and average lead levels, nor was any pattern detected relating to age group.

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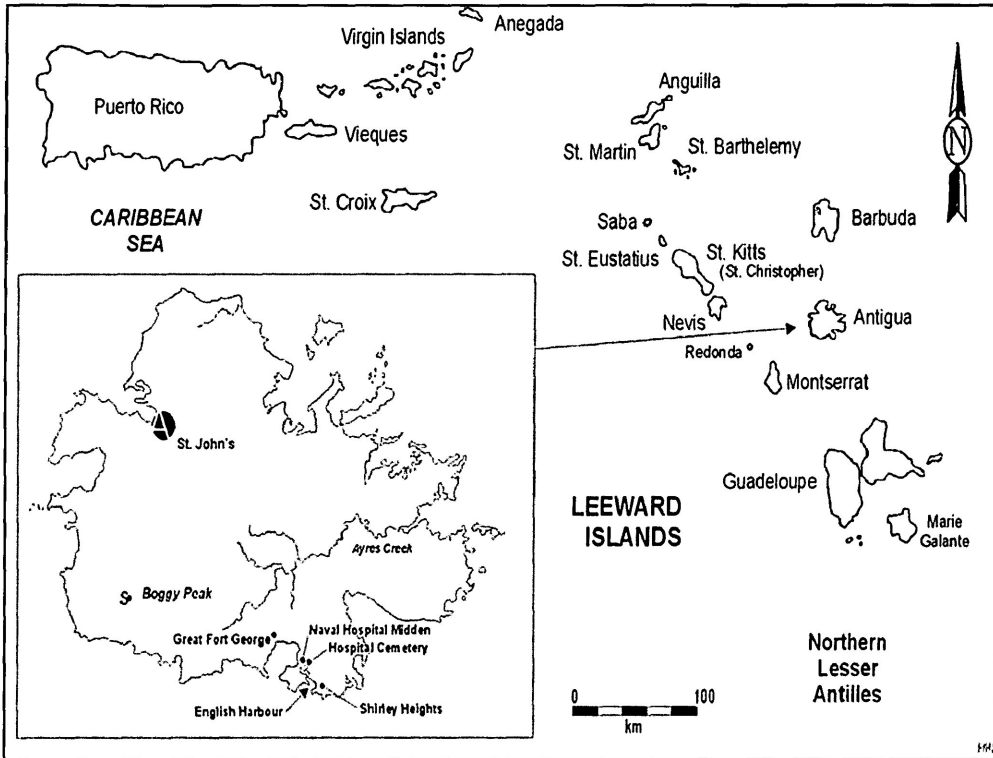


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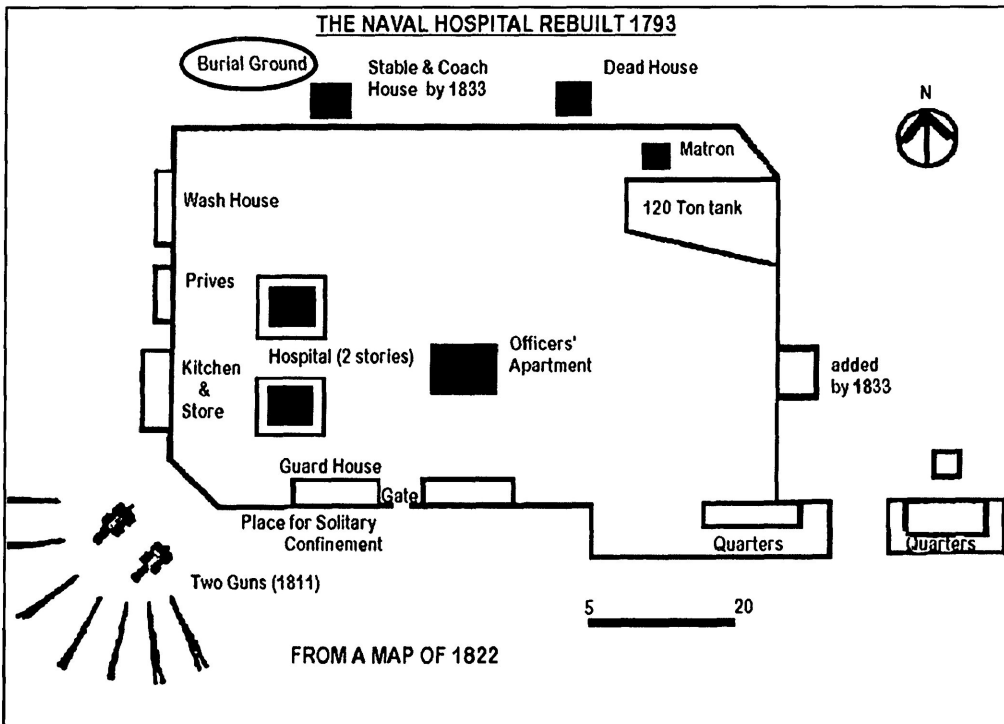
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## **Chapter 1 – Introduction**

This thesis focuses on the analysis of bone lead levels in human skeletal remains from the Colonial period. The remains were excavated from the cemetery belonging to the British Royal Navy Hospital at Nelson's Dockyard in English Harbour, Antigua, West Indies following the cemetery's discovery during the construction of residential housing (see Figures 1.1 and 1.2). Historical documentation indicated that the hospital operated between 1793 and 1822CE (Nicholson, 1993). Cranio-facial analyses of the remains performed on-site during excavation determined the presence of individuals of both African and European ancestries (Varney, 2003). The presence of both ancestries in one burial ground from that time period is believed to be unique in the West Indies, which is historically a region of significant European colonization and enslavement of individuals of African ancestry (Varney, 2003). The internment of both ancestries in one cemetery allowed for more equivalent comparisons of the skeletal material, as they shared a common burial environment. Further analyses revealed that all the individuals were males and that their ages ranged from infancy up to approximately 50-60 years of age (Varney, 2003). Archival information indicated that the individuals interred were low-ranking naval personnel and members of a specialized group of enslaved labourers, the King's Negroes (Varney, 2003).



**Figure 1.1 - Map of West Indian Isles with Antigua inset**



**Figure 1.2 – Map of the Royal Navy Hospital with cemetery indicated in upper left hand portion (Nicholson, 1993)**

Lead can be detected in cortical bone samples from the remains using both inductively-coupled plasma mass spectrometry (ICP-MS) and synchrotron X-ray fluorescent imaging (SR-XFI). The application of SR-XFI allows for visualization of the location of lead in bone and ICP-MS analysis gives us the bulk quantity of lead in bone. The quantitative analysis of lead in bone can be significantly impacted by diagenesis, alterations in bone material resulting from chemical exchanges within the burial site. This could potentially lead to a loss or gain of material either to or from the depositional environment. The use of both ICP-MS and SR-XFI together can be shown to support a biogenic origin of the detected lead and allow for more meaningful interpretations of the data collected.

Aufderheide et al. (1981) found that a Colonial era plantation owner and his family had much higher bone lead levels than did the plantation's enslaved labourers. The researchers also found that the enslaved labourers' lead levels roughly increased with the age-at-death of the individual. Corruccini et al. (1987) found that a group of enslaved labourers from a Colonial Barbadian plantation had bone lead levels that were more comparable to the high lead levels seen in the plantation owner's family than the enslaved labourers in Aufderheide et al. (1981). Additionally, Corruccini et al. (1987) found that the lead levels in the labourers increased with age-at-death. Based on these similar studies that compared lead levels between ancestries and amongst different age groups, it is hypothesized that there will be a significant difference between the mean lead levels of the two different ancestries and that there will be an increase of lead burden in bone associated with increasing age at death.

The use of lead was commonplace during the Colonial period, and it is believed that a significant portion of the British armed forces were suffering from lead poisoning, which is also known as plumbism (Buckley, 1998; Howard, 2000). Significant numbers of medical reports

from that time period also indicated that naval personnel suffered from symptoms that were consistent with lead poisoning (Trapham, 1679; Hughes, 1750; Blane, 1785; Turnbull, 1806; Lloyd & Coulter, 1961; Buckley, 1998). Due to lead's toxicity, if lead levels of naval personnel were high, this would have resulted in damage to their overall state of health and wellness. This work will either help to support or refute this supposition by examination of the lead levels of the individuals interred in the British Royal Navy Hospital cemetery.

The first chapters in the following literature review will outline the history of the British Royal Navy Hospital at Nelson's Dockyard in English Harbour, Antigua, West Indies, the use of lead in the Colonial era British Royal Navy and the effects of lead on the human body. Succeeding chapters will outline the studies that have been performed on lead in bone. It will begin with a chronological discussion of the analysis of lead in archaeological bone to demonstrate the development of methodologies and understanding of complicating factors such as diagenesis. The application and method development of the later-developed ICP-MS to analyzing lead in bone will follow and the concluding literature review chapter will cover the use of SR-XFI to analyze lead in bone. In the final two chapters, special focus will be given to those studies involving archaeological bone material.

## **Chapter 2 – History of the British Royal Navy Hospital at Nelson’s Dockyard in English Harbour, Antigua, West Indies**

In order to better understand the outcomes of analyses, it is beneficial to give archaeological samples as much historical context as possible. The following chapter briefly summarizes the development of Nelson’s Dockyard in English Harbour, Antigua West Indies as well as the Royal Naval Hospital and what is known of its operations. Accounts of individuals whom were known to have been treated within the hospital or to have died there will be stated. The findings of the archaeological investigations and excavations of the hospital grounds and its cemetery will also be outlined.

The small island which came to be known as Antigua was first spotted on Columbus’ second voyage to the New World (Oliver, 1894). It was first colonized by the British in 1632 after Sir Thomas Warner, the founder of the British colonies on the Leeward Islands, sent a group of settlers there from St. Kitts (Oliver, 1894). Part of the Lesser Antilles, the island was initially planted with crops of cotton and tobacco (Sanders, 1994). Soon, the crop of choice became sugar cane. Sugar cane was grown initially in 1655 and the first successful sugar plantation, Betty’s Hope, was built in 1674 (Jane, 1982). These crops required significant amounts of labour, and the plantation owners began importing enslaved labourers from Africa in order to meet the demands (Jane, 1982). The population of Antigua swelled; by 1678 half of the population was comprised of enslaved labourers (Nellis, 2013). By 1705, most of the island was planted with sugar cane (Jane, 1982). Antigua was to become the most profitable Leeward Island in the West Indies during the 18<sup>th</sup> century (Jane, 1982). In fact, profits in the West Indies exceeded those brought in from the North American colonies; the West Indies contributed more than £400,000 compared to the approximately £380,000 pounds from all of the North American

colonies (Jane, 1982). The British sent their Royal Navy to the colonies in order to protect their assets in the Caribbean (Jane, 1982).

English Harbour, Antigua, was first used as a location to careen and trim ships (Nicholson, 1991). It was discovered that it provided significant shelter from storms in 1671 when a yacht harboured there survived a hurricane (Nicholson, 1991). The successful careenings and safe harbour provided by the area led to the recommendation that a naval yard be built there, and construction was begun in 1725 (Nicholson, 1991). A small number of structures, including a battery, wharf and cisterns, were some of the initial developments (Nicholson, 1991). The dockyard at English Harbour became one of two permanent stations in the West Indies used by the British Royal Navy, particularly during hurricane season; the other was located at Port Royal, Jamaica (Jane, 1982). The dockyard extended to the west side of the harbour in 1745 and the naval hospital was built at about this time at the north end of the harbour (Nicholson, 1993). A hurricane in 1772 completely destroyed the hospital, and, as a result of much back and forth correspondence with England; it was not rebuilt until after 1780 (Nicholson, 1993). The 1770s and 80s saw significant development of English Harbour under the watches of both Governor Shirley and Captain Shipley (Jane, 1982; Nicholson, 1991). Governor Shirley made mention of the location of the hospital, as well as other dockyard constructions in a letter in 1781 (Oliver, 1894). Following Captain Horatio Nelson's stay at English Harbour and his triumphs in the Napoleonic wars, the dockyard was named Nelson's Dockyard (Sanders, 1994).

The development and workings of the dockyard were largely accomplished by the efforts of enslaved labourers of African ancestry (Nicholson, 1991). Most frequently, the Navy would borrow slaves from plantations for menial work, but eventually they also purchased their own group of enslaved labourers; these individuals became known as the King's Negroes (Nicholson,

1991; Buckley, 1998). The majority of labourers purchased by the Navy were specially trained, working as sailmakers, sawyers, masons and blacksmiths, as well as other specialized trades (Nicholson, 1991; Buckley, 1998). The need for the King's Negroes was significant; tradespeople of European ancestry were disinclined to work in the West Indies, particularly English Harbour, due to the extent and deadliness of the illnesses present there (Crewe, 1993). By 1780, 70% of the dockyard's workforce was of African descent, and the majority were enslaved (Nicholson, 1991)

Following reconstruction, the Royal Naval Hospital at Nelson's Dockyard was in use from 1793 until 1822CE and operated under civilian contract with the Navy (Nicholson, 1993). It was not well-regarded (Crewe, 1993). An account from 1808 from one of the Royal Navy's surgeons, John Augustus Waller, made mention of its poor location, given that the harbour had poor air circulation due to the surrounding hills and it was situated windward of a swamp, as well as the hospital's high death rate, second only to Barbados at that time (Oliver, 1894).

Regardless of its reputation, its facilities were used by the officers and soldiers of many regiments and ancestries, as well as by enslaved labourers. The diversity of its patients has been indicated and supported by both archaeological evidence and historical documentation. The naval hospital's midden was discovered during the construction of a private home in 1980 (Nicholson, 1993). Little time was available for excavation, but numerous items were found (Nicholson, 1993). Among the artifacts was a badge from the shaker hat belonging to an individual from the 8<sup>th</sup> West Indian regiment, indicating that patients of African descent were treated there, as these regiments were comprised of men of African ancestry (Nicholson, 1993). Buttons from the uniforms of officers and even French prisoners of war were also recovered (Nicholson, 1993). DV Nicholson performed archival research for his 1993 *Mud and Blood*



publication, revealing documents from St. Philips Church that indicated that the hospital chaplain baptized an infant on hospital grounds; the infant was the child of a woman who was owned by the Hospital watchman's wife (Nicholson, 1993). Further exploration of records by DV Nicholson also revealed that the January 28<sup>th</sup> 1819 Antigua Gazette contained an advertisement that announced that inoculations would be taking place at the hospital at English Harbour, referring to it as the General Detachment Hospital (Nicholson, 1993). The final death record for the hospital states that Tom Spanker, a sailmaker who was one of the King's Negroes, died there in 1822 (Nicholson, 1993). Excavation of a portion of the cemetery belonging to the hospital starting in 1998 revealed the remains of 31 individuals in 26 graves; 24 were adults while the other individuals were subadults, including 5 individuals under the age of 5 (Varney, 2011). Few items were associated with the graves, but those that were found assisted in establishing the nature of the cemetery (Varney & Nicholson, 2001). A nail bearing the Devil's Claw mark, which indicated government or military ownership, was found in burial 5, and burials 4 and 6 contained bone buttons consistent with those found on the trousers of naval personnel at the time of internment (Nicholson, 1993; Varney & Nicholson, 2001). One grave was marked as belonging to Alexander Bernard, a Royal Navy Surgeon's assistant (Varney & Nicholson, 2001). Fourteen of the twenty two adult individuals were able to be assessed for ancestry due to good preservation of the skull; seven of them were found to be of European ancestry and seven were of African ancestry (Varney, 2011). Archival information indicates that the interred individuals were low-ranking naval personnel and individuals belonging to the King's Negroes (Varney, 2003). The discovery that the cemetery was unsegregated was surprising; no other contemporary cemetery in the Caribbean is known to contain individuals of both European and African ancestries (Varney, 2011).

The unsegregated nature of the British Royal Naval Hospital cemetery at English Harbour, Antigua allows for studies of more accurate comparison of aspects of the lives of the individuals that were interred within it. An isotopic study comparing the diet of those interred was undertaken (Varney, 2011). It was found that the individuals of African and European ancestry interred in the cemetery had significant overlap of stable carbon and nitrogen isotopic ratios (Varney, 2011). When isotopic ratios from probable slaves interred on other West Indies islands were compared against the two populations from Antigua there was little to no overlap with the Antiguan European group, but significant overlap with the Antiguan African group (Varney, 2011). These findings likely indicate that there was similarity in dietary staples between the African-descended enslaved labourers at the dockyard and the European naval personnel (Varney, 2011). The Antiguan dockyard workers appear to have had a similar protein source as the European Naval personnel, but they overlapped with the other enslaved groups in their staple carbohydrate source (Varney, 2011).

Previously, studies have been conducted that compared the bone lead levels of colonial era, African-descended enslaved labourers and their owners of European ancestry (Aufderheide, et al., 1981; Corruccini, et al., 1987). The cemeteries from which the remains for Aufderheide's and Corruccini's studies were excavated were both segregated, and differing post-mortem conditions presented a challenge to these studies (Aufderheide, et al., 1981; Corruccini, et al., 1987). Considering that the extensive use of lead in the West Indies during the Colonial period has been well documented, and that the British Royal Naval Hospital in Antigua cemetery was unsegregated, it was determined that this avenue of investigation was warranted in regards to this unique set of remains as it would allow for more direct comparison with less need for the consideration of differing burial environments.

### **Chapter 3 - Lead and the British Royal Navy**

The interest in analyzing the skeletal remains from the British Royal Navy's English Harbour hospital cemetery is rooted in medical records from the Colonial period. A significant number of accounts from medical practitioners of the time indicate that many naval personnel suffered from symptoms that are consistent with lead poisoning (Trapham, 1679; Hughes, 1750; Blane, 1785; Turnbull, 1806; Lloyd & Coulter, 1961; Buckley, 1998). Mass lead poisoning during the Colonial era is not outside of the realms of possibility as the use of lead was commonplace at the time. Lead is a soft, pliable metal that could easily be worked into many forms; these characteristics resulted in it being a component of 18<sup>th</sup> century water systems such as cisterns, gutters and troughs (Handler, et al., 1986). It was used as a pigment enhancer and commonly found in house paints, pottery glazes, hair dyes and cosmetics (Handler, et al., 1986; Nicholson, 1993). It could also be used as an antiseptic, preservative, and as a sweetener (Nicholson, 1993).

While lead's use was widespread, its toxicity was also well known by that time. Hippocrates is considered the first to have described lead poisoning, but Nicander is credited with the first clinical account in the second century BCE (Waldron, 1973). James Hardy wrote in 1778 of colics arising from lead poisoning as a result of lead leaching into alcohols from the vessels in which they were contained (Hardy, 1778). Lead poisoning epidemics were described throughout history and given names such as the Colic of Poitou, Devonshire colic, entrapado of Spain, and the bellain of Derbyshire (Waldron, 1973).

The presence of lead in spirits is considered one of the most probable sources of lead intoxication in the West Indies (Handler, et al., 1986). Most of the islands were dedicated to sugar production, a significant portion of which was processed into rum, and initially the

distillation equipment required for this process had many components which contained lead, including the boiling cauldron, condenser coil and still head (Handler, et al., 1986). Additionally, the heat required and acidity created increased the likelihood of lead leaching into the liquid (Handler, et al., 1986). 'New' rum, also known as low-wine, was a particular problem. This unrefined beverage was a primary distillation product (Handler, et al., 1986; Howard, 2000). Accordingly, it contained a high percentage of alcohols, some of which were poisonous and it also contained a significant amount of lead (Handler, et al., 1986). It was relatively inexpensive as compared to aged rum, and so it was more often consumed by those of low socioeconomic status (Handler, et al., 1986). While aged rum appears to have a reduced impact on health, it still contained appreciably toxic amounts of lead (Handler, et al., 1986).

Most of the officers and soldiers of the British Royal Navy consumed rum regularly. After 1740, a half pint was a part of all regiments' daily allotment (Lloyd, 1969). They consumed it more than willingly, and much of the administration was relatively tolerant of the general drunken state of the crew (Thomas, 1968, Dyde, 1997). Rum was believed to be an antiscorbutic and preventative of fever, and given the prevalence of scurvy, malaria and yellow fever in the West Indies, a substance that might mitigate any of these conditions was acceptable (Lloyd, 1969; Duffy, 1987; Dyde, 1997). Rum consumption may also have been elevated due to thirst resulting from the presence of salted meat as a regular part of their weekly rations (Dyde, 1997). Since Antigua was a particularly dry island, only two natural springs could be found on it at the turn of the 18<sup>th</sup> century; access to fresh water for thirst abatement was limited (Oliver, 1894). Boredom also contributed to excessive alcohol consumption. Aaron Thomas, a seaman in the British Navy in 1794 theorized that the regular daily rum consumption from a young age eventually induced alcoholism (Thomas, 1968).

The link between rum consumption and ill health in the Navy and the Army was made early on and by the late eighteenth century, the connection had also been made to lead. Dry bellyache, caused by constipation, was observed in Jamaica in 1670 by the physician Dr. Thomas Trapham and he likened it to the bellain of Derbyshire, although he made no specific mention of lead (Trapham, 1679). The linkage of dry bellyache to lead poisoning was made over the next one hundred years (Handler, et al., 1986). In fact, Dr. William Turnbull listed the dry bellyache as the third most common disease of Navy personnel in the West Indies and compared its symptoms to the Devonshire colic which was then known to have been caused by lead (Turnbull, 1806). Dr. Griffith Hughes noted that many sufferers of dry bellyache were rum distillers and sugar boilers, but that most of the sufferers drank excessive amounts of new rum (Hughes, 1750). John Augustus Waller, a Navy surgeon, lamented in 1808 that rum was too easy for the men to procure and believed that its excessive consumption also contributed to the increased incidence of fever (Oliver, 1894). Sir Gilbert Blane, a Navy physician, also believed that the consumption of new rum increased the likelihood of acquiring yellow fever and, additionally, that the lead stills used for making it were responsible for the many fluxes (dysentery or gastroenteritis) experienced by the seamen (Blane, 1785; Lloyd & Coulter, 1961). He eventually convinced the Navy to replace rum with wine (Lloyd & Coulter, 1961).

Hospitalization did not necessarily reduce the exposure to lead. While daily rations inside the hospital did not include rum, it was often smuggled in to the patients by comrades, so much so that a prickly pear hedge was constructed around the hospital to help keep it out of the yard (Crewe, 1993; Nicholson, 1993). The presence of rum in the hospital was supported by the discovery of bottles consistent with liquor bottles in the hospital midden excavated in 1980 (Nicholson, 1993). Lead was also present in medications. Litharge, a lead oxide that was a

common component of a surgeon's chest, was used as an adhesive and, ironically, a treatment for colic (Anon., 1846; Lloyd, 1969; Needleman, 1999). Lead could also be used as an antiseptic (Nicholson, 1993). A small ceramic pot containing a white powder that was later identified as lead acetate was found in the hospital midden in 1980, supporting the presence of lead in medical practice of the time (Nicholson, 1993). Additionally, exposure to lead may have resulted from foods stored in lead-glazed pots, as multiple sherds were also found in the same midden, and lead has been found to leach into food from these types of ceramics (Avila, et al., 1991; Nicholson, 1993).

