

**Examining Biogenic and Diagenetic Lead Exposure with Synchrotron Radiation X-ray
Fluorescence Imaging of Experimentally Altered Bone**

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By

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Abstract

Trace elements, including the toxic trace metal of lead (Pb), have both the potential to provide valuable insights into past human lifeways, as well as a strong affinity for bone and dental tissues, making the analysis of them potentially useful to bioarchaeology. However, trace element analysis of archaeological skeletal remains is constantly hindered by diagenesis, the post-mortem chemical, physical, and biological transformations of skeletal remains, as these processes can interfere with the biogenic (lifetime) chemical composition of bone and teeth. New approaches may aid in overcoming some of these limitations. Synchrotron radiation X-ray fluorescence imaging (SR-XFI) can generate maps of trace metals, including Pb, in bone on a microstructural scale, and it has been proposed that this could be used to distinguish biogenic from diagenetic Pb exposure and provide insights into the individual life histories of Pb exposure. Recent technological improvements in SR-XFI, particularly the use of confocal optics, has permitted higher spatial resolution in element maps and optical, rather than physical, sectioning of fragile archaeological bone samples. The aim of this thesis was to experimentally test whether there are spatial differences in the distribution of Pb for diagenetic and biogenic modes of uptake in bone, and evaluate individual life histories of biogenic Pb exposure in a cadaveric population sampled from Saskatoon, Saskatchewan. To address these aims, this study used inductively coupled plasma-mass spectrometry (ICP-MS) and SR-XFI on bone samples from eighteenth to nineteenth century archaeological sites from Antigua and Lithuania representing biogenic and diagenetic Pb exposure, respectively, and experimentally altered modern bone samples donated to the Body Bequeathal Program (University of Saskatchewan, Saskatoon, SK). Pb concentrations in the cadaveric bone ranged from 1.2 to 7.1 $\mu\text{g/g}$. By contrast, the bulk Pb concentration of the Antigua sample was 253.94 $\mu\text{g/g}$ and the bulk Pb concentration of the Lithuania individual was 125 $\mu\text{g/g}$. SR-XFI

results demonstrated that there are marked differences in the spatial distribution of Pb corresponding to biogenic versus diagenetic uptake for both archaeological and experimentally altered modern samples. The modern Saskatchewan sample demonstrated a pattern of relatively low Pb exposure with higher levels of Pb exposure occurring in mature bone structures that formed earlier in life, likely during the era of leaded gasoline (pre-1980s).

Plain Language Summary

Though it is an extremely harmful and toxic substance, lead (Pb) has been widely used by humans for thousands of years in everything from paint and cosmetics to plumbing and food containers. Most of the Pb we ingest becomes stored in our bones and teeth, where it can remain for several years or decades. Therefore, it is an important and useful topic to the field of bioarchaeology (the study of past humans based on their skeletal remains). There are several techniques that can measure the levels of Pb (and other trace elements) in bones and teeth, but these techniques often encounter limitations. First, it is difficult to account for the effects of *diagenesis* (the changes that bones and teeth undergo in the burial environment); in other words, how much of the Pb within a bone or tooth is from a person's exposure to Pb during their lifetime, and how much Pb has seeped into the bone from the burial environment? Second, these techniques often cannot give us information about the timeline of Pb exposure. Was someone exposed to Pb consistently throughout their lifetime, or did it occur in a few events? Did the exposure occur earlier on in life or right before death?

This thesis used synchrotron radiation X-ray fluorescence imaging (SR-XFI), a technique that maps the distribution of Pb in bone. By examining which structures (e.g. newly formed versus mature structures) in bone contain Pb, it is possible to discover a timeline of Pb exposure. It may also be possible to use this technique to determine whether diagenesis has contaminated a bone sample with Pb from the burial environment. Bone samples from modern human donors were collected and part of each sample was exposed to Pb in a simulated diagenesis treatment. Bone samples from archaeological individuals were also collected: one, from colonial Antigua, where it is suspected that the British Royal Navy personnel stationed there experienced Pb poisoning, and

another, from a nineteenth-century Lithuanian crypt, where diagenetic Pb contamination is known to have taken place.

The Pb levels of each bone sample was determined with a technique called Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) and the distribution of Pb in each bone sample was mapped with SR-XFI. The ICP-MS results showed that the archaeological bone samples had Pb levels roughly 29 to 59 times higher than the modern humans from Saskatchewan. The SR-XFI maps showed that lifetime (*biogenic*) versus diagenetic Pb exposure distributes differently in bone and that the lifetime Pb exposure for the individuals from modern Saskatchewan were mainly exposed to Pb early on in life, likely when leaded gasoline was common (pre-1980s). This research is promising at demonstrating how these methods can be applied to help identify diagenesis in bone and understand the timeline and severity of Pb exposure in humans, past and present.

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Chapter 1—Introduction

1.1 Research Background

Concentrations and isotopic ratios of the trace elements from human skeletal remains have the potential to convey a vast array of information about past populations. Due to its long history of exploitation by humans and its toxic health implications, lead (Pb) in particular can provide rich insights into the health, industry, occupations, provenance, habitual activities, and social dynamics of past humans (Jarcho, 1964; Aufderheide et al., 1981, 1985; Reinhard & Ghazi, 1992; Gulson et al., 1997; Nakashima et al., 2011; Giffin et al., 2017; Laffoon et al., 2020). While Pb was used more extensively in the past, it remains a global public health issue today, including in North America, due to the continued existence of leaded pipe and house paint infrastructure (Health Canada, 2013; Safruk et al., 2017) and the environmental perseverance of Pb from tetraethyllead gasoline dating from the 1920s–1980s (Nriagu, 1990; Flegal et al., 2010; Health Canada, 2013).

Pb has a strong affinity for the skeleton; as a divalent cation, Pb can substitute for calcium (Ca) in the hydroxyapatite crystal of bones and teeth and can bind to non-collagenous proteins such as osteocalcin and osteopontin (Pemmer et al., 2013). Pb becomes incorporated into regions of the skeleton that are actively mineralizing during the time of exposure; for dental enamel and primary dentin, Pb incorporation is therefore restricted to childhood, but for bone, dental cementum, and secondary dentin, this can occur throughout an individual's lifetime.

Archaeological bone chemistry research is subject to the limitations posed by diagenesis—the chemical, biological, and physical alteration of skeletal remains occurring in the depositional environment (Hedges, 2002). From a bioarchaeological Pb perspective, the biogenic (lifetime) Pb composition of bones and teeth can be permanently obscured by the diagenetic incorporation of Pb into the skeleton from the depositional environment (e.g. Waldron, 1981; Wittmers et al., 2008;

King et al., 2020). Despite the potential utility of trace elements for addressing bioarchaeological questions, trace element research in bioarchaeology has waned over the past few decades because of the uncertainty cast on the discipline by the impacts of diagenesis (Price, 2008). Implementing new or different approaches in bioarchaeological trace element analysis that may help identify diagenesis and recover biogenic information is therefore key to the discipline.

This thesis stems from a larger SSHRC-funded project, “Investigating Lead Exposure Patterns in Royal Naval populations from the Colonial Era,” led by Dr. Tamara Varney (P. I.). This project has applied historical records, bioarchaeological methods, and chemical analytical techniques to examine the social determinants of Pb poisoning in both a colonial British Royal Navy population from Antigua and in contemporaneous colonial populations from Montserrat, Newfoundland, and Arctic Canada. Fueled by historical documentary evidence and preliminary trace element data indicating that Pb poisoning was prevalent and commonplace among navy personnel in the West Indies, this project has used ICP-MS in conjunction with synchrotron radiation X-ray fluorescence imaging (SR-XFI) to examine biogenic Pb exposure in greater detail.

X-ray fluorescence involves the X-ray excitation of a sample and subsequent release of X-rays characteristic of the atoms from which they originated, which can be detected and from which a spatial map of elemental distribution can be generated. Utilizing synchrotron radiation X-rays as the excitation source increases the precision and spatial resolution of the resulting map, given the high photon flux, small source size, and low beam divergence of synchrotron radiation (Iida, 2013). Previous research conducted under this project has postulated that biogenic Pb accumulates in cortical bone heterogeneously according to one’s life history of exposure, with particular enrichment occurring in the cement lines and central (Haversian) canals of secondary osteons (Haversian systems), the fundamental microscopic units of bone (Swanston et al., 2012, 2018;

Choudhury et al., 2016, 2017). By contrast, it has been proposed that diagenetic Pb exposure would result in a microdistribution pattern distinguishable from that of biogenic exposure (Swanston et al., 2012, 2017) and be present in bone as a different element species than biogenic Pb (Choudhury et al., 2017). Experimental research is required to support the inference that diagenesis and biogenicity do indeed produce different spatial patterns of uptake for Pb in bone.

1.2 Research Questions and Hypotheses

(1) When the two processes are controlled for experimentally, do biogenic and diagenetic Pb exposure result in different spatial patterns of uptake?

To answer this research question, diagenesis was experimentally simulated in modern bone samples and SR-XFI maps of Pb were compared against their unaltered counterparts as well as against Pb maps from archaeological examples. The null hypothesis of this research question is that the two processes would not produce different spatial patterns of enrichment. The alternative hypothesis is that biogenic and diagenetic sources do indeed result in different spatial patterns of uptake and SR-XFI could be a useful tool for differentiating biogenic and diagenetic Pb exposure in bioarchaeological remains.

(2) What does the spatial distribution of Pb in modern bone samples from a contemporary Saskatchewan population say about biogenic Pb exposure experienced by this population?

To answer this question, ICP-MS Pb data and SR-XFI maps of Pb from unaltered modern bone samples (reflecting solely biogenic Pb exposure) were analyzed and compared with archaeological examples to contextualize Pb exposure. The main hypothesis for this research question is that the majority of Pb exposure for each modern individual sampled would have occurred years ago,

during the era of leaded gasoline (1920s–1980s). Alternatively, Pb exposure could have continued into the present due to extrinsic sources of Pb exposure in modern society and/or intrinsically, from the reintegration of mobilized Pb into bone.

1.3 Thesis Significance

By investigating spatial differences in biogenicity and diagenesis, this thesis contributes new insights into the body of literature on diagenesis. As a complex, pervasive, and unpredictable suite of processes impacting skeletal remains, diagenesis represents a significant hurdle to the discipline of bioarchaeological bone chemistry. Insights into the behaviour of diagenesis and developing methods for identifying and addressing diagenesis are therefore critical to this discipline. The novelty of this project stems from its experimental, rather than observational, research design. Several diagenesis studies involve observing patterns in archaeological remains (e.g. Klepinger et al., 1986; Elliott & Grime, 1993; Hedges et al., 1995; Nielsen-Marsh & Hedges, 2000; Berna et al., 2004; López-Costas et al., 2016) and inferring diagenetic behaviour; this study, like other experimental diagenesis work similar to it (e.g. Von Endt & Ortner, 1984; Nicholson, 1996; McNulty et al., 2002; Harbeck & Grupe, 2009; Fisk et al., 2019; Krajcarz, 2019), permits more control over variables and consequently, can help support or refute archaeological inferences about diagenesis. Unlike other experimental diagenesis work, however, this study specifically investigates diagenetic Pb uptake, which is an under-researched topic relative to other trace elements and other aspects of diagenesis.

Using donated bone samples from modern human cadavers as opposed to an animal model for this experimental project presented the unique opportunity to simultaneously gain insights into the biogenic nature of Pb exposure among individuals sampled from a Saskatchewan population.

Up to this point, Pb exposure research in Canada has been limited to blood Pb levels (Neri et al., 1988; Clark et al., 2007; Tsuji et al., 2008; Wong & Lye, 2008; Bushnik et al., 2010; Health Canada, 2013; Levallois et al., 2013; Ngueta, 2014, 2016; Safruk et al., 2017), with few exceptions (Gamblin, 1984; Kowal et al., 1989, 1991; Samuels et al., 1989; Tsuji et al., 1997). Blood lead levels represent a short-term time scale of Pb exposure while bone Pb research has the potential to convey much more long-term information about life histories of Pb exposure. This study represents the first application of ICP-MS and SR-XFI to investigate biogenic Pb exposure in modern Saskatchewan.

1.4 Thesis Structure

Following the introductory chapter, this manuscript-style thesis contains three standalone manuscripts: two background review article chapters and one research article chapter. Chapter 2 frames this research within the larger discipline of bioarchaeological trace element analysis by reviewing the history and contemporary prospects for the discipline. This chapter provides an in-depth literature review on limitations of trace element analysis, including the impacts of diagenesis, the simplification of complex element uptake processes, and the use of unsuitable elements for dietary reconstruction. This review also highlights cutting-edge techniques in trace element analysis that have growing momentum in bioarchaeology, including micro-sampling, element mapping, and non-traditional stable isotope analysis, and assesses how these approaches may address the common limitations faced by the discipline. A version of this review article has been submitted to *Archaeological and Anthropological Sciences* (June 2020).

Chapter 3 is a methods-based review article providing an overview of the application of SR-XFI and X-ray absorption spectroscopy (XAS) to the study of Pb in bioarchaeological remains.

This chapter focuses on technological advancements to these methods, including the use of confocal optics, and how they have helped develop this approach. A version of this review article has been previously published in a special issue, “Synchrotron Radiation in Art and Archaeology,” of *Synchrotron Radiation News* (Simpson et al., 2019).

Chapter 4 is a research article manuscript under preparation that outlines the application of ICP-MS and SR-XFI to experimentally modified cadaveric and archaeological bone samples to address the two main objectives of this thesis and contextualize the findings in the larger body of diagenesis and Canadian Pb exposure research. A conclusion chapter (Chapter 5) follows.

Chapter 2— Historical Perspectives and New Directions in Bioarchaeological Trace

Element Analysis: A Review

This chapter has been submitted as a review article to *Archaeological and Anthropological Sciences* as of June 2020 (Simpson, R., Cooper, D. M. L., Swanston, T., Coulthard, I., & Varney, T. L. Historical perspectives and new directions in bioarchaeological trace element analysis: a review). RS, DC, and TV conceptualized this review article. Research was funded by a SSHRC Insight Grant (TV, P.I.). RS was responsible for writing the manuscript. The manuscript was revised by RS, DC, TS, IC, and TV.

Abstract

Given their strong affinity for the skeleton, several trace elements are often stored in the bones and teeth long-term. Diet, geography, health, disease, social status, activity, and occupation are some factors which may cause differential exposure to, and uptake of, trace elements, theoretically introducing variability in their concentrations and/or ratios in the skeleton. Trace element analysis of bioarchaeological remains has the potential, therefore, to provide rich insights into past lifeways. This review provides a historical overview of bioarchaeological trace element analysis, commenting on the current state of the discipline by highlighting approaches gathering momentum in this area. Popularity for the discipline surged following preliminary studies in the 1960s to 1970s that demonstrated the utility of strontium (Sr) as a dietary indicator. During the 1980s, Sr/Ca ratio and multi-element studies were commonplace in bioarchaeology, linking trace elements with dietary phenomena. Interest in using trace elements for bioarchaeological inferences waned following a period of critiques in the late 1980s to 1990s that argued the discipline failed to account for diagenesis, simplified complex element uptake and regulation processes, and used several unsuitable elements for palaeodietary reconstruction (e.g. those under homeostatic regulation,

those without a strong affinity for the skeleton). In the 21st century, trace element analyses have been primarily restricted to Sr isotope analysis and the study of toxic trace elements, though small pockets of bioarchaeology have continued to analyze multiple elements. Techniques such as micro-sampling, element mapping, and non-traditional stable isotope analysis have provided novel insights which hold the promise of helping to overcome limitations faced by the discipline.

2.1 Introduction

Bones and teeth act as reservoirs for several minor and “trace” elements that circulate through the body, and consequently, the skeleton represents a record of one’s lifetime element exposure. Trace element analysis of human skeletal remains can be useful from a bioarchaeological standpoint because trace elements can provide rich insights into past human diet, health, status, occupation, and activities. While theoretically invaluable for interpreting the past, bioarchaeological trace element analysis has previously faced numerous critiques, primarily regarding diagenesis and the reliance upon unsuitable elements. This review will analyze the state of bioarchaeological trace element analysis of bone and teeth by providing a historical overview of the field and by highlighting approaches with growing momentum in bioarchaeology.

Works cited in later sections (2.6, 2.7) are primarily restricted to research conducted during the last twenty years, with greater emphasis on research conducted in the last ten years, in an effort to highlight patterns and trends in the discipline of bioarchaeological trace element analysis. The exception to this inclusion criteria comes through earlier cited works in these sections that provide historical or methodological context. As the focus of this review is bioarchaeology, the majority of research cited pertains to human remains from past populations, though some examples from faunal remains and modern populations are also highlighted in cases where such works show potential for bioarchaeological trace element analysis of human remains.

2.2 Background

Bone consists of approximately 60% inorganic mineral, 30% organic matrix, and 10% water (Feng, 2009). The organic matrix of bone is primarily comprised of collagen fibres that are in turn studded with inorganic hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) mineral crystals. Teeth similarly

consist of an organic matrix and inorganic hydroxyapatite, but the inorganic to organic ratio varies on a tissue-specific level (approximately 96% inorganic constituents and 4% organic constituents and water in enamel, 70% inorganic constituents, 20% organic constituents, and 10% water in dentin, and 45–55% inorganic mineral and 50–55% organic matrix and water in cementum; Bhaskar, 1991; de Dios Teruel et al., 2015). Trace elements circulating through the body can become incorporated into actively forming regions of skeletal tissues. In the case of remodeling bone, trace element incorporation occurs throughout an individual's life, as bone undergoes an ongoing natural cycle of turnover consisting of osteoclastic bone resorption followed by osteoblastic bone formation. Initial secretion of organic matrix is followed by the two-fold process of mineralization, in which 50–70% of hydroxyapatite crystals are rapidly added during the process of primary mineralization (6 months in ewe [*Ovis aries*] animal model) and continue to be gradually added and mature during the process of secondary mineralization (30 months in ewe animal model and estimated to be couple years in humans; Bala et al., 2010; Ruffoni et al., 2007).

Individual bone remodeling rates may vary according to a number of factors, including bone type, loading patterns, sex, age, and disease (Lanyon, 1984; Recker et al., 2004; Shea & Miller, 2005; Cho et al., 2006). That being said, studies have shown that bone has the ability to survive up to several decades. Hedges and colleagues (2017) used radiocarbon (residual in bone from 1960s to 1970s bomb testing) as a tracer to determine bone collagen turnover rates in 67 adults ranging from 40 to 97 years of age at death. They discovered that mean femoral bone collagen turnover rates were lower than previously thought for males (1.5–3% per year) and females (3–4% per year), with bones from some individuals still containing collagen that formed during adolescence. In sum, the majority of trace elements are incorporated into actively forming

regions of bone undergoing primary mineralization and bone as a whole represents a composite mosaic of trace element exposure dating back years to decades.