Lead exposure may also have resulted from the water systems at English Harbour. Since Antigua was an almost dry island, cisterns were built at the dockyard to collect rainwater for drinking and cooking (Nicholson, 1991). The rain gutters and the lining of the cistern itself were made from lead, as was typical at the time (Handler, et al., 1986). Given the high temperatures of the island that would have resulted in repeated cycles of evaporation and condensation within the cisterns, higher levels of lead may have leached into the cisterns' contents. Other possible sources of lead for Navy personnel include food storage vessels and servingware (Handler, et al., 1986).

Enslaved labourers also had regular access to rum, particularly new rum (Handler, et al., 1986). Rum would not have necessarily been a daily allotment, however, but it would have been available through other means (Handler, et al., 1986). They would likely also have consumed water from the same sources. Most of the menial labour came from plantation slaves, and as this work may have included working in a still, their exposure could have been significant (Handler, et al., 1986; Nicholson, 1991). Since the King's Negroes belonged to the Navy, their provisions would likely have come from similar sources as the other Navy personnel.

Given that the remains from the Royal Naval Hospital cemetery at English Harbour are of both European and African ancestry, it was deemed prudent to analyze and compare the results of the bone lead quantification of the individuals of known ancestry in order to ascertain if there is any significant difference in mean levels or variance between the two populations or amongst the different age groups.

## Chapter 4 - Effects of Lead on Human Biology

In order to understand the potential impact of lead on naval personnel, it is necessary to outline both how it is introduced into the human body and its effects once it has been absorbed. Lead, Pb, in its inorganic form, exists as a divalent cation. It can also be found in organic forms such as tetraethyl lead, which historically was added to gasoline as an antiknock agent (Anon., 1928). Lead can enter the human body in either form, primarily through ingestion and inhalation, but organic forms may also be absorbed through the skin. If inhaled, about 30-50% of the inhaled lead is absorbed into the adult body (Morrow, et al., 1980). In adults, about 90% of ingested lead is excreted, predominantly in urine; the other 10% is absorbed by the body (Bogen, et al., 1976). Children absorb a higher percentage of ingested lead: 50% of the lead they ingest is absorbed (Ziegler, et al., 1978). It has been shown that regardless of the source concentration, the ingestion and inhalation absorption rates generally remain relatively consistent (Flanagan, et al., 1982; Morrow, et al., 1980). The amount of lead absorbed by an adult through ingestion can increase dramatically, upwards of 60%, during periods of fasting (Heard & Chamberlain, 1982).

Once the lead has been absorbed, it is taken up into erythrocytes using calcium transporters and anion exchange (Calderon-Salinas, et al., 1999; Bannon, et al., 2000). Ninety nine percent of it becomes bound to blood proteins, almost half of it to hemoglobin (DeSilva, 1981; Philip & Gerson, 1994). The half-life of lead in the blood is approximately 36 days (Rabinowitz, et al., 1977). From there it is distributed to soft tissues and bone. While the human body does not require lead for any of its functions, it very frequently uses other divalent cations such as calcium, zinc and magnesium to assist in achieving physiological requirements. Due to its similarity with the other divalent cations, once it has been absorbed, lead can easily take the



place of these ions, often resulting in a detrimental effect (Godwin, 2001). It is thought that this is in part due to lack of lead activity regulation in the body, as humans evolved with little exposure to lead (Bridges & Zalups, 2005; Garza, et al., 2006). Regulation of cationic activity of zinc and calcium enzymes is achieved through selectivity based on electronegativity, active site atoms, concentration, size, coordination number and affinity (Katz, et al., 1996). Since lead was not prevalent at the time that these mechanisms evolved, no mechanisms were created that would exclude lead from the active sites, and due to lead's characteristics, it often has greater affinity for these sites than do the natural cation cofactors. The sheer number of enzymes which utilize divalent cations also contributes to the problem; once lead has entered into the human body there are many proteins with which it can interact.

Divalent cation transporter I (DCT1), a metal transporter molecule, is capable of facilitating lead uptake into cells and certain voltage-gated calcium channels have also been shown to allow lead entry into cells (Gunshin, et al., 1997; Simons & Pocock, 1987). Divalent metal transporter I (DMT1), another metal transporter molecule, has also been implicated (Bannon, et al., 2002). The endothelial cells of the blood-brain barrier have been shown to preferentially accumulate lead, and high levels of lead can result in disruption of this barrier when the calcium that is normally required for the cells' tight junctions' integrity is replaced by lead (Toews, et al., 1978; Bridges & Zalups, 2005; Silbergeld, et al., 1980). Lead is also preferentially deposited in astroglial cells in the brain (Thomas, et al., 1973).

Once within the cell, lead can be found within the mitochondria and the nucleus (Hitzfield & Taylor, 1989; Silbergeld, et al., 1980). Lead can disrupt the mitochondria in such a way that too much calcium is released into the cell, which ultimately leads to cell death (Fox, et al., 1997). This effect has been well-studied in retina cells, and may explain the loss of vision

that is often experienced by both adults and children who are suffering from lead poisoning (Fox, et al., 1997). Lead can also inhibit the action of Apel nuclease, which is responsible for DNA repair; the inhibition of this enzyme contributes to the development of cancers (McNeill, et al., 2004).

Lead has a high affinity for zinc enzymes. One of the most extensively studied interactions is between lead and  $\delta$ -aminolevulinic acid dehydratase (ALAD). When lead binds to ALAD instead of zinc, the enzyme is inhibited; this disrupts the heme synthesis pathway, thus contributing to the anemia seen in individuals with high blood lead levels (BLLs) (Warren, et al., 1998). Lead can also interact with the zinc finger motifs found in some DNA transcription factors and inhibit their activity as well (Hanas, et al., 1999; Huang, et al., 2004). Inhibition of transcription factors will deregulate some aspects of genetic expression.

Lead will commonly bind to the sites for calcium in helix-loop-helix structures and C2 domains of enzymes (Markovac & JW, 1988; Habermann, et al., 1983; Boutton, et al., 2001). Enzymes like protein kinase C and calmodulin are activated by lead, more so than by their regular cation cofactor, calcium, causing drastic changes in cellular signal cascades (Kern, et al., 2000; Neal, et al., 2010). Synaptotagmin, which is involved in vesicle transport of neurotransmitters, is also partially activated by lead, leading to loss of control of neurotransmitter release (Boutton, et al., 2001; Neal, et al., 2010). In contrast, lead inhibits the function of  $\text{Ca}^{2+}$ -ATPase which results in the loss of calcium from the endoplasmic reticulum (ER) of cells (Hechtenberg & Beyersmann, 1991). Many proteins associated with the ER are dependent on calcium to achieve their proper structure, and the reduction in calcium concentration within the ER will disrupt their overall function (Shirabe & Hirano, 1977; Qian, et al., 2000).

Elevated BLLs will produce symptoms of lead poisoning, but these levels differ significantly between adults and children (Murata, et al., 2009). Children have been shown to demonstrate neurological impairment at BLLs below 10 $\mu$ g/dL, and recently the Centers for Disease Control and Prevention in the United States stated that a level of 5 $\mu$ g/dL is cause for intervention, but no amount of lead is in fact considered safe (Canfield, et al., 2003; Anon., 2004). Adults, on the other hand, seem to start experiencing adverse effects from lead at the 20 $\mu$ g/dL level, as shown by a metadata study done by Murata et al. in 2009 (Murata, et al., 2009).

The adverse effects differ due to the nature and route of the exposure. Acute symptoms that can arise from a concentrated exposure include abdominal colic, constipation, fatigue, anemia, numbness or pain in the hands and feet, and issues with the central nervous system (Cullen, et al., 1983). The most intense exposures can result in coma, convulsions and swelling of the optical discs due to intracranial pressure (Cullen, et al., 1983). More moderate exposures may present as headaches and personality changes (Cullen, et al., 1983). The effects of acute lead exposure are often permanent (Byers & Lord, 1943). Chronic exposure to lower levels of lead has a wide range of effects as well. Inhibition of ALAD contributes to anemia, as does lead's inhibition of ferrochelatase, another enzyme in the heme synthesis pathway (Hernberg, et al., 1970; Roels, et al., 1976). Nerve conduction velocity is slowed and there can be tremors, numbness and pain in extensor muscles as well as poor reflexes (Seppalainen, et al., 1985; Landrigan & Todd, 1994). Intelligence quotient deficiencies have been observed in children demonstrating no other clinical symptoms (Needleman, et al., 1979). Kidney disease leading to kidney failure is a classic symptom of long-term lead intoxication (Landrigan & Todd, 1994). Several studies have shown that there is a two to three hundred percent increase in deaths from

renal disease amongst workers who were occupationally exposed to lead (Cooper & Gaffey, 1975; Malcolm & Barnett, 1982; McMichael & Johnson, 1982; Selevan, et al., 1985). Elevated blood pressure is associated with lead absorption (Victory, et al., 1982). Chronic exposure to lead has reproductive effects as well; lower sperm counts in males and increased rates of spontaneous abortion both in female workers and in the wives of occupationally-exposed workers of have been documented (Hamilton & Hardy, 1974; Lancranjan, et al., 1975).

Blood and soft tissue contain only a very small part of the lead that is absorbed by the body. Ninety to ninety five percent of the absorbed lead will become deposited in bone, taking the place of calcium and becoming incorporated into the hydroxyapatite crystal that comprises the mineral component of bone (Spadaro & Becker, 1970; Barry, 1975). The lead deposited in bone can reside there for many years. Lead has been shown to have a half-life in bone between 2-27 years; variations occur as a result of metabolism, age and type of bone in which the lead is deposited (Hyrhorczuk, et al., 1985; Rabinowitz, 1991; O'Flaherty, 1993; Schutz, et al., 1987). The turnover rate for cortical bone is only about 1% per year, and trabecular bone turns over at a rate of about 8% per year; once lead has been deposited in cortical bone, it resides there much for a much longer period (Gulson & Gillings, 1997).

Wittmers et al. (1988) examined the distribution of lead in bone by analyzing specific regions of the tibiae, vertebrae, ribs, ilia and skulls of 134 individuals ranging in age from 0-98yrs. Study subjects were all Caucasians: 81 were male and 53 were female (Wittmers, et al., 1988). The overall purpose of the study was to determine if sample site had an impact on the lead quantity found (Wittmers, et al., 1988). Each of the sites tested, except for the skull, showed increasing lead content with age up until the age of 75yrs (Wittmers, et al., 1988). Bones containing more trabecular bone (ribs, vertebrae and ilia) began to show decreases at this

age, and the degree of loss seemed to correlate with the amount of trabecular bone (Wittmers, et al., 1988). The skull had slightly higher lead levels in youths between 14 and 20yrs than in the next age bracket of 21-35yrs and the researchers surmised that this was due to the diploic nature of the skull (Wittmers, et al., 1988). The researchers found no statistically significant differences along the diaphysis of the tibia (Wittmers, et al., 1988). They did not detect differences between the right and left diaphyses following comparison of 12 pairs of tibiae (Wittmers, et al., 1988). Differences in lead distribution resulting from load bearing were analyzed by comparison of the lead content of two lumbar vertebrae and a thoracic vertebra with no significant difference detected (Wittmers, et al., 1988). A comparison of the lead content of trabecular bone and cortical bone in the metaphysis and diaphysis of the tibiae of 31 males and 17 females revealed that while there was no significant difference in the lead content of the cortical bone from both regions, the trabecular bone had a higher lead level than both cortical areas (Wittmers, et al., 1988). Wittmers et al. (1988) then evaluated each of the five sampled bones' ability to predict total skeletal lead content and determined that the tibia produced the most accurate results and suggested that other bones that were predominantly comprised of cortical bone would produce similar results as well. Trabecular bone appeared to be significantly affected by developmental stage, and potentially by disease states such as osteoporosis as well, and would be less likely to produce an accurate reflection of total skeletal lead burden (Wittmers, et al., 1988). Considering the fragmentary or incomplete nature of skeletal remains in archaeological contexts, this study's findings are very significant. Variations in skeletal element tested could lead to incorrect assumptions and conclusions. Testing cortical bone provides the most accurate picture of long-term exposure to lead.

Once the lead has been deposited in bone, it can be mobilized back into the blood. Bone is not a permanent repository; toxic effects can be experienced long after exposure has ceased (Schutz, et al., 1987). Stable isotope studies have demonstrated that 40-70% of the lead found in blood can be from mobilized bone lead (Gulson, et al., 1995; Smith, et al., 1996). Other changes can also induce lead's departure from bone. Joint replacements and pregnancy have been shown to further increase the release of bone lead into blood (Smith, et al., 1996; Gulson, et al., 1997). Since bone lead can be reintroduced into circulation and cause further damage to the nervous system and kidneys, it is indicative of a disease state which has the potential to inflict serious detrimental effects upon the individuals afflicted with lead poisoning. When lead is detected in archaeological remains, it can suggest that the health of that population may have been compromised. The effects of lead poisoning may not have been understood during the time period that the individuals lived. Even though Colonial era doctors noticed the similarity of the naval personnel's symptoms to lead poisoning, the diagnosis of lead poisoning was not made in that time period (Trapham, 1679; Turnbull, 1806). Lead poisoning, depending on its severity, could have even influenced their way of life as a result of its impacts on the population's health.

## **Chapter 5 - Analysis of Lead in Archaeological Bone**

### **5.1 Introduction**

With the understanding that lead becomes deposited in bone and that those lead levels could, to some degree, indicate the health status of a population, investigators became interested in analyzing skeletal bone lead levels in archaeological bone samples as well as in modern bone samples. The efforts to study lead levels in past populations were also used to empirically establish the baseline lead level in bone through time. These studies were important contributions to the scientific community's understanding of lead in the environment and its potential impact on health.

This chapter will chronologically examine some of the first forays into the analysis of lead in archaeological bone in order to establish the development of methodologies and accumulation of knowledge regarding lead in bone analysis. As well, the difficulties encountered and insights gained during investigations of archaeological materials will also be reviewed. Many studies involved establishing baseline lead levels and many others compared ancient and modern samples in order to determine the degree of modern lead burdens. A variety of methods were employed during initial investigations, including atomic absorption spectroscopy (AAS), thermal ionization mass spectrometry (TIMS), arc emission spectroscopy (AES), isotope dilution mass spectrometry (IDMS), anodic stripping voltammetry, dithizone colorimetry, inductively coupled plasma atomic emission spectroscopy (ICP-AES) and inductively coupled optical emission spectroscopy (ICP-OES). The wide range of techniques was largely due to both accessibility to technologies and the experimental nature of these analyses.

Archaeological bone investigations present unique challenges. Sample sizes, the skeletal elements used, and their state of preservation can affect outcomes and limit inter-study comparisons. Result comparability issues can arise due to the employment of different analytical techniques particularly if different units of measurement are involved. Some common units are equivalent to one another, including parts per million (ppm) and microgram per gram ( $\mu\text{g/g}$ ), but if ratios or other units are used, it becomes more difficult to compare values. One of the major concerns for archaeological sample investigators, however, is diagenesis, alterations to the biological material resulting from chemical exchanges within the depositional site, which can cause the transfer of lead from the environment into the bone. As the understanding of lead in the environment grew, it became apparent that lead from the depositional environment was mobile under certain conditions. Methodologies which attempted to account for this possibility were developed as a result. New technologies and techniques that were more capable of differentiating diagenetic and biogenic lead eventually arose, such as inductively coupled plasma mass spectrometry (ICP-MS) coupled with X-ray fluorescence (XRF). A review of original investigations of lead levels in archaeological bone using ICP-MS and XRF can be found in chapters 6 and 7 of this thesis.

## **5.2 Investigations of Lead in Archaeological Bone**

The results of Jarcho's (1964) study highlighted some of the difficulties experienced when analyzing archaeological remains: small sample size, difficulty in acquiring comparable samples and incomplete contextual information. This was one of the first studies of lead in archaeological remains. Prehistoric remains of individuals from areas known to use lead-glazed pottery were examined to determine if they would show evidence that the individuals had suffered from lead poisoning (Jarcho, 1964). Two archaeological sites in Arizona provided the



remains: forty six bone fragments from Kinishba, where lead-glazed pottery was produced and used, and 33 fragments from Point of Pines, where lead-glazed pottery was used but not produced, were examined for lead content (Jarcho, 1964). Initial examination was done by XRF in order to choose samples that would be likely to have detectable levels of lead for AAS (Jarcho, 1964). Eleven samples were chosen for AAS and the results were examined (Jarcho, 1964). To the researcher's surprise, higher levels were observed in the Point of Pines samples as compared to the Kinishba samples (Jarcho, 1964). The investigator acknowledged that the occupations of the Kinishba individuals were not known, and it was possible that the individuals tested did not have occupational exposure (Jarcho, 1964). He also recognized that the use of multiple skeletal elements may have further confounded the results, as it was already known that different bones accumulated lead at different levels (Jarcho, 1964). The levels found in both sets were not indicative of individuals suffering from plumbism, lead poisoning, according to data published at that point in time; they were all less than 18ppm lead (Jarcho, 1964).

In an effort to determine natural trace element composition, Becker et al. (1968) examined both modern and archaeological bones. The archaeological samples included five long bones (either tibia or femur) from remains excavated from the Peruvian desert and one femur from an aboriginal burial ground in Pennsylvania; the samples were estimated to be between 500 and 600 years old (Becker et al., 1968). Five long bone samples were chosen for the modern group as well, and all samples were ashed prior to analysis (Becker et al., 1968). Arc emission spectroscopy was chosen as the method of analysis (Becker et al., 1968). Low levels of lead were found in the ancient bones: the Peruvian samples had between 1 and 5ppm, and no lead was found in the North American aboriginal sample (Becker et al., 1968). Comparatively, between 5 and 110ppm lead was found in the modern samples (Becker et al., 1968). Due to the variation of

lead levels, the authors hypothesized that lead was not an essential trace element in bone, that it was likely a contaminant, and as such cautioned that the lead fumes from modern gas combustion were a likely source (Becker et al., 1968). Studies of this nature were (and are) important in establishing element levels in modern and archaeological remains, giving a frame of reference which can be addressed by further research.

Jaworowski (1968) also explored the increase of lead concentrations with time in the environment and in human bones. The study was one of the first attempts at empirically determining the so-called “physiological zero”. It had been previously calculated theoretically by Patterson to be  $0.85\mu\text{g/g}$  (Patterson, 1965). Finding the zero point was a necessary step in being able to determine if individuals would have experienced lead effects in their lifetimes, indeed to even have an understanding of what “normal” might be. Jaworowski (1968) examined glacier ice from two locations and found that mean ice lead levels had increased from about  $5.0\mu\text{g/L}$  in the 1860s to  $75\mu\text{g/L}$  in contemporary samples. Bones from 45 sets of Polish remains with origins ranging from 1700YBP to contemporary times were examined for lead using dithizone colorimetry (Jaworowski, 1968). Very low lead levels were found in the 3<sup>rd</sup> century CE bones and contemporary bones, whereas the 11<sup>th</sup>-18<sup>th</sup> century CE bones were relatively high in lead (Jaworowski, 1968). Jaworowski (1968) hypothesized that the low contemporary levels indicated that atmospheric contamination was not the most significant source of lead in the Polish population, and that items contributing to dietary lead such as tin utensils may have been more significant in the past. Additionally, because the peoples whom inhabited the region now known as Poland in the 3<sup>rd</sup> century CE were not known to have had contact with items containing lead, he surmised that the levels found in their remains indicated the natural lead level in the human body (Jaworowski, 1968).

Mackie et al. (1975) strove to correlate historical knowledge of the Roman use of lead with data regarding the lead levels found in 77 rib bones recovered from a Romano-British cemetery in York. The samples were analyzed by AAS, and no trend was found with regards to sex or age (Mackie et al., 1975). The authors acknowledged that the soil was unavailable for testing, but noted that the results of one child interred in a stone coffin had results not dissimilar to the mean; the child's lead level was  $45.1\mu\text{g/g}$  and the overall mean for all ages and sexes was  $63.5\pm 31.6\mu\text{g/g}$  (Mackie et al., 1975). Due to the consistency of this result, the investigators hypothesized that the soil had had little impact on their results (Mackie et al., 1975). This analysis of lead in bone demonstrated a few of the issues experienced when analyzing archaeological remains, such as small sample size and loss of contextual material which could provide supportive data (e.g. grave soil).

Grandjean (1975) published an extensive study regarding lead in which he examined the usage and levels of lead in Danish people through history to modern times. The investigator analyzed 126 modern and 109 archaeological vertebrae samples (dating from the Stone Age to the 1940s) for their levels of lead using AAS. He found that lead concentration roughly increased over time, with increased exposure to lead-containing sources, up until contemporary times when the levels fell (Grandjean, 1975). He correlated lead values with likely practices of the time, including the use of pewter dishes (Grandjean, 1975). Interestingly, Grandjean posited that low levels in older bones may have been the result of lead leaching out of the bones and into the surrounding soil (1975). He believed that the decrease in lead levels from the 1940s to the 1970s was as a result of the 1940s samples having become contaminated in a maceration process and that their levels were not in fact representative of their true value (Grandjean, 1975).

Grandjean was unable to determine age or sex-related patterns of lead content in the

archaeological samples as these characteristics were not able to be determined for many of these samples (Grandjean, 1975). While this study was broad in its scope, it still suffered the constraints experienced in other investigations of archaeological materials such as small sample size and incomplete historical data, and in the case of the 1940s remains, added issues due to sample processing that may have introduced contamination. Overall patterns are still, however, apparent and can provide a basis for further research.

Waldron et al. (1976) began investigating the lead levels in bones in the UK dating to the Roman era. The authors wished to add further metric data from the archaeological remains to support the historical information surrounding the extensive use of lead by the Roman people. Two hundred and two rib samples, 161 from Poundbury in Dorset and 41 from Henley Wood in Somerset, were analyzed (Waldron et al., 1976). Elevated lead levels were found in both cemeteries; means of 112.7 and 65.7 $\mu\text{g/g}$  were found for Poundbury and Henley Wood, respectively (Waldron et al., 1976). The mean level of lead in males in Poundbury was greater than that in females and the difference was statistically significant (Waldron et al., 1976). A general increase of lead level with age was also found in the Poundbury samples (Waldron et al., 1976). The researchers concluded that the lead would not have come from the soil because conditions required for lead mobility, for instance, acidity, would not be suitable for the observed bone preservation (Waldron et al., 1976). Comparisons of the bone lead levels found in this study were made with studies of modern-day bones, including Hislop and Parker (1971), Shroeder and Tipton (1968), Lanzola et al. (1973) and Barry (1975). Using the values found in the modern studies, the investigators hypothesized that several of the individuals, children in particular, were likely suffering from plumbism (Waldron et al., 1976).