Barring the surface of the tooth crown, which fluctuates through periods of mineralization and demineralization due to interaction with saliva (Abou Neel et al., 2016), teeth do not remodel, and the time sequence of trace element incorporation is tissue dependent. Primary dentin, the inner portion of teeth comprising the root and majority of the crown, and enamel, the hard outer covering the tooth, incrementally form layers during childhood. Secondary dentin continues to gradually form layers once roots are complete (Hillson, 1996, p. 194). By contrast, cementum, the outer layer of the tooth root, incrementally forms throughout one's lifetime. Therefore, primary dentin and enamel trace element composition represent a record of childhood trace element exposure whereas secondary dentin and cementum represent a linear record of lifetime trace element exposure.

The majority of trace elements of bioarchaeological relevance (e.g. barium [Ba], calcium [Ca] copper [Cu], fluorine [F], iron [Fe], lead [Pb], magnesium [Mg], manganese [Mn], sodium [Na], strontium [Sr], and zinc [Zn]) contribute to the inorganic component of bone though there are a few exceptions—for example, bromine (Br) and selenium (Se) preferentially bind to bone collagen (Brätter et al., 1977). While the Ca to phosphate ratio of the mineral component of bone remains relatively fixed (Burton, 2008), “bone seeking” trace elements, such as Ba, Pb, and Sr have a strong affinity for the inorganic phase of the skeleton; consequently, up to 99% of the total body burden of these elements is contained within bones and teeth, where they remain sequestered for years to decades or a lifetime, respectively. Elements with divalent cations (+2) are capable of substituting for Ca ions in the hydroxyapatite structure of bones and teeth given their chemical similarities to Ca. Other elements such as Pb and Zn are also capable of binding to non-collagenous proteins like osteocalcin or osteopontin that are particularly rich in the cement lines and central

(Haversian) canals of bone (Pemmer et al., 2013). Table 2.1 provides an overview of several trace elements commonly studied in bioarchaeology contexts, along with pertinent characteristics.

TABLE 2.1—*Properties of trace elements studied in bioarchaeology.*

	Element	Atomic Number	Atomic Mass (amu)	Ions	Stable Isotopes (Radiogenic Isotopes)	% of Body Levels in Bone	Mechanism of Incorporation	Essential?	Toxic?
Alkaline Earth Metals	Calcium (Ca)	20	40.08	+2	⁴⁰ Ca, ⁴² Ca, ⁴³ Ca, ⁴⁴ Ca, ⁴⁶ Ca, (⁴⁸ Ca)	99% ¹	Component of hydroxyapatite mineral	Yes	No
	Barium (Ba)	56	137.3	+2	¹³⁰ Ba, ¹²³ Ba, ¹³⁴ Ba, ¹³⁵ Ba, ¹³⁶ Ba, ¹³⁷ Ba, ¹³⁸ Ba	90% ²	Substitute for Ca ions in hydroxyapatite	No	No*
	Magnesium (Mg)	12	24.31	+2	²⁴ Mg, ²⁵ Mg, ²⁶ Mg	50–60% ³	Minor component of hydroxyapatite; substitute for Ca ions in hydroxyapatite	Yes	No
	Strontium (Sr)	38	87.62	+2	⁸¹ Sr, ⁸⁴ Sr, ⁸⁶ Sr, ⁸⁸ Sr, (⁸⁷ Sr)	99% ⁴	Substitute for Ca ions in hydroxyapatite	No	No
Transition Metals	Copper (Cu)	29	63.55	+2	⁶³ Cu, ⁶⁵ Cu	Unknown	Collagen cross-linking function ⁸ ; binds to hydroxyapatite? ⁹	Yes	No
	Iron (Fe)	26	55.85	+3, +2	⁵⁴ Fe, ⁵⁶ Fe, ⁵⁷ Fe, ⁵⁸ Fe	Unknown	Collagen synthesis cofactor ¹⁰ ; substitutes for Ca ions in hydroxyapatite	Yes	No
	Mercury (Hg)	80	200.6	+2, +1	¹⁹⁸ Hg, ¹⁹⁹ Hg, ²⁰⁰ Hg, ²⁰¹ Hg, ²⁰² Hg, ²⁰⁴ Hg	Unknown	Substitute for Ca ions in hydroxyapatite	No	Yes
	Manganese (Mg)	25	52.94	+7, +4, +2	⁵⁵ Mn	43% ⁵	Role in regulating bone remodeling ¹¹ ; substitute for Ca ions in hydroxyapatite	Yes	No

	Vanadium (V)	23	50.94	+5, +4, +3	⁵¹ V	Unknown	Incorporates into hydroxyapatite framework ¹²	No	No
	Zinc (Zn)	30	65.39	+2	⁶⁴ Zn, ⁶⁶ Zn, ⁶⁷ Zn, ⁶⁸ Zn, ⁷⁰ Zn	28% ⁶	Metallothionein co-factor ¹³ ; attachment to non-collagenous proteins ¹⁴ ; substitute for Ca ions in hydroxyapatite	Yes	No
Other Metals	Lead (Pb)	82	207.2	+2	²⁰⁴ Pb, ²⁰⁶ Pb, (²⁰⁷ Pb), (²⁰⁸ Pb)	78-96% ⁷	Substitute for Ca ions in hydroxyapatite; attachment to non-collagenous proteins ¹⁴	No	Yes
Metalloids	Arsenic (As)	33	74.92	+5, +3	⁷⁵ As	Unknown	Arsenate (AsO ₄ ³⁻) substitutes for phosphate ions in hydroxyapatite ¹⁵	No	Yes

* Barium (Ba) not toxic at environmental levels; certain compounds may be toxic (e.g. BaCl)

¹Paterson, 1978, p. 246; ²WHO, 2001; ³Jahnen-Dechent & Ketteler, 2012; ⁴Cabrera et al., 1999; ⁵O'Neal et al., 2014;

⁶Hambidge et al., 1986; ⁷Saltzman, 1990; ⁸Opsahl et al., 1982; ⁹Laurencin et al., 2011; ¹⁰Tuderman et al., 1977;

¹¹Strause et al., 1987; ¹²Petit et al., 2017; ¹³Hoadley et al., 1988; ¹⁴Pemmer et al., 2013, p. 188; ¹⁵Dani, 2013, p. 542.

2.3 Early Trace Element Analysis of Human Skeletal Remains (1950s to mid-1970s)

Mid-twentieth century clinical and archaeological efforts of chemical skeletal analyses aimed to define (1) the trace elements present in bone, (2) which of these trace elements are essential, and (3) the normal and abnormal levels for each element (Drea, 1935; Hodges et al., 1950; Fore & Morton, 1952; Brudevold & Steadman, 1955; Sowden & Stitch, 1957; Taylor, 1959). Medically, these research questions were dictated by societal concerns for the radioactive impacts of Cold War nuclear testing on human health. As such, Sr and Ba were of primary interest, given their potential radioactive forms. Hodges and colleagues (1950) and Sowden and Stitch (1957)

found similar levels of Sr between all bone samples, and a positive relationship between Sr content and age. Ba levels were lower than Sr levels, demonstrating a preferential affinity of Sr for bone (Sowden & Stitch, 1957).

Chemical analyses of archaeological human remains were previously concerned mainly with investigating the permeation of trace elements from the soil into ancient bones and teeth (Steadman et al., 1959), and by extension, the potential for chronologically dating remains through analysis of their chemical composition (Heizer & Cook, 1952; Cook & Heizer, 1953). It was not until the mid-1960s, however, that trace element analysis of archaeological or fossil bone was used to infer past behaviour. Toots and Voorhies (1965) were the first to apply Sr to Ca (Sr/Ca) ratios in fossil bone to make paleodietary inferences. Their study was based on Odum's (1957) finding that mammalian metabolisms follow a Ca biopurification process, in which gastrointestinally, Sr is selectively discriminated against and Ca is preferentially taken up; therefore, Sr/Ca ratios markedly decrease with each increase in trophic level. Toots and Voorhies (1965) proposed that fossil animals' diets and trophic positions could then be inferred through analysis of the Sr/Ca ratio; carnivores would have a low Sr/Ca ratio when compared with herbivores, and herbivore Sr/Ca ratios would vary according to the type of vegetation typically consumed by a species (i.e. leafy greens are high in Sr while grasses are low in Sr). Similar to Sr, dietary Ba and Pb also follow a Ca biopurification process, and therefore, Ba/Ca and Pb/Ca ratios also decrease with each increasing trophic position (Elias et al., 1982), though while Pb is abundantly taken up into body tissues via contaminated food and drink, it has a number of additional pathways of entry, such as through inhalation or skin (Schroeder & Tipton, 1968).

Antoinette Brown's (1973, 1974) doctoral research constituted the first application of trace element analysis of Sr in human skeletal remains to reconstruct paleodiet. Brown analyzed bulk

Sr levels in skeletal samples from the Huitzo village site in Oaxaca, Mexico, arguing that differences in Sr concentrations related to differences in plant and meat consumption and that, consequently, social stratification can be inferred by individuals' differential access to meat protein. In his dissertation work, Robert Gilbert (1975) proposed the measurement of Cu, Mg, Mn, Sr, and Zn to reconstruct paleodiet. According to Gilbert (1975), low Zn levels represent primarily plant consumption and high Zn levels represent high meat consumption, though he suggested that low Zn levels may also be indicative of high cereal and grain consumption, because the phytate compound in these plants may interfere with Zn absorption. In a similar vein, Wessen and colleagues (1978) proposed the use of Ba as a determinant of the animal origin of bone artifacts, finding significant differences in seal and terrestrial animal Ba content likely based on their dietary differences.

Jarcho (1964) first measured Pb in bone samples from two prehistoric North American sites in Arizona: Kinishba, where Pb-glazed pottery was known to be manufactured, and Point of Pines, where it was not. He found no significant differences in Pb concentrations between bones from Kinishba and Point of Pines, further it was impossible to determine the manufacturers and users of Pb-glazed pottery from the remains at Kinishba. These early efforts of using trace elements to recreate past lifeways set the stage for a new era of chemical analysis in bioarchaeology.

2.4 Peak of Trace Element Analysis (mid-1970s to 1990s)

2.4.1 Trace Elements and Paleodiet

Beginning in the late 1970s and extending into the 1990s, the popularity of bioarchaeological bone chemistry exploded. Improvements in chemical analytical methods allowed for a wider range of elements to be detected (Price et al., 1985) as well as improved

element detection limits. Following Brown's (1973, 1974) application of Sr and Gilbert's (1975) proposed use of Cu, Mg, Mn, Sr, and Zn for paleodietary reconstruction, numerous trace elements were used to infer paleodiet. Sr was considered a staple of trace element paleodietary reconstruction and was used to infer dietary constituents, diachronic changes in diet, and sex- and status-related differences in diet. Echoing Brown's (1973, 1974) seminal work on human remains, Schoeninger (1979) used Sr concentrations to infer social status in an ancient population from Chalcatzingo, Mexico (1150–550 BCE), reporting that high status individuals (associated with jade funerary objects) had low bone Sr indicative of high meat consumption, while low status individuals (not associated with grave goods) had high bone Sr indicative of high plant consumption.

Sr concentrations and Sr/Ca ratios became popular in establishing diachronic subsistence shifts—for example, the introduction and increased reliance upon cultivated plants. As iterated above, Sr is impacted by the biopurification of Ca among most mammals and therefore is potentially indicative of trophic level. Price and Kavanagh (1982) used Sr/Ca ratios to examine diachronic changes in diet, arguing that there were increases in plant consumption between the Late Archaic, Middle Woodland, and Mississippian periods among prehistoric Wisconsin sites. Similarly, Katzenberg (1984) and Katzenberg and Schwarcz (1986) used bone Sr levels and stable carbon (C) and nitrogen (N) isotopes in their analysis of Southern Ontario populations to assess changes in plant consumption and the possible introduction of maize. Based on Sr and stable C and N isotope values, the introduction of maize in Southern Ontario was not accompanied by a significant decrease in animal protein consumption, contrary to previous speculation (Katzenberg & Schwarcz, 1986).

While initially high Sr concentrations or Sr/Ca ratios were thought to be exclusively indicative of plant consumption, later studies demonstrated this was not as simple as initially proposed. Within plants, leaves are higher in Sr than stems, and because certain plants like maize and squash are notably depleted in Sr, relatively low bone Sr levels may also be indicative of maize or squash horticulture (Katzenberg, 1984). Consumption of other low-Sr or Ca-rich foods may further complicate Sr/Ca ratios; for example, mollusc consumption obscured Sr evidence of high plant consumption, causing prehistoric agriculturists to have unexpectedly low Sr (Schoeninger & Peebles, 1981).

During the 1980s, multielement analysis was extremely popular in trace element analyses of archaeological remains (e.g. Katzenberg, 1984; Beck, 1985; Hatch & Geidel, 1985; Byrne & Parris, 1987; Francalacci, 1989; İşcan et al., 1989; White & Schwarcz, 1989; Arrhenius, 1990; Liden, 1990). Early practitioners examined correlations and relationships between different element concentrations and ratios, leading to several elements being proposed for use in paleodiet reconstruction studies. In addition to the continued widespread use of Sr as an indicator of trophic level, Ba, Cu, Mg, Mn, V, and Zn were used to make a variety of paleodiet inferences.

A popular application of some elements was to establish trophic level. Following a similar biopurification process to Sr (Elias et al., 1982), Ba/Ca ratios conceivably reflect trophic level, and by extension, plant versus meat consumption. Scholars also employed Ba/Sr ratios as an indicator of marine food consumption, due to seawater's low Ba levels (Burton & Price, 1990; Gilbert et al., 1994). Some scholars proposed that Zn enrichment occurs with each increasing trophic level, due to the naturally high levels of Zn in blood and soft tissue (Gilbert, 1975; Rheingold et al., 1983). As such, high Zn levels were interpreted as an indicator of high meat consumption, and low levels

an indicator of high plant consumption. Beck (1985) used Sr and Zn concentrations to attempt to classify populations with hunter-gatherer, agricultural, and horticultural subsistence strategies.

Scholars also proposed numerous trace elements for interpreting nut consumption. First proposed by Gilbert (1975), Mg was used in paleodiet reconstruction as an indicator of nut consumption. According to Hatch and Geidel (1985), Mn and V, like Sr, are indicators of plant consumption, but nuts are low in V; therefore, vegetarian and meat-rich diets with or without nuts could potentially be differentiated on the basis of V in conjunction with Cu, Mn, Sr, and Zn levels. Mn was later interpreted as an indicator of diagenetic contamination (Francalacci, 1987), due to its very low naturally biogenic levels in humans.

Cu was proposed as an indicator of meat consumption (Schroeder et al., 1966). Arrhenius (1990) argued that Cu levels could also reflect arthropod, specifically maggot, consumption, due to a Cu hemocyte in insects. In her study of Cu, Se, and Sr concentrations in Scandinavian hunter-gatherer and medieval bone samples, Arrhenius (1990) argued that the elevated Cu levels in the hunter-gatherer populations reflect a subsistence strategy heavily weighted in gastropods, molluscs, and arthropods, and hypothesized that these populations intentionally grew maggots for subsistence.

2.4.2 Toxic Trace Element Exposure

While most trace element studies in bioarchaeology focused on paleodiet and paleoecology, toxic trace elements were, to a lesser extent, also analyzed to make inferences about population exposure. Pb concentrations can inform our understanding of populational Pb use, health, industry, occupation, and social status, as well as differentiate commingled remains or distinguish ancient from modern skeletal material (Aufderheide et al., 1988). Several early Pb analyses in prehistoric populations were driven by research questions of how abnormally elevated

modern Pb exposure was relative to physiologically “natural” human levels (Patterson, 1965; Ericson et al., 1979; Grandjean et al., 1979; Jaworowski et al., 1985). For example, Ericson and colleagues (1979) determined Pb/Ca ratios in ancient Peruvians (c. 1,600 BP), reporting that “natural” Peruvian levels were one hundredth the levels observed in modern England and USA.

Since the late 1970s, scholars have used bioarchaeological trace element analysis to track the history of human Pb exploitation, demonstrating that there was a high pre-industrial peak of Pb production during antiquity (Wittmers et al., 2002). Specifically, Pb was widely exploited by—and even proposed as a contributor to the downfall of—the Roman Empire (Nriagu, 1983) and consequently, Pb of Roman and post-Roman skeletal material has been the subject of much scrutiny (e.g. Mackie et al., 1975; Ahlgren et al., 1980; Molleson et al., 1986; Vuorinen et al., 1990; Aufderheide et al., 1992).

The 1980s saw an increasing focus on the social determinants of Pb exposure (Aufderheide et al., 1981, 1985; Handler et al., 1986; Corruccini et al., 1987). Did certain social groups have differential exposure to Pb? Variation in Pb exposure within a population can be due to a number of social factors, such as habitual activities or access to luxury goods. Aufderheide and colleagues (1981) argued that plantation owners and enslaved individuals from a colonial Virginia cemetery could be differentiated based on bone Pb concentrations, because plantation owners had elevated Pb exposure from consuming food and drink from luxury pewter dinnerware. Exceptions to this pattern came in the form of a white individual with low bone Pb concentrations interred alongside the black enslaved individuals, who likely had similar living conditions, and a black female with an abnormally high Pb concentration (96 $\mu\text{g/g}$) in her bones, which may be indicative of work as a housemaid (Aufderheide et al., 1981).

First proposed by Waldron (1981), Pb isotopes can be used to investigate the material source of Pb exposure, due to regional geological variability in Pb isotopic signatures. For example, Reinhard and Ghazi (1992) used Pb isotopes to infer the source of exposure in an ancient Omaha population. Ultimately, they argued that the primary source of Pb exposure originated from Pb- and cinnabar-based cosmetic pigments applied to the body during mortuary rituals (Ghazi et al., 1994). Pb isotopes were also used to infer mobility; among individuals interred in the Roman cemetery at Poundbury Camp, Dorset, the Pb isotopic composition in most individuals was consistent with a British origin, though one child's Pb isotope signature was identical to Pb ores from Laurion, Greece (Molleson et al., 1986). So similar were these bone and ore Pb values that the authors argued the child immigrated from Laurion, rather than simply consuming imported food and drink from the region (Molleson et al., 1986).

2.5 Critiques of Trace Element Analysis (late 1980s–1990s)

During the late 1980s and 1990s, several scholars raised critiques in the field of trace element analysis, particularly regarding (1) erroneous or simplified assumptions regarding the effects of diagenesis on human remains (Radosevich, 1993), (2) the simplification of complex element uptake processes (Radosevich, 1993), and (3) the use of elements inappropriate for paleodietary reconstruction (Klepinger, 1990; Ezzo, 1994).

The process of diagenesis is the suite of post-mortem physical, chemical, and biological alterations to skeletal remains occurring in the depositional environment (Hedges, 2002). With regards to the impact of diagenesis on the trace element composition of skeletal remains, the inorganic phases of bones and teeth are of primary interest. Groundwater ions and organic compounds in the depositional environment can be deposited in remains by filling pores or cracks

in bone or teeth, or by becoming more permanently incorporated into the hydroxyapatite crystalline structure (Hedges & Millard, 1995). Conversely, biogenic elements from the bones and teeth can leach into the depositional environment (Price et al., 1992). Microbial activity can also diagenetically impact the chemical composition of remains; soil fungi and bacteria and/or intrinsic gut bacteria can physically degrade the integrity of skeletal remains via tunneling and can alter the chemical composition of bone by introducing elements from the exterior sediment or by depositing chemical metabolic byproducts (Grupe & Piepenbrink, 1988, 1989; White & Booth, 2014).

The extent of diagenesis can be exacerbated by high temperatures (Von Endt & Ortner, 1984), acidic soil pH (Gordon & Buikstra, 1981), moist and humic soils (Krajcarz, 2019), and poor site drainage and hydrological movements (Hedges & Millard, 1995; Nielsen-Marsh & Hedges, 2000), as well as a myriad of other biological, chemical, and physical taphonomic factors (Nielsen-Marsh et al., 2007). With respect to bone, smaller bones are more susceptible to diagenetic degradation, leading to an underrepresentation of small fauna and infants in the bioarchaeological record (Von Endt & Ortner, 1984; Buckberry, 2000). Bone pore structure—namely, the size, volume, shape, and distribution of bone pores—also impacts the pervasiveness of diagenesis (Nielsen-Marsh & Hedges, 2000) and contributes to the underrepresentation of osteoporosis in the bioarchaeological record (Bartosiewicz, 2008). The physical and/or microbial breakdown of bone and consequent alteration of pore structure can further increase susceptibility to diagenesis, as heavy metals in the depositional environment can enter the bone through cracks and pores (Hedges & Millard, 1995; Rasmussen et al., 2019). Due to this multiplicity of factors, diagenesis does not behave in a linear or predictable manner in skeletal remains (Klepinger et al., 1986).

Diagenesis became a very critical topic of discussion for scholars in the heyday of trace element analysis; however, many such scholars “explained away” the problem of diagenesis by

arguing that (1) certain trace elements and skeletal tissues were resistant to diagenesis, (2) diagenesis could be identified by the presence of certain elements and alterations to the crystallinity index, or (3) diagenetic alteration could be removed from skeletal tissues. Gilbert (1975) and Schoeninger (1979) proposed that Sr and Zn are resistant to diagenesis. This assumption was rejected by Sillen (1981), who found a homogenization effect in Sr concentrations of Natufian and Aurignacian era fossil remains at the Hayonim Cave, Israel, potentially indicative of diagenesis. Nonetheless, this assumption persevered into the 1980s (e.g. Lambert, 1984, 1985). Pb was also previously thought to be immobile in soil if the soil pH was basic (Zimdahl & Skogerboe 1977); however, Waldron (1981) found an exceedingly Pb concentration (10,228 µg/g) in a bone sample from a Pb-lined coffin, and Wittmers and colleagues (2008) found that diagenetic Pb contamination had completely obscured the biogenic Pb signal bones from in a First African Baptist Church population (Philadelphia, PA). While it has been clearly demonstrated that virtually any element can be affected by diagenesis, scholars have also effectively demonstrated that some skeletal tissues are more resistant to diagenesis than others. Parker and Toots (1980) showed that due to its small pores and tightly-packed structure, tooth enamel is more resistant to diagenetic effects than either bone or tooth dentin, and this has been confirmed by Kyle (1986). Additionally, cortical bone is relatively less affected by diagenesis than the extremely porous trabecular portion of bone (Price et al., 1992).

On a related note, other scholars have argued that certain elements in bone are unlikely to have a biogenic origin, and therefore are indicative of probable diagenesis. For example, Lambert and colleagues (1984, 1985) suggested that aluminium (Al), Ba, Cu, Fe, Mn, potassium (K), uranium (U), and V are likely to diagenetically contaminate bone, whereas Ca and Na are susceptible to leaching out of bone. Katzenberg (1984) argued that rare earth elements (REEs)

such as zirconium (Zr) and yttrium (Y), and Ca/P ratios are indicative of diagenesis; Zr and Y are unlikely to be biogenically incorporated into the skeleton, and an abnormally high Ca/P ratio is indicative of carbonate contamination in bone. Evaluating REEs and Ca/P ratios continues to be a popular means of identifying possible diagenetic contamination in skeletal remains today (e.g. Willmes et al., 2016; Giffin et al., 2017; Özdemir et al., 2017; Kamenov et al., 2018).

Comparing element concentrations in archaeological bone and *in situ* soils is one method used to attempt to identify diagenesis; Lambert and colleagues (1979, 1984) and Nelson and Sauer (1984) argued that if the concentrations of an element in the soil and remains were inconsistent, then diagenesis did not take place. However, diagenetic activity is not this simple. “Bone seeking” elements do not often behave according to a simple concentration gradient between the bone and soil; rather, bones act as a “trace element sink” (Kohn & Moses, 2013, p. 422), accumulating elements disproportionately from the surrounding depositional environment. In fact, bone is often used as a medium to extract heavy metals from water and soil (Hodson et al., 2001; Chen et al., 2008). According to Pate and Hutton (1988), a simple soil-bone comparative approach also fails to consider the quantity of soluble and exchangeable ions actually available in the soil and under what conditions. In sum, while there is value in extracting soil samples for the study of diagenesis, the intricacies of bone-soil dynamics need to be considered.

Another approach to identify the presence of diagenesis in skeletal remains is to examine the crystalline integrity of bone, through methods such as Fourier Transform Infrared Spectroscopy (FTIR). Hydroxyapatite crystal imperfection is a sign that diagenesis has degraded the bone and as such, various crystallinity indices have been proposed to aid in assessing the extent of diagenesis (Shemesh, 1990; Person et al., 1995). Trueman and colleagues (2008) later argued that while

crystallinity values may inform early diagenetic alterations to bone, they are less reliable for long-term diagenetic changes, which are often unpredictable and site dependent.

It has been consistently demonstrated that diagenetic contamination is often most concentrated at the periosteal and endosteal surfaces of bone (Price et al., 1992; Wittmers et al., 2008; Rasmussen et al., 2019). Price and colleagues (1992) reviewed and evaluated the efficacy of methods such as mechanical cleaning, chemical cleaning, and washing with a reducing agent for removing diagenetic alteration. They found that the success of such methods depends on both the extent of diagenetic contamination and the element in question. Leaching bone samples in a weak acid has been shown to remove some diagenetic Sr, though this can only be said for Sr occupying pores in bone; if Sr has pervaded the bone and become incorporated into the hydroxyapatite crystalline structure of bone, then an acidic soak is unable to remove it (Beard & Johnson, 2000). A weak acid treatment has also been shown to be quite effective at removing diagenetic Sr from enamel pore spaces (Hoppe et al., 2003).

A second critique of trace element analysis during the late 1980s and 1990s was the widespread simplification of complex element uptake processes for paleodietary inferences. For example, it has been demonstrated that Sr can widely vary in response to dietary factors, but is also subject to vary in response to local geological fluctuations, demographic variables, and culinary practices (Katzenberg et al., 2000) and the Sr/Ca ratio can be swayed by high Ca foods and minor dietary contributions (Burton & Wright, 1995), becoming convoluted in mixed, omnivorous diets. It was previously assumed that Sr/Ca ratios reflect trophic level, and that humans following omnivorous diets would have Sr/Ca ratios midway between herbivore and carnivore values; however, Runia (1987) showed that plants may discriminate between Sr and Ca more than initially thought; certain plants, such as cereals and grains, have a high Sr/Ca signature

that may produce values higher than herbivores. Furthermore, the bioavailability and mobility of Sr for uptake into plants varies according to regional microbial ecology and soil condition factors, such as pH and temperature (Burger & Lichtscheidl, 2019). Sr values also vary according to an animal or human's sex and age (Sillen, 1988), with notable Sr elevation occurring in pregnant and lactating females (Price et al., 1986; Blakely, 1989).

A third major critique of the field of trace element analysis was an overzealous optimism for using certain trace elements for paleodietary reconstruction, despite a lack of a scientific justification. According to Ezzo (1994), for an element to carry any paleodietary significance, it must satisfy the following conditions. First, the element must have a mode of biogenic incorporation into bone and ideally, the majority of the body burden for that element should be in the skeleton. Second, levels in bone must relate in some way to the levels in diet. Third, the element must not be under homeostatic control. However, many of the trace elements used in the era of multielement paleodiet reconstruction do not satisfy these conditions; many commonly-studied trace elements have crucial biological functions and are therefore homeostatically regulated. Klepinger (1990) experimentally demonstrated this by feeding two groups of pigs (*Sus scrofa domestica*) identical diets with high and low Mg supplementation, respectively. Despite one group consuming almost twice the amount of Mg as the other, there were no significant differences in bone Mg levels between the two groups, demonstrating the effect of homeostatic regulation on Mg metabolism.

In his critique of the widespread use of Zn in trace element analysis, Joseph Ezzo (1994) argued that unlike Sr, there was currently insufficient medical and scientific evidence that skeletal Zn levels are related to certain dietary components or to trophic level, yet a general uncritical acceptance for Zn's paleodietary utility existed among many bioarchaeologists. Additionally, Zn

is known to be a metalloenzyme cofactor that is under homeostatic regulation (Hoadley et al., 1988; King et al., 2000) and the skeleton's Zn stores represent only 28% of the entire body burden of Zn (Hambidge et al., 1986). To further complicate the matter, virtually all bioarchaeological studies implementing Zn concentrations for paleodietary inferences cited the same few works in the literature (i.e. Gilbert, 1975, 1977; Lambert et al., 1979, 1982; Blakely & Beck, 1981; Hatch & Geidel, 1983; Beck, 1985) as their scientific rationale. The majority of these cited works treated correlational multielement data in archaeological remains as empirical evidence, rather than drawing upon the physiological literature or controlled scientific studies (Ezzo, 1994). Ezzo (1994) emphasized that more controlled biomedical, ecological, and environmental studies needed to be undertaken before Zn could feasibly be applied to paleodietary reconstruction. Following this period of critiques, trace element analysis of bioarchaeological remains for paleodietary reconstruction largely fell out of favour. Since then, stable isotope analysis of bone has primarily been the focus of archaeological bone chemistry, though Sr and toxic trace elements continue to be an exception.

2.6 Post-Critique: Bioarchaeological Trace Element Analysis in the 21st Century

Following the abundant critiques of the field of trace element analysis, there has been a shift toward employing trace elements strictly for mobility and element exposure studies, commonly through evaluating the radiogenic isotope ratios of Sr and Pb. Of the four Sr isotopes present in bedrock, ^{87}Sr is a radiogenic product of the decay of ^{87}Rb , whereas ^{86}Sr is a stable, non-radiogenic Sr isotope. $^{87}\text{Sr}/^{86}\text{Sr}$ isotopes in bones and teeth originate from the food and water consumed by the individual, which in turn, reflect the Rb to Sr isotopic composition of the local bedrock. Weathering releases Sr sequestered in bedrock into the local stream water, atmosphere,

and soils, where it can be incorporated into plants and consumed by other living things (Bentley 2006). “Isoscapes” based on regional $^{87}\text{Sr}/^{86}\text{Sr}$ variability in bedrock and water systems can be constructed to infer provenance (Bataille & Bowen, 2012). Consequently, in assessing $^{87}\text{Sr}/^{86}\text{Sr}$ composition of bioarchaeological remains, there is the potential for inferring mobility and migration in past populations, specifically by interpreting nomadic or migratory behaviour, identifying social residence patterns, or differentiating “locals” from “foreigners” (Table 2.2). Variation in childhood vs. adult Sr isotope signatures can be established by comparing values in enamel vs. bone (Ericson, 1985).

TABLE 2.2—A selection of recent examples of applications of $^{87}\text{Sr}/^{86}\text{Sr}$ isotope analysis to studying mobility and migration in archaeological skeletal remains.*

	Application	Study	Location/ Time Period	Objective
Mobility	Cultural/ Subsistence Strategies (e.g. foraging, pastoralism)	Tofuri et al. (2006)	Holocene Libya (9000 BP–100 CE)	Examined mobile pastoral settlement pattern and related kinship system shifts in the Fezzan
		Haverkort et al. (2008)	Middle Holocene Siberia (4650–3950 BP)	Differentiated between resource use/mobility foraging models and explored inter- individual variation in Sr signatures
		Ventresca Miller et al. (2018)	Bronze Age Kazakhstan (2500–1400 BCE)	Analyzed variation in mobility range and resource use in pastoralists from Middle to Late Bronze Age
		Machicek et al. (2019)	Late Bronze Age to Turk/Uighur Period Mongolia (1400 BCE–840 CE)	Predicted extent of mobility and subsistence practices for nomadic pastoralists in two micro-regions
	Trade/ Exchange Networks	Sjögren et al. (2009)	Neolithic Sweden (3300–3000 BP)	Explored interactions with neighbouring regions via Sr isotope analysis of human remains and imported fauna
		Roberts et al. (2012)	England	Traced spread of treponemal disease along trade networks from Sweden
		Eriksson et al. (2013)	Neolithic Sweden (3400–3200 BCE)	Modeled “Neolithization” of Baltic region against backdrop of mobility and cultural contact
Migration	Political Dynamics (e.g. colonization)	White et al. (2007)	Teotihuacan, Mexico (200–400 CE)	Established geographic provenance for non-local Moon Pyramid sacrificial victims
		Tung & Knudson (2011)	Peruvian Andes (600–1000 CE)	Explored Wari expansionism by differentiating between locals, voluntary migrants, and captives in Conchopata
		Shaw et al. (2016)	Roman Britain (40–400 CE)	Identified geographic origins of <i>Londinium</i> individuals in context of Imperial Roman expansionism
		Barbarena et al. (2017)	Andes (2300 BP to present)	Re-evaluated the “high residential mobility” hypotheses for Andean populations
		Winter-Schuh &	Early Middle Ages Germany	Examined post-Roman Empire political dynamics and settlement contact via human mobility

		Makarewicz (2019)	(5 th –6 th centuries CE)	
Large-Scale Migration (e.g. forced migration, diaspora)		Schroeder et al. (2009)	Barbados (17 th -19 th c. CE)	Reconstructed geographic origins and dietary shifts among enslaved Africans
		Kootker et al. (2016)	South Africa (1750–1827)	Analyzed mobility, age at forced migration, and geographic provenance for enslaved individuals
Social Status		Turner et al. (2009)	Late Horizon Peru (1428–1532 CE)	Established provenance of migrants in Machu Picchu to infer elite versus labourer social class
		Müller Sheeßel et al. (2020)	Iron Age Germany (620–250 BCE)	Examined possible causes (e.g. low status enslaved social group, deviant modes of death) for cave site burial
Kinship and Residence Patterns		Haak et al. (2008)	Germany (4600 BP)	Examined nuclear kinship and patrilocality within multiple burials in conjunction with ancient DNA (aDNA) analysis
		Eerkens et al. (2014)	Middle Period California (500 BCE–700 CE)	Explored potential for post-marital matrilocality residence in the San Francisco Bay

*This table is by no means exhaustive. Given the breadth of research in this area, this table has been restricted to contain two to four examples of each migration and mobility sub-category.

While Sr isotope analysis has dominated twenty-first century trace element studies, Sr/Ca ratios have occasionally continued to be used in weaning and diet studies. Because breastmilk is depleted in Sr relative to Ca, but weaning foods contain more Sr, Sr/Ca ratios have utility in reconstructing breastfeeding and weaning patterns in past populations. By comparing Sr/Ca ratios against estimated age-at-death for juvenile individuals, Mays (2003) reported that the average weaning age among the medieval Wharram Percy population was between one and two years. From a dietary perspective, Sr/Ca ratios have been used in conjunction with stable C, N, and sulphur (S) isotope data to analyze the diet of gladiators and civilians from a Roman-era cemetery in Turkey (Lösch et al., 2014). The authors reported that the highly elevated Sr/Ca ratios in gladiator bone relative to civilians that is likely indicative of the gladiators' frequent consumption of a plant ash beverage used as a post-fight remedy (Lösch et al., 2014).

Pb has continued to be an important subject of study, with continued focus on the history of human Pb exploitation and Pb's impact on human health (e.g. Nakashima et al., 2011; Montgomery et al., 2012; Pastorelli et al., 2014; Giffin et al., 2017; Laffoon et al., 2020; Scott et al., 2020), though increasing efforts have focused on its use as a Sr-esque indicator of provenance (Kamenov & Gulson, 2014; Shaw et al., 2016; Laffoon et al., 2020). Laffoon and colleagues (2020) attempted to use Pb isotopes to infer geographic origin among enslaved individuals interred on a Barbadian plantation. However, in many cases, the childhood to young adult geogenic signature of Pb was obscured by anthropogenic Pb exposure, whether biogenic or diagenetic in origin.

Environmental, social, and occupational determinants of human exposure to toxic elements beyond Pb have also been the subject of recent bioarchaeological study. For example, Swift and colleagues (2015) studied arseniasis in human remains in the Atacama Desert, Chile during the Late Chinchorro to Inca periods (3867–474 BP), finding that over several millennia, approximately one third of the population suffered from chronic arsenic (As) poisoning as a result of the contaminated drinking water in this region. Population-specific occupational and social practices can further increase the extent of element exposure. For example, high levels of mercury (Hg) were found among Danish monks interred at the Cistercian Abbey cemetery at Øm but not at the Franciscan Friary of Svenborg (Rasmussen et al., 2008). This pattern was attributed to Cistercian monks treating leprosy and syphilis patients with Hg-containing medicine, or alternatively, from using Hg ink in the Abbey's scriptorium (Rasmussen et al., 2008). Similarly, Emslie and colleagues (2019) analyzed biogenic Hg exposure in numerous Neolithic to Bronze Age Iberian populations that were known for their extensive use of cinnabar-based pigments, finding that interestingly, humeri disproportionately contained greater concentrations of Hg, which the authors speculated could be caused by greater blood flow to this skeletal region and/or higher, load-

induced remodeling rates in the humerus. Rasumussen and colleagues (2020) tracked Cu exposure across pre- and post-medieval Denmark, finding that urban individuals experienced significantly higher copper exposure than their rural counterparts, likely due to social status and the availability of metallic household goods.

Despite the 1990s critiques, small pockets of scholars continue to use trace elements for paleodietary inferences. Some of these studies (e.g. González-Reimers et al., 2001; Corti et al., 2013; İzci et al., 2013; Bianchi et al., 2017; Bocca et al., 2018) have continued to rely upon the correlational evidence used in the 1980s to suggest that elements such as Ba, Cu, Fe, Mg, Mn, nickel (Ni), Sr, and Zn can be used to make direct inferences about paleodiet. For example, İzci and colleagues (2013) analyzed Cu, Fe, Mg, Mn, molybdenum (Mo), Ni, Pb and Zn in skeletal samples from a Roman-era cemetery in Turkey to infer sex differences and higher vegetable consumption relative to meat consumption.