Considering the potential impact of diagenesis on analytical outcomes, the report made by Waldron et al. (1979) is of significant importance to archaeological investigations regarding lead, as it indicates that certain environments are not conducive to lead mobilization in soil. Two soil samples were taken from a total of 41 graves from the Romano-British cemetery at Poundbury (Waldron et al., 1979). Each of the soil samples, along with a rib sample from each grave was analyzed for lead by AAS (Waldron et al., 1979). No correlation was found in the collected data, and the authors concluded that, at least in the case of this particular environment, the soil's lead had had no effect on the lead content of the bones analyzed (Waldron et al., 1979).

Like several other contemporaneous studies, the data from archaeological materials and remains in Ericson et al.'s (1979) study were needed in order to establish baseline levels in humans, so as to understand the degree to which lead pollution was affecting the modern environment. Hoping to minimize controversy associated with contamination issues, Ericson et al. (1979) analyzed the lead in bone and teeth from the remains of ancient Peruvians. The researchers posited that all remains in the Old World were subject to contamination due to the extensive lead processing that occurred there from 4500YBP until the present (Ericson et al., 1979). The investigators planned to compare the values obtained from pre-metalworking ancient Peruvians to individuals who lived and died after copper cupellation had begun on the South American continent, as well as compare the ancient values to modern American and British values (Ericson et al., 1979). Lead was analyzed in the various skeletal elements of seven individuals: one pre- and one proto-metalworking individual, four individuals from the metallurgical age and one ancient Egyptian (Ericson et al., 1979). The tooth enamel of four of these individuals, the pre- and proto-metalworking individuals as well as the two of the metallurgical age individuals, were also analyzed for lead (Ericson et al., 1979). Analyses were

done using IDMS and TIMS (Ericson et al., 1979). The ratios of Pb/Ca in bone and teeth samples were compared against the ratios of Pb/Ba (Ericson et al., 1979). The investigators believed that ratios of Ba/Ca in teeth and bone were generally similar to one another across many geographical areas and therefore if the ratio of Ba to Ca increased, diagenesis was indicated (Ericson et al., 1979). The pre- and proto-metalworking samples and one of the metallurgical age samples were thus eliminated as their bone values and ratios suggested contamination by soil (Ericson et al., 1979). The vertebral sample from the ancient Egyptian mummy was also dismissed because it had a high Pb/Ca ratio; the authors believed it was likely contaminated due to extensive smelting and mining in the Mediterranean regions (Ericson et al., 1979). Ericson et al. found that the ratio of Pb/Ca in ancient Peruvian bones was about  $6.0 \times 10^{-8}$ , as compared to modern American levels at  $3500 \times 10^{-8}$  and modern British at  $2100 \times 10^{-8}$  determined in previously published studies on modern American and British lead levels, and following corrections for age and methodology (Ericson et al., 1979). The investigators cautioned that considering the theoretical ratio of lead to calcium had been calculated to be  $2 \times 10^{-8}$ , and that their ancient population of low-exposure Peruvian bones had a Pb/Ca ratio of  $6.0 \times 10^{-8}$ , the levels seen in modern populations should be addressed (Ericson et al., 1979).

In another effort to demonstrate the increasing lead burden on modern humans, Grandjean et al. (1979) analyzed the lead levels in the bones and teeth of 105 ancient Nubians (5300 – 1250YBP) and compared them against the bone and teeth lead levels of 17 individuals from contemporary Denmark, as well as tooth lead from six individuals from the contemporary US (Grandjean et al., 1979). Temporal bone samples were analyzed using flameless AAS and teeth were analyzed using anodic stripping voltametry (Grandjean et al., 1979). Their studies revealed that the ancient populations' bones and teeth had much lower levels of lead overall than

the contemporary populations (Grandjean et al., 1979). The median Ancient Nubian level of lead in bone ranged from 0.6 to 2.0 $\mu\text{g/g}$ , depending on the time period that the population lived, and the median lead found in the dentine ranged from 0.9 to 5.0 $\mu\text{g/g}$  (Grandjean et al., 1979). The contemporary population from Denmark had a median bone lead level of 5.5 $\mu\text{g/g}$ , but a median dentine level of 25.75 $\mu\text{g/g}$ , and the dentine of contemporary American individuals had a median level of 175.05 $\mu\text{g/g}$  (Grandjean et al., 1979). While the differences in bone lead levels were marginal, but still statistically significant, the differences in tooth lead levels were quite extreme, suggesting that childhood exposure levels in modern populations are much greater than those for ancient populations. It also provides support for the suggestion that higher lead levels in tissue have resulted from changing cultural practices, as opposed to being the result of natural occurrences. Ideally however, comparisons should involve ancient and contemporary populations from the same geographical regions in order to better factor in the latter possibility.

Barry and Connolly (1981) presented findings of lead in mediaeval-era bones from a monastery cemetery that demonstrated post mortem alteration of lead levels in bones. Remains from 28 individuals were tested, and the minimum level of lead found was 360ppm (Barry & Connolly, 1981). It was determined that the high content of lead in the soil, which ranged from 100-13,000ppm, in combination with the known, repeated flooding of the cemetery area had likely created the elevated levels found analytically (Barry & Connolly, 1981). The repeated flooding increased the likelihood that the lead would be mobile in the soil. The authors cautioned that data of this nature should be carefully examined prior to forming conclusions (Barry & Connolly, 1981). This investigation is a prime example of why there is a need to be able to determine levels of biogenic lead. While it is known that the monks of this monastery

used many lead-containing products, no inferences can be made regarding their exposure or state of health due to the masking by diagenetic lead.

Following a study into Romano-British remains found in the UK, Waldron decided to further investigate post-mortem chemical alteration of bones (Mackie et al., 1975; Waldron et al., 1976; Waldron, 1981). The study by Waldron (1981) helped to illuminate the additional techniques that can be employed to determine the origin of lead found in archaeological human remains. The remains of four individuals who had been interred in lead-lined coffins were examined: three had been buried in Poundbury, Dorsetshire and the other in Southwark, London (Waldron, 1981). Rib samples were analyzed for lead levels using AAS; additionally, lead distribution was analyzed by the electron microprobe analysis of a section of a metacarpal from the Southwark remains (Waldron, 1981). The Southwark sample was extremely high in lead: 10,228 $\mu\text{g/g}$  (Waldron, 1981). Even remains contained in a wooden coffin from the same cemetery, analyzed as a control, were very high at 1,165 $\mu\text{g/g}$ . The soil in the Southwark cemetery had lead levels ranging from 100 to 800 $\mu\text{g/g}$  (Waldron, 1981). The Poundbury rib samples had lower lead levels than the Southwark sample: 2820, 276 and 296 $\mu\text{g/g}$  (Waldron, 1981). Two hundred twenty other rib samples from this site had been analyzed previously to have a mean lead value of 108 $\mu\text{g/g}$  (Waldron, 1981). The authors noted that one of the sets of remains from the group of 220 which had shown a high level of lead had been interred close to a lead-lined coffin, and hypothesized that the soil in the area could have been contaminated by the coffin (Waldron, 1981). The soil lead levels at Poundbury were otherwise relatively low at 20 $\mu\text{g/g}$  (Waldron, 1981). The electron microprobe analysis of the medial surface of the first left metacarpal from the Southwark skeleton showed that the lead was concentrated at exposed surfaces. It was thus concluded that the lead detected in the remains tested had been diagenetic,



that the lead had been introduced as a result of the remains being interred in a lead coffin (Waldron, 1981). This investigation also indicates that low lead soil results alone cannot discount the possibility of diagenetic alteration.

In 1981, comparisons of lead level in bone were made between two distinct colonial era socioeconomic groups: plantation owners and the enslaved labourers of whom they claimed ownership (Aufderheide et al., 1981). Multiple skeletal elements from 16 individuals who had been interred at the plantation were analyzed for lead by graphite furnace AAS (Aufderheide et al., 1981). The mean levels of the landowner group and the labourer group were extremely different, 185 $\mu\text{g/g}$  and 35 $\mu\text{g/g}$ , respectively. The lead level range for the plantation owner's family was between 128 and 258 $\mu\text{g/g}$ , while the labourers' lead levels ranged from 8 to 96 $\mu\text{g/g}$  (Aufderheide et al., 1981). There was age correlation found in the labourer group, which suggested to the authors that their exposure was steady and moderate (Aufderheide, et al., 1981). In contrast, the lack of age correlation in the landowner group implied to the authors that high exposure began with the landowners' arrival at the plantation, as opposed to having steady high exposures throughout their lifetimes (Aufderheide et al., 1981). It was known that at this point in time, wealthy landowners used pewter-based tableware, serving ware and storage vessels quite extensively, and thus the researchers were able to connect the high lead levels of the landowners with contamination from their daily life (Aufderheide et al., 1981). It was also known that labourers had significantly less exposure; their contact with lead-containing items was minimal (Aufderheide et al., 1981). Aufderheide et al. (1981) recognized that the significant amount of historical and archaeological information available to them regarding this site was beneficial to them in regards to drawing conclusions from the data. This investigation demonstrated just how

much different daily activities could profoundly affect lead levels detected in archaeological materials and why contextual information provides significant insight in data interpretation.

Difficulties can be encountered when comparing remains from multiple depositional environments, or if samples that are being compared are not treated and analyzed by equivalent methods or if results are quoted in different units. Drasch (1982) set out to determine both the physiological zero level for lead and the levels of lead burden throughout Germany's history in his 1982 publication. Using data collected from the analysis of 26 ancient Peruvian bones, he found that the average amount of lead in the ancient Peruvian population was  $0.56\mu\text{g/g}$  (Drasch, 1982). Notably there was variation in Pb levels within this population when different regions of Peru were considered; samples from Mochica were considerably lower than those from other regions within Peru (Drasch, 1982). The investigator acknowledged that the difference may have been due to the use of the femur for analysis whereas samples from the other Peruvian regions had been taken from crania (Drasch, 1982). The author performed calculations to correct for the different skeletal elements, but corrected values increased the discrepancy and the author posited that there may have been cultural practices that differed in this region that may have led to the differences (Drasch, 1982). Diagenesis was not believed to have occurred due to the extremely arid conditions that the remains had experienced; many of the individuals were mummified (Drasch, 1982). Following the investigator's establishment of the physiological zero, the remains from Germany were tested. The oldest sets, dating from 3800 to 2500YBP, had a mean level that was higher than that of the ancient Peruvians,  $1.92\mu\text{g/g}$  as compared to  $0.56\mu\text{g/g}$  (Drasch, 1982). The researcher could not discount the possibility of post mortem contamination unfortunately, as 36 of the 40 individuals tested had been buried in a pit in a manner that would have allowed exposure to seepage and ritual objects that may have contained

lead (Drasch, 1982). Analysis of 121 individuals from the period of Roman occupation yielded a mean bone lead level of  $4.7\mu\text{g/g}$  (Drasch, 1982). Drasch believed this to be a biogenic value due to the known and documented use of lead objects during this period. He nonetheless acknowledged the possibility of diagenesis due to water seepage and items found in the graves (Drasch, 1982). He performed several tests that he believed would illustrate the presence of diagenesis, or the lack thereof (Drasch, 1982). These tests included comparison of lead levels by age, sex, and left and right skeletal elements (Drasch, 1982). It was concluded that since there was no difference between male and female levels, nor any difference between right and left femora, and that levels increased with age as expected, that no diagenesis had occurred (Drasch, 1982). Eighty eight individuals from the Middle Ages were the next group to be examined (Drasch, 1982). Values found supported a continuing, increasing use of lead-containing products, such as the Queen's tin and lead glazes on china and earthenware (Drasch, 1982). The final group that was analyzed was composed of 57 individuals from contemporary Germany (Drasch, 1982). The lead levels found were more than twice those found in Roman- and Middle Ages-era remains (Drasch, 1982). The investigator also found that individuals living in urban areas had higher lead levels than those living in rural areas, and cautioned that this likely indicated uptake as a result of leaded gas fume inhalation (Drasch, 1982). Unfortunately, the results were called into question later because the contemporary bones analyzed were not dried first (Jaworowski, et al., 1985). The samples' dry weights were determined by calculation of correction factors that were considered dubious (Jaworowski et al., 1985).

Jaworowski et al. (1985) undertook a large scale study in which they explored heavy metal levels, including lead, in 180 human bones spanning from the Neolithic to the modern era, as well as Pleistocene-era and contemporary animal bones (Jaworowski et al., 1985). They

found that the older specimens, i.e. the Neolithic human bones and the Pleistocene animal bones contained very low levels of lead, and they hypothesized that fossilization had replaced any detectable level of lead (Jaworowski et al., 1985). Human Bronze Age samples showed detectable levels of lead but the researchers could not state with certainty that these levels were not the result of diagenesis (Jaworowski et al., 1985). Ancient Roman samples had appreciable lead levels, as did those from the Merovingian period (1200-1500YBP), but the Middle Ages saw a very significant rise in lead levels (Jaworowski et al., 1985). Jaworowski et al. (1985) attributed these levels to the multiple sources of lead contamination that had become available to humans at that time, including smelting sites, servingware, food storage vessels, water pipes, paints and cosmetics. Modern human samples had modest lead levels by comparison, at least an order of magnitude lower than those from the Middle Ages, indicating the absence of many of the previous sources of contamination (Jaworowski et al., 1985). Contemporary animals from this study showed no detectable lead, but previous animal studies conducted by the authors had shown that contemporary animals have lead levels comparable to the contemporary human levels found in this study, possibly indicating a general level of environmental contamination (Jaworowski et al., 1985). The investigators hypothesized that the additional of calcium supplementation into this study's contemporary animals' feed may have reduced their absorption of lead (Jaworowski et al., 1985). Ultimately, using data collected in this and other lead level studies, the researchers concluded that establishing baseline lead levels for humankind was very difficult due to global variation in natural geochemical levels (Jaworowski et al., 1985). This study highlighted the requirement for consideration of geography when undertaking studies of archaeological remains, as differing practices and natural resources can significantly impact the lead levels found in human bones.

A study performed by Aufderheide et al. (1985) suggested that, during the Colonial Period, social and economic status played an important role in an individual's exposure to lead in their lifetime (Aufderheide, et al., 1985). Remains from four different sites were examined: Catoctin Furnace, an iron working facility in Maryland, College Landing, a commercial area and supply point in Virginia, several Governor's Land sites in Virginia near Jamestown, and Irene Mound, a plantation site close to Savannah, Georgia (Aufderheide, et al., 1985). Approximately 60 individuals were analyzed for bone lead by graphite furnace AAS, half of them were of African ancestry and the other half of European ancestry (Aufderheide, et al., 1985). They found that social status appeared to correlate more strongly with bone lead levels than did the occupation of the individual (Aufderheide, et al., 1985). European tenant farmers had higher levels than enslaved labourers of African ancestry, but not as high as the European landowners (Aufderheide, et al., 1985). During the Colonial Period, most lead exposure occurred in the home, and much of it was due to pewter service-ware or lead-glazed ceramics for storing food (Aufderheide, et al., 1985). Wealthier individuals had more of these types of items, while enslaved labourers or domestic servants often ate from wooden tableware instead (Aufderheide, et al., 1985). Quite interestingly, the females from Catoctin Furnace had a higher mean lead level than did the males, 40.3ppm in females as compared to 14.1ppm in males, suggesting that they may have been exposed to more lead by being in European families' homes, perhaps as enslaved domestic servants (Aufderheide, et al., 1985). The individuals from College Landing, believed to be free people of African descent, had mean lead levels consistent with that of the women from Catoctin Furnace (Aufderheide, et al., 1985). This suggests some exposure to lead, but the exact source was not determined. Skeletal markers on individuals from College Landing indicated a more artisan lifestyle as compared to the Catoctin Furnace workers (Aufderheide, et

al., 1985). It was noted that more urban environments such as College Landing had more possible sources of lead than did plantation or rural environments, including various types of alcohol (Aufderheide, et al., 1985). All the individuals from the Governor's Land sites were of European descent (Aufderheide, et al., 1985). Burial methods were used to aid in determination of social status where possible; individuals found shrouded and in coffins were believed to be of higher social and economic status (Aufderheide, et al., 1985). These individuals generally had higher bone lead content than those believed to be enslaved labourers or domestic servants (Aufderheide, et al., 1985). One female that was believed to be a domestic servant had high bone lead, however (Aufderheide, et al., 1985). Her bone lead level, 264.8ppm, suggested regular, high exposure to lead and the investigators hypothesized that she may have been the cook which would have meant constant exposure to the lead-containing items (Aufderheide, et al., 1985). The researchers also commented on soil Pb's relationship to bone Pb. Catoctin Furnace soil samples were very high in lead, but the bone levels were quite low comparatively (Aufderheide, et al., 1985). Aufderheide et al. (1985) believed that while they could not rule out diagenesis as a contributing factor, it had not had a large impact on the lead levels that they found in the bones.

Kosugi et al. (1986) examined the elemental composition of ancient Japanese bones from fifty sites spanning five different eras using ICP-AES and AAS. The highest level, 12.2µg/g ashed bone was found in the most recent, or Edo, era, which was more than six times the amount found in remains from any other era (Kosugi, et al., 1986). The authors acknowledged that location, and thus diagenesis, may have influenced these results as the Edo remains were collected from cemeteries that are now in Tokyo, a very large city in Japan (Kosugi et al., 1986). Kosugi et al. (1986) expressed the need to analyze soil from these areas in order to validate their

results. The Edo era was known to have higher exposure to lead than previous eras however, due to the use of lead-based cosmetics, lead ammunition for guns, and an increased demand for silver, resulting in lead smelting works that were present on the land that is now occupied by Tokyo (Kosugi, et al., 1986). Additionally the investigators noted that other elements known to be soil-borne did not accumulate in the bone to the same degree as the lead, giving further support to its biogenic presence via consumption, inhalation and absorption (Kosugi, et al., 1986). This study outlined the benefits of having significant environmental data as well as historical knowledge to supplement elemental analysis of archaeological remains.

In order to expand on historical information regarding the lives of enslaved labourers in Barbados during the Colonial period, Corruccini et al. (1987) examined lead levels found in remains from the Newton Plantation cemetery. Cortical bone from 48 individuals was tested and all samples were analyzed by graphite furnace AAS (Corruccini et al., 1987). The mean lead content of the individuals was high,  $117.6\mu\text{g/g}$  with a high standard deviation of  $94.9\mu\text{g/g}$  with a range from 0 to  $424\mu\text{g/g}$  (Corruccini et al., 1987). Analysis of the data indicated that there was no significant difference as a result of sample site or sex, although a high degree of variation was noted in the female set (Corruccini et al., 1987). This was posited to be due to differences in the females' duties (Corruccini et al., 1987). Upon noting that 81% of the individuals above 30 years of age had  $100\mu\text{g/g}$  or more of lead, the researchers analyzed this data further and found that the lead level generally increased with age (Corruccini et al., 1987). Correlations were also made that demonstrated that individuals who were brought to Barbados from Africa as adults had much lower levels of lead overall; these individuals were identified through dental mutilation and burial orientation (Corruccini et al., 1987). This suggests that the lead contained within the remains resulted from exposures that occurred on Barbadian soil (Corruccini et al., 1987). This

study clearly demonstrated that lead levels experienced by enslaved labourers were highly variable and likely dependent on the tasks required of them and their place of birth, especially when the results are compared with those of Aufderheide et al. (1985) which also looked at lead levels of enslaved labourers of African ancestry. Corruccini et al.'s 1987 study also highlights that exposure to lead is not necessarily consistent within the same site, as all the remains tested came from individuals who laboured at the Newton Plantation.

Hisanaga et al. (1988) examined lead levels in ancient and contemporary Japanese bones using AAS in order to evaluate lead burden through Japan's history. The sites chosen were in southern Japan; in total 41 ancient rib samples and 11 contemporary ribs were analyzed, 25 of them from males and 16 from females all aged between 40 and 60 years of age (Hisanaga et al., 1988). Similarly to Kosugi et al. (1986), the researchers found that the Edo era bones showed elevated lead as compared to the preceding eras, with levels at least 3.5X greater (Hisanaga, et al., 1988). Modern bones showed elevated lead levels in comparison to samples from all other eras except the Edo. The bones from contemporary Japan had a mean Pb of 4.47 $\mu$ g/g dry bone where the Edo era bones had a mean of 7.42 $\mu$ g/g dry bone (Hisanaga et al., 1988). They also found that in the Edo era, the females had a higher molar ratio of Pb, and suggested that the significant use of lead-based cosmetics in this period may have been the cause of this difference (Hisanaga et al., 1988). While this may be the case, other factors may also have contributed. Female metabolism is significantly altered during pregnancy as demonstrated by May et al. (1999) when they compared lead levels of pregnant and non-pregnant swine placed on the same diets. The pregnant swine consistently showed higher bone lead levels, indicating that exposure was not the only determining factor (May et al., 1999). Without knowing the child-bearing history of the women whose remains were studied, these impacts cannot be appropriately



evaluated. Given that in archaeological studies this information may not be available, studies that involve comparisons by sex may have bias that cannot be mitigated.

An investigation into the fate of the members of the Franklin expedition was made by Kowal et al. in 1989. The trace element analysis of one crewman's bone by Beattie in 1985 had revealed unexpectedly high levels of lead -  $125\mu\text{g/g}$  (Kowal, et al., 1989). This high level indicated that lead poisoning may have been a possible contributing factor to the demise of the members of the expedition. Using graphite furnace AAS, Kowal et al. (1989) analyzed 29 bones from members found in two different locations, Beechey Island and King William Island. Additionally, they analyzed bones from King William Island Inuit from the same time period and caribou bones that had been found commingled with the remains of the expedition members (Kowal, et al., 1989). The results indicated that expedition members had elevated bone lead levels (Kowal, et al., 1989). The Beechey Island remains had levels ranging from  $69\text{-}183\mu\text{g/g}$  Pb and the King William Island remains had Pb levels ranging from  $87\text{-}223\mu\text{g/g}$  (Kowal, et al., 1989). Importantly, the Inuit bones from the same area and time period had Pb levels ranging from  $1\text{-}14\mu\text{g/g}$  and the 2 caribou bones had a mean Pb level of  $2.0\mu\text{g/g}$  (Kowal, et al., 1989). The Inuit bone levels, and particularly the caribou bone results, due to the commingling, suggest that diagenesis would not likely be a contributing factor to the Pb found in the Franklin expedition members. Since the Pb levels found were high; it is possible that lead contributed to the deterioration and death of the members of the Franklin expedition, although significant questions remain regarding the source of the lead found in the bones (Martin, et al., 2013). Investigations of this manner demonstrate the ability of analytical techniques to contribute information to the causes and outcomes of historical events.