Some studies have successfully employed trace elements in conjunction with other lines of evidence to make more careful and comprehensive paleodietary inferences (Arnay-de-la-Rosa et al., 2011; Lazzati et al., 2016; Lugli et al., 2017). For example, Sr/Ca and Ba/Ca ratios in conjunction with pollen, stable C and N isotopes, and historical data were used to infer the onset of maize cultivation in Italy (Lugli et al., 2017). In a study on the medieval Italian site of Caravete, Lazzati and colleagues (2016) analyzed the population's diet by through multi-collector (MC) ICP-MS analysis of 22 trace elements in dental calculus (tartar) samples, supplemented with optical and scanning electron microscopy of the phytoliths within calculus. Based on both the trace element and phytolith evidence, they hypothesized that this population primarily consumed fish protein and carbohydrates belonging to dicot and monocot (specifically Poaceae, or grasses) plant families. This novel approach to using dental calculus for trace element paleodietary reconstruction

potentially overcomes the regulatory effects of metabolism on trace elements in bones and teeth, because calculus deposits on teeth secondarily. However, this particular study was perhaps premature; before this approach should be further applied, additional research should be conducted on the relationship between diet and element values in calculus, as well as the influence of diagenesis on the elemental composition of calculus.

Despite bleak prospects for the field of trace element analysis following the late 1980s to 1990s critiques, there is promise for the field of trace element analysis in terms of reconstructing mobility and element exposure. That being said, a small number of studies have continued to use trace elements in skeletal remains for paleodietary information, with variable scientific validity. Technological innovations and novel approaches have added further analytical techniques to the bioarchaeologist's toolkit that may once again extend the applicability of trace elements to bioarchaeological questions.

2.7 Resurgence of Trace Element Analysis? Techniques with Growing Momentum for the Field

2.7.1 Micro-Sampling Techniques

The “biostratified nature” of skeletal remains means that they contain a record of growth, modeling, and modeling drift events (Maggiano et al., 2016, p. 191), and by extension, trace element exposure dynamics. However, because most solution-based chemical analytical methods require skeletal sample amounts in the order of milligrams to grams (Castro et al., 2010), these conventional methods often produce element concentrations and ratios reflecting a chemical average of up to several years. Approaches to counteract this limitation and gain temporal specificity have included comparing the chemical composition of enamel and bone samples (Ericson, 1985), sampling from different bone density fractions (Bell et al., 2001), comparing

trabecular and cortical bone values from multiple bones with different turnover rates (Skytte & Rasmussen, 2013; Rasmussen et al., 2013, 2016) or comparing samples from the developed diaphysis and developing metaphysis of a bone (Waters-Rist et al., 2011). Micro-sampling techniques (e.g. micro-sectioning, micro-milling/micro-drilling, laser ablation) are another means of accessing bioarchaeological element data with greater specificity by allowing for the elemental analysis of extremely small sample amounts that potentially correspond to discrete microstructures in teeth and bone (Outridge et al., 1995). Such techniques are by no means novel, with initial applications dating back several decades, but they represent an area with growing momentum in bioarchaeological trace element analysis. Micro-sampling is advantageous to the field as a whole by minimizing the extent of destruction to precious skeletal material (Stadlbauer et al., 2007), but serial micro-sampling from distinct bone and dental microstructures provides the potential for (1) assessing an individual's "chemical life history" (Skytte & Rasmussen, 2013) with higher temporal resolution, and (2) combatting diagenesis.

Micromilling or microdrilling techniques allow for small samples to be extracted that potentially corresponding to discrete bone and tooth microstructures and studied with solution-based chemical analytical techniques (e.g. TIMS, ICP-MS). Improved sensitivity at sampling from discrete structures and an improved ability to analyze elemental composition from smaller samples with solution-based techniques have paved the way for this approach. Such approaches have been successfully and widely applied in both lighter stable isotope studies of both human dentin increments (e.g. Fuller et al., 2003; Beaumont et al., 2013; Kwok et al., 2018) and bone density fractions (e.g. Bell et al., 2001, as iterated above), as well as seasonal migration studies analyzing $^{87}\text{Sr}/^{86}\text{Sr}$ in archaeological faunal enamel (e.g. Balasse et al., 2002; Britton et al., 2009; Valenzuela et al., 2016). However, the application of micromilling and microdrilling for serial microsampling

in bioarchaeological trace element analysis of human skeletal remains is quite limited, and laser ablation methods are preferred.

Further spatial (and consequently, temporal) sensitivity can be attained with laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) or laser ablation-multi-collector-inductively coupled plasma-mass spectrometry (LA-MC-ICP-MS), in which a laser with a spot size of as little as five microns extracts a micro-sample from a bone or tooth section and analyze the concentrations or isotopic ratios of elements (e.g. Humphrey et al., 2007; Scharlotta et al., 2013; Dudgeon et al., 2016).

To study hunter-gather mobility, Scharlotta and colleagues (2013) used LA-MC-ICP-MS to analyze intra-osteon $^{87}\text{Sr}/^{86}\text{Sr}$ isotopic ratios in bone samples from the Khuzhir-Nuge XIV cemetery (c. 4650–4250 years BP) of the Cis-Baikal region, Siberia, wherein it was suspected that the remains were partially diagenetically altered. The authors visually identified diagenetically-altered regions of each sample and laser ablated, with a 6 μm spot size, several non-overlapping lines (each line representing a timespan between one month and one year of life) within osteons either identified as “intact” or “diagenetic.” In doing so, the authors both recovered biogenic Sr information and characterized diagenetic Sr chemical profiles. These novel studies demonstrate the utility of micro-sampling in the face of common limitations in the chemical analysis of skeletal remains—specifically, improving temporal resolution to gain insights into individual “chemical life histories” and combatting the potential impacts of diagenesis on biogenic signatures.

These same micro-sampling approaches have been used to examine trace element dynamics in skeletal tissues from modern human populations (e.g. Goodman et al., 2003; Dolphin et al., 2005, 2009; Castro et al., 2010; Farrell et al., 2013), which in turn, may help to provide insights into interpreting trace element patterns observed in remains from past populations.

Modern studies often have the ability to study these element dynamics and patterns in conjunction with demographic and life history variables from the study populations, the results from which may be successfully extrapolated to trace element phenomena in the bioarchaeological record. Such studies have examined trace element deposition patterns within discrete skeletal structures in relation to factors such as development, nutrition, physiological stress, disease, and environmental pollution. In doing so, some of these studies have begun to address some of the limitations posed by earlier critics of bioarchaeological trace element analysis (e.g. Ezzo et al., 1994) regarding the lack of biomedical research on trace element dynamics, particularly for homeostatically-controlled elements such as Zn. For example, Dolphin and colleagues (2009) examined micro-variation in Zn accumulation in modern deciduous enamel from children in Solís, Mexico, particularly in relation to social status, maternal nutrition (e.g. maize, phytate, Ca consumption), and adaptive absorption strategies and their impacts on the Zn metabolism of mothers and children. Research in this area has produced several valuable takeaways for the bioarchaeological interpretation of trace element variation within specific developmental junctures.

2.7.2 Element Mapping

Similarly, element mapping can aid in differentiating the nature of element uptake in skeletal remains by improving temporal resolution or distinguishing biogenic from diagenetic exposure. In bone, osteonal microstructures can be relatively “dated”; interstitial osteon fragments represent mature bone that survived remodeling events, whereas intact osteons are relatively newer formations that are superimposed on older structures, and younger osteons may be hypomineralized (having not completed secondary mineralization) and/or have a larger central canals relative to mature secondary osteons. In teeth, the incrementally-forming layers of enamel,

dentin, and cementum can be clearly delineated microscopically. Because trace elements are only incorporated biogenically into actively-forming and -mineralizing bone and dental structures, a visual distribution of trace elements in relation to structures represents a record of lifetime exposure. Additionally, it would be expected that biogenic and diagenetic trace element distributions would vary due to their different mechanisms of uptake. Element mapping of bone and tooth samples can be achieved through techniques such as synchrotron radiation X-ray Fluorescence Imaging (SR-XFI), LA-ICP-MS, or Proton Induced X-ray Emission (PIXE).

Compared with most solution-based analytical techniques, SR-XFI is a relatively less destructive technique that uses a very focused X-ray microbeam to two-dimensionally map multiple trace elements within a given sample. Some of the earliest applications of SR-XFI to map the distribution of trace elements in skeletal tissues date back to the late 1980s and early 1990s (e.g. Jones, 1989; Bockman et al., 1990), but these scans had extremely poor spatial resolution, caused in part by higher beam spot sizes and fluorescence emitting from multiple depths. In their early exploratory study, Martin and colleagues (2007) used SR-XFI to study the distribution of Br, Ca, Pb, and Zn in ancient Peruvian bone and tooth samples, finding that Zn and Pb disproportionately accumulated in the cementum and periosteal surface of bone, while Br accumulated in Ca-deficient regions of bone and the central canal surfaces of bone. As it was hypothesized that navy personnel in colonial Antigua experienced Pb toxicity due to leaded rum distillation equipment, Swanston and colleagues (2012) used SR-XFI to generate a two-dimensional fluorescence map of the microdistribution of Pb in bone samples from a British Royal Navy hospital cemetery, the spatial resolution later improved upon with the addition of confocal optics (Choudhury et al., 2016; Swanston et al., 2018; Figure 2.1). Pb was unevenly distributed across each bone sample, though focused in the cement lines, central canals, and interstitial

fragments of osteons, which provided evidence for biogenic Pb exposure sustained over a long period of time. Swanston and colleagues (2015) used the same approach to map the distribution of biogenic Hg in a bone sample from an individual with an unusually high bone Hg concentration (94.6 $\mu\text{g/g}$). The biogenic nature of Hg was confirmed by employing X-ray absorption spectroscopy (XAS), a synchrotron technique which can identify the organic, inorganic, or elemental forms of a given element, some of which are more likely to be biogenic or diagenetic in origin.

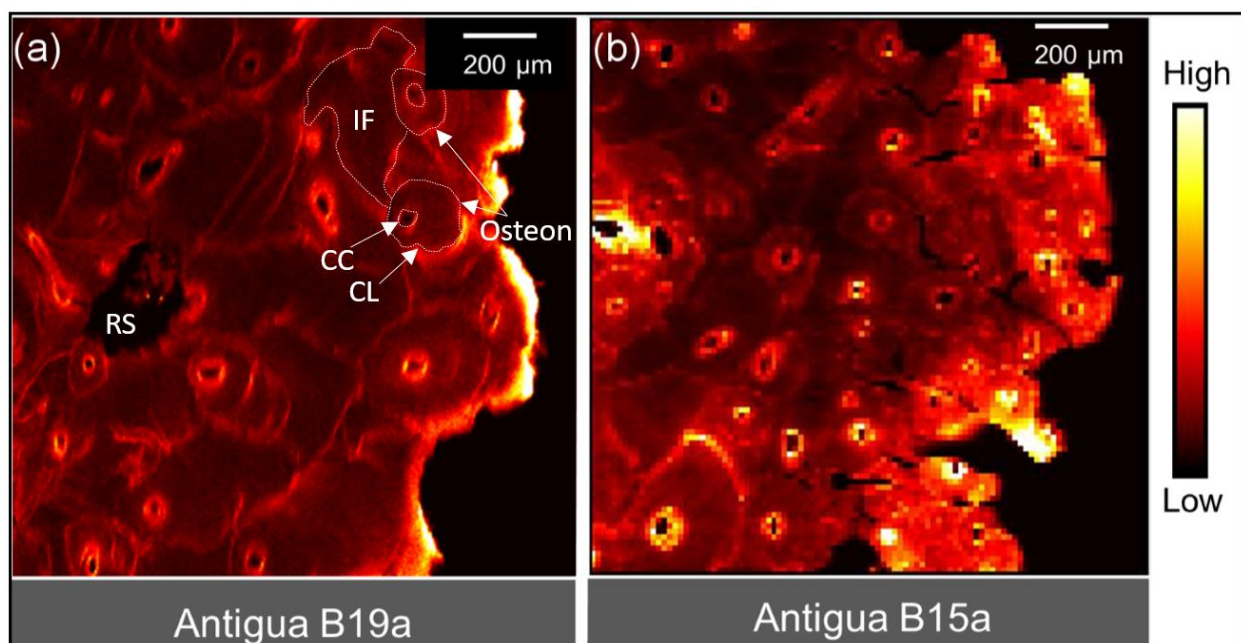


Figure 2.1—SR-XFI elemental map of Pb from cortical bone samples from two British Royal Navy personnel from colonial Antigua. The intensity of Pb varies in accordance with bone microarchitecture, wherein cement lines (CL) and central canals (CC) of osteons are enriched. Interstitial fragments (IF) of former osteons and primary lamellae are similarly enriched. Resorption spaces (RS) represent active regions of bone remodeling. This naval population experienced high levels of sustained Pb exposure (reproduced and modified from Swanston et al., 2018, PLOS ONE, under Creative Commons Attribution (CC BY) license).

SR-XFI of teeth has similarly provided insights into metabolism, diet, and the element exposure. Dean and colleagues (2018, 2019) used SR-XFI to map variation of Ca, Sr, and Zn in fossil primate and modern human teeth, finding that Sr varies pre- and postnatally due to

differences in serum levels, and that cementum increments and the neonatal line were particularly rich in Zn. Studies have also used SR-XFI to spatially examine Br and Pb in cementum in order to elucidate marine dietary components and the timing of element exposure, respectively (Dolphin et al., 2013; Swanston et al., 2018).

LA-ICP-MS has also been used to spatially map the distribution of elements by taking numerous sequential ablation spots (Kang et al., 2004; Galiová et al., 2013; Rasmussen et al., 2019). Galiová and colleagues (2013) used LA-ICP-MS to map Ba, Ca, P, Sr, and Zn in a tooth root from a brown bear fossil specimen. In doing so, they reconstructed seasonal migration and feeding trends, finding an elevation in the Sr/Zn ratio in winter season bands corresponding to hibernation (Figure 2.2). LA-ICP-MS and PIXE mapping can also be used to identify diagenetic trace element uptake and/or recover biogenic data from archaeological bones and teeth (Goodwin et al., 2007; Dudgeon et al., 2016; Willmes et al., 2016; Rasmussen et al., 2019).

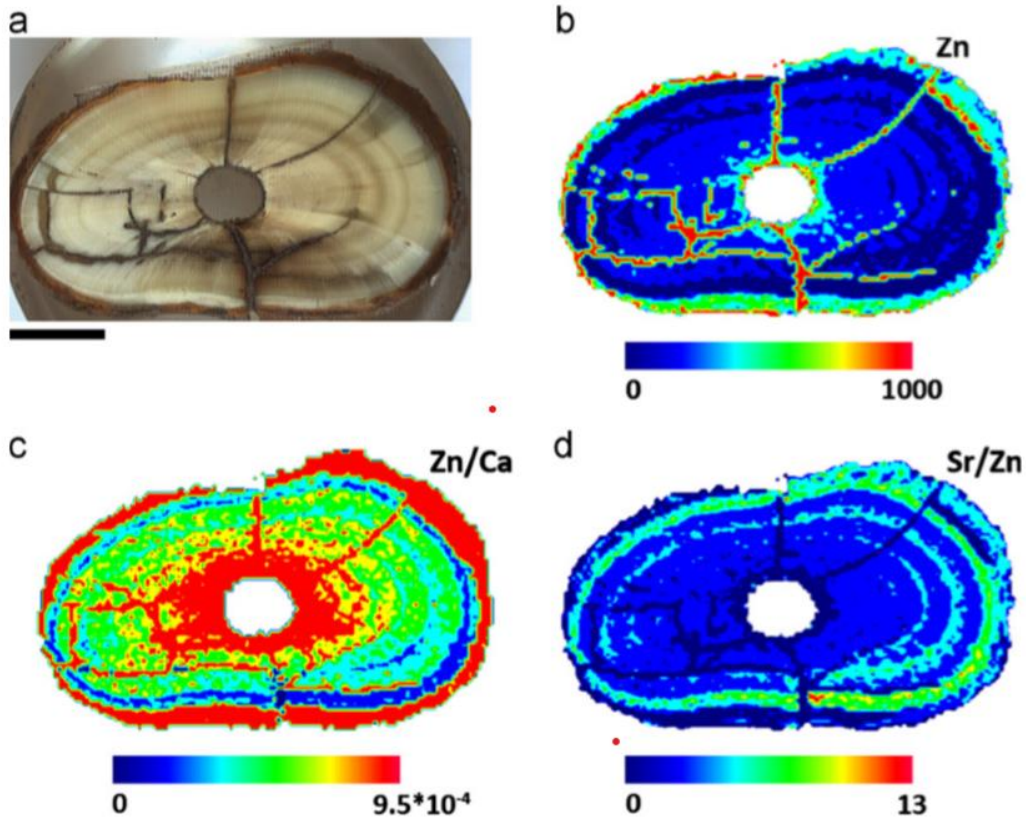


Figure 2.2—Within the brown bear (*Ursus arctos*) canine tooth root pictured in (a), element distribution maps of Zn (b), Zn/Ca ratios (c), and Sr/Zn ratios (d) were compiled using LA-ICP-MS. Zn, Sr, and Ca distributions in dental increments were used to infer seasonal migration, feeding, and hibernation patterns (originally published in Galiová et al., 2013 and reproduced with permission from Talanta).

SR-XFI is relatively less destructive than LA-ICP-MS, but quantification of elements is more refined with LA-ICP-MS. Employing SR-XFI and LA-ICP-MS requires a sufficiently thin sampling “thickness” so that a high degree of certainty of co-localization between the elements of interest and bioarchitecture of the bone/teeth is achieved. This co-localization certainty is somewhat greater for confocal SR-XFI given the ability to achieve typically smaller beam focus.

Element mapping of bone and dental samples from modern humans has also provided valuable insights into human trace element metabolism and interactions between trace elements and diet or disease. Such research has provided insights into biogenic “baselines” and metabolic patterns of trace element accumulation in bone and dental tissues (Kang et al., 2004; Zoeger et al.,

2005, 2006; de Souza-Guerra et al., 2010, 2014; Pemmer et al., 2013; Wang et al., 2017). Use of SR-XFI to map the disproportionate distribution of trace elements such as Pb and Zn in modern bone with respect to diseases such as osteoarthritis and osteosarcomas (Zoeger et al., 2008; Rauwolf et al., 2017) may aid in palaeopathological interpretations, potentially including cases where the diseases were still in early stages at death.

2.7.3 Non-Traditional Stable Isotopes

Conventionally, the “traditional” non-radiogenic stable isotopes of carbon ($\delta^{13}\text{C}$), nitrogen ($\delta^{15}\text{N}$), sulphur ($\delta^{34}\text{S}$), and oxygen ($\delta^{18}\text{O}$), and radiogenic stable isotopes of strontium ($^{87}\text{Sr}/^{86}\text{Sr}$) and lead ($^{206}\text{Pb}/^{204}\text{Pb}$) have been used to establish paleodiet, migration patterns, weaning, and social status in past populations (Burton, 2008; Katzenberg & Waters-Rist, 2019). However, innovative developments in multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS) and thermal ionization mass spectrometry (TIMS) have allowed for the differentiation of isotopes from heavier trace metals, such as copper ($\delta^{65}\text{Cu}$), iron ($\delta^{56}\text{Fe}$, $\delta^{57}\text{Fe}$), magnesium ($\delta^{25}\text{Mg}$, $\delta^{26}\text{Mg}$), zinc ($\delta^{66}\text{Zn}$, $\delta^{67}\text{Zn}$, $\delta^{68}\text{Zn}$), and non-radiogenic strontium ($\delta^{88}\text{Sr}$; Jaouen & Pons, 2017).