Further efforts to determine the physiological zero point of lead were performed in 1991 through comparisons of lead levels in tooth enamel, rib and femur samples from pre-contact aboriginal peoples in the American Southwest (Patterson et al. 1991). They posited that the levels found in remains of peoples in Europe may have been subject to contamination due to the amount of lead smelting and lead goods produced in the area, whereas those found in the American Southwest pre-contact populations would be unlikely to be contaminated as there were no known sources of lead contamination available at the time (Patterson et al., 1991). Following the analysis by TIMS of 23 individuals, 10 from the Canalina-Chumash tribe and 13 from the Kayenta-Anasazi Pueblo tribe, their calculations determined that the natural level of lead was  $40\mu\text{g}/70\text{kg}$  adult, or 0.57ppb (Patterson et al., 1991). The authors believed that due to the variation in levels seen between the tooth, rib and femur analyses, and the chemical behaviours of lead and apatite, diagenesis had occurred in the remains they examined, although they could not pinpoint a source when the lead isotopes were tested, nor did they consider the known distribution of lead in the body based on tissue type as shown in Wittmers et al.'s 1988 publication (Patterson et al., 1991; Wittmers et al., 1988). When examining archaeological remains, many factors must be incorporated into any conclusions that are drawn, otherwise, contributing influences may be overlooked.

Ericson et al. (1991) also attempted to find basal metal levels in humans, and met with results very similar to other studies in this area. Comparison with modern day levels indicated that modern humans were being exposed to much more environmental contamination likely due to increased anthropogenic production of lead (Ericson et al., 1991). The researchers additionally wanted to examine if there were hemispheric differences in lead uptake by comparing their results to a previous study involving ancient Peruvians (Ericson et al., 1991;

Ericson et al., 1979). Differing bones from the skull were chosen from 14 individuals of both sexes and varying ages from Pecos Pueblo in what is now New Mexico (1300 and 1823 ACE) (Ericson et al., 1991). Both inner and near-surface (outer) bone fragments samples were chosen for analysis by graphite furnace AAS; the outer bone fragments samples included both cancellous and cortical bone whereas the inner fragments samples were comprised only of cortical bone (Ericson et al., 1991). The outer samples were included to provide an indication of the degree of diagenesis. Interestingly, the authors acknowledged that the differing levels of metals that were found in the inner and outer bone fragments may have arisen due to metabolic differences, but they believed that the metal level variation, including lead, was due to diagenetic contamination (Ericson et al., 1991). The inner sample values were chosen as preferable for establishing basal levels, but, for lead, 5 of the 14 samples were eliminated because they were believed to be contaminated as well (Ericson et al., 1991). They reported a mean basal lead level of  $0.65 \pm 0.32 \mu\text{g/g}$ , and added that it may in fact be high as they were unsure that the samples used for the establishment were free of contamination, as no patterns related to age or sex could be found (Ericson et al., 1991). They found this mean value to be similar to the lead values found in ancient Peruvian bones in their 1979 study, which ranged from 0.11 to 2.7ppm (Ericson, et al., 1979; Ericson, et al., 1991).

A novel area of investigation was approached when Keenleyside et al. (1996) used X-ray excitation from a  $^{109}\text{Cd}$  source to detect lead in bones recovered from a site on King William Island. These remains had been attributed to the lost members of the Franklin Expedition (Keenleyside et al., 1996). The investigators selected 52 of the bones, which included an assortment of skeletal elements: pelvic bones, skull bones, long bones, vertebrae and calcanei (Keenleyside et al., 1996). Using previously established correlations and the known similarity

between right and left skeletal bone pairs, the researchers measured the lead concentrations in the bones and then used these concentrations to identify the number of individuals that were represented in the set of 52 bones that they had selected (Keenleyside et al., 1996). Lead concentrations in these bones were elevated. The lowest value, 61.8ppm was found in a tibia and the highest value, 1739.8ppm was found in a vertebra (Keenleyside, et al., 1996). Based upon differences between pelvic bones and vertebrae, they were able to identify 11 individuals within their sample set (Keenleyside et al., 1996). This was an interesting development, as the site was initially believed to have had eight individuals present, based on the retrieval of 8 mandibles (Keenleyside et al., 1996). This study shows that lead detection may be useful for assignation of individuality. Considering that archaeological sites often have commingled remains, this publication demonstrated that lead concentrations could prove to be a useful tool for identifying the number of individuals present.

Arnay-de-la-Rosa et al. (1998) compared the lead levels of 15 ancient, 14 historical (18<sup>th</sup> century) and 25 modern individuals from the Canary Islands. Using AAS, they found that the modern population's lead level was significantly higher than those of the historic and ancient samples. It was noted that the ancient and historical populations were a mix of males and females whereas the modern population selection contained only males, and that tibial samples were used in all cases but six of the ancient individuals, for whom pelvic bones were used instead (Arnay-de-la-Rosa, et al., 1998). Soil testing from the burial sites of the historical and ancient populations indicated moderate levels of lead: 19.7-21.3ppm for the historical site and 5.1-14.2ppm for the ancient sites (Arnay-de-la-Rosa, et al., 1998). The authors believed that the lead in the soil arose from environmental pollution created by the significant lead smelting performed by the Roman Empire on the mainland which had begun 2000 years prior to the

establishment of populations on the Canary Islands. Diagenesis was not excluded as a possible contributor to the lead found in these two populations, but their values were still significantly lower than that of the modern population. The mean lead values in the bones were  $18.57 \pm 14.80$  ppm for the modern population,  $5.57 \pm 6.44$  ppm for the historic population and  $4.12 \pm 4.77$  ppm for the ancient population (Arnay-de-la-Rosa, et al., 1998). While the sample sets were small and the female sex underrepresented in the modern population, this publication added further to the considerable information regarding the increased lead burden of modern populations in comparison to populations whom had minimal contact and trade with lead utilizing peoples or with natural sources of lead.

The difference in lead levels between modern and ancient populations of the Canary Islands was further examined by Gonzales-Reimers and colleagues (1999). In this study, the investigators analyzed 19 modern male bone samples and 40 bone samples from pre-Hispanic Canarians ranging from approximately 875 to 1740 YBP, 33 of them from males and 7 from females. Bone lead analysis by AAS revealed that the mean lead levels of the two populations differed significantly (Gonzalez-Reimers, et al., 1999). The authors also found that lead levels between older and younger individuals of the modern era also differed significantly, with lead levels increasing with age, however, no such relationship was demonstrated within the pre-Hispanic population (Gonzalez-Reimers, et al., 1999). Diagenesis was not considered to be a significant factor, as the ancient peoples were not interred, rather they were laid to rest on stone or vegetal matter in caves (Gonzalez-Reimers, et al., 1999). The researchers compared the lead results of their ancient population with other studies of archaeological populations and found that their results were somewhat higher than what would be expected of an isolated population that did not have natural exposure to lead (Gonzalez-Reimers, et al., 1999). They noted that their

results may have been influenced by the nature of their method as they had not ashed their samples prior to analysis (Gonzalez-Reimers, et al., 1999). Gonzales-Reimers et al. (1999) also posited that trade winds passing the contemporaneous Roman smelting sites in Southern Spain may have introduced lead into this otherwise isolated population. The differences in analytical method are important to note as comparisons of data acquired by differing methodologies may not truly be possible. Different sample preparation techniques can yield vastly different outcomes on the same samples (Jaworowski et al., 1985). An isotopic study of the lead found may shed some light on possible sources of the lead in the ancient remains as well.

In their 2002 review on lead analysis' contribution to archaeology, Wittmers et al. published the findings from two new studies. The remains of 27 males from the War of 1812's Snake Hill battle site were analyzed (Lalich & Aufderheide, 1991). It was found that the overall mean lead content of their bones was  $31.3 \pm 24.3 \mu\text{g/g}$  and that the mean lead content of the bones increased with age, suggesting chronic low exposure (Wittmers et al., 2002). It was believed that due to the relatively low lead values, these individuals were likely of lower socioeconomic class (Wittmers et al., 2002). Two individuals, whose results were much higher, 82.9 and 113.1, were believed to have been either from a high socioeconomic class or that they had had significant occupational exposure (Wittmers et al., 2002).

In 2003, Gonzalez-Reimers et al. returned to studying lead levels in modern and ancient Canary Island populations, this time using AAS. The researchers compared lead levels between the two eras, but in this study, long bones of goats, sheep and pigs were analyzed along with human tibial epiphyses (Gonzalez-Reimers, et al., 2003). The lead levels found in modern Canarians were again significantly higher than those of the pre-Hispanic Canarians and the animal bone lead levels also showed an increase from ancient to modern eras, but their levels

were much lower than the human levels (Gonzalez-Reimers, et al., 2003). Interestingly, there appeared to be a similarity between the degree of difference seen between modern and ancient humans, and modern and ancient animals, which suggested to the authors that a similar source may be responsible (Gonzalez-Reimers, et al., 2003; Gonzalez-Reimers, et al., 2005).

Additionally, the age of the animals at death may also have had an impact on the lead levels observed as lead has been seen to increase with age, however, no data on the ancient animals' age was available to substantiate this hypothesis (Gonzalez-Reimers, et al., 2003).

Further work on lead levels in the Canary Islands was performed by Arnay-de-la-Rosa (2003) by comparing lead levels between 18<sup>th</sup> century inhabitants of two different islands, Tenerife and Gran Canaria, and then comparing those levels to that of modern Tenerife inhabitants. Tibial epiphyses were analyzed by AAS (Arnay-de-la-Rosa, et al., 2003). The modern population's lead levels were significantly higher than those of both of the 18<sup>th</sup> century populations, but the two 18<sup>th</sup> century island populations' lead levels did not significantly differ from one another (Arnay-de-la-Rosa, et al., 2003). This likely indicated similar practices and exposures from one island to another, as differing practices can have large impacts on lead levels even within the same geographical region (Aufderheide et al., 1981; Aufderheide et al., 1985; Arnay-de-la-Rosa, et al., 2003). The significantly higher bone lead levels of modern Canarians when compared with the bone lead levels of historic or pre-Hispanic Canarians has been consistent through several studies (Arnay-de-la-Rosa, et al., 1998; Gonzalez-Reimers, et al., 1999; Arnay-de-la-Rosa, et al., 2003; Gonzalez-Reimers, et al., 2003).

Lead levels between ancient populations from across the Canary Islands archipelago were compared by graphite furnace AAS (Gonzalez-Reimers, et al., 2005). The lead values of tibial epiphyses showed that there were significant differences between mean lead levels of the ancient

populations of the different islands (Gonzalez-Reimers, et al., 2005). El Hierro had the lowest level, and it differed significantly from all of the other islands. The authors hypothesized that the islands' distribution of lead levels supported the possibility that trade winds had brought lead contamination from human sources to the islands, as the ancient population levels found in the Canary Islands were higher than levels found in other remote ancient populations with no known natural lead sources. El Hierro is the island that is furthest from both Southern Spain and North Africa where lead smelting was known to occur during the first millennium BCE (Gonzalez-Reimers, et al., 2005).

The changes in lead levels through time in Cartagena, Spain were presented by Martinez-Garcia in 2005 (Martinez-Garcia, et al., 2005). Eighty samples from multiple skeletal elements of 38 individuals were obtained and analyzed using anodic stripping voltammetry or flame AAS (Martinez-Garcia, et al., 2005). The researchers found that bone lead levels were very low in the Neolithic, peaked between the Roman and Byzantine eras due to silver mining, and then fell through the following centuries until the present day where they are higher than the Neolithic but much lower than the Roman peak values.

Wadi Faynan, in present-day Jordan, was the location of extensive copper mining in the Byzantine era; lead was known to be a by-product of copper extraction from ores (Grattan, et al., 2005). Lead and copper levels of 25 individuals interred at the ancient site were analyzed by AAS, of which 11 samples were chosen for further examination; different parts of the skeleton, including various long bone regions, calcanei, vertebrae and ilia, were analyzed for levels to determine if skeletal partitioning occurred (Grattan, et al., 2005). No patterning of lead partitioning was observed; indeed further analysis of three points of the humerus indicated that dispersion was similar across the bone, supporting earlier work by Wittmers et al. (1988).



Copper, however, was found to be preferentially taken up in the humerus in nine of the eleven individuals tested, particularly in the medial epicondyle (Grattan, et al., 2005). The authors believed that this supported the possibility that since copper is an essential trace element its deposition may be more controlled, whereas since lead is non-essential its deposition would be more less selectively distributed (Grattan, et al., 2005).

The impact of the mining activities at Wadi Faynan was assessed by analyzing bone lead and copper of Bronze Age and Byzantine humans, ancient goats and sheep, and modern goats and sheep, using flame AAS (Pyatt et al., 2005). Associated sediments were also analyzed and found to have low levels of copper and lead (Pyatt et al., 2005). They found that the animal levels were still high, but had decreased through the passage of time, and that greater distance from the mining activities' waste heaps contributed to lower levels as well, as samples collected 12km from the mining sites showed the lowest levels (Pyatt et al., 2005). Bronze Age humans had lower levels than did the Byzantine humans, but the levels in the skeletons of both ages were high: 98 and 196ppm in the outer femora, respectively (Pyatt et al., 2005). The lead level of this Byzantine individual's femur is roughly four times that of the femora of the eleven individuals analyzed by Grattan et al. (2005). Pyatt et al. (2005) believed that these high femoral lead levels supported the known increase in mining activities of the Byzantine era. Diagenesis was not considered to be a major factor due to the comparatively low sediment levels and arid nature of the depositional environment (Pyatt et al., 2005). This archaeological investigation is an interesting demonstration of how profoundly past activities can affect modern conditions.

In 2005, Bower et al. examined lead isotopes in the remains of 15 individuals in an effort to trace the individuals' migrations (Bower et al., 2005). The remains were discovered on the grounds of a mental health institute in Pueblo, Colorado that had been in operation for over a

century (Bower et al., 2005). Research determined that the individuals had lived in the 19<sup>th</sup> century and had likely been buried between 1879 and 1899 ACE (Bower et al., 2005). The investigators hypothesized that by examining the lead isotope signature of different tissues - teeth, bone and bone callus - from healing fractures, they would be able to illuminate details of the individuals' migration (Bower et al., 2005). Ore smelting was known to have taken place in the Pueblo, Colorado area and the researchers hoped to connect these individuals with the associated lead isotopes (Bower et al., 2005). Additionally, they sampled burial soil from two locations at grave level to assess diagenetic influence (Bower et al., 2005). ICP-OES and TIMS analysis of the lead in the human and soil samples found that isotope ratios of the grave soil were not consistent with the isotope ratios found in the human remains; from this the investigators extrapolated that diagenesis had not been a significant contributor to the lead found in the human remains (Bower et al., 2005). Furthermore, they provided evidence that modern lead would have been unlikely to have contaminated the bones based on soil alkalinity, Pb mobility rates and radioactivity (Bower et al., 2005). Several individuals who had been morphologically classified as having African and Hispanic ancestries had isotopic signatures corresponding to their likely places of birth (Southeastern and Southwestern USA, respectively) (Bower et al., 2005). Individuals identified as Caucasians had isotopic ratios consistent with known sources in the northern US (Bower et al., 2005). Certainly Bower et al. (2005) were able to uncover some interesting data, but this study also demonstrated some limitations experienced when analyzing archaeological populations. If the same skeletal element is not available for each individual tested, then results become less comparable. Having complete, accurate, historical data would go far to supporting results, in this case birthplace records, but this information is rarely available.

With this in mind, conclusions can be drawn, provided room is left for uncertainty and the introduction of further information that may change the results' implications.

The discovery of more burials at the Pueblo, Colorado mental hospital's cemetery prompted Bower et al. to expand their 2005 work. The lead content and lead isotope ratios of long bones, predominantly femora, were analyzed using ICP-OES, as well as TIMS and multiple collector inductively coupled plasma mass spectrometry, respectively (Bower et al. 2007). Teeth and grave soils were analyzed as well, along with callus from three fractures. Differences in lead isotopic ratio data between the bones and the soil, lack of lead-containing grave artifacts and lead mobility rates were used to discount diagenesis as the lead source in the bones. Statistically significant differences were found between the lead isotope ratios of cortical bone and callus, which indicates that the lead exposure sources changed between the time when the bone tissue was initially laid down and the time of the fracture. Analysis of records indicated that a higher percentage of the individuals came from counties with smelters than would be expected according to census data. The authors also calculated, using bone data and hospital records that specified lead intoxication as a cause of admission, that approximately 6.6% of the hospital's population was suffering from negative effects due to increased lead exposure (Bower et al., 2007).

### **5.3 Summary**

While comparing these investigations of archaeological materials presents complications due to small sample sizes, sample variability, and differences in methodology, there is still valuable information to be gleaned from them. Notably, Waldron et al.'s 1981 study incorporated more than one analytical technique in order to determine if diagenesis had had any impact on the lead detected in the bones that were tested. Merely quantifying lead in bone is not

sufficient. Researchers should employ techniques that can illuminate both the nature and the quantity of the lead contained within archaeological bone.

The reviewed studies by Aufderheide et al. in 1981 and 1985, Corruccini et al. in 1987, Kowal et al. in 1989 and Keenleyside et al. in 1996 are the most pertinent and comparable to the present work. Aufderheide et al.'s 1981 study compared lead levels between individuals of African and European ancestry whom lived on a plantation in the Colonial period. The research found that European individuals had higher lead levels, and the investigators believed this resulted from food and drink contamination due to the use of pewter tableware (Aufderheide, et al., 1981). The enslaved labourers on the Virginian plantation did not have high lead levels, and it was believed that neither their labours nor food provided significant exposure. Aufderheide et al.'s 1985 study examined the correlation between lead levels and social status, and the results supported earlier work; the higher lead levels were observed in individuals of higher social status. Additionally it was observed that lead levels tended to increase in age (Aufderheide, et al., 1985). Corruccini et al.'s investigation involved individuals who lived on Barbados in the Colonial period. Barbados, like Antigua, is in the West Indies and its economy was also based on sugar export. Rum production was significant (Handler, et al., 1986). Like many of the islands in the Colonial Caribbean, most of the residents of Barbados were enslaved labourers of African descent (Corruccini, et al., 1987). The analyses of the remains from a Barbadian plantation's enslaved labourer cemetery showed unexpectedly high lead levels, and it was determined that the distillation processes involved in converting sugar cane to rum were the likely reason (Handler, et al., 1986; Corruccini, et al., 1987). Due to the similarities in the Colonial populations of Antigua and Barbados, and the presence of different ancestries in the British Royal Navy hospital cemetery, significant differences between the two ancestries were

expected, as well as an increase of lead burden with increasing age at death. Kowal et al.'s 1989 publication and Keenleyside et al.'s 1996 work also have strong similarities to the analyses in this thesis. Both studies involved investigations into the lead content of bones from historical individuals whom had been part of the Franklin Expedition, an ill-fated attempt to find the Northwest Passage. When analysis of a single bone indicated high levels of lead, questions arose regarding the fate of the Expedition's sailors, particularly whether or not they were affected by lead poisoning (Kowal, et al., 1989). This work also attempts to address a historical question, in this case regarding the extent of lead poisoning in the British Royal Navy in the West Indies during the Colonial period.

Analysis of the studies in this chapter indicates that ancient bone lead levels are generally lower than those of modern populations. Some historical populations known to have significant contact with lead-containing items had higher levels of lead in their bones, such as individuals from the time of the Roman Empire, the Edo era and the Colonial period. Small sample sizes, the use of multiple skeletal elements and diagenesis sometimes resulted in difficulty interpreting lead in bone data. Comparability amongst the findings can be made difficult by the employment of different analytical techniques and the presentation of data values in various different units of measurement. Examination of these investigations reveals that many factors were found to influence lead levels, including degree of contact with anthropogenic sources such as environmental contamination from mining and smelting activities, differing cultural practices, differing places of birth and ancestry, and different socioeconomic statuses.

## **Chapter 6 - Inductively-Coupled Plasma Mass Spectroscopy Analysis of Lead in Bone**

### **6.1 Introduction**

Earlier studies of lead in bone used methods like AAS and TIMS for quantification and isotopic differentiation. In the nineteen eighties, new and improved technologies like ICP-MS became available. ICP-MS is an analytical method capable of ultra-trace detection of elements; it is able to quantitate at levels as low as 1ng per litre, which is equivalent to one part per quadrillion (ppq) (Thomas, 2004; Taylor, 2001). This high sensitivity, along with its speed, multielement capability, and its ability to differentiate isotopes makes ICP-MS preferable to other analytical techniques including flame AAS, electrothermal atomization and ICP-OES. ICP-MS is highly accurate and precise and does not significantly suffer from interference resulting from target analyte spectra overlap, strengthening the validity of the data that it produces (Taylor, 2001). If appropriate methods are used, including internal standards or dilution, then interferences related to suppression by the analyte matrix can be overcome (Wolf, 1997). Since hydroxyapatite in bone can cause significant interference due to an overwhelming calcium signal, a technology capable of reliably analyzing relatively trace elements was highly desirable. Additionally, ICP-MS is highly customizable; many combinations of ion measurement technology and sample introduction techniques are available (Taylor, 2001). These combinations give researchers and analysts better ability to find methodologies that suit their samples of interest.

## 6.2 Studies of Lead in Bone by ICP-MS

The detection of lead in archaeological bone using ICP-MS was first performed by Reinhard and Ghazi in 1992 in their examination of lead levels found in skeletal remains of 18<sup>th</sup> century Omaha Indians. Their results showed elevated bone lead levels when compared with the soil, and some were remarkably high. The remains of one subadult contained 2567.0ppm Pb (Reinhard & Ghazi, 1992). It was later determined that a significant portion of the lead that was found was likely introduced to the remains through burial practices, as Pb was found in both artifacts and paint pigments associated with the remains (Ghazi, et al., 1994). Some the lead that was found could be not be ascribed to post-mortem contamination due to differences in isotopic composition (Ghazi et al., 1994). Skeletons with no detectable pigment or associated lead artifacts still had Pb levels that were elevated as compared to the soil (Ghazi, et al., 1994). Therefore, it was believed by investigators that these Omaha had ingested or inhaled lead while still alive, as lead-glazed pottery was being used at that time (Ghazi, et al., 1994). The outcomes of these studies underline the need to be able to differentiate between lead that was introduced pre- and post-mortem.

In order to validate ICP-MS's utility for analyzing lead in bone, comparisons to previously established methods became necessary. In 1995, Yoshinaga et al. published a research study in that area. Using inductively coupled atomic emission spectroscopy (ICP-AES), atomic absorption spectroscopy (AAS) and ICP-MS, they examined levels of multiple elements in ribs obtained following autopsies of elderly Japanese who had died in hospital (Yoshinaga, et al., 1995). Through comparison to AAS analysis, their findings indicated that their ICP-MS data was more reliable than their ICP-AES for Pb determination due to issues with the latter method's sample preparation requirements (Yoshinaga, et al., 1995). Their results also implied that there

was a relationship between lead level and cancer, but as their sample set was small, 45 individuals were tested, further research in this area was recommended (Yoshinaga, et al., 1995). Ultimately, Yoshinaga et al. (1985) found that, without substantial sample pre-treatment, the sensitivity and detection capabilities of ICP-MS significantly exceeded those of both ICP-AES and AAS. In 1998, Hinners et al. (1998) published an interlaboratory comparison of the analysis of the National Institute of Standards and Technology's 1400 Bone Ash Standard Reference Material (SRM) using both ICP-MS and thermal ionization mass spectrometry (TIMS). Their findings indicated that the TIMS data from one of the labs was the most precise measurement of Pb, determinations by all of the labs made using ICP-MS were statistically equivalent to the values found by TIMS (Hinners, et al., 1998).

Beyond method comparisons, further methodological advancements in the detection of lead in calcified tissues such as bone were needed as well. Using bone reference material, Outridge et al. (1996) found that hydroxyapatite significantly suppressed Pb signals in ICP-MS analysis and in order to deal with these interferences, recommended the use of isotope dilution or external calibration with internal standardization as modes of measuring Pb in materials with high calcium content.

Yoshinaga continued research into elemental analysis using ICP-MS by next examining its capabilities regarding isotopic differentiation. In 1996, Yoshinaga examined the isotopic distribution in hair and in bone samples of both modern and ancient origin (Yoshinaga, 1996). He found that ICP-MS was more than capable of precise and accurate measurement of isotopic distribution in human biological materials through comparisons to expected values of SRMs (Yoshinaga, 1996)



Methodological development was continued by Lee et al. in 1999. The investigators demonstrated that the standard addition technique gave comparable results to the isotopic dilution method that has been standardly employed for ICP-MS analyses (Lee, et al., 1999). The validation of this method was significant to lead analysis in tissues like bone because sample preparation was much simpler, and it also prevented dilution of the target which could result in an inability to detect very low concentrations in a highly interfering matrix such as hydroxyapatite (Lee, et al., 1999).

By 2000, ICP-MS had become a relatively common method of measuring low level elements, such as lead, in bone. In fact, ICP-MS could be used comparatively in order to validate other measurement techniques. Aro et al. (2000) validated the use of K X-ray fluorescence in this manner for *in vivo* and *in vitro* bone lead detection and quantification. By comparing the concentrations of lead found in cadaver tibias, both within and separated from surrounding tissues, to those concentrations found by ICP-MS analysis of bone ash, the researchers were able to demonstrate that K X-ray fluorescence was a suitable method for analysis of lead levels in bone (Aro, et al., 2000).

Djingova et al. (2004) proposed and validated an analytical method for ICP-MS analysis of elements in bone. The researchers had noted that there were few published papers describing methods for ICP-MS multielement analysis of bone (Djingova, et al., 2004). The validation was accomplished through repeated analysis of a SRM and by comparison of elemental concentrations found through AAS, ICP-AES and ICP-MS (Djingova, et al., 2004). The parameters set for the method achieved ICP-MS results that demonstrated its comparability with AAS and ICP-AES as well as its capability of recovering expected element concentrations in a SRM (Djingova, et al., 2004). The method was then applied to three samples of archaeological

bone from the fourth century before the Common Era (Djingova, et al., 2004). Low levels of lead, <9mg/kg, were found in all three samples (Djingova, et al., 2004). The method was developed to allow for an investigation of the diet of Bulgarians during the Hellenistic period through analysis of skeletal remains (Djingova, et al., 2004).

With ICP-MS's utility established, researchers observed the necessity for improving accuracy validation and inter-study comparison through the development of SRMs. Bellis et al. (2008) developed four candidate reference materials (CRMs), hoping to address the need for SRMs with higher lead concentrations and concerns regarding the preparation of the bones used in the National Institute of Science and Technology NIST SRMs. CRMs are materials that are being tested for suitability for usage as SRMs. In order to characterize the CRMs, samples of the CRMs were sent out and analyzed by 29 different laboratories using known and established methodologies (Bellis, et al., 2008). Additionally, the research group analyzed the CRMs and a NIST SRM using double isotope dilution ICP-MS in order to validate the method's approach (Bellis, et al., 2008). Ultimately, three of the four CRMs had lead concentrations higher than NIST SRMs, and the double isotope dilution ICP-MS method achieved expected lead recoveries with suitable precision when compared to both the certified and interlaboratory data (Bellis, et al., 2008). This indicates that three of the four high lead CRMs would be suitable as SRMs and that the double isotope dilution ICP-MS method was a valid analytical technique.

Using ICP-MS analysis also has benefits regarding its greater adaptability. Modification to the technique allowed for new methods and applications of lead analysis. In 1998, Budd et al. used laser ablation ICP-MS (LA-ICP-MS) to explore lead distribution in dental tissues (Budd, et al., 1998). Laser ablation is a minimally destructive technique that allows for surface sampling; the sample does not have to be removed from the whole in order to be analysed. Budd et al.'s

results suggested that enamel did not tend to absorb lead from diagenesis, as unerupted and modern teeth showed highly similar patterns of lead distribution (Budd, et al., 1998). The slightly decreased Pb/Ca ratios in the archaeological teeth suggested to the researchers that enamel may actually lose Pb to the burial environment instead of gaining it (Budd, et al., 1998). Further advancements to LA-ICP-MS analysis of lead in bone were made in 2006. Bellis et al. prepared pellets from recognized reference materials and then used the formed pellets to calibrate the LA-ICP-MS for spatial distribution quantification (Bellis, et al., 2006). Once the calibration was completed, the investigators then applied LA-ICP-MS to a goat metacarpal and were able to determine both the concentration and approximate location of lead within the bone (Bellis, et al., 2006). Their results showed that there was more lead in the trabecular bone versus the cortical bone, and that discrete areas of both bone types had greater concentration than others (Bellis, et al., 2006). A different modification was developed by De Muynck et al. (2008a). They published a method validation study for the analysis of lead isotopes in bone, and other materials as well, using single collector ICP-dynamic reaction cell-MS (De Muynck, et al., 2008a). While the method was of lower precision than the multicollector method previously mentioned, it used less toxic reagents than other methods, the lead separation chromatography columns that were developed to isolate the lead were reusable, and, most importantly it could be applied to a wider range of materials, allowing for better comparisons and source attribution accuracy (De Muynck, et al., 2008a).

ICP-MS has been the technique employed in several animal studies of bone lead levels that have implications for humans and studies of archaeological remains as well. In 1999 it was used to examine the absorption of lead by pregnant mammals and their unborn fetuses by analyzing the lead levels in swine tissues including bone (May, et al., 1999). Their data showed

that pregnant females absorbed fifty percent more lead than non-pregnant females, and that while fetal concentrations were lower than maternal concentrations, there was a linear dose-response curve (May, et al., 1999). The fetal femora lead levels ranged from 6.25 parts per billion (ppb) to 103ppb whereas the maternal femora ranged from 46.8 to 396ppb Pb (May, et al., 1999). The lead dosages ranged from 0 to 1000 $\mu\text{g}/\text{kg}/\text{day}$  (May, et al., 1999). The increase in lead uptake in the pregnant females is likely due to the increased need for calcium during pregnancy, partially due to the formation of skeletal material in the fetus(es). Since lead can take the place of calcium, and enzyme affinity for lead is often greater than it is for calcium, then if lead is available the pregnant female will likely preferentially accumulate lead (Kern, et al., 2000; Neal, et al., 2010). Interestingly, in a low-dosage group, 10 $\mu\text{g}/\text{kg}/\text{day}$ , it was found that more than seventy percent of the lead in the fetal tissues arose from lead that had been mobilized from the mother's bones; in the high dose group, 1000 $\mu\text{g}/\text{kg}/\text{day}$ , only 1% of the lead in fetal tissues was mobilized maternal lead and the remainder came from the given dose (May, et al., 1999). This data suggests that human maternal bone lead levels can have great impact on the bone lead levels of their unborn children, which may confound results of archaeological remains. Young children are not generally expected to have high levels of lead in their bones, but if they do, their mother's bone lead levels should be checked as well, if possible, to help ascertain the source of the children's bone lead. Seltzer et al. (2006) used LA-ICP-MS to compare bone lead levels and distribution in the femora of wild and captive American alligators. Their data revealed that the captive alligators had remarkably higher lead levels when compared to those in the wild (Seltzer, et al., 2006). More pointedly, the researchers were able to connect some of the animals' behaviours to the bone lead levels; the captive alligators were actually showing symptoms of lead poisoning (Seltzer, et al., 2006). This study aptly demonstrates the difference that

environment can make on the bone lead levels of the same species. Thomas et al. (2009) used ICP-MS's ability to distinguish lead isotopes in a study of wild red grouse (*Lagopus lagopus scoticus*) on both the Yorkshire and Scottish moors in 2009. Leg and foot bones from 196 grouse from two different estates were tested, and the results showed that the Yorkshire estate grouse contained lead consistent with both lead shot and the historic galena mines in the area, whereas the Scottish grouse bone lead appeared to arise from exposure to lead shot only, as no mines had been historically associated with the Scottish estate areas (Thomas, et al., 2009). The isotopic composition of the grouse bones also indicated that the lead contained within did not likely result from leaded gasoline nor from the air pollution (Thomas, et al., 2009). It appears that the birds were consuming the shot. This research reveals lead's ability to contaminate through environmental pollution and underscores its ability of lead to enter our food chain. Further evidence of lead's ability to contaminate the environment was provided when Jerez et al. (2013) used ICP-MS to investigate the elemental levels in bone and other tissues of penguin chicks in 2013. They were interested in examining the impact of pollutants on an isolated environment (Jerez, et al., 2013). The lead levels they detected were not so high as to cause sickness in the seabirds, but they were comparable with birds which spent more time in areas known to be polluted, suggesting that the pollutants had made their way into the penguins' environment and food chain (Jerez, et al., 2013). This indicates that detected lead may not have resulted from direct exposure to a lead source. In archaeological terms, if human remains are analyzed and found to be containing lead, there may not be a direct link, contextually or historically, to the lead's origin. This study's findings are consistent with findings from studies of human bone lead levels in the Canary Islands (Arnay-de-la-Rosa, et al., 1998; Gonzalez-Reimers, et al., 1999; Arnay-de-la-Rosa, et al., 2003; Gonzalez-Reimers, et al., 2003). ICP-MS

was employed to develop an elemental level profile of eel products that originated in Japan, China or Taiwan by Iguchi et al. (2013). Eel bones from fillets were analyzed and the lead content between the bones was very different (Iguchi, et al., 2013). The fillets from Japan showed 0.10 $\mu\text{g/g}$  of lead, as compared to 0.61 $\mu\text{g/g}$  in those from China and 0.46 $\mu\text{g/g}$  in the fillets from Taiwan (Iguchi, et al., 2013). The investigators used the values obtained from the ICP-MS analysis to develop a model that could allow for the differentiation of product origin based on elemental levels (Iguchi, et al., 2013). Unfortunately, a model such as this is neither definite nor permanent, as changing practices and environmental conditions could drastically affect the lead levels found at any given point in time.

Several studies of modern human bone by ICP-MS have implications for archaeological remains as well. ICP-MS's sensitivity was utilized by Roberts et al. (1996) to detect lead and other elements in bone. The investigators were interested in examining if elemental composition correlated to bone fracture (Roberts, et al., 1996). Femora of modern-day, previously healthy individuals, femora from modern-day individuals whom had suffered from osteoarthritis and also femora from modern-day patients that had suffered femoral head fractures were analyzed (Roberts, et al., 1996). The data showed that there was no significant composition difference amongst all the femora, except in the case of lead, which was significantly lower in the osteoarthritic femora (Roberts, et al., 1996). The researchers surmised that this difference was a result of elevated bone resorption (Roberts, et al., 1996). The influences of disease on lead levels in skeletal remains pose a difficulty when studying archaeological remains. Even with a complete skeleton, diseases which could influence the results are not always easy to diagnose.

Some human bone lead level studies using ICP-MS give insight into the exposure patterns of a specific population. ICP-MS was used by Llobet et al. (1998) to examine the levels

of multiple elements, including lead, in tissues from twenty autopsies of fifteen men and five women. The study was undertaken in order to help establish baseline levels in residents of Tarragona, Spain prior to the installation of a waste incinerator (Llobet, et al., 1998). The researchers compared each of the target elements' concentrations in kidney, brain, lung, liver and bone (Llobet, et al., 1998). Their analyses determined that, of all the examined tissues, bone contained the highest concentrations of lead (Llobet, et al., 1998). A mean bone lead level of 3.71 µg/g wet weight was found for the 20 individuals' ribs tested (Llobet, et al., 1998). They also observed that there were no statistically significant differences in the lead levels between men and women, amongst persons living near oil refineries or in urban areas, or even between adults aged 35 to 65 and those older than 65 (Llobet, et al., 1998). Elemental levels of autopsied individuals from Tarragona, Spain were revisited by Garcia et al. (2001). Their research indicated that the kidney lead levels found in the 78 individuals autopsied were similar to those found in other studies of the same tissue; three of the compared studies took place previously in Tarragona Spain and three others were done in Japan, Europe and the United States (Garcia, et al., 2001). Bone had the highest concentration of lead, with levels twenty times greater than other tissues (Garcia, et al., 2001). They also noted an overall increase of lead level in bone with age with a simultaneous decrease in kidney lead levels (Garcia, et al., 2001). Unlike other lead level studies that had been performed, no difference in lead levels between the sexes was observed (Garcia, et al., 2001). Hess et al. (2013) undertook an exploration on the different lead exposure experienced by South African males (Hess, et al., 2013). The femora of 101 individuals who died between 1960 and 1998 were tested; 72 were black and 29 were white (Hess, et al., 2013). The levels found were contrary to what has been observed in most of the other current publications that examine lead exposure amongst different socioeconomic groups

(Hess, et al., 2013). The white males showed a higher level of lead in their bones, the median value was 10.04 $\mu\text{g/g}$ , where the median value of lead in the black males' bones was 3.80 $\mu\text{g/g}$  (Hess, et al., 2013). The white males from this study were believed to have had greater contact with lead due to their urbanized residential areas, mandated by apartheid, which put them in contact with significant atmospheric pollution from lead-containing gasoline (Hess, et al., 2013). Results of this nature had also been found by Aufderheide et al. (1981); plantation proprietors of European ancestry had shown significantly increased lead levels as compared to the enslaved plantation labourers of African ancestry. The plantation owners' lead high levels were believed to have arisen from increased contact with lead containing objects. Lead-containing pewter tableware was a mark of status in 18<sup>th</sup> century culture (Aufderheide, et al., 1981). Other than Aufderheide et al.'s 1981 publication, most previous studies of this nature have indicated that typically it is those occupying the lower socioeconomic groups that have higher lead concentrations in their tissues, often due to labour related exposure (Corruccini, et al., 1987; Hess, et al., 2013). Alternatively, elevated levels in lower socioeconomic classes has been seen to be related to substandard housing conditions, poor food quality, contamination of food storage vessels and nutritional deficiencies (Brown & Longoria, 2010). When the relationship of lead level to age was examined by the researchers, their data showed a weakly positive correlative relationship; as age increased, the concentration of lead increased as well (Hess, et al., 2013). This correlation between increasing lead level and increasing age-at-death has also been observed in other studies including Aufderheide et al. (1981), Aufderheide et al. (1985), and Corruccini et al. (1987).

The application of ICP-MS to study lead in archaeological remains has been sporadic. After the initial investigations by Reinhard and Ghazi in 1992 into the Omaha Indians, there



were no publications relating to ICP-MS analysis of archaeological skeletal material until 1997. Keenleyside et al. (1997) analyzed human remains associated with the ill-fated Franklin Expedition following their discovery in 1992 at a site near King William Island in Nunavut, Canada designated NgLj-2. The lead content of nine cortical bones was analyzed to determine if the NgLj-2 remains had high lead levels like those found by AAS in remains from King William and Beechey Islands (Kowal, et al., 1989; Keenleyside, et al., 1997). A range from 49ppm Pb to 204ppm Pb was found in the seven right femora and the left and right tibia that were analyzed, indicating a high lead burden in the individuals which was consistent with Kowal et al.'s findings in the remains from the other sites (Keenleyside, et al., 1997). Research into comparative lead levels and isotopic distribution in bone using ICP-MS was performed by Yoshinaga et al. in 1998. The researchers sampled bones from the prehistoric Jomon, historic Edo and modern periods, as well as deciduous teeth from contemporary Japanese children (Yoshinaga, et al., 1998). The investigation showed that the highest levels were found in the bones from the Edo period, while the lowest levels were found in the Jomon and modern deciduous teeth bones and that the isotope ratios of the Jomon and Edo period bones differed significantly from those of the contemporary bones and deciduous teeth (Yoshinaga, et al., 1998). The authors indicated that the source of the Edo period contamination has yet to be determined, but cosmetics have been considered a possible source as lead levels in females from this period were elevated in comparison with those of the males (Yoshinaga, et al., 1998). Notably, the investigators mentioned the possibility of diagenetic lead contamination and the difficulties associated with its removal, but they presumed that at least part of the lead found was biogenic, due to the agreement in lead levels between their study and two previous studies of bones from the same eras but differing burial environments. (Yoshinaga, et al., 1998). Grattan et al. (2002) used ICP-

MS to assess the lead and copper content of the remains of 36 individuals from the Byzantine era whom had died at Wadi Faynan, an ancient copper mining site in present-day Jordan. In the process of smelting, lead was released from the ores that were being refined for their copper content (Grattan, et al., 2002). The investigators found that the individuals had a range of lead concentrations; values between 1.0 and 289.2 $\mu\text{g}$  Pb/g bone, with a mean of 42.49 $\mu\text{g}/\text{g}$  were found (Grattan, et al., 2002). The soils from the area that were tested ranged between 0.28 and 3.56 $\mu\text{g}/\text{g}$ , which supported the hypothesis that the lead values found were reflective of the living individuals' skeletal lead burden (Grattan, et al., 2002). Additionally, the authors noted that the graves that were excavated were located well above water sources and rainfall in the area was minimal, which indicated that the conditions required for cation exchange between bone and soil were unlikely to be significant (Grattan, et al., 2002). The lead concentrations found were compared against those found from individuals living in Silesia, which is considered a modern toxicological disaster zone due to industrial pollution (Grattan, et al., 2002). The results were very similar, indicating that the conditions experienced at Wadi Faynan were likely also toxic due to the heavy mining and subsequent environmental damage that occurred (Grattan, et al., 2002). This analysis was an interesting reversal; modern data gave a frame of reference for the archaeological information, allowing a better understanding of the conditions experienced by past peoples. Stadlbauer et al. (2001) attempted to verify that a cranium housed in the Stiftung Mozarteum in Austria could in fact be the remains of Mozart the composer. They analyzed the Stiftung cranium, skulls and femora from four of Mozart's 18<sup>th</sup> century relatives, and femora from three modern individuals to allow for comparison of 18<sup>th</sup> century lead levels with those of the modern population (Stadlbauer, et al., 2007). Their results in that regard were inconclusive, but their data did show that individuals from Mozart's era had significantly higher lead levels in

their bones when compared against the same tissue from the modern individuals (Stadlbauer, et al., 2007). Then, in 2008, Shafer et al. employed high resolution (magnetic sector) ICP-MS, HR-ICP-MS. The study described the elemental analysis of eighty Iron Age bone samples along with methods to correct for diagenetic alteration of the elemental levels (Shafer, et al., 2008). Their findings indicated that modern bones usually had much higher levels of Pb than did bone samples from the Iron Age, and the researchers went on to state that much of the lead mobilization that has been observed is anthropogenic in origin (Shafer, et al., 2008).

Isotopes of lead in bone were analyzed by De Muynck et al. (2008b) using multicollector ICP-MS in order to help ascertain the source of the lead. Infant bones from a Roman era cemetery were analyzed, along with soil from each site where bones were excavated, as well as lead objects and fish bones excavated from the site (De Muynck, et al. 2008b). The isotopic analysis showed that little of the lead present in the infant bones was consistent with the soil lead and more consistent with the lead objects and fish bones from the site, suggesting that diagenesis was not the main source of the high lead levels found in the infant bones (De Muynck, et al. 2008b).

Lead detection in bone was used to help illuminate the circumstances surrounding the deaths of 17 Swedish sealers whom had died while sheltering in a cabin over the winter of 1872 (Aasebo & Kjaer, 2009). It was believed that the men had died from scurvy, even though there were foods present that would have prevented the disease's onset, and they had been given instruction by a physician on how to avoid scurvy (Aasebo & Kjaer, 2009). Aasebø and Kjær believed that lead poisoning may have been responsible after discovering lead-soldered cans at the cabin site, especially given the findings regarding the Franklin expedition that had been published in 1996 by Keenleyside et al. (Aasebo & Kjaer, 2009; Keenleyside, et al., 1996). A

scapula, clavicle, humerus, and two cervical vertebrae from one individual were collected from a permafrost grave (Aasebo & Kjaer, 2009). Upon macroscopic examination, none of the diagnostic signs of scurvy were discovered (Aasebo & Kjaer, 2009). Using ICP-SF-MS, the bones were analyzed for lead and found to contain 65ppm Pb in the dried bone (Aasebo & Kjaer, 2009). The associated sedimentary material was found to have 16ppm Pb; combining this data with the skeleton entombment in permafrost suggests that diagenesis would not have been a contributing factor (Aasebo & Kjaer, 2009). The authors extrapolated blood lead concentrations from the dried bone levels, and, given that they also had documentation to support the timeframe in which the lead consumption occurred, concluded that the individual would have been suffering from acute lead poisoning at their time of death (Aasebo & Kjaer, 2009). Even though the lead level of 65ppm does not seem high relative to other values seen in this review, the authors are suggesting that this exposure occurred over a brief span of time, one winter, and for this accrual to have occurred in such a brief period, the individual's blood level would have had to have been disastrously high. In cases such as this, detection of high levels of lead in bone may indicate a possible cause of death, when data supporting other causes cannot be found.