As discussed above, a key critique faced by the field of trace element analysis was the use of elements (e.g. essential elements) inappropriate for paleodiet reconstruction. Variation in element concentrations of consumed plants does not directly correspond to element concentrations of bone if said elements are regulated by homeostatic control mechanisms (e.g. Ca, Cu, Fe, Mg, Zn), except in cases of extreme deficiency or toxicity. Still in its infancy, the analysis of so-called “non-traditional” stable isotopes (Jaouen & Pons, 2017) is a potential means of addressing this limitation because bone isotopic ratios are more likely to reflect dietary factors. Unlike element concentrations subjected to a host of regulatory processes, a body’s isotopic ratios are impacted

by both isotopic ratios in food and metabolic fractionation mechanisms; therefore, by comparing the isotopic signature of an element in remains against the isotopic signatures of local bioavailable foods and known human isotope fractionation processes, it may be possible to gain interesting insights into paleodiet. Utilizing “non-traditional” isotopes for paleodiet construction has the added benefit of using hydroxyapatite-based elements in cases where the organic preservation is poor, such as in reconstructing hominin diet (Jaouen, 2018).

Several studies have provided insights into the ecological dynamics of several metal isotopes. For example, Jaouen and colleagues (2013) studied the fractionation of Cu, Fe, and Zn isotopes in mammalian food webs, reporting that plant consumption results in the preferential absorption of heavier Zn isotopes. Jaouen and colleagues (2016a, 2016b) found a marked trophic level effect for $\delta^{66}\text{Zn}$ values in terrestrial and marine mammals in the Turkana Basin and Canadian Arctic, respectively. With regards to Fe isotopes, plant metabolisms vary with respect to light versus heavy Fe isotopes and employ one of two uptake strategies that may be useful in differentiating specific plants in one’s diet (Guelke & von Blanckenburg, 2007). Von Blanckenburg and colleagues (2013) established discrete $\delta^{56}\text{Fe}$ isotopic ranges for vegetables, grain crops, and animal products.

Some scholars have proposed applications of certain “non-traditional” stable isotopes to bioarchaeology beyond the realm of paleodiet reconstruction. Similar to Sr and Pb, geographic isotopic variation in Ca, Mg, and Zn have been found (Martin et al., 2014; Jaouen et al., 2016a, as cited in Jaouen et al., 2017), demonstrating the potential utility of these isotopes for establishing provenance. Assessing the isotopes of essential elements under homeostatic control may also provide insight into cases of nutritional deficiency or metabolic dysregulation (Jaouen et al., 2017). Interestingly, Jaouen and colleagues (2012) found significant sex differences in $^{56}\text{Fe}/^{54}\text{Fe}$ and

$^{65}\text{Cu}/^{63}\text{Cu}$ isotopic ratios in a 17th to 18th century French population and suggested that, with further research on animal models and human populations, these findings could potentially be translated into an alternative approach for sex estimation of skeletal remains. The authors proposed that this sex-specific variation in bone likely reflects a metabolic phenomenon in blood reported by Walczyk and von Blanckenburg (2002), in which ^{56}Fe and ^{65}Cu are depleted among females when compared to males.

While still in its infancy, the emerging field of “non-traditional” stable isotopes is a promising direction for bioarchaeological trace element analysis that may re-open the door to trace element analysis of chemicals previously deemed unsuitable. Hopefully bioarchaeologists will be influenced by the cautionary tales of overzealous multielement analyses of the 1980s, however, and continue to carefully research these isotopes before widely applying them to the bioarchaeological record.

2.8 Conclusions

This review has provided a historical overview of the field of trace element analysis and analyzed the current state of the field by investigating common trends and new innovative approaches. Mid-twentieth century efforts to characterize human bone chemistry and interpret diet from Sr in skeletal remains culminated in the widespread use of a multiplicity of elements for bioarchaeological interpretations during the 1980s. The early optimism for several trace elements to carry significance about past human lifeways was called into question, however, by critics who argued that many scholars failed to take into account the impacts of diagenesis and the complexities of geological, ecological, and metabolic trace element dynamics. This period of critiques resulted in the relative abandonment of trace elements for paleodietary reconstruction,

though the use of trace elements for reconstructing mobility and geological origins has proved fruitful in the twenty-first century. New developments in element micro-sampling techniques, element mapping methods, and “non-traditional” stable isotope analysis of trace elements may help combat previous critiques of the field of trace element analysis, however, and provide a means to revisit earlier thinking. Micro-sampling and element mapping of bones and teeth present potential means to account for diagenesis, recover biogenic information, and provide better temporal specificity into the “chemical life histories” of individuals. Typically unaffected by homeostatic regulation, “non-traditional” isotopes may re-open the door to the study of essential and regulated elements previously deemed “unsuitable” by critics.

Chapter 3—Investigating Past Lead Exposure in Bioarchaeological Remains with Synchrotron X-Ray Fluorescence and Absorption Spectroscopy

This chapter was previously published in the special issue, “Synchrotron Radiation in Art and Archaeology” (Simpson, R., Varney, T. L., Swanston, T., Coulthard, I., & Cooper, D. M. L. (2019). Investigating past lead exposure in bioarchaeological remains with synchrotron X-ray fluorescence and absorption spectroscopy. *Synchrotron Radiation News*, 32(6), 11–16).). RS, TV, and DC conceptualized the manuscript. The manuscript was written by RS. RS, TV, TS, IC, and DC were all involved in the revision of the manuscript.

Abstract

Trace element analysis of bone often contributes valuable information to bioarchaeology. Bones and teeth are an excellent proxy for trace element exposure during an individual’s life as many trace metals, such as lead (Pb), become incorporated into actively mineralizing sites of the skeleton and dentition, acting as a record of an individual’s lifetime trace element exposure. Unfortunately, many conventional methods of trace element analysis are hindered by an inability both to account for the effects of diagenesis (the post-mortem modification of remains), and to access any information regarding the timing or chronicity of trace element exposure. Synchrotron radiation X-ray fluorescence imaging (SR-XFI) and Confocal X-ray Absorption Spectroscopy (CXAS) represent two potential means to mitigate these limitations by allowing the user to construct maps of element microdistribution in bones and teeth and speciate elements in localized regions of bones and teeth, respectively. This article will evaluate the current state of research in this area by reviewing successful applications of these techniques to the study of past human Pb exposure, with a focus on methodological improvements.

3.1 Introduction

Bioarchaeology, the sub-discipline of biological anthropology concentrating on human remains, involves the study of skeletal tissues to make inferences about lifeways in the past. Trace metals such as lead (Pb), strontium (Sr), mercury (Hg), and barium (Ba) have a strong affinity for bones and teeth and consequently accumulate in these tissues during different timeframes of an individual's life. Bones, which constantly undergo the process of remodeling, reflect an individual's relatively recent exposure (years to decades) to trace elements (Hedges et al., 2007), whereas dental tissues, which do not remodel, reflect trace element exposure during their formation during childhood. Trace element analysis of archaeological bones and teeth has the potential to provide the bioarchaeologist with insights into past human diet, health, environment, mobility, occupation, habitual activities, and burial conditions (Burton, 2008). Pb is historically significant given the breadth of its use throughout the human past as well as its toxic health impacts on populations. First exploited by humans as early as the sixth millennium BCE (Martínez-Cortizas et al., 2016), Pb has been widely used by humans in paint, ammunition, cosmetics, plumbing, and as a food additive (Lessler, 1988) with prominent peaks in Pb use occurring in antiquity, the Middle Ages, and following the European Industrial Revolution (Lessler, 1988; Jaworowski et al., 1985). As such, Pb has been the focus of numerous previous bioarchaeological studies (e.g. Jarcho, 1964; Aufderheide et al., 1985; Patterson et al., 1991; Giffin et al., 2017). Methods conventionally used to assess Pb in skeletal remains are limited in their ability to discern the nature and timing of Pb uptake into bone. This article will review the strides that synchrotron X-ray microbeam techniques have made regarding the analysis of Pb in bioarchaeological remains by summarizing key studies and methodological advancements, such as the addition of confocal optics.

3.2 Conventional Trace Element Analytical Techniques

Skeletal tissues, bone and dental, act as a reservoir for many trace metals. Pb is taken up into actively mineralizing regions of bone where it can bind with non-collagenous proteins or substitute for calcium in the hydroxyapatite mineral that largely constitutes the inorganic portion of bone (Pemmer et al., 2013). Bone is continually remodeled throughout an individual's life by resorption events occurring in concert with the formation of new secondary bone. In the dense outer cortex of bone this process creates osteons (also referred to as Haversian systems) which are cylindrical structural units of bone consisting of a central (Haversian) canal for blood vessels and nerves surrounded by layers of lamellae and bordered by a mineral- and protein-rich cement line (Figure 3.1).

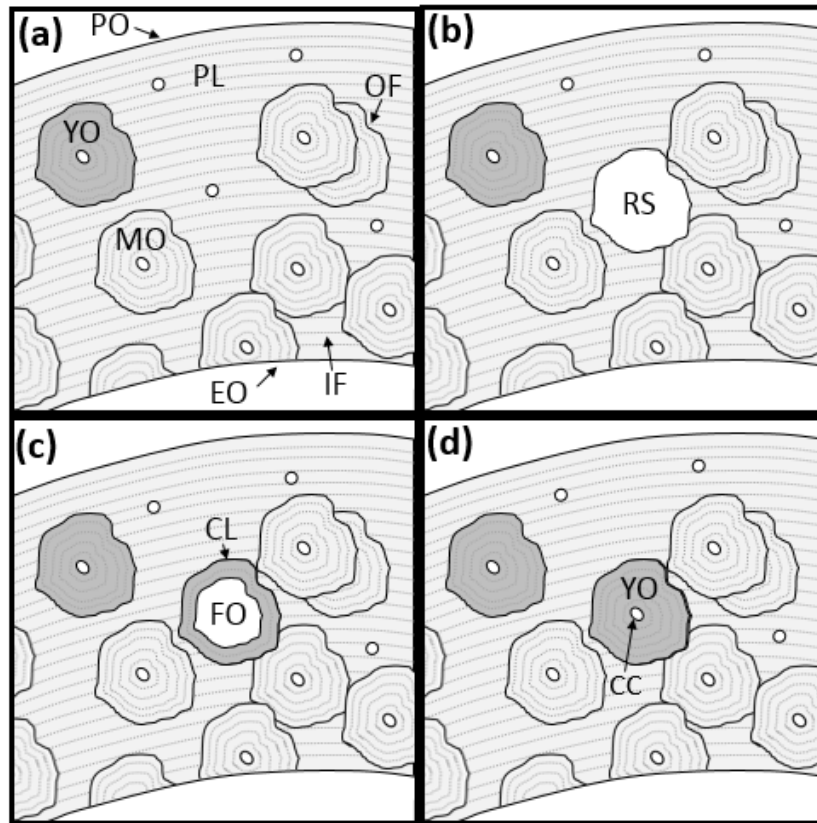


Figure 3.1—Schematic representation of human bone microarchitecture (spanning a timeframe of years to decades up until death) and the remodeling process. (a) Bone contains an outer periosteal (PO) and an inner endosteal (EO) surface. During growth, layers called primary lamellae (PL) form on either surface and are gradually replaced by secondary osteons during remodeling. Mature osteons (MO) have a mineral density comparable with surrounding bone tissue while young osteons (YO) are relatively hypomineralized. As remodeling progresses, resorption of existing bone may leave behind interstitial fragments (IF) of bone and osteon fragments (OF). (b) Remodeling begins with creation of a resorption space (RS) cutting through existing bone microstructure. (c) New forming osteons (FO) form within resorption spaces, bordered by cement lines (CL). (d) New bone formation continues until a new young osteon (YO) has formed, containing a central canal (CC) for neurovascular structures. Modified and reproduced with permission from Swanston et al. (2018; PLOS ONE).

As with bone, Pb can be substituted into the hydroxyapatite crystal of actively forming dental tissues. Teeth are comprised of three distinct mineralized tissues: dentin, enamel, and cementum. Enamel forms the outer layer of the tooth crown which is visible inside the mouth and is underlain by dentin whereas dentin also forms the inner layer of tooth roots covered by a thin

layer of cementum. Both deciduous (a.k.a. baby) and permanent tooth enamel and dentin form during fetal and childhood development and do not remodel, therefore acting as a childhood record of element exposure. Cementum rings continue to grow incrementally throughout an individual's life, thereby carrying the potential for information on timing of element exposure throughout one's life.

Conventionally, trace element analysis of Pb in bioarchaeology is undertaken either by determining bulk elemental concentrations of Pb (e.g. ppm, ppb, $\mu\text{g/g}$) or by differentiating Pb isotopes. This can be achieved through use of techniques such as atomic absorption spectrometry (AAS), thermal ionization mass spectrometry (TIMS), or inductively coupled plasma mass spectrometry (ICP-MS). One key limitation associated with these conventional analytical techniques is their inability to determine the nature of elemental uptake; they cannot account for the effects of diagenesis or highlight temporal information regarding the timing and nature of trace element exposure. For example, bulk analysis can identify evidence of Pb uptake but cannot differentiate between acute high-level Pb exposure and chronic low-level Pb exposure.

In bioarchaeology, the term *diagenesis* typically refers to the suite of post-mortem alterations affecting skeletal remains in their depositional environment. Specifically, in the context of trace element research, diagenesis often refers to the chemical modification of bone caused by elements leaching in or out of the bone. According to Rasmussen and colleagues (2019), trace elements often become diagenetically deposited in bone via diffusion across the outer (periosteal) or internal (endosteal) surfaces (Figure 3.1), vascular pores, taphonomic openings (such as cracks or microbial damage), or into interior, porous surfaces of bone following post-mortem deterioration of outer and internal surfaces.

The biogenic uptake of elements, referring to elements incorporated into bones and teeth during an individual's lifetime, is of primary interest to archaeologists, but the inability to distinguish biogenic from diagenetic element uptake has cast uncertainty over previous trace element studies of Pb. Studies have previously attempted to account for the effects of diagenesis by analyzing site soil samples for Pb, mechanically or chemically cleaning samples prior to analysis, or screening for other diagenetic indicators (Pate & Hutton, 1988; Price et al., 1992). While these methods are sometimes successful at identifying probable diagenesis, they often cannot ascertain the extent of diagenetic alteration.

3.3 SR-XFI of Archaeological Bones and Teeth

Invaluable information regarding the nature of trace element uptake can be gained by accessing the spatial distribution of elements in relation to microstructures of bone and dental tissues. Synchrotron radiation X-ray fluorescence imaging (SR-XFI) uses the principle of X-ray fluorescence, in which the 'dropping in' of outer shell electrons to fill the vacancy posed by an expelled inner shell electron results in the emission of an X-ray with an energy level characteristic of the element from which it originated. Secondary X-ray fluorescence originating from multiple elements within the sample are simultaneously emitted and detected to generate a two-dimensional element map. Other non-synchrotron techniques can also be used to map trace elements in bones and teeth (Elliot et al., 1993; Goodwin et al., 2007; Guimarães et al., 2016; Rasmussen et al., 2019); however, SR-XFI is advantageous in that it can attain high sensitivity and spatial resolution and involves less destruction and processing of precious archaeological samples compared to conventional methods of analysis. SR-XFI involves taking thin sections of bone whereas other methods may involve grinding bone into a powder, digesting bone samples, embedding bone

sections, and/or ablating spots or lines from a section. That being said, X-rays may destroy the integrity of DNA within a given sample, and thus, any DNA sampling from the same section should take place prior to SR-XFI analysis. Another limitation to take into account is that SR-XFI is a highly specialized technique with limited availability and access, so the widespread applicability of this method is constrained and when limited in the achievable thickness of samples, as is the case with fragile archaeological bone, the ability to quantify element concentrations is limited due to the release of fluorescence from multiple depths within the sample.

Synchrotron radiation X-ray Absorption Spectroscopy (XAS) approaches, such as X-ray Absorption Near Edge Structure (XANES), are a complementary means of investigating trace elements in bioarchaeological remains. Microbeam XAS techniques involve analyzing the edges of element spectra to determine the nature of chemical species present, such as oxidation state, potentially providing insights into the source and nature of trace element exposure.

3.3.1 Distinguishing Biogenic and Diagenetic Pb with Microbeam Techniques

Differentiating biogenic elemental uptake in bone from diagenetic contamination has been a critical problem in trace element analysis. Due to the spatial dimension of SR-XFI, studies have argued that it may be possible to distinguish between biogenic and diagenetic sources of Pb in archaeological bone by analyzing the distribution of trace elements in relation to bone and tooth microarchitecture (Martin et al., 2007; Wittmers et al., 2008; Swanston et al., 2012); over the past few years, great technological strides have been made with regards to the specificity and spatial resolution of fluorescence maps (Choudhury et al., 2016, 2017).

At the Advanced Photon Source (APS) 20-ID-B beamline and the National Synchrotron Light Source (NSLS) X27A beamline, Martin and colleagues (2007) used XFI to map Pb, calcium (Ca), zinc (Zn), and bromine (Br) in a rib and tooth sample from a pre-colonial Peruvian individual.

Pb enrichment occurred along the tooth surface, in the tooth dentin and cementum, and within the bone, Pb was particularly enriched on the periosteal surface. The maps had insufficient spatial resolution to allow the authors to ascertain whether the source of the Pb was biogenic and/or diagenetic, but they argued that contemporaneous metal smelting practices in the area might have been the source of Pb exposure.

In a study of a nineteenth century Philadelphia population from the First African Baptist Church, Wittmers and colleagues (2008) used SR-XFI at the National Synchrotron Light Source (NSLS) X26 X-ray Microscope (XRM) to analyze variation in the ratio of Pb to Ca from the periosteal to endosteal layers of bone in samples from this cemetery. This was carried out in conjunction with AAS of the bone samples and adjacent soil samples. They argued that the bone samples were highly contaminated with diagenetic Pb, given that the infant skeletons were high in Pb, the Pb content of each sample was positively correlated with histological damage, and some samples showed a surface “peak” of Pb on the periosteal or endosteal surfaces. The authors argue that the effects of diagenesis completely obscured any biogenic evidence of Pb within the remains.

Due to the British Royal Navy’s extensive use of Pb in the nineteenth century, it has been proposed that Pb was a significant contributor to morbidity and mortality in the West Indies at that time. Swanston and colleagues (2012) set out to test this hypothesis by performing conventional mass spectrometric techniques in conjunction with histology and SR-XFI at the Canadian Light Source (CLS) VESPERS beamline on a bone sample from a British Royal Navy cemetery site in Antigua (1793–1822). They found that the bulk Pb level for this sample was 253.94 ppm, remarkably high when compared with 26.91 ppm for a pre-contact Antiguan sample. By overlaying the XRF image on a matching histological image (Figure 3.2a), it became clear that certain structures, such as the central canal surfaces and cement lines, were heavily enriched in Pb

relative to other structures, which is consistent with patterns from SR-XFI studies of modern bone (e.g. Zoeger et al., 2005, 2008; Pemmer et al., 2013). Swanston and colleagues (2012) concluded that given the heterogenous distribution of Pb across the bone sample, there was strong evidence for biogenic uptake corresponding to temporal variation in Pb exposure throughout the individual's life. It would be expected that diagenetic exposure would result in a more homogenous pattern of Pb with potential enrichment along the periosteal surface or endosteal surface of bone.