ICP-MS is not always successful on its own. Castro et al. (2010) used elemental levels, including lead, in bone and teeth to attempt to discriminate between individuals. They employed laser ablation sampling and a sector field based ICP-MS (LA-ICP-SF-MS) which improved resolution and detection capabilities (Castro, et al., 2010). Their methods were moderately successful, when only femur fragments were considered the fragments were grouped correctly for 75% of the individuals (Castro, et al., 2010). Humerus fragment comparisons gave correct assignments in 63% of the individuals (Castro, et al., 2010). However, if the humerus and femur fragments were analyzed together, the grouping was only correct for 50% of the individuals

(Castro, et al., 2010). Given the fragmentary nature of many archaeological remains, and that certain regions of the same bone can have varying levels of a given trace element when analyzed by LA-ICP-MS, this suggests that elemental levels are not sufficient as the sole method for identifying reuniting skeletal elements of individuals (Bellis, et al., 2006). The complementation of ICP-MS with other techniques can yield more definitive results.

### **6.3 Summary**

Many of the analyses discussed in this chapter outline the methodological development of ICP-MS, particularly in regards to determinations of lead levels in bone. Eventually, due to its sensitivity and adaptability, ICP-MS became a common analytical technique for lead in bone analysis. ICP-MS was used in studies of lead in animal and human bones, and the results of some of these have implications for studies of archaeological bone material, including the effects of disease and pregnancy (Roberts, et al., 1996; May, et al., 1999; Iguchi, et al., 2013; Jerez, et al., 2013). Another lead in human bone studies using ICP-MS indicated that environment had a significant impact on bone lead levels, and that socioeconomic status plays a part in the type of environment to which an individual is exposed (Hess, et al., 2013).

Archaeological bone lead studies and those involving historic mysteries are the most relevant to this thesis. Reinhard and Ghazi (1992) found elevated levels in ancient Omaha remains. Keenleyside et al. (1997) showed results similar to those found by AAS and X-ray excitation in earlier studies (Kowal, et al., 1989; Keenleyside, et al., 1996). The bone lead levels in the Franklin expedition crew were elevated and may have contributed to their deaths (Kowal, et al., 1989; Keenleyside, et al., 1996; Keenleyside, et al., 1997). ICP-MS was used to analyze bone lead from a Swedish sealer who was part of a group of seventeen that died mysteriously when stranded in a cabin over a 19<sup>th</sup> century winter (Aasebo & Kjaer, 2009). The lead levels

found in the bone material indicated that lead poisoning from lead-soldered tins was a possible contributing cause of the death of the Swedish sealers (Aasebo & Kjaer, 2009).

Some of the limitations of ICP-MS analysis of lead in bone were also revealed. Reinhard and Ghazi (1992) demonstrated that ICP-MS quantitation of lead in bone could not by itself determine the nature of the lead, i.e. whether it was introduced through diagenesis or biogenesis. Isotopic differentiation and knowledge of cultural practices of the Omaha were required to help determine the source of the lead (Ghazi, et al., 1994). It was found that certain methods, such as isotope dilution, were needed to assess trace elements in order to overcome matrix interference from hydroxyapatite in bone (Lee, et al., 1999). Quantitative analysis via ICP-MS of different bones cannot reliably allow for discrimination of individuals (Castro, et al., 2010).

Ultimately, however, despite some of ICP-MS's limitations regarding detection of lead in bone, it was found to be a more reliable method than ICP-AES and more sensitive than both ICP-AES and AAS for the determination of the levels of lead in bone (Yoshinaga, et al., 1995).

## Chapter 7 – X-Ray Fluorescence and Synchrotron X-Ray Fluorescent Imaging

### 7.1 Introduction

One of the techniques that can be used to complement ICP-MS in the analysis of bone is X-ray fluorescence (XRF). XRF uses X-rays to excite electrons which results in the ejection of inner shell electrons (Verma, 2007). When this ejection occurs, an outer shell electron drops to the inner shell to fill the vacancy, but in order to drop, the electron must lose some of its energy, which is then emitted as X-rays (Verma, 2007). The energy of the emitted X-ray is dependent upon the difference between the energy levels of the inner and outer shell of the atom involved, and the intensity of the beam is related to the quantity of the element detected (Verma, 2007).

XRF has been known to the scientific community for a significant period of time. The abilities of XRF to detect and quantify were first demonstrated in 1913 by Moseley, but it took a significantly longer time before the technology was widely available (Adams, et al., 1998). The first commercial X-ray products became available at the end of WWII, and following this, X-ray technologies became a standard method for multielement analyses (Adams, et al., 1998). The application of XRF to biological materials began early on, but several issues were encountered (Jones, et al., 1988). The matrix would sometimes overwhelm a trace level target analyte, or the heterogeneity of the sample could cause difficulties with interpretation (Jones, et al., 1988). Some samples were not compatible with vacuums, which were required in initial instrumentation setups, and some needed extensive preparation (Jones, et al., 1988).

Nonetheless, XRF was a useful tool for semiquantitative determination of lead levels in bone. It was determined that it was viable as both an *in vivo* and *in vitro* method by Ahlgren and Mattson (1979), where they used XRF of finger bones to determine lead levels in metal industry workers.

The development of synchrotron radiation (SR) sources and technologies was able to address some of the difficulties encountered with conventional XRF. Synchrotrons are large storage rings that contain accelerated electrons (Bertrand, et al., 2012). The electrons are injected into a linear accelerator which boosts their speed and then, often, are sent to a smaller booster synchrotron which further increases their speed (Bertrand, et al., 2012). Following the boost, the electrons are injected into the main storage ring where their path can be directed by bending magnets or insertion devices such as undulators and wigglers (Bertrand, et al., 2012). The bending magnets or devices allow the created radiation to be emitted (Bertrand, et al., 2012). The generated radiation is linearly polarized and it is directed outwards, tangential to the electrons' path (Adams, et al., 1998). The X-rays produced are much more powerful and more focused than those from conventional X-ray sources which leads to increased sensitivity, and samples can be left at room temperature and pressure (Jones, et al., 1988).

One of the major benefits of the use of synchrotron radiation X-ray fluorescence imaging (SR-XFI) to analyze lead in bone is its ability to determine the location of lead in bone, allowing for better interpretations of factors such as the general timing of lead exposure (Zoeger, et al., 2006; Swanston, et al., 2012; Martin, et al., 2013). Additionally, lead's location within bone can give indications regarding the nature of its deposition, whether or not it may have been introduced during diagenesis or during the individual's lifetime (Smith & Walker, 1964; Waldron, et al., 1979; Swanston, et al., 2012).

## **7.2 SR-XFI Analysis of Lead in Modern Bone**

Gilfrich et al. (1983) established initial SR method detection limits (MDLs) for several elements, including lead at the Stanford Synchrotron Radiation Laboratory, also known as the SSRL. The study of biological materials using SR was initiated in 1987 by Hanson et al. (1987)



when they applied SR-induced X-ray emission to samples of gelatin, pepperbrush and hair at the National Synchrotron Light Source (NSLS). Their work was furthered by the application of SR-XRF to materials such as animal bone, human hair, pepperbrush and river sediment that was undertaken by Jones et al.(1988) at the NSLS . Jones et al.'s work revealed that using SR as an X-ray source effectively addressed the major issues encountered with biological specimens when examined using conventional XRF devices (Jones, et al., 1988).

The application of SR to human bone began when Jones et al. (1990) examined the distribution of lead in a human tibia that had been amputated from a 54 year-old male. Their investigation demonstrated that while calcium and strontium were evenly distributed across the bone section examined, lead and zinc were not. The latter pair of elements, however, tended to be distributed in similar patterns and that, concordant to studies performed by Lindh, the lead tended to be deposited at the periosteum and associated with osteons (Lindh, et al., 1978; Lindh, 1980; Jones, et al., 1990). This distribution pattern suggests that Pb is taken up into bone only when it is present in the system, and that while it may replace calcium, perhaps due to lead's greater affinity; it is not the standard metal that is incorporated into hydroxyapatite.

In 1997, Jones et al. used synchrotron radiation induced X-ray emission (SRIXE) to examine the lead distribution in the tibia bones of chicks (Jones, et al., 1997). Two weeks prior to the SRIXE analysis, the chicks were fed diets either normal or Ca deficient and half of each of these two groups was fed a diet either enriched with Pb or containing no Pb (Jones, et al., 1997). The SRIXE analysis of the tibia showed that the chicks whose diet was deficient in Ca and enriched in Pb had depleted trabecular bone growth, a very thin growth plate, and cartilage cores that were significantly reduced in size relative to the chicks not exposed to Pb (Jones, et al., 1997). The results described demonstrate the impacts of early exposure to high lead levels and

indicate what investigators could observe in archaeological remains if the living individual(s) experienced that condition.

Xie et al. (2003) employed SR-XRF at the Beijing Synchrotron Radiation Facility to study elemental distribution in Adelie penguin bones. The researchers wished to take advantage of SR-XRF's trace-level, multielement capabilities, exceptional resolution, the minimal sample preparation required, and the minimal sample damage incurred (Xie, et al., 2003). Their study determined varying elemental levels and correlations, including some elements known to be toxic, such as mercury (Xie, et al., 2003). The investigators recommended that further animal bone studies be performed in order to create a database of SR-XRF profiles to allow further comparison and evaluation of animal bone data (Xie, et al., 2003). The requirement of a template for comparison of trace elements was revealed; without an understanding of the biologically normal levels, researchers cannot draw conclusions from their data.

In 2006, Zoeger et al. examined femoral heads and patellae from modern individuals who had died of short-term illnesses and who had no known history of lead exposure or bone disease. They used SR-induced micro XRF (SR  $\mu$ -XRF) to examine the distribution of Pb in the bone and articular cartilage. In each bone segment analyzed, the Pb was found in highest concentrations in the area between the calcified and non-calcified cartilage (Zoeger, et al., 2006). The SR  $\mu$ -XRF also showed a strong correlation to the distribution of Pb and Zn (Zoeger, et al., 2006). That Pb was found at its highest level in the calcification area indicates that it was likely a relatively recent exposure, and thus positioning of Pb in these zones may be able to give a rough estimate when the lead exposure occurred.

Zoeger et al. (2008) revisited Pb accumulation in joint bones in 2008. Along with the conventional SR- $\mu$ XRF that the researchers had used in their 2006 study, they employed SR

absorption and fluorescence tomography, backscattered electron imaging, and confocal SR- $\mu$ XRF to get a better visual representation of the lead's distribution in the femoral heads and patellae (Zoeger, et al., 2006; Zoeger, et al., 2008). The data produced clearly shows that Pb was strongly associated with the tidemark, the calcification front, supporting the association of Pb accumulation and osteoarthritis (Zoeger, et al., 2008). If Pb is implicated as a contributing factor for the development of osteoarthritis, then archaeological remains that demonstrate characteristics consistent with the disease may warrant further investigation of their lead levels.

Synchrotron radiation has also been demonstrated to be useful for examining the impact of disease in bone. Zhang et al. (2005; 2007) observed elemental loss in trabecular bone of individuals suffering from osteoporosis, and Fei et al. (2007) noted a similar outcome in studies of rats with induced diabetes mellitus. While trace elements did not change significantly in Fei et al.'s (2007) study, Ca showed a substantial decrease. This indicates that both osteoporosis and diabetes may have an impact on lead uptake from the environment and that the presence of these diseases may serve as confounding factors in bone lead analysis, since it appears that lead takes the place of calcium in the hydroxyapatite crystal. Additionally, increased porosity resulting from disease increases the likelihood of diagenesis as there is more surface area available for exchange of materials (Lambert et al., 1985; Grupe, 1988). Rao et al. (2009) analyzed the impact of varying the energy levels of the synchrotron X-ray beam from 8keV to 12keV on elemental detection in rat lumbar vertebrae. The rats were from three age groups: 8 weeks, 56 weeks and 78 weeks old (Rao, et al., 2009). The spectra showed that while increasing the energy level increased detection capability for some trace elements, scattering also increased, particularly in the 78 week old rats (Rao, et al., 2009). These results may indicate that samples

belonging to individuals of greater age may produce more complex spectra at higher energies and may require more focused techniques for accurate detailed analyses.

While previous research had established the presence of lead at the tidemark between calcified and non-calcified cartilage in joints, Meirer et al. (2011) went further by examining the chemical nature of the Pb that was detected (Zoeger, et al., 2006; Zoeger, et al., 2008). Using SR- $\mu$ XANES (X-ray absorption near edge structures) at the Ångströmquelle Karlsruhe (ANKA) in Austria, the investigators analyzed the spectra of the tidemark lead in femoral heads and patellae and compared them to those of several lead compounds (Meirer, et al., 2011). The closest match found was to the spectrum of a synthetically generated Pb-doped carbonated hydroxyapatite, which indicates that the Pb may be directly integrated into the crystal structure of hydroxyapatite (Meirer, et al., 2011).

### **7.3 SR-XFI Analysis of Lead in Archaeological Bone**

The utility of applying synchrotron radiation to archaeological materials was addressed by Harbottle et al. in 1986, but it was not until 1999 that bone material was investigated using synchrotron radiation (Harbottle, et al., 1986; Janssens, et al., 1999). Janssens et al. (1999) wanted to take advantage of several aspects of SR-XRF analysis: its sensitivity, quantitative capabilities, minimal sample preparation requirements and that it would be non-destructive to the samples. The bones were fossilized: an equid and a reptile from Olduvai Gorge in Tanzania (Janssens, et al., 1999). They were analyzed for the presence of rare earth elements using the Hasylab synchrotron in Hamburg, Germany, and the data they collected was found to be in agreement with previously published laser ablation microprobe ICP-MS and instrumental neutron activation analysis (Janssens, et al., 1999).

In 2006, small angle X-ray scattering (SAXS) using SR at the European Synchrotron Radiation facility (ESRF) was utilized to examine the crystalline structure of archaeological bone to help illuminate the results of diagenesis (Hiller & Wess, 2006). Crystal changes between modern and archaeological samples were revealed and some of these changes correlated to biomolecular preservation in the archaeological bone samples (Hiller & Wess, 2006).

Martin et al. (2007) used SR-XRF at the NSLS to examine Br, Zn and Pb levels in ribs and teeth from an individual from Cabur, an archaeological site from pre-Columbian Peru. Pb was detected in the cementum and dentine of the teeth and at the periosteum and cortex of the rib (Martin, et al., 2007). The investigators could not rule out diagenetic change, but suggested that the Pb found in the remains may have originated from air contamination due to smelting of galena (PbS) on the northwest coast that was occurring at the time of internment (Martin, et al., 2007).

SR-XRF was one of the techniques employed by Wittmers et al. in 2008 to assist in determining the source of high bone lead levels in human remains from the nineteenth century First African Baptist Church cemetery site in Pennsylvania (Wittmers, et al., 2008). The investigators were trying to ascertain whether the lead found was of biogenic or diagenetic origin (Wittmers, et al., 2008). SR-XRF was used to examine the pattern of lead distribution through bone samples of eleven of the excavated skeletons (Wittmers, et al., 2008). No discernible pattern was detected; two had higher concentrations at the periosteal surface, two were higher at the endosteal surface and the remaining seven had an irregular Pb distribution (Wittmers, et al., 2008). The SR-XRF data collected using their methodologies and facility and instrumentation could not conclusively indicate the nature of the bone lead's origins (Wittmers, et al., 2008).

SR-XRF was utilized, along with other forms of spectral analysis, by Kuczumow et al (2010) to study fossilization in bovid bones from two sites in Africa that ranged in age from about 3000 to 1.5 million years old (Backwell, 2010; Kuczumow, et al., 2010). The SR-XRF was used to scan for heavier elements, such as lead, and allowed mapping of the mineralization that occurs during fossilization (Kuczumow, et al., 2010).

Dumont et al. exploited SR- $\mu$ XRF in an attempt to study the elemental composition of fossilized *Brachiosaurus* bones (Dumont, et al., 2009). Unfortunately, their examination was only able to determine that diagenetic changes prevented the researchers from gaining any useful insight into the physiology and biology of the sauropods (Dumont, et al., 2009). Elemental analysis by SR- $\mu$ XRF indicated that a high concentration of minerals had invaded natural openings and had created microscopic fissures in the bones, and then the minerals spread further into the created cracks (Dumont, et al., 2009). These findings imply that the preservation state of the sample material must be considered prior to analysis. If the microstructures are found to have been compromised by taphonomic changes, then techniques that might allow the researchers to differentiate diagenetic and biogenic material should be employed, such as SR- $\mu$ XRF.

#### **7.4 Employing Complementary Methodologies**

Given that diagenesis can be a confounding factor in the outcomes of bone analysis, methods by which to detect it would be best employed concurrently when examining remains that are affected by it in order to make more informed deductions from acquired data. Additionally, combining techniques gives more valuable information than any one method on its own, particularly when a method reinforces or complements the other(s) employed, as is seen

with results in the following studies obtained by Martin, et al.(2013), Reiche and Chalmin (2008), Reiche, et al. (2010), Swanston, et al. (2012) and Zougrou, et al. (2014).

Two synchrotron techniques that were developed could serve as useful complements to other methodologies in the analysis of lead in bone. The first was a specialized type of spectroscopy called X-ray absorption near edge structure (XANES). It was utilized at the ESRF to determine the chemical origin of blue, grey and black-coloured bone from several different sites in France and Mexico dating from 40000YBP up to about 4400YBP (Reiche & Chalmin, 2008). The technique allowed the researchers to determine the oxidation state of the manganese in the sample whose presence had been determined by other methodologies (Reiche & Chalmin, 2008). They found that blue colours corresponded specifically to the  $Mn^{5+}$  oxidation state whereas the grey and black colours could arise from a variety of manganese oxides (Reiche & Chalmin, 2008). These results indicate that the state of an element, and not just its presence, impacts the conclusions that can be drawn from analytical data. The oxidation state gives the researcher a more precise analysis of the element, which can assist in determining its source; XANES may give clues as to whether the element is diagenetic or biogenic. The second complementary technique that was developed was the use of the synchrotron to determine bone preservation state. The ability of synchrotron radiation micro FTIR (SR- $\mu$ FTIR) to detect bone preservation state was evaluated by Reiche et al. (2010). The collagen, phosphate crystallinity, and carbonate levels of an archaeological bone sample, dated to 5000YBP, were examined and compared against a modern bone sample at the Berliner Elektronen-Speicherring Gesellschaft Gesellschaft für Synchrotronstrahlung (BESSY) (Reiche, et al., 2010). The results suggested that the archaeological bone was well-preserved in the area examined; the numbers were very consistent with those found for the modern bone sample (Reiche, et al., 2010). The researchers

concluded that SR- $\mu$ FTIR was an effective method to evaluate preservation state and that further work on samples in varying preservation states and from different burial contexts should be evaluated (Reiche, et al., 2010).

The two preceding studies are both examples of synchrotron techniques that could be used in combination with other methodologies. If either technique is employed along with another lead analysis method, more information can be gained. The first, the application of XANES, could allow researchers to determine the oxidation state of lead, which would help to determine the type of compound that the lead is in, for instance lead oxide or lead phosphate. Determining the preservation state prior to analysis could assist in the researchers' evaluation of the sample prior to analysis. If the preservation state is determined to be poor, different approaches may be required in order to ensure the accuracy of the lead analysis results or the sample can be rejected.

Other studies demonstrated the value of the concurrent employment of methodologies. Swanston et al. (2012) used SR-XRF at the Canadian Light Source (CLS) to support the biogenic uptake of Pb and Sr into the fibria of an individual excavated from a late 18<sup>th</sup>/early 19<sup>th</sup> century cemetery in Antigua. By combining the synchrotron data with histological images obtained through light microscopy, the researchers were able to map the localization of these elements into some, but not all, osteons, supporting the hypothesis that the elements were taken up during the individual's lifetime (Swanston, et al., 2012). The methods employed by Swanston et al. supports that combining techniques allows for better quality analyses. Without the employment of both light microscopy and SR-XRF together, the information would have been less contextual. Light microscopy alone shows only physical structure and SR-XRF gives information about



elemental make-up. Performed together, these techniques allow for more precise location of elements on or in a given material.

Further investigation into the fate of the individuals from the Franklin expedition was made by Martin et al. (2013). Using SR- $\mu$ XRF at the NSLS to examine the lead distribution and LA-ICP-MS to test isotope ratios, Martin et al.'s data contradicted previous conclusions made by Kowal et al. in 1991; it appeared unlikely that tin solder from canned food was the cause of lead poisoning of the crew (Kowal, et al., 1991; Martin, et al., 2013). Martin et al. (2013) found that the distribution of lead through the skeletal elements tested was consistent with long-term exposure, not acute exposure, and the isotopes found in the bones differed significantly from the isotopes found in the tin solder, thereby negating canned food as the cause of lead poisoning. With the employment of both techniques, Martin et al. (2013) were able to gather sufficient evidence to question the conclusions previously drawn by Kowal et al. (1991).

The BESSY II and DORIS synchrotrons in Germany were employed by Zougrou et al. (2014) to assist in examining the diagenetic effects on the fossilized bones of an artiodactyl and a perissodactyl excavated in Greece (Zougrou, et al., 2014). The researchers used light and scanning electron microscopy along with SR-XRF, EXAFS and XANES to fully analyze the diagenetic changes that occurred in the bones (Zougrou, et al., 2014). They found that Fe and Mn were the most prevalent metal impurities in the bones and that they gained access via the periosteum, medullar cavities and Haversian canals (Zougrou, et al., 2014). Analysis of the depositional material indicated a source of sulphur. Given the presence of Fe in the soil and the sulphur in both the collagen and the rock formation in which the fossils were deposited, the observation of ubiquitous pyrite inclusions was explained (Zougrou, et al., 2014). Application of

techniques such as these together can give archaeologists a fuller picture of the taphonomic changes that have affected the specimens or remains they are investigating.

## 7.5 Summary

The initial investigations described in this chapter described the foundations of elemental analysis of biological materials using SR-XFI. Studies involving detection of lead in modern human bone soon followed. The next group of analyses focused on the distribution of lead in either animal or human bones. It was found that human bones tended to have discrete locations of lead, and that it was particularly associated with the calcification tidemark (Zoeger et al., 2006; Zoeger et al., 2008). Using SR- $\mu$ XANES, lead was also found to have spectra consistent with incorporation into the hydroxyapatite crystal (Meirer et al., 2011). The impact of diseases and age on human bone was also examined by SR-XFI and it was found that these factors have an impact on porosity and focusing techniques, respectively (Zhang et al., 2005; Fei et al., 2007; Rao et al., 2009). These results have implications regarding archaeological material as well. Bones of diseased individuals may be more subject to diagenetic change due to their increased porosity and there may have also been elemental loss during the individual's lifetime, which would result in decreased accuracy in assessment of total skeletal burden of a given trace element (Lambert et al., 1985; Grupe, 1988).

SR-XFI studies involving archaeological bone often examine the effects of fossilization. Due to SR-XFI's ability to determine the location of lead in bone, it was used in several studies to help ascertain the origin of the detected lead, whether it had been introduced during the individuals' lifetimes or introduced diagenetically from the depositional environment.

The final group of investigations in this chapter each describe the use of an SR-XFI method in combination with another analytical method. The use of complementary techniques is better able to contextualize information than the use of any one method alone.

## **Chapter 8 – Methods**

### **8.1 Sample Preparation**

#### **8.1.1 Age-at-death estimations and ancestry determination**

The skeletal remains of 30 individuals excavated from the English Harbour, British Royal Navy hospital cemetery were analyzed by Tamara Varney in order to estimate age-at-death and determine ancestry (Varney, 2003). Ancestry was assessed using craniofacial features and following methods outlined in Gill and Gilbert, as well as Rhine (Varney, 2003; Gill & Gilbert, 1990; Rhine, 1990). Due to lack of skull preservation, only 14 of the individuals could be considered for ancestry determination (Varney, 2003). Seven individuals were found to be of European ancestry and 7 were of African ancestry (Varney, 2003). The ages of the interred ranged from newborn to over 50 years of age (Varney, 2003).

The analysis of these human skeletal remains has been approved by Dr. Reginald Murphy of National Parks Antigua, the curator of the remains, as well as by the Lakehead University Research Ethics Board as part of project 042 13-14.

#### **8.1.2 Sample selection**

Twenty four male individuals of the 30 sets of remains were selected for analysis because they had determinable age ranges. This set of 24 included the 14 individuals who had determinable ancestry (see Table 8.1 for details). The individuals under five years of age were not selected due to several factors. Metabolic and bone growth rates of very young individuals would potentially complicate interpretation of bone lead data. Since no potential mothers were interred in the cemetery, it would also be difficult to ascertain whether the lead was developed in utero or following birth. In addition, their bone material diameter was both too thin and too friable to allow for informative scanning by SR-XFI.

The fibula was chosen for trace element analysis, as it was generally the best represented and best-preserved long bone amongst the remains. Cortical bone is preferred over trabecular bone for studies of long term lead exposure, due to the lower turnover rate of cortical bone, as compared to trabecular bone, it is considered more reflective of total skeletal burden (Wittmers et al., 1988). Cortical bone is also less subject to diagenetic change than trabecular bone due to its reduced surface area as compared to trabecular bone (Lambert et al., 1985; Grupe, 1988).

**Table 8.1- Individuals excavated from the cemetery of the British Royal Navy Hospital cemetery in English Harbour, Antigua, West Indies selected for ICP-MS analysis**

<b>Burial Number</b>	<b>Age (years)</b>	<b>Ancestry (A=African, E=European, ND=non-determinable)</b>
B1	45-49	A
B2	25-29	E
B3	30-34	E
B4	50-60	A
B5	16-18	E
B6	25-29	E
B8-1	14-15	ND
B8-3	20-29	ND
B9a	18-20	ND
B9b	20-29	ND
B12a	35-39	A
B13	45-49	A
B14	35-39	ND
B15a	35-39	E
B16	16-18	ND
B17	35-39	ND
B18	30-35	E
B19a	40-45	E
B19b	20-24	ND
B22	20-29	ND
B23	35-39	A
B24	25-29	A
B25	20-25	A
B26	14-18	ND

### **8.1.3 Bone cleaning**

All bone samples were gently cleaned with a clean toothbrush, using water filtered first by reverse osmosis and then further purified by a Millipore Synergy<sup>®</sup> unit, to remove residual materials from excavation.

### **8.1.4 Bone cutting**

Bone sections of approximately one inch were cut from the fibulae using a Ryobi BS903 band saw at Lakehead University in ON, Canada and then sent to the University of Saskatchewan for further analysis.

Following methods used in Swanston et al. (2012), prior to synchrotron analysis thin bone sections of 300 $\mu$ m thickness were cut from the larger sections using a Buehler<sup>®</sup> Isomet<sup>®</sup> Low Speed Saw using a Norton Company diamond wheel. These thin sections were then ground to 100 $\mu$ m using sandpaper (2000 grit, 3M) after which residual material was removed using ultrasonication for 10min.

## **8.2 Sample Analysis**

### **8.2.1 ICP-MS**

ICP-MS analysis was performed to quantitate the lead within the fibulae. It was the method of choice due to its sensitivity, particularly since lead is a trace element in bone, and also because ICP-MS's utility, accuracy and precision when analyzing lead in bone has been well-established in published investigations.

Preceding ICP-MS analysis, bone sections were heated to dryness at 60°C in a slide dryer, dependent on state of preservation (Swanston, 2014). The dried bone samples were then ground to powder between two plastic weigh boats before being sent to the laboratory for ICP-MS (Swanston, 2014).

Inductively-coupled plasma-mass spectrometry was performed at the University of Saskatchewan following the methods outlined in Stefanova et al. (1990), Jenner et al. (1990), and Jackson et al. (1990). Briefly, approximately 0.1g of bone sample was digested in a hydrofluoric acid (HF) and nitric acid (HNO<sub>3</sub>) mixture in Teflon bombs and then evaporated to dryness, dissolved a second time in HNO<sub>3</sub> and evaporated to dryness again, and then suspended in 2 or 3mL of 8M HNO<sub>3</sub> (Jenner et al., 1990). The aliquot was transferred to a 125mL bottle and diluted with double distilled water to a total mass of 90g (Jenner et al., 1990). An autosampler, rinsed with 1M HNO<sub>3</sub> was used to transfer the samples into the ICP-MS (Jenner et al., 1990). Lens settings were such that sensitivity was maximized and oxide formation was minimized (Jenner et al, 1990). Instrument settings were as per outlined in Jackson et al. (1990) and Jenner et al. (1990). The run was comprised of 24 samples, and also included two repeat analyses and a triplicate analysis of the BCR2 reference material (Jenner et al., 1990). Internal standards were also employed (Swanston, et al., 2012). Data was acquired on manufacturer software (Jenner et al., 1990). Correction factors for matrix and drift were applied to calculations of element concentration (Jenner et al., 1990).

### **8.2.2 SR-XFI**

The presence and location (distribution) of lead was detected in the thin bone sections using SR-XFI at the Advanced Photon Source (APS) in Argonne, IL. Lead's location within bone can give an idea of the timing of exposure(s) and assist in assessing the nature of the deposited lead, i.e. whether it is diagenetic or biogenic in origin. The thin bone sections previously analyzed by light microscopy were mounted between two Thermanox<sup>®</sup> metal-free coverslips (Cedarlane, Burlington, ON) which were secured together using double-sided Scotch tape. The coverslips were then secured to the detector's stage. The detector used was a silicon

drift diode with a polycapillary snout attached, set at a 45° angle to the sample. The 20 ID beamline at the APS, modified by an undulator device to increase signal strength, was set at 16.5keV to allow for strontium detection, as multiple element maps were being analyzed for each bone section. The scan area on the sections was set to 2mm by 2mm in most cases, some larger scans were taken for some samples; the scan areas chosen included the periosteal surface. L $\alpha$ 1 lead signal was found at about 10.5keV; Pb L $\beta$  and L $\gamma$  lines were also monitored.

Only three scans were performed for this investigation. The intention of the SR-XFI analysis is to provide supporting evidence that the lead detected by ICP-MS in the fibulae samples was deposited during the individuals' lifetimes and not introduced by the depositional environment. ICP-MS and SR-XFI were used together in order provide both the quantity and the distribution of lead within the bone samples.

Raw data were captured, and using custom software, the data were converted into images. ImageJ was used to for image editing ([www.rsbweb.nih.gov/ij](http://www.rsbweb.nih.gov/ij)).

### **8.2.3 Statistical analysis**

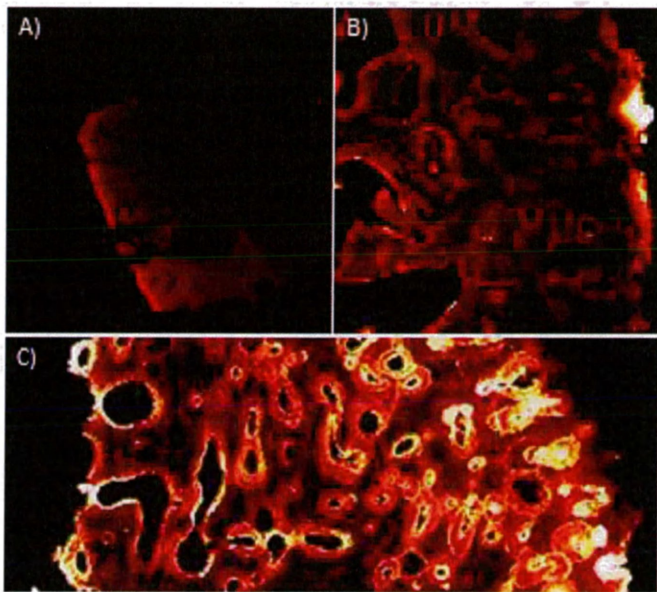
Lead levels determined by ICP-MS were analyzed using Microsoft Excel. The mean lead levels and standard deviations for the individuals of European ancestry and individuals of Africa were calculated. The means (distributions) were then compared using a nonparametric statistical method, the Wilcoxon Rank Sum Test, due to the small data set (n<30). Box and whisker plots were generated to display the means, distributions and ranges of lead levels of the two ancestries. The 24 individuals were separated into different age groups, defined by the decade they were in at their time of death, i.e.  $\leq$ 20yrs, 20-29yrs, 30-39yrs, 40-49yrs, as well as 50yrs and over. The mean lead level and standard deviation of each group was calculated. Box and whisker plots



were generated (where possible) to display the means, distributions and ranges of lead levels of the age groups in order to visualize any pattern that may be occurring.

## **Chapter 9 - Results**

Figure 9.1 shows three images captured at the Advanced Photon Source in Argonne, Illinois, USA. The Haversian canals and cement lines of osteons are visible in B as they fluoresce red. The locations of Haversian canals fluoresce brightly in C and some cement lines are still visible. Note that the images show increasing lead fluorescence as the concentration of lead increases from samples A to C. The uneven distribution of lead through the bone microstructure, more evident in images B and C, supports biogenic introduction of lead.



**Figure 9.1 – Images generated by SR-XFI at 10.5keV by the 20ID beamline at the APS showing lead fluorescence: A) burial 2 - 22.18ppm Pb by ICP-MS, B) burial 19a – 101.85ppm Pb by ICP-MS and C) burial 4 – 163.1ppm Pb by ICP-MS. Increasing brightness (from black to red to white) indicate increasing levels of lead.**

The ICP-MS data collected below in Table 9.1 shows a significant spread in the lead concentrations of the cortical bone. The lowest value, 10.08ppm Pb, belongs to an individual who was between the ages of 20 and 29 years at their time of death. The highest value, 251.49ppm Pb, belongs to an individual who was between 35 and 39 years at their time of death.

**Table 9.1 – ICP-MS-determined lead levels, ancestry and age of individuals excavated from the British Royal Navy Hospital cemetery at English Harbour, Antigua, West Indies**

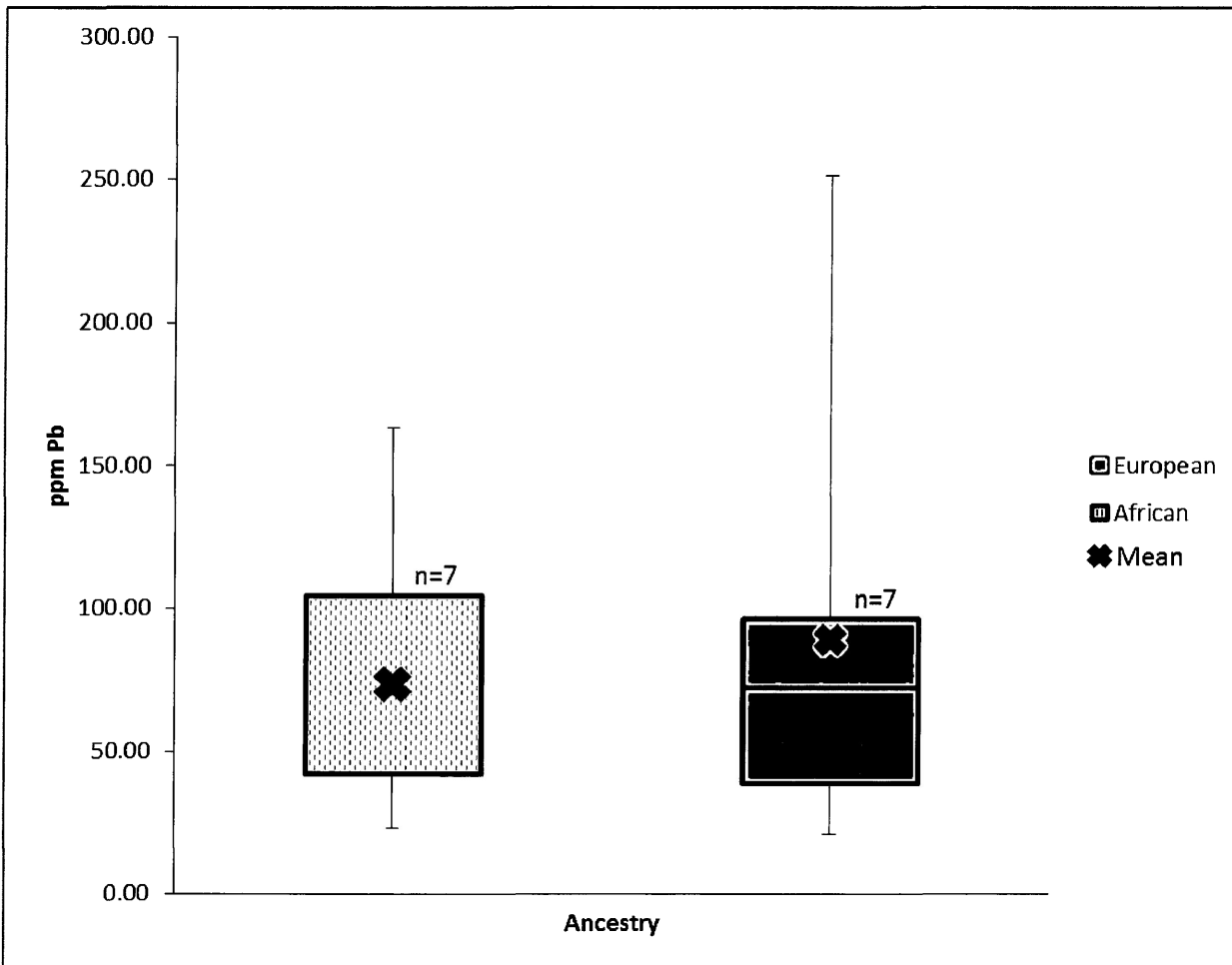
<b>Burial Number</b>	<b>Age (years)</b>	<b>Ancestry (A=African, E=European, ND=non-determinable)</b>	<b>Pb (ppm)</b>
B1	45-49	A	41.19
B2	25-29	E	22.18
B3	30-34	E	72.25
B4	50-60	A	163.10
B5	16-18	E	90.18
B6	25-29	E	21.03
B8-1	14-15	ND	214.77
B8-3	20-29	ND	36.89
B9a	18-20	ND	149.04
B9a (duplicate)	18-20	ND	147.14
B9b	20-29	ND	151.92
B12a	35-39	A	86.93
B13	45-49	A	41.97
B14	35-39	ND	30.90
B15a	35-39	E	251.49
B16	16-18	ND	73.19
B17	35-39	ND	15.99
B18	30-35	E	54.72
B19a	40-45	E	101.85
B19b	20-24	ND	61.19
B22	20-29	ND	10.08
B23	35-39	A	121.77
B24	25-29	A	42.09
B25	20-25	A	23.08
B26	14-18	ND	21.70
B26 (duplicate)	14-18	ND	21.73

Graphical comparison of the lead level means of the African and European ancestries show a difference between their values, as was expected. The standard deviations, shown in Table 9.2 and the ranges (standard error) shown in the bars in Figure 9.2, demonstrate the wide distribution of values found in both ancestries. Figure 9.2 also demonstrates that individuals of European ancestry had a slightly higher mean and a larger standard deviation than the individuals of African ancestry, but there is noticeable skew in the African data towards values of Pb that are

higher than the median. Calculations for quartile points in the box and whisker plots were performed using Microsoft Excel 2010.

**Table 9.2 – Mean lead levels by ancestry of individuals excavated from British Royal Navy hospital cemetery at English Harbour, Antigua, West Indies**

Ancestry	Number of Individuals	Mean Pb (ppm)	Standard Deviation (ppm)	Minimum Pb (ppm)	Median Pb (ppm)	Maximum Pb (ppm)
African	7	74.30	51.80	23.08	42.09	163.10
European	7	87.67	78.63	21.03	72.25	251.49



**Figure 9.2 – Lead levels by ancestry of the individuals excavated from the British Royal Navy hospital cemetery at English Harbour, Antigua, West Indies. The bars indicate standard error,**

In order to test to see if the observed difference in mean lead levels by ancestry was significant, a Wilcoxon Rank Sum Test was performed to test for a difference between the distributions of the two means. This test is the equivalent of a mean comparison (t-test) for nonparametric data (Mendenhall, et al., 2009).

### Wilcoxon Rank Sum Test

Hypothesis: the mean Pb levels of the two ancestries are significantly different

Null Hypothesis: there is no significant difference between the mean Pb levels of the two ancestries

Where:

- sample set 1 = individuals of African ancestry
- sample set 2 = individuals of European ancestry
- $n_1$  = the number of observations in sample set 1
- $n_2$  = the number of observations in sample set 2
- $T_1$  = the rank sum of the sample set 1
- $T_1^* = n_1(n_1+n_2+1)-T_1$

The smaller value (of  $T_1$  and  $T_1^*$ ) is chosen as the test statistic,  $T$

**Table 9.3 - Wilcoxon ranking of individuals with known ancestry**

Rank	Ancestry	Pb (ppm)
1	E	21.03
2	E	22.18
3	A	23.08
4	A	41.19
5	A	41.97
6	A	42.09
7	E	54.72
8	E	72.25
9	A	86.93
10	E	90.18
11	E	101.85
12	A	121.77
13	A	163.10
14	E	251.49

$$T_l = 52$$

$$T_l^* = 53$$

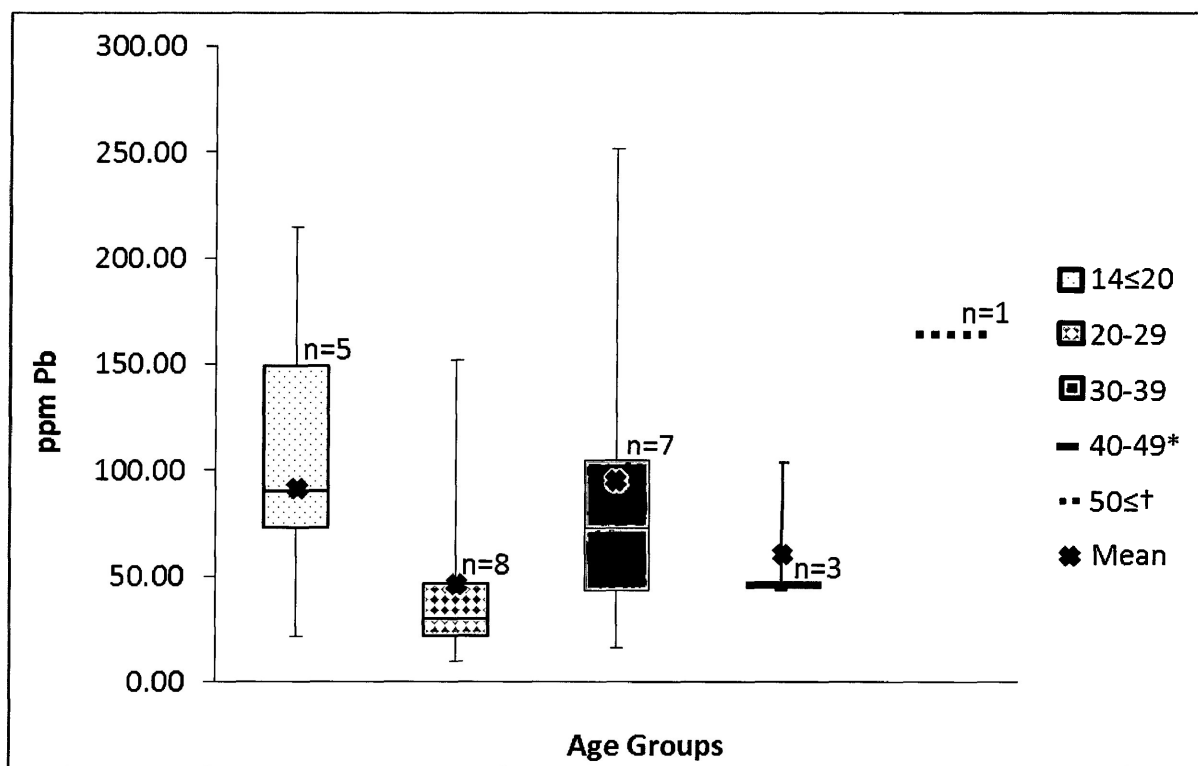
Therefore  $T_l$  is chosen as the test statistic  $T$ .

The test statistic  $T$  is greater than all critical values for  $\alpha$ 's ranging from 0.25 to 2.5% (Mendenhall, et al., 2009). Therefore the null hypothesis cannot be rejected; the means are not significantly different between the two ancestries.

The mean lead levels according to age groupings (Table 9.4) were compared graphically in order to determine if there was a pattern according to age. An increase according to age was expected, but none was observed. While the mean lead levels differed amongst the age groups, no pattern was found. The standard deviations, shown in Table 9.4, and the ranges, as shown by the error bars in Figure 9.3, demonstrate the broad distribution of lead levels within each age group. Calculations for quartile points in the box and whisker plots were performed using Microsoft Excel 2010.