The effectiveness of synchrotron radiation microbeam techniques relies upon producing thin sections of bones and teeth; while thick sections do emit fluorescence, the superposition of structures within the three-dimensional volume of the sample produces a blurry image. This is reflected in early applications of SR-XFI to bioarchaeological remains (e.g. Wittmers et al., 2008; Swanston et al., 2012) in which thicker bone sections (ranging from 50–100 μm thick) had to be used due to the fragility of poorly preserved archaeological bone. Resulting images from these preliminary works had comparatively lower spatial resolution to maps of modern tissues due to fluorescence emitting from multiple depths within each sample. Use of confocal optics can mitigate this problem; by placing a polycapillary or collimating channel array (CCA) optic between the sample and the detector, it is possible to optically, rather than physically, section the sample by collecting fluorescence emitted only at a specific depth within a three-dimensional volume and reject extraneous scatter (Choudhury et al., 2016).

In 2016, Choudhury and colleagues re-scanned bone samples from the British Royal Navy cemetery site in Antigua and scanned bone samples from two contemporaneous naval sites using both a standard XFI setup (as published in Swanston et al., 2012) as well as a confocal setup, implementing an XOS® polycapillary optic with a 25 μm diameter spot size. The addition of confocal geometry produced a marked improvement in spatial resolution in which variation in the

presence of Pb in relation to bone microstructure features could be clearly delineated. As outlined in Swanston and colleagues' (2012) paper, this variation is inferred to be evidence of biogenic uptake.

In 2017, Choudhury and colleagues (2017) published a follow-up testing the effectiveness of collimating channel array (CCA) optics, employing a silicon optic with a depth resolution of 7 μm and a germanium optic with a depth resolution of 2.5 μm . They compared Pb, Ca, and Sr maps generated with CCA optics against maps generated with and without a confocal polycapillary optic (Figure 3.2). The CCA optics produced superior image resolution as a result of the improved depth resolution. Variation in the intensity of Pb across various microstructures is particularly evident, potentially demonstrating variation in the extent of Pb exposure during the times in which these features formed.

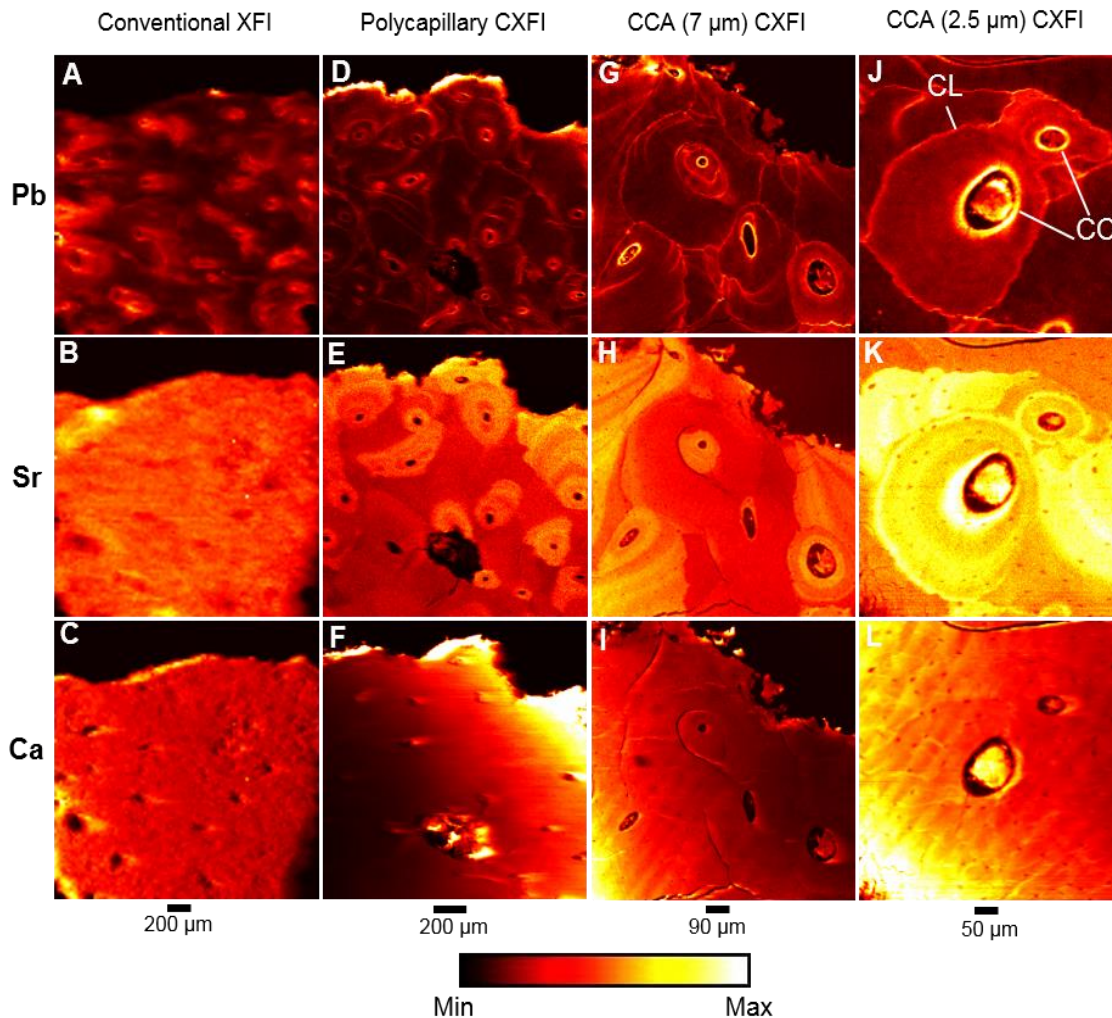


FIGURE 3.2—*Pb, Sr, and Ca element maps from British Royal Navy (Antigua) bone samples using conventional, polycapillary, and CCA (7 and 2.5 μm) SR-XFI modes. CC: central canal of osteon, CL: cement line of osteon. Modified and reproduced with permission from Choudhury et al. (2017; JAAS).*

But is it possible to distinguish biogenic from diagenetic Pb if both processes have occurred in the same sample? Wittmers and colleagues (2008) argued that if pervasive diagenetic contamination were present, it would completely obscure the biogenic distribution of Pb. However, further developments in distinguishing biogenic from diagenetic Pb have arisen due to the adoption of Confocal X-ray Absorption Spectroscopy (CXAS) techniques. By employing an additional focusing optic in an XAS setup, it becomes possible to speciate elements corresponding to

localized microfeatures. Choudhury and colleagues (2017) used a 2.5 μm CCA optic in their XAS setup to discern subtle differences in Pb spectra corresponding to a slightly different species of Pb present along the periosteal surface, potentially corresponding to a diagenetic origin (Figure 3.3).

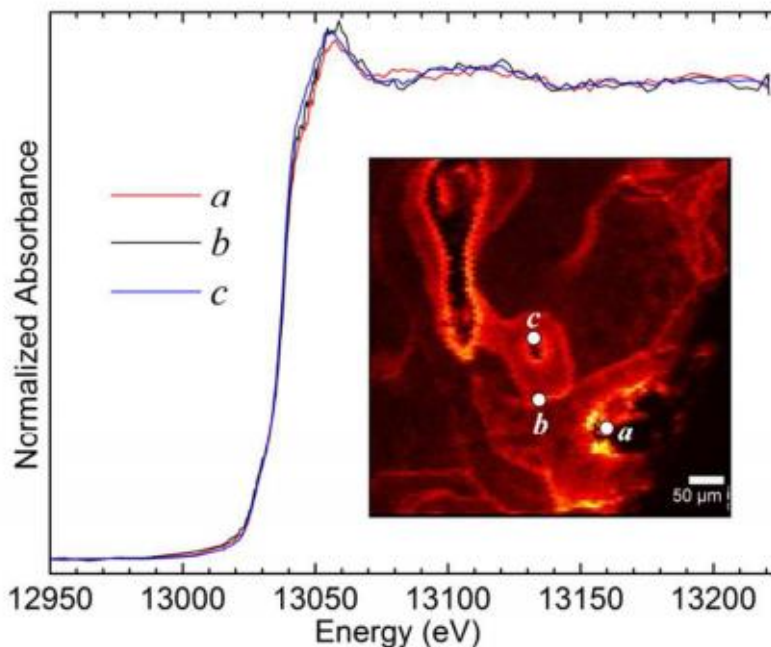


FIGURE 3.3—Pb L_3 -edge CXAS spectra collected using 2.5 μm CCA optic from British Royal Navy (Antigua) bone sample in which location *a* refers to the sample surface, location *b* refers to the cement line, and location *c* refers to the canal wall. Reproduced with permission from Choudhury et al. (2017; JAAS).

3.3.2 Temporal Resolution: The Franklin Expedition

A second limitation of many conventional forms of trace element analysis is an inability to access information regarding the timing or chronicity of trace element exposure. Bulk element levels or isotopic ratios cannot reveal any temporal information; however, by analyzing the spatial distribution of an element, it becomes possible to gain insights into research questions of this nature. SR-XFI studies investigating the death of the Franklin expedition illustrate this capability.

In 1845, Sir John Franklin led a British Royal naval expedition attempting to navigate the Northwest Passage across the Arctic; however, the entire crew perished, the reasons for which are

widely debated (e.g. Kowal et al., 1991; Horowitz, 2003; Mays, 2015). A popular explanation for the demise of the Franklin crew is Pb poisoning, given the elevated Pb levels observed in recovered tissues from some of the crewmembers and isotopic similarities between the Pb of the solder sealing food cans that provisioned the expedition and Pb in the bones of crewmembers (Kowal et al., 1991).

Martin and colleagues (2013) set out to test the hypothesis that heightened Pb exposure during the expedition caused the death of Franklin's crew by employing SR-XFI of bone samples from the crew members and by conducting Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS) of Pb isotopes in the bone and solder seals. The resulting fluorescence maps showed an overall uniformity in Pb with subtle variation across the sample, meaning that Pb exposure was likely sustained across a long period of time. Comparison of Pb isotopes from the crewmembers' bones and the tin solder seals revealed subtle but significant differences, meaning that tin solder was not the primary source of Pb for these individuals.

Swanston and colleagues (2018) revisited this hypothesis using confocal SR-XFI of bone and tooth samples belonging to crewmembers of the Franklin expedition to generate higher resolution maps. If the crew experienced elevated Pb exposure during the expedition, those known to have died in the first winter (Beechey Island) would exhibit signs of lower Pb exposure and those who died the following year or later (King William Island) would exhibit signs of more sustained Pb exposure, and samples from the Franklin Expedition crewmembers would exhibit elevated Pb when compared with contemporaneous populations.

Within the bone samples, Swanston and colleagues (2018) assessed whether Pb was enriched in microstructures forming around the time of death as opposed to mature structures by assessing degree of mineralization for each osteon (via assessment of Ca fluorescence images) and

size of the central canal; younger, newly forming osteons would be hypomineralized and have a comparatively large central canal while mature secondary osteons may have relatively smaller central canals and a mineral density similar to surrounding interstitial bone (Figure 3.1). Pb was present in many osteons irrespective of their relative ages and little variation existed in comparing Beechey Island and King William Island samples (Figure 3.4). The tooth cementum scans showed evidence of sustained Pb exposure throughout life, rather than a peak in Pb in the outermost cementum layers just prior to death, also demonstrating that crewmembers had been chronically exposed to Pb.

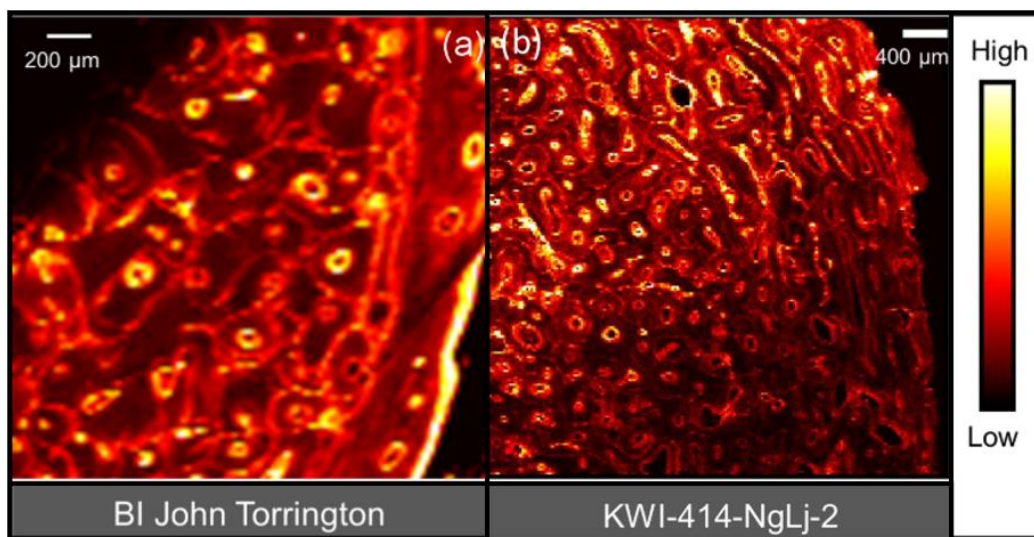


FIGURE 3.4—SR-XFI Pb spatial map for a Beechey Island bone specimen (John Torrington) (a) and a King William Island bone specimen (b) from the Franklin Expedition. Modified and reproduced with permission from Swanston et al. (2018; PLOS ONE).

In comparing the Pb maps from the Franklin expedition crewmembers with samples from the roughly contemporaneous British Royal Navy cemetery in Antigua, there are consistencies in both bulk Pb content and in the general patterns observed in Pb fluorescence maps, suggesting that sustained Pb exposure was common throughout the British Navy at this time. Overall, this study

is consistent with Martin and colleagues' (2013) conclusion that there is not evidence of elevated Pb exposure immediately prior to death to constitute the demise of the Franklin crew.

3.4 Conclusion

This paper has reviewed the key studies using synchrotron microbeam techniques to analyze Pb exposure in the bioarchaeological study of past human remains. Confocal SR-XFI and XAS techniques have helped overcome many of the limitations posed by conventional trace element analytical techniques. Visualizing the distribution of Pb in relation to bone and tooth microstructure and speciation of elements at localized regions provide the bioarchaeologist with the potential to differentiate between biogenic and diagenetic sources of Pb and gain insights into the timing and extent of element exposure. Advancements in confocal optics have vastly improved research in this area and broadened the scope of research questions that can be addressed using synchrotron microbeam techniques. These techniques will likely continue to deepen our insight into past human exposure to Pb.

Chapter Four—Synchrotron Radiation X-ray Fluorescence Imaging of Lead in Experimentally-Altered Bone: Insights into Biogenic and Diagenetic Exposure

This manuscript is in preparation for publication involving the collaboration of Rachel Simpson, Tamara L. Varney, David M. L. Cooper, Ian Coulthard, Treena Swanston, Vaughan Grimes, T. Jessica A. Munkittrick, and Rimantas Jankauskas. The target venue for the manuscript will be an archaeological science journal. The study was conceptualized by DC, TV, and RS and funded by a SSHRC Insight Grant (TV, P.I.) and the Canadian Research Chairs Program (DC). Modern samples were acquired by RS and archaeological samples were acquired by TV and RJ. VG and TM conducted ICP-MS of Lithuania sample and RS, IC, and TS conducted SR-XFI analysis. The manuscript was written by RS. All co-authors will be involved in manuscript revision.

Abstract

The toxic element lead (Pb) has had critical repercussions for human populations in the past and remains a worldwide health concern today. The trace element analysis of Pb from archaeological skeletal remains is hindered by diagenesis, the post-mortem chemical, physical, and biological transformations of skeletal remains, as these processes can interfere with the biogenic (lifetime) chemical composition of bones and teeth. Synchrotron radiation X-ray fluorescence imaging (SR-XFI) generates maps of Pb in bone on a microstructural scale, potentially providing insights into the biogenic or diagenetic nature of its uptake, as well as aspects of an individual's life history of biogenic Pb exposure. The aim of this study was to (1) test the hypothesis that there are spatial differences in the distribution of Pb for diagenetic and biogenic modes of uptake in bone, and (2) characterize lifetime biogenic Pb exposure in a modern sample of cadaveric tissues donated in Saskatoon, Saskatchewan, Canada. To address these aims, this study used inductively coupled plasma-mass spectrometry (ICP-MS) and SR-XFI on bone samples from eighteenth to nineteenth

century archaeological sites from Antigua and Lithuania and experimentally altered modern bone samples donated to the Body Bequeathal Program (University of Saskatchewan, Saskatoon, SK). Results from the modern cadaveric samples support the hypothesis that there are marked differences in the spatial distribution of Pb corresponding to biogenic versus diagenetic uptake that correspond to patterns observed in the archaeological samples from Antigua and Lithuania. Diagenetic Pb is mainly confined to the periosteal edge of each sample with some enrichment of cracks and sub-periosteal canals. These results may be useful in the future for differentiating diagenetic from biogenic Pb accumulation and for informing sampling strategies. The modern Saskatchewan sample demonstrates a pattern of relatively low Pb exposure with higher levels of Pb exposure occurring in bone structures of a relatively older age that formed earlier in life, likely during the era of leaded gasoline (pre-1980s).

4.1 Introduction

Given its grave health implications and its extensive exploitation by humans for millennia, lead (Pb) plays an integral role in our understanding of the past. Pb is a naturally occurring element, though exposure to it is known to cause severely toxic neurological, cognitive, gastrointestinal, renal, reproductive, and cardiovascular effects if ingested, absorbed, or inhaled (Victery, 1988; Loghman-Adham, 1997; Benoff et al., 2000; Schwartz et al., 2000; Shiri et al., 2007; Luo, 2012). The severity of Pb exposure is further compounded in children, as even trace amounts of Pb can have critical developmental repercussions (Mushak et al., 1989; Finkelstein et al., 1998). Despite many governments' efforts to drastically reduce Pb exposure in many modern societies, it remains an important health concern worldwide, including in Canada (Ngueta et al., 2016; Safruk et al., 2017; Juric et al., 2018), especially with prevailing Pb pipe and paint infrastructure (Troesken & Beeson, 2003; Foley et al., 2011; O'Connor et al., 2018).

Studies estimate that between 90% and 97% of the total body burden of Pb becomes sequestered in the skeleton (Barry, 1975). While Pb, as a divalent cation, is believed to substitute for calcium (Ca) in the hydroxyapatite mineral of bones and teeth, this is not a simple substitution and more research is needed (Montgomery et al., 2010: 211). Pb can also bind to non-collagenous proteins such as osteopontin and osteocalcin (Pemmer et al., 2013). Minerals and osteogenic proteins are especially rich along the osteon central (Haversian) canal surfaces and in the cement lines (Figure 4.1), i.e. the interfaces between osteons and surrounding interstitial bone (Schaffler et al., 1987; Skedros et al., 2005). Pb is typically only incorporated into actively mineralizing surfaces of bone; then, following localized osteoclastic remodeling activity of bone, Pb can be released back into the bloodstream, where it can intrinsically act as a source of Pb exposure and become reintegrated into newly formed bone once more (Rabinowitz, 1991). Bone constantly remodels throughout an individual's life (Figure 4.1) and as such, represents a long-term, chronic

record of trace element exposure spanning years to decades. Pb is often mobilized from bone at an increased rate in disease states such as osteoporosis (Silbergeld et al., 1988; Nash et al., 2004), or during pregnancy and lactation (Manton et al., 2003; Gulson et al., 2016).