**Table 9.4 - Lead levels by age group of the individuals excavated from the British Royal Navy hospital cemetery at English Harbour, Antigua, West Indies**

Age Group (yrs)	Number Of Individuals	Mean Pb (ppm)	Standard Deviation (ppm)	Minimum Pb (ppm)	Median Pb (ppm)	Maximum Pb (ppm)
14≤20	5	91.48	74.24	21.70	90.18	214.77
20-29	8	46.06	45.60	10.08	29.98	151.92
30-39	7	90.58	79.19	15.99	72.25	251.49
40-49*	3	61.67	34.79	41.19	41.97	101.85
50≤†	1	163.1	N/A	163.1	163.1	163.1



**Figure 9.3 – Lead levels by age group of the individuals excavated from the British Royal Navy hospital cemetery at English Harbour, Antigua, West Indies. The bars indicate standard error.**

\*the 40-49 group has no quartile positions as there were only 3 individuals in this group

†the 50≤ group has only one individual and therefore only one data point

## Chapter 10 - Discussion and Conclusions

### 10.1 Discussion

The skeletal remains of 24 individuals recovered from the cemetery belonging to the Colonial period British Royal Navy hospital at English Harbour, Antigua, West Indies, were successfully analyzed for lead. The cemetery is believed to be unique in the Caribbean for the colonial time period as it was the unsegregated burial ground for individuals of either European or African ancestry. The interred were low-ranking members of the British Royal Navy and individuals believed to be the King's Negroes, a group of specialty-trained enslaved labourers who belonged to the Navy (Varney, 2003). Historical documents support the use of the hospital by all naval personnel including enslaved labourers (Nicholson, 1993).

Diagenesis is a valid and prevalent concern for research involving lead levels in archaeological bone. Early studies involving skeletal materials from the Roman era were found to be significantly contaminated by the surrounding soil, which had likely been contaminated by lead coffins interred nearby (Waldron, 1981). It was found that testing burial soil for lead quantity and/or lead isotopic ratio could help to address the issue of diagenesis, as could the location of the lead on the bone itself (Waldron, et al., 1976; Waldron, et al., 1979; Barry & Connolly, 1981; Wittmers, et al., 2008). No soil samples were retrieved at the time of the Royal Navy Hospital's cemetery excavation; instead the technique of synchrotron X-ray fluorescence (SR-XRF) was applied to ascertain the nature of the lead in the bones. Swanston et al. (2012) demonstrated that SR-XRF can be used to determine the location of lead in bone by comparing the generated elemental maps to histological sections produced by digital light microscopy. Figure 9.1 shows the result of three of the SR-XRF scans generated as part of this study, with increasing brightness (from black through red to white) indicating increasing amounts of lead. Other than the edge artifact, seen as the lit edge of the bone sample due to the geometry of the



beam and the absorbing tendency of the sample, individual A has minimal lead visible in the bone microstructure, individual B has significant microstructure outlining including cement lines and individual C has very bright microstructure outlining (Swanston, et al., 2012). These scans are consistent with the associated ICP-MS data: individual A has 22.18ppm Pb, individual B has 101.85ppm Pb and individual C has 163.10ppm Pb in their respective fibulae. The incorporation of the lead into the microstructure supports the hypothesis that the lead in the bone is likely biogenic in origin. The variable levels of fluorescence across the bone microstructure also support a biogenic origin rather than diagenetic deposition (Swanston et al., 2012). High lead concentrations at the periosteal surfaces of individuals B and C do not necessarily indicate diagenesis either, as bone remodelling tends to occur at the periosteal surface; if the exposure to lead was perimortem, the outer surface would be an expected location for lead (Smith & Walker, 1964).

The ICP-MS data shown in Table 9.1 reveals a broad spectrum of Pb levels in the twenty four individuals tested. The minimum level found is 10.08ppm, found in an individual of indeterminate ancestry who was between 20 and 29 years of age. The highest level, 251.49ppm, was found in a European individual who was between the ages of 35 and 39 yrs. The wide-ranging levels found in the individuals tested indicate that the lead exposure experienced by each individual was varied.

The lead levels found in this study, as well as the degree of variation amongst individuals, are not uncommon in archaeological studies similar to this work. Table 10.1 summarizes the ranges of lead levels found in the studies performed by Aufderheide et al. (1981), Aufderheide et al. (1985), Corruccini et al. (1987), Kowal et al. (1989), Keenleyside et

al. (1996), Keenleyside et al. (1997) and Aasebo and Kjaer (2009). Detailed information regarding each of these investigations can be found in chapters 5, 6 and 7 of this study.

**Table 10.1 - Lead level ranges in select studies of archaeological bone**

Study Author(s)	Year	Site	Individuals	Number of Individuals Analyzed	Range (ppm)
Aufderheide et al.	1981	The Clifts Plantation	Proprietor's family <sup>1</sup>	5	128-258
			Enslaved labourers <sup>2</sup>	11	8-96
Aufderheide et al.	1985	Catoctin Furnace	Enslaved labourers <sup>3</sup>	24	0-233.1
		College Landing	African descent	17	9.9-93.3
		Governor's Land	European descent	23	5.7-264.8
		Irene Mound	Plantation owners <sup>1</sup>	16	4.9-183.8
Corrucini et al.	1987	Newton Plantation	Enslaved labourers <sup>3</sup>	48	0-424
Kowal et al.	1989	Beechey Island	Franklin expedition crew <sup>1</sup>	3	69-183
		King William Island	Franklin expedition crew <sup>1</sup>	8-15	87-223
			Inuit Caribou	17 samples 2 samples	1-14 N/A
Keenleyside et al.	1996	King William Island	Franklin expedition crew <sup>1</sup>	11+	61.8-1739.8
Keenleyside et al.	1997	King William Island	Franklin expedition crew <sup>1</sup>	7-9	49-204
Aasebo & Kjaer	2009	Swedish House at Kapp Thordsen	Swedish sealer <sup>1</sup>	1	N/A

<sup>1</sup>European ancestry

<sup>2</sup>European and African ancestries (1 individual and 10 individuals, respectively)

<sup>3</sup>African ancestry

The causes of the variance are worthy of consideration, but the contributing elements are complex. Many factors affect lead levels. Metabolism has multiple effects on the amount of lead detected in bone. Adults incorporate about 90% of the lead they absorb into their bone tissue and subadults incorporate about 95% of the lead they absorb, but the absorbance rates are significantly different; adults absorb 10% of the ingested lead whereas subadults absorb 50% of the lead ingested, so lead absorbance is impacted by age (Bogen, et al., 1976; Ziegler, et al., 1978). The source and length of exposure will impact the lead levels detected. The metabolic rate of the individual has an effect as well. Lead levels are also impacted by the personal habits of the individuals during their lifetimes. Sources are not indicated by quantitation of bone lead

levels alone. In order to gain some insight into where the lead originated, isotopic ratio studies need to be undertaken. Sourcing also requires a template; there must be something to which the isotopic ratios can be compared (Aasebo & Kjaer, 2009; Kowal, et al., 1991; Bower, et al., 2005; Bower, et al., 2007). The length of exposure that an individual received has not yet been determinable. Bulk lead levels are not easily correlated with length of exposure. Lead doses can be acute or chronic, and depending on the level of either exposure type, and the time span over which either occurred, similar amounts of incorporation into bone can be seen (Rabinowitz, 1991). A large acute dose cannot yet be distinguished from a moderate to low chronic dose as the amount of lead that will be deposited in the bone could be very similar. Bone tissue maintenance is also an ongoing process through life. Bone is resorbed and laid down continually, so natural resorption, i.e. routine bone remodelling, may lower bone lead level. If lead exposure is no longer occurring then routine remodelling of bone would release some of the bone lead into the bloodstream, and the new bone that would be laid down would not have as much lead incorporated into it, thereby distorting total lead burden results (Schutz, et al., 2005). Confounding of results may also be seen if the lead source changes to one of lower intensity. Additionally, lead mobilized from bone into the blood can then be reabsorbed back into bone, so new lead exposure is not required to introduce lead into bone. Most of the lead that re-enters the bloodstream will be re-deposited in bone but a small percentage will be excreted in urine (Bogen et al., 1976). Malnutrition and disease states can also severely impact lead metabolism and bone resorption rates, and since the individuals tested were of low socioeconomic status and likely in hospital when they died, their overall health must be considered as well (Varney, 2003; Tolouian, et al., 2010; Heard & Chamberlain, 1982). Periods of fasting or low food intake can result in the lead absorption rate increasing to 60% of the lead ingested, when the normal level is

10% (Heard & Chamberlain, 1982). Individual practices and behaviours also contribute to lead levels significantly. In view of the fact that lead has been experimentally found to be a contaminant of rum from the West Indies in the Colonial period and that rum was a ration from the British Royal Navy, the drinking habits of an individual would have had an impact on their levels as (Lloyd, 1969; Hunter, 1785 cited in Handler, et al., 1986). Without knowing each individual's complete history or doing further testing, it is therefore challenging to postulate on the causes of the variation seen amongst all of the determined lead levels.

Table 9.2 and Figure 9.2 indicate the mean lead levels found in the individuals of European and African ancestry. The mean lead level of the individuals of African ancestry was found to be  $74.30 \pm 51.80 (69.72\%)$  ppm and the mean European lead level was found to be  $87.67 \pm 78.63 (89.69\%)$  ppm. The Wilcoxon rank sum test determined that the lead level distributions of individuals of African and European descent were not significantly different.

This result differs from previous studies of contemporaneous populations where the lead levels of individuals of European and African ancestry were analyzed. Aufderheide et al. (1981) examined skeletal remains from a segregated burial at a Virginian plantation and found that the mean lead level of the individuals of European ancestry, 185 ppm, was significantly higher than that of the individuals of African ancestry, 35 ppm. Corruccini et al. (1987) found a different result in their study of remains from a cemetery for enslaved labourers belonging to a Barbadian plantation; the individuals of African ancestry had significantly high levels. A mean bone lead level of 117.6 ppm was found (Corruccini et al., 1987). In both cases, the practices and behaviours of the respective groups were found to be the most probable source of the differences between the exposure levels (Aufderheide, et al., 1981; Corruccini, et al., 1987). Aufderheide et al. (1981) believed that the European practice of eating food and drinking from pewter dishes

may have resulted in their high levels of lead. The food itself may have already been contaminated by lead-glazed storage vessels which were commonplace at the time (Aufderheide, et al., 1981). The enslaved labourers of the same plantation did not use the same serving ware for their own food consumption (Aufderheide, et al., 1981). The Barbadian enslaved labourers, however, were believed to have been exposed to lead either while working in a rum distillery or by consuming rum (Corruccini, et al., 1987). It had been found that enslaved labourers had relatively easy access to ‘new rum’ which was the primary distillation product in sugar processing and known to be heavily contaminated with lead and dangerous chemicals (Handler, et al., 1986). Simply working in a distillery posed risks as well. Rum distilleries were common on sugar plantations, which were found throughout the colonial Caribbean (Handler, et al., 1986). Much of the distillation equipment was composed of lead and the vapours that came off of the boiling cane liquid were toxic (Handler, et al., 1986). The results found in this study of skeletal remains from a Colonial period British Royal Navy hospital cemetery in Antigua are different again. The mean lead levels of individuals of African and European ancestry are not significantly different from one another. This may be the result of similar practices and habits amongst the two ancestral groupings, including rum consumption. Rum was a daily ration given by the Navy to its personnel (Lloyd, 1969). Rum, particularly “low-wine”, the primary rum distillation product, was easily accessible on the island to anyone, including enslaved labourers (Handler, et al., 1986; Oliver, 1894). Many sailors drank to excess frequently, and also willingly donated some of their supply to younger members whom were not given the rum ration (Thomas, 1968; Oliver, 1894). Rum found its way onto the hospital grounds frequently enough that a cactus hedge was constructed to deter the practice (Nicholson, 1993). Lead-glazed pottery used by the hospital was found in an associated midden, and a lead compound used in medical

practice at the time, lead acetate, was also found in a vessel in the same refuse pile (Nicholson, 1993). Food sources were likely very similar as well, as the King's Negroes were owned and sustained by the Navy, and it is possible that the storage vessels were contaminated with lead (Nicholson, 1991; Handler, et al., 1986). Additionally the drinking water at the naval dockyard was also likely contaminated as many of the water catchment system's troughs and cisterns were made from or contained lead (Handler, et al., 1986). So even if an individual did not consume rum as a beverage, lead would have contaminated their water or tea.

It is worthy to note that the more similar means and distributions of the two ancestries in this study may also result from greater similarities in experiences between the social statuses of these two specific groups. Archival documentation found at the time of the cemetery's excavation indicated that the individuals interred in the Nelson Dockyard's hospital cemetery were low-ranking officers, sailors, and labourers (Varney, 2003). It is believed that the individuals of African ancestry were part of the King's Negroes, as these were the only enslaved labourers that belonged to the British Royal Navy and it was known they were treated at the dockyard's hospital (Nicholson, 1991; Nicholson, 1993; Varney, 2003). The King's Negroes were given specialized training, such as sail-making, as European tradesmen were disinclined to work in the West Indies due to the level of disease in the area (Crewe, 1993). Nutritional studies involving stable nitrogen and carbon isotope analysis of the individuals interred in the cemetery indicates that both ancestries shared a common protein source, but differed more in their carbohydrate staple (Varney, 2003). The nutritional studies, the King's Negroes' specialized training and treatment at the dockyard hospital along with European naval officers and, finally, interment in an unsegregated cemetery, all provide evidence of greater similarity in experiences

between the socioeconomic statuses of the individuals of European and African ancestry that were interred in the British Royal Navy hospital cemetery in English Harbour.

The wide range of values, as indicated by the large standard deviations in the ancestral groups, supports considerable differences in labour duties, practices and behaviours, and, thereby, exposures of individuals. The length of time an individual had belonged to the Navy would likely also have an impact. Their duties within the Navy would likely have had a significant effect. If they had worked as smiths or with piping their lead levels would likely be higher than those of workers holding positions with less exposure to metals on a regular basis.

Table 9.4 and Figure 9.3 display the results of the lead level comparison according to age grouping. The twenty four individuals were subdivided into five groups. The first group was comprised of subadults between 14 and 20yrs. The remaining individuals were separated according to which decade of age they were in at their time of death.

The mean lead levels according to age group can be found in Table 9.4. Typically, when lead levels of a population are grouped according to age, a positive correlation is seen as lead levels increase with age (Wittmers, et al., 1988). This pattern is not seen in the population from the British Royal Navy hospital cemetery at English Harbour. In the tested population, the group of younger individuals had a high mean lead level and then there is a sharp drop to the mean lead level in individuals aged 20-29yrs. The 30-39yrs individuals' mean lead level increases from the group before it, but the following group, those 40-49yrs, is again lower. The single individual that was aged 50-59yrs had a high level of lead, 163.10ppm, but as there were no other individuals in this age group, his level may not be representative of individuals of that age group.

Certainly, the small sample set size limits the confidence that can be given to the accuracy of the data. There is evidence to support the results seen in this study, however. The

high mean lead levels in the two groups of young individuals are not surprising, considering their higher lead absorption rate and higher rate of bone deposition. Subadults absorb 50% of the lead they ingest or inhale, as compared to the 10% that is absorbed by adults (Bogen, et al., 1976; Ziegler, et al., 1978). Therefore, exposure to the same lead source by a subadult and by an adult would result in a higher lead dose to the subadult. Lead was present in the dockyard's drinking water, and while subadults were not given rum rations directly by the navy, compatriots would often share their supply (Thomas, 1968; Handler, et al., 1986). The consumption of either would have greater effect on the subadults than on adults. In regards to the variable mean lead levels of the older age groups, in order for lead levels to continually increase with increasing age, the sources must remain constant (Wittmers, et al., 1988). The lack of patterning suggests that their exposures were not constant, as the mean bone lead levels did not steadily increase with age. The high standard deviations also imply that there was significant variation in individual habit and behaviour. This may result from multiple factors. While rum was a ration from the navy, not all individuals consumed it (Thomas, 1968; Lloyd, 1969). Press-ganging was a known method of naval recruitment, and it was relatively indiscriminate (Lloyd, 1969). Therefore, even if sources within the navy were constant, the age of the seamen's entry was not necessarily so. Additionally, due to a lack of historical documentation, we know little of these men's backgrounds. The accrual of lead in bone over time may have started at any age for the individuals tested in this study. At this time, there is no known method to determine at what point in the individual's lead exposure occurred by analyzing bone tissue (Rabinowitz, 1991).

The prevalence of lead poisoning in the British Royal Navy cannot be directly answered with the results of this study. The bone lead levels of several of these individuals do however indicate a likelihood that they had suffered from lead poisoning at some point. While BLLs and



bone lead levels are not easily correlated due to bone remodelling, it has been found that tibial bone accrues  $1\mu\text{g Pb}$  per g of bone per year if the BLL is at  $20\mu\text{g/dL}$  (Coke et al., 1994 cited in Keenleyside et al. 1996). Fibulae would likely accumulate lead at a similar rate as tibiae, as they are both long bones made of predominantly cortical bone (Wittmers, et al., 1988). The highest lead level observed in the individuals tested was  $251.29\mu\text{gPb/g}$  of bone (i.e. ppm), and his age at death was estimated to be between 35 and 39 years. If his lead accumulation was averaged over his lifetime, this would equate to an accrual of a minimum of  $6.44\mu\text{g Pb}$  per g bone for each year of his life. If the relationship of blood lead to bone lead was assumed to be linear, this would indicate a yearly BLL of  $129\mu\text{g/dL}$ . Serious symptoms of lead poisoning, including vomiting, convulsions, colic and weakness begin to appear once the BLL reaches about  $80\mu\text{g/dL}$  and more severe forms of these symptoms begin to appear at approximately  $120\mu\text{g/dL}$  (Handler, et al., 1986). This individual very likely suffered acute lead poisoning at some point during his lifetime. The same could be said for at least five other individuals of the twenty four individuals tested which equates to 25% of the tested population. This indicates that a significant number of this small population had substantial lead burdens. Unfortunately, it is unknown to what degree the lives and health of these individuals were impacted by the lead. The lack of clear lead level patterning associated with age and the great variability of lead levels observed within this population are of significant interest, particularly within the context of previous studies of contemporaneous populations.

While this study was successful in determining the lead levels of twenty four individuals from a British Royal Navy hospital cemetery (1793-1822CE) at English Harbour, Antigua, West Indies, there are some constraints that must be considered. The small sample set limits the applicability of the conclusions that can be drawn from the data. There may also be bias in this

sample set. All of the individuals examined in this study were interred at a hospital cemetery, which indicates that their state of health prior to their death was likely poor, and it may have been in decline for some time. It is also possible that some of the individuals buried in this cemetery had been hospitalized due to symptoms of lead poisoning, and that, ultimately, these effects resulted in death.

This investigation does still provide a window into a time period about which little is known. Few historical accounts of the Colonial period in Antigua were written, and as such, there has been a gap in the knowledge base regarding the European expansion into the West Indies. This study found a wide range of lead levels in the bone samples analyzed by ICP-MS. This breadth of range is not uncommon in studies of lead in archaeological bone, as can be seen in Table 10.1, and can be attributed to many factors, including metabolism, habits, labour duties and health. The SR-XFI scans of three bone sections supports a biogenic origin of the lead detected by ICP-MS, as the lead is incorporated in the microstructure of the bone, but its distribution is uneven across the section. The mean lead levels do not significantly differ between the individuals of African and European ancestry. This suggests that the sources of lead, including rum and water, were equally available to both groups, and that while individual levels varied significantly, the overall behaviours and practices of the two groups were comparable. It is also possible that a greater degree of similarity in experiences of their socioeconomic status groups contributed to their more similar values, as compared to the differences seen between ancestries in Aufderheide et al. (1981) and Hess et al. (2013). The lack of positive correlation between lead level and age suggests that exposure is not at a constant rate, and the high variability within each age group indicates significant individual variation. We lack information regarding both the age at which these individuals entered the British Royal Navy and

their lives prior to entry, so exposure may have occurred at any point in their lifetime, and their ages, individual habits and health would have significant effect on the lead levels detected.

Finally, analysis of the bone lead data indicates that it is likely that a minimum of 25% of this population suffered from severe lead poisoning symptoms at some point in their lifetime. These findings indicate that it is likely that a significant percentage of the British Royal Navy personnel suffered from varying severities of lead poisoning during their service.

## **10.2 Future Directions of Research**

There is yet more information yet available from the skeletal remains tested in this study. X-ray absorption near edge structure (XANES), a synchrotron technique, permits Pb speciation which may assist in determining whether the lead observed at periosteal surfaces is biogenic or diagenetic. Isotopic studies could be undertaken to examine the sources of the lead found in these individuals, but in order to do, Colonial era lead sources would be required for comparison. Higher resolution synchrotron imaging would allow for more detailed examination of the osteon. Through comparison to scans of prepared standards, concentrations of lead in specific microstructures, i.e. osteons, may be determined; this would give researchers insight into the dose an individual experienced at a point in time. The ability to determine the timing of exposure would be of significant benefit as well, as it may allow researchers to interrelate the individuals' results within a specific time period.

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## Appendix I

### Sample Calculations

#### Mean Calculation

Where:

$\bar{x}$  = mean of sample readings

n = number of readings

$$\bar{x} = (x_1 + x_2 + x_3 + x_4 + x_5 + x_6 + x_7) / n$$

$$\bar{x} = (41.19 + 163.10 + 86.93 + 41.97 + 121.77 + 42.09 + 23.08) / 7$$

$$\bar{x} = 74.30 \text{ ppm Pb}$$

#### Standard Deviation

Where:

$x_i$  = sample Pb (ppm)

$\bar{x}$  = mean of sample readings

s = standard deviation

n = number of readings

Pb (ppm)	$x_i$	$x_i - \bar{x}$	$(x_i - \bar{x})^2$
1	41.19	-33.11	1096.272
2	163.10	88.80	7885.44
3	86.93	12.63	159.5169
4	41.97	-32.33	1045.229
5	121.77	47.47	2253.401
6	42.09	-32.21	1037.484
7	23.08	-51.22	2623.488
Sum	N/A	N/A	16100.83

$$s = \sqrt{\frac{\sum(x_i - \bar{x})^2}{n - 1}}$$

$$s = \sqrt{\frac{16100.83}{6}}$$

$$s = 51.80$$

### Wilcoxon Rank Sum Calculations

Where:

sample set 1 = individuals of African ancestry

sample set 2 = individuals of European ancestry

$n_1$  = the number of observations in sample set 1

$n_2$  = the number of observations in sample set 2

$T_1$  = the rank sum of the sample set 1

$$T_1^* = n_1(n_1 + n_2 + 1) - T_1$$

$T_1$  = the rank sum of the sample set 1

$$T_1 = 3 + 4 + 5 + 6 + 9 + 12 + 13$$

$$T_1 = 52$$

$$T_1^* = n_1(n_1 + n_2 + 1) - T_1$$

$$T_1^* = 7(7 + 7 + 1) - 52$$

$$T_1^* = 53$$