Due to its historical significance and strong affinity for the skeleton, Pb and its impact on past populations can be studied through chemical analysis of the skeleton. Bioarchaeologists have frequently used bulk Pb concentrations and ratios of Pb's four stable isotopes (^{204}Pb , ^{206}Pb , ^{207}Pb , ^{208}Pb) from skeletal remains to make inferences about past human health (Nakashima et al., 2011; Giffin et al., 2017), industry (Jarcho, 1964), social status (Aufderheide et al., 1981, 1985; Handler et al., 1986; Corruccini et al., 1987), and geographic origins (Reinhard & Ghazi, 1992; Carlson, 1996; Montgomery et al., 2005, 2010; Laffoon et al., 2020). However, these efforts have often been hindered by an inability to account for the effects of diagenesis—the suite of chemical, physical, and biological changes that degrade and alter bone post-mortem—on the chemical composition of archaeological bone. This chemical transformation of bone can occur via several different processes. Ions may adsorb onto the surface of bone, diffuse into bone, substitute into (and potentially recrystallize) the hydroxyapatite fraction of bone, or precipitate new phases in bone (Dudás et al., 2016). It is important to note that there is a degree of element specificity in the mechanism of diffusion, wherein different elements vary with respect to the strength of their diffusion gradients, a phenomenon related to the electrochemical similarity of an ion to either calcium or phosphate (Dudgeon et al. 2016), and consequently, research focusing on the diagenetic behaviour of Pb is needed. In some previous bioarchaeological studies (e.g. King et al., 2020; Molleson et al., 1986; Waldron, 1981; Wittmers et al., 2008), a “diagenetic overprint” (Hinz & Kohn, 2010: 3228) has obscured the biogenic (lifetime) Pb signature. Problems associated with

diagenesis are often compounded by the unpredictability of diagenesis and lack of controls on mortuary conditions.

Visualizing the distribution of trace elements, such as Pb, in relation to bone microarchitecture may represent a valuable means of overcoming this critical limitation, such as through synchrotron radiation X-ray fluorescence imaging (SR-XFI), laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS), or particle-induced X-ray emission (PIXE). SR-XFI is a quick and relatively less destructive method of mapping multiple elements in a sample with remarkably high spatial and depth resolution. For this method, a microbeam of synchrotron X-rays interacts with a given sample, resulting in the excitation and expulsion of inner shell electrons; outer shell electrons “drop in” to fill the vacancy, resulting in the release of radiation (fluorescence) with an energy level characteristic of the atom from which it originated. Consequently, spatial data on a microscopic scale can be simultaneously collected for multiple elements, even those present in only minute quantities. SR-XFI has previously been carried out on both modern (Jones et al., 1989; Zoeger et al., 2005, 2008; Pemmer et al., 2013; Roschger et al., 2013) and archaeological (Martin et al., 2007, 2013; Wittmers et al., 2008; Swanston et al., 2012, 2018; Choudhury et al., 2016, 2017; de Winter et al., 2019) bone and dental samples to determine the microdistribution of Pb. In visually analyzing Pb in archaeological bone, different spatial patterns have emerged that scholars have previously attributed to “biogenic” versus “diagenetic” element exposure (Swanston et al., 2012, 2017; Rasmussen et al., 2019). Based on archaeological observations, it was hypothesized that biogenic Pb exposure results in an uneven, heterogeneous distribution across different bone microstructures, with central canal surfaces and cement line structures of osteons disproportionately enriched, while diagenetic Pb exposure would be

manifested predominantly as enrichment at the periosteal (exterior) or endosteal (interior) surfaces, pores, or cracks in bone.

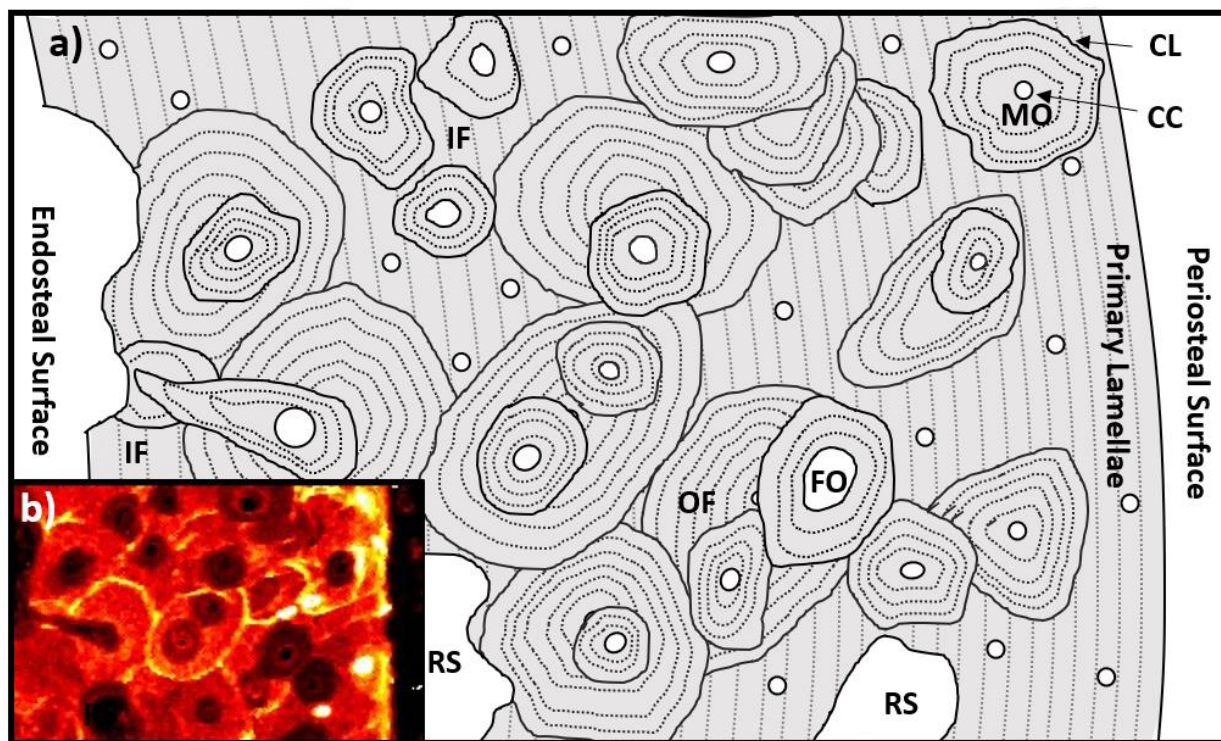


FIGURE 4.1—Schematic of cortical bone microarchitecture and remodeling in humans (a) and a SR-XFI map of Pb in human cortical bone with corresponding microarchitecture (inset; [b]). Human cortical bone contains a periosteal surface (exterior) and an endosteal surface (interior). Layers of primary lamellae develop on these surfaces during growth, to be gradually replaced by secondary osteons (or Haversian systems)—cylindrical structures of bone containing concentric lamellae and a central (or Haversian) canal (CC) for neurovascular structures, bound by a hypermineralized cement line (CL). Secondary osteons form following localized osteoclastic bone resorption events. These resorption spaces (RS) are gradually filled in by forming osteons (FO), which continue to develop into mature osteons (MO). Surrounding bone alongside existing osteons consists of hypermineralized osteon fragments (OF) and interstitial fragments (IF) of former osteons and lamellar bone that survived focal remodeling events. In the SR-XFI map inset, dark regions represent areas of low Pb uptake whereas bright red to yellow regions represent high Pb uptake. Note that in Pb map inset, the intensity of Pb does not necessarily correspond to age of structures. Age of structures can be relatively inferred on the basis of the microstructure superimposition; younger structures are superimposed on older structures, and interstitial osteonal fragments are relatively older structures. Figure 3.1 provides a more detailed overview of timing inferences based on bone microarchitecture and calcium density.

The aim of this study is twofold. First, it aims to determine experimentally, using SR-XFI, whether there are indeed spatial differences for biogenic and experimentally induced diagenetic

Pb exposure in modern cadaveric bone, and whether these differences correspond with what has been observed and interpreted archaeologically. The null hypothesis is that these two processes would not produce different spatial patterns of enrichment; Pb, if permeating through the entire bone sample, may potentially enter via large pores and bind to osteogenic proteins and hydroxyapatite minerals rich in the canals and cement lines, mimicking the so-called “biogenic” pattern.

The second aim of this study is to use the spatial distribution of Pb in modern bone to help characterize Pb exposure in a sample of individuals from a contemporary Saskatchewanian population and contrast this pattern of Pb exposure with the patterns observed in past populations. The issue of Pb exposure has become an increasingly relevant topic in urban Saskatchewan, given concerns that a portion of residential water pipes rely on old lead-containing infrastructure and recent reports that Pb levels in tap water exceed the recommended limit of 5 parts per billion (ppb) (Wilson & Ackerman, 2019). However, it is hypothesized that each modern Saskatchewan’s lifetime Pb exposure has changed, on the scale of decades, with higher exposure occurring in the past during the era of leaded gasoline (1920s–1980s). This would mean that Pb would likely be primarily confined to microstructures that had formed during this era. However, the Pb could alternatively also enrich newly formed structures with previously stored Pb that had temporarily returned to the bloodstream and then become re-incorporated into bone recently.

4.2 Materials and Methods

Modern human femoral bone samples were acquired from 14 individuals donated to the Body Bequeathal Program (Department of Anatomy, Physiology, Pharmacology, University of Saskatchewan, Saskatoon, SK). This sample assemblage consisted of 8 males (aged 69–96) and 6 females (aged 53–97). The femur was selected for analysis because it reflects a longer turnover

rate duration (mean 1.5–3% turnover per year in adult males, mean 3–4% turnover per year in adult females; Hedges et al., 2007), and thus a longer timeframe for lifetime Pb exposure. In keeping with the program’s anonymity standards, no information apart from age and sex were imparted for each individual. Biomedical ethics approval was obtained (BIO ID# 970) from the University of Saskatchewan. Mid-diaphyseal femoral cross sections approximately 5 cm in length were cut using an autopsy saw.

Controlled experiments implementing aqueous solutions with other elements, such as fluorine, have previously proved effective in simulating chemical transformations in bone (Aufort et al., 2019). In this study, diagenesis was experimentally simulated in half of each cadaveric sample by soaking the bone in an aqueous lead acetate ($\text{Pb}(\text{CH}_3\text{COO})_2$) solution. This procedure aimed to mimic general mechanisms of Pb diagenesis caused by water in the depositional environment, including ion adsorption, exchange, and diffusion onto and into bone (Dudás et al., 2016).

Lead acetate concentrations and the duration of the soak for this experiment was first determined by exposing portions of a bovine (*Bos taurus*) femur to varying Pb concentrations for different lengths of time and analyzing the final Pb content in these femoral sections with ICP-MS (Appendix 1). Because the goal of this research is rooted in confirming inferred ‘biogenic’ and ‘diagenetic’ lead patterns of uptake previously observed in an archaeological British Royal Navy population from colonial Antigua, experimental conditions of a 200 $\mu\text{g}/\text{L}$ (ppm) Pb concentration and a time period of 7 days were selected because it resulted in a post-experimental bone Pb concentration of 200 $\mu\text{g}/\text{g}$ that was comparable with the biogenic levels observed in the Antigua population.

A small portion of each cadaveric bone sample was sacrificed for inductively coupled plasma-mass spectrometry (ICP-MS) to determine Pb concentrations. This portion of each sample was cut using an IsoMet low-speed saw (Buehler, Lake Bluff, IL) using a diamond wafer blade. The blade was cleaned and the water was changed between each sample to prevent trace element contamination between samples. Samples were ground into a powder using a Spex liquid nitrogen freezer mill (Lakehead University, Thunder Bay, ON). Samples were digested with microwave digest using 10 mL of 65% nitric acid. 2% butanol was added to all samples and standards to digest remaining organic components. For Pb calibration, calibration blanks were used, and standards were run at 1 µg/L, 10 µg/L, and 100 µg/L concentrations. ICP-MS of the human cadaveric bone and diagenetically-altered bovine bone was carried out at the Saskatchewan Research Council Environmental Analytical Laboratory using an Agilent 8800 ICP-MS with an Agilent ISIS discrete sampling system for sample introduction.

An archaeological fibula sample belonging to a 40–50-year-old male of European descent was acquired from a British Royal Navy Hospital Cemetery site in English Harbour, Antigua (c. 1768–1822), which represents a population with a documented history of high and sustained Pb exposure (Giffin et al., 2017). This sample was previously screened for Pb using ICP-MS (Neptune, Thermofisher) at the Geological Sciences Department, University of Saskatchewan, Saskatoon, SK (Giffin et al., 2017). An archaeological femoral sample was also acquired from an adult female entombed in an above-ground wooden coffin in the Tiškevičius (Tyskiewicz) family crypt in Kretinga, western Lithuania (c. 1830–1891). ICP-MS was previously used to determine the bulk Pb concentration of this bone sample using a PerkinElmer Elan DRC II ICP-MS at Memorial University, St. John's, NL. Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) was previously conducted on multiple spots within the sample with a

Finnigan Element XR High resolution double focusing magnetic sector inductively couple plasma mass spectrometer and GEOLAS 193 nm excimer laser system at Memorial University, St. John's, NL (as presented and described in Swanston et al., 2017).

To prepare samples for synchrotron SR-XFI, bone samples were sectioned into 1–2 mm thin sections using an IsoMet low-speed saw and a diamond wafer blade. No additional sample preparation was required. At the Advanced Photon Source (APS; Argonne National Laboratory, Lemont, IL) Sector 20-ID-B beamline, SR-XFI of the Lithuania individual was performed in August 2017 using a rapid scan mode and SR-XFI of the modern cadaveric and Antigua samples was performed in November 2019. The obtained incident beam energy at Sector 20-ID-B was 16,299.983 eV (target incident beam energy 16,300 eV). The beam spot size was 3 μm . It has consistently been demonstrated that confocal optics can optimize spatial resolution of element maps of bone by reducing the amount of fluorescence emitted from multiple depths (Choudhury et al., 2016, 2017; Rauwolf et al., 2017). Additionally, confocal optics enable optical, rather than physical, sectioning thereby minimizing the need for sample preparation steps such as grinding and polishing (Choudhury et al., 2016, 2017). A polycapillary confocal optic was employed to reach a depth of 150 μm within each sample. Each sample was mounted onto a glass microscope slide with double sided tape, which was mounted onto a sample holder with double sided tape. The sample was placed in front of the beam with the periosteal surface of the sample oriented away from the detector. A 0.5 mm-thick sheet of aluminium foil was placed over the beam aperture to preferentially decrease Ca counts due to its lower photon energy. The scan areas for each sample were 2 mm x 2 mm, or 1.6 mm x 2.5 mm if the cortical envelope was thin, and contained more trabeculae. Using multi-channel analysis (MCA), spatial data was simultaneously collected for Pb $K\alpha$ and $K\beta$, Ca, zinc (Zn), strontium (Sr), copper (Cu), and iron (Fe).

Samples were analyzed using ImageJ software (Rasband & ImageJ, 1997–2018) and a Sector 20 input-output plug-in. A 0.5-pixel median filter was applied to improve the signal to noise ratio of images. A non-linear logarithmic calibration scale was used for the diagenetically-altered images to normalize Pb counts. Brightness and contrast were adjusted to maximize visibility of bone structures enriched with Pb and ensure consistency between scans.

4.3 Results

ICP-MS results of bulk Pb for cadaveric bone are presented in Table 4.1. The procedural blank for Pb was $<0.1 \mu\text{g/L}$. Iridium was used as an internal standard for Pb. Pb concentrations in the unaltered cadaveric bone samples range from 1.2 to 7.1 $\mu\text{g/g}$ with a mean of 4.28 $\mu\text{g/g}$ (± 1.95). The mean Pb concentration for females is 3.47 $\mu\text{g/g}$ (± 1.90) while for males, the mean Pb concentration is 4.90 $\mu\text{g/g}$ (± 1.71).

TABLE 4.1—Sex, age, and ICP-MS results of Pb concentrations for unaltered cadaveric bone.

Individual	Sex	Age	Bulk Pb Levels (µg/g)
1366	M	90	6.2 ± 0.6
1550	F	87	3.6 ± 0.5
1565	F	53	7.1 ± 0.7
1587	M	86	3.6 ± 1.0
1627	F	85	4.2 ± 0.6
1627	M	91	7.0 ± 2.0
1632	M	86	6.9 ± 0.7
1640	M	74	4.4 ± 0.7
1646	F	97	2.8 ± 0.4
1647	M	96	2.4 ± 0.4
1648	M	77	5.3 ± 0.5
1649	F	93	1.2 ± 0.2

According to the ICP-MS results, the Antigua sample has a bulk bone Pb content of 253.94 µg/g (Giffin et al., 2017). The Lithuania bone sample had a bulk Pb concentration of 125 µg/g. LA-ICP-MS analysis of the Lithuania sample revealed that the outer periosteal edge had a Pb concentration exceeding 1,000 µg/g (Swanston et al., 2017).

Figures 4.2–4.8 depict the unaltered and/or corresponding experimentally-altered SR-XFI Pb maps for cadaveric bone samples. Figures 4.9 and 4.10 represent Pb maps of the Antigua sample and the Lithuania sample, respectively. Fluorescence maps of Pb in unaltered cadaveric images demonstrate heterogeneity across the internal bone microarchitecture of each sample. Commonly, cement lines and central canals of osteons are enriched with Pb relative to concentric lamellar layers of osteons (e.g. Figure 4.2).

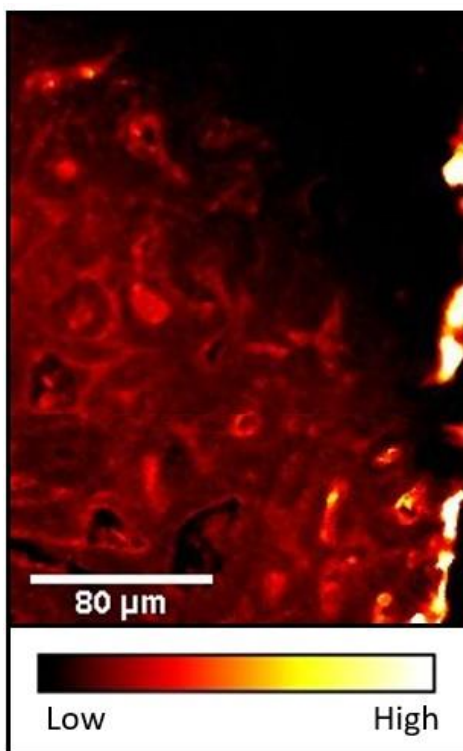


Figure 4.2—SR-XFI map of Pb for modern Saskatchewan bone sample 1550 (87-year-old female): unaltered bone sample with biogenic Pb level of 3.6 $\mu\text{g/g}$.

The presence of lead is somewhat uniform across intact osteons and interstitial fragments for Sample 1550a (Figure 4.2). Among Samples 1647a (Figure 4.3a), 1628a (Figure 4.4a), and 1627a (Figure 4.8a), interstitial fragments from remodeled osteons are typically more enriched in Pb compared to intact osteons.

SR-XFI maps of experimentally altered cadaveric bone samples (Samples 1647b, 1628b, 1565b, 1649b, 1366b, 1627b; Figures 4.3b–4.8b) consistently demonstrate a thick band of Pb enrichment along the periosteal edge of the sample. For initial scans (Samples 1647, 1628, 1565; Figures 4.3–4.5), internal bone microarchitecture within diagenetically-altered samples was obscured because the sensitivity of the scan was lower than the signal-to-noise ratio. Consequently, the diagenetic Pb enrichment along the periosteal edge is visible, but the interior of bone contains too much noise to align with its corresponding unaltered biogenic Pb map.

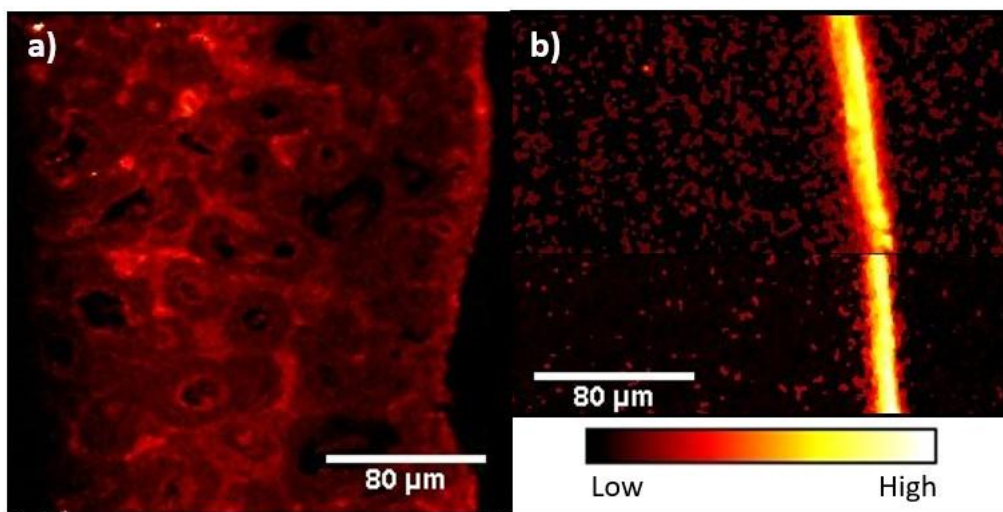


Figure 4.3—SR-XFI maps of Pb for modern Saskatchewan bone sample 1647 (96-year-old male): a) unaltered (2.4 µg/g Pb), and b) diagenetically altered with 200 µg/g Pb for 1 week. Variation is evident in the spatial resolution of internal bone microarchitecture between 4.3a) and 4.3b) due to poor beam sensitivity during scan 4.3b), causing low levels of Pb to not be detected with adequate spatial resolution.

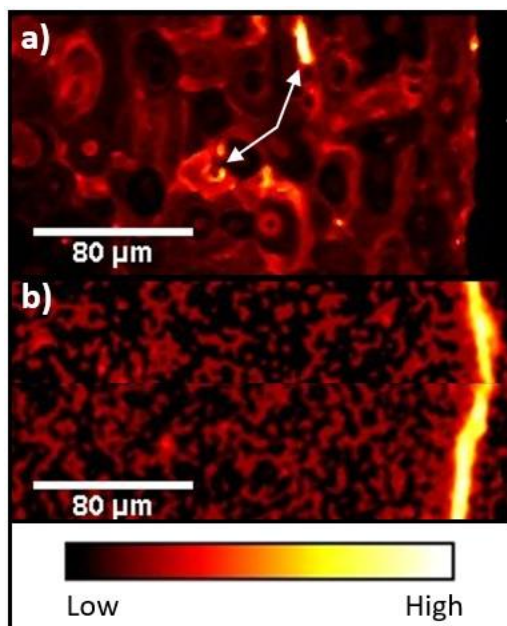


Figure 4.4—SR-XFI maps of Pb for modern Saskatchewan bone sample 1628 (91-year-old male): a) unaltered (7.0 µg/g Pb), and b) diagenetically altered with 200 µg/g Pb for 1 week. Arrows on Figure 4.4a) indicate structures of high Pb enrichment. Variation is evident in the spatial resolution of internal bone microarchitecture between 4.4a) and 4.4b) due to poor beam sensitivity during scan 4.4b), causing low levels of Pb to not be detected with adequate spatial resolution.

An exception to this consistent pattern of low signal to noise for early scans of diagenetically-altered bone was Sample 1565b. Sample 1565b exhibits Pb enrichment of two structures near to the endosteal surface of the sample (Figure 4.5a), which correspond with two large pores on the Ca map (Figure 4.5b).

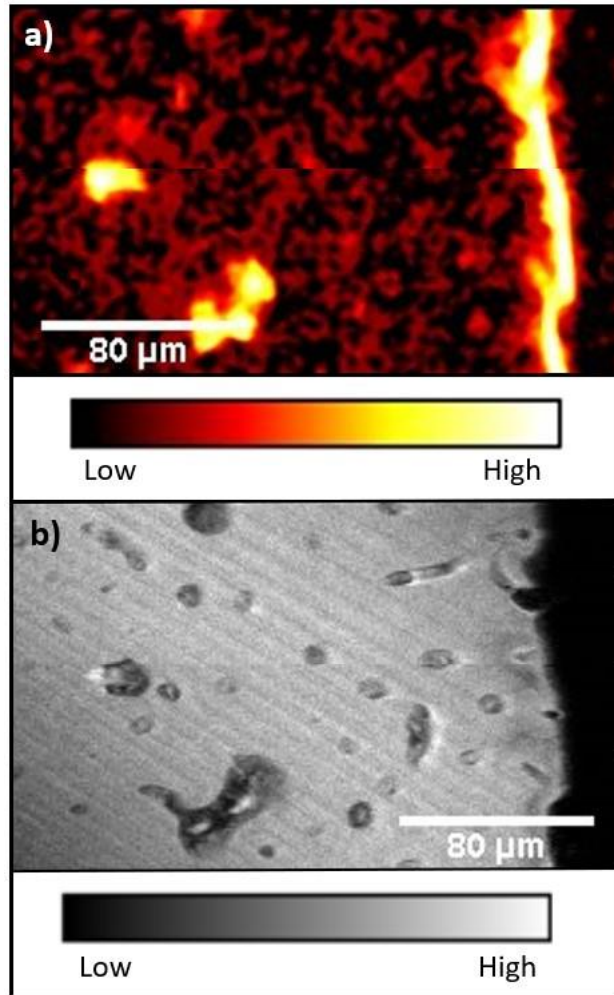


Figure 4.5—SR-XFI maps of a) Pb, and b) Ca for modern Saskatchewan bone sample 1565B (53-year-old female, 7.1 $\mu\text{g/g}$ Pb), diagenetically altered with 200 $\mu\text{g/g}$ Pb for 1 week. Internal Pb enrichment on 4.5a) corresponds with two large pores visible on the Ca map (4.5b).

Greater element sensitivity and spatial resolution was achieved following recalibration of beamline conditions. This increase in beam sensitivity allowed the internal bone microarchitecture of diagenetically-altered samples to be observed alongside the Pb-rich periosteal edge (Samples 1649b, 1366b, 1627b; Figures 4.6b–4.8b). Greater sensitivity also permitted closer observation of the effects of diagenetic alteration of Pb on internal bone microarchitecture. Diagenetically altered samples 1649b, 1366b, and 1627b (Figure 4.6b–4.8b) exhibit the enrichment of small pores near to the periosteal surface with Pb; these features are lacking in each image’s unaltered counterpart

(1649a, 1366a, 1627a; Figures 4.6a–4.8a). In unaltered bone samples from these later scans (1649a, 1366a, 1627a; Figures 4.6a–4.8a), interstitial fragments from remodeled osteons continue to be more enriched in Pb compared to intact osteons.

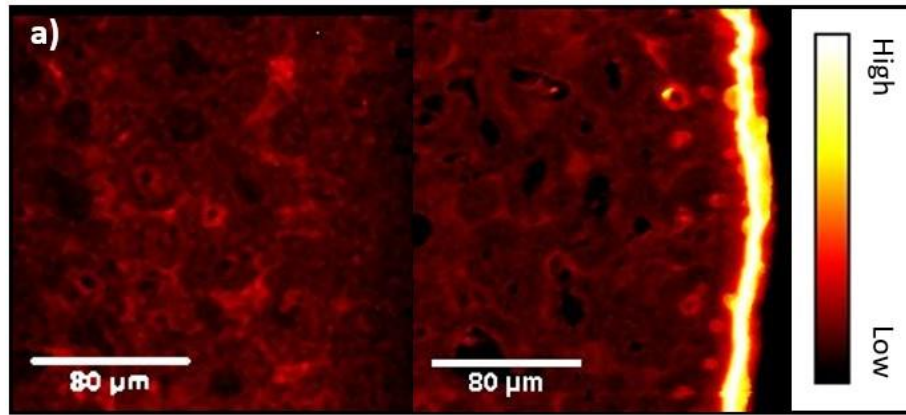


Figure 4.6—SR-XFI maps of Pb for modern Saskatchewan bone sample 1649 (96-year-old female): a) unaltered (1.2 µg/g Pb), and b) diagenetically altered with 200 µg/g Pb for 1 week. Scan took place following recalibration of beam, allowing internal bone microarchitecture of 4.6b to be adequately detected.

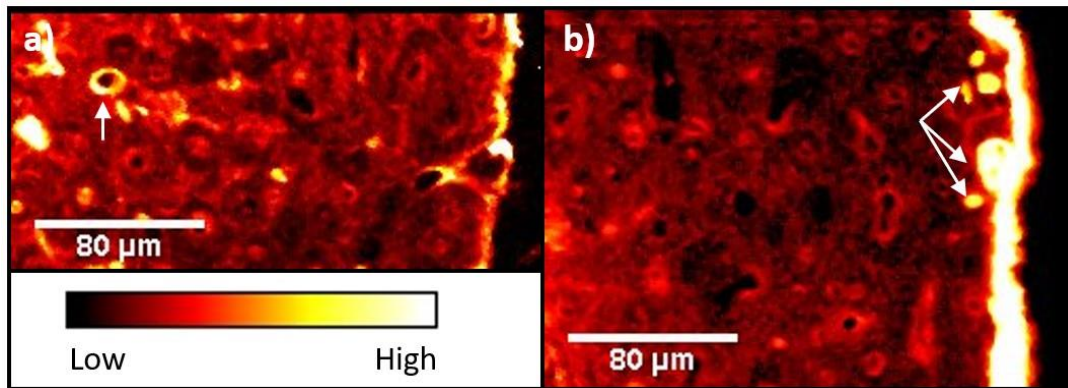


Figure 4.7—SR-XFI maps of Pb for modern Saskatchewan bone sample 1366 (90-year-old male): (a) unaltered (6.2µg/g Pb), and (b) diagenetically altered with 200 µg/g Pb for 1 week. Arrow in Figure 4.7a indicates canal with high lead enrichment. Arrows in Figure 4.7b indicate enriched pores close to periosteal surface. Scan took place following recalibration of beam, allowing internal bone microarchitecture of 4.7b to be adequately detected.

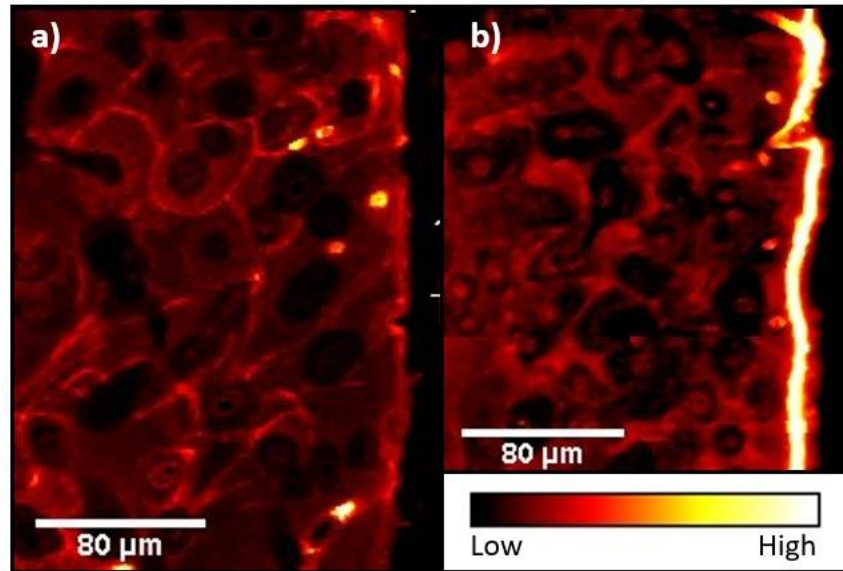


Figure 4.8—SR-XFI maps of Pb for modern Saskatchewan bone sample 1627 (85-year-old female): a) unaltered (4.2 µg/g Pb), and b) diagenetically altered with 200 µg/g Pb for 1 week. Scan took place following recalibration of beam, allowing internal bone microarchitecture of 4.8b to be adequately detected.

The SR-XFI Pb map of the Antigua sample (Figure 4.9) demonstrates vast heterogeneity with cement lines and central canals of osteons and osteonal fragments disproportionately enriched with Pb. Certain osteonal structures are far more enriched with Pb than others. Post-mortem cracks are visible on the Pb map as dark lines with no Pb signal. The Lithuania sample (Figure 4.10) demonstrates a remarkably high intensity of Pb at the periosteal edge of the bone sample. The Pb signal permeates past the periosteal surface via cracks and pores.

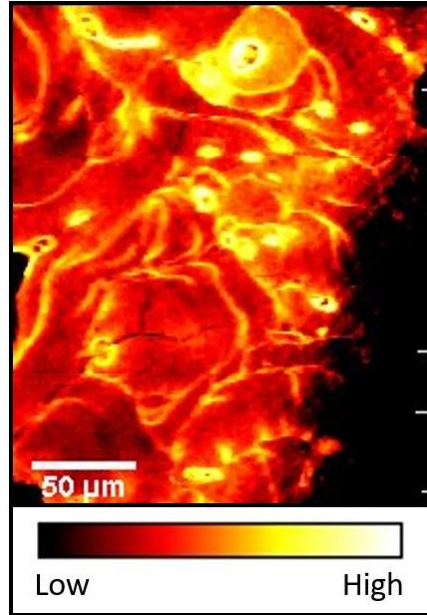


Figure 4.9—SR-XFI map of Pb for the archaeological Antigua bone sample (male, estimated to be 40–50 years old). This sample has a Pb concentration of 253.94 $\mu\text{g/g}$ (Giffin et al., 2017).

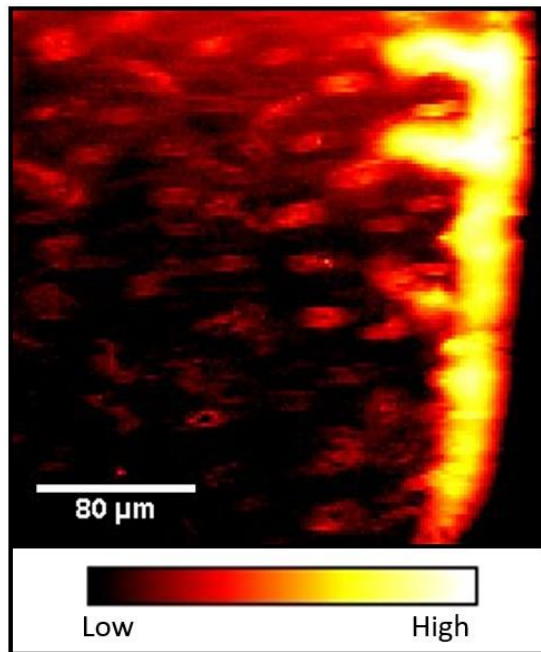


Figure 4.10—SR-XFI map of Pb for the archaeological Lithuania bone sample (adult female). This sample has a bulk Pb concentration of 125 $\mu\text{g/g}$.

4.4 Discussion

4.4.1 Confirming the Spatial Distribution of Biogenicity vs. Diagenesis

Diagenesis of bone is a prevailing concern for the field of archaeological bone chemistry. Diagenetic contamination and recrystallization of skeletal tissues by ions in the groundwater of a mortuary site can sometimes interfere with or obscure the natural biogenic signal of Pb in bone (Hedges & Millard, 1995). Like many trace elements, Pb cations in the depositional environment may become mobilized by groundwater and adsorb onto bone surfaces or enter bone via pores, cracks, and other spaces where it may substitute for Ca in the Ca(I) or Ca(II) positions in hydroxyapatite mineral and ultimately recrystallize the hydroxyapatite (Hedges & Millard, 1995; Dudás et al., 2016; Keenan, 2016). When assessing bulk Pb concentrations alone, diagenetic contamination of bone may be obvious when Pb concentrations exceed, for example 1,000 µg/g. However, this may be less possible to discern when the concentration is within a biologically feasible range (e.g. <1 to 350 µg/g) previously observed in both modern and archaeological studies (Barry 1975; Green, 1978; Ericson et al., 1979; Drasch, 1982; Gamblin, 1984; Corruccini et al., 1987; Patterson et al., 1987; Kowal et al., 1989, 1991; Kniewald et al., 1994; Rasmussen et al., 2015; Giffin et al., 2017). Vast efforts within the field have been devoted toward developing methods of recognizing and analyzing diagenesis, such as through studying the elemental composition of soil, rare earth element (REE) concentrations in apatite, crystallinity of bone, or Ca/P ratios (e.g. Nelson & Sauer, 1984; Wright et al., 1984; Shemesh, 1990; Price et al., 1992; King et al., 2011). Meanwhile, other scholars have proposed methods of recovering the biogenic signal from diagenetically-altered remains, such as surface abrasion and micro-sampling from uncontaminated regions of bone (e.g. Lambert et al., 1990; Scharlotta et al., 2013). The primary aim of this study was to examine differences in the spatial patterning of biogenic and diagenetic

Pb exposure in both archaeological and experimentally altered modern bone, potentially aiding in both endeavours.

Based on observations from archaeological bone, previous studies have posited that biogenic and diagenetic trace elements accumulate in skeletal remains in different distribution patterns (Swanston et al., 2012, 2017; Rasmussen et al., 2019; de Winter et al., 2019). Previous SR-XFI and LA-ICP-MS studies have observed that Pb tends to accumulate heterogeneously in cortical bone and concentrates heavily on the central canal surfaces and in the cement lines of osteons (Swanston et al., 2012, 2018; Pemmer et al., 2013; Rasmussen et al., 2019). Given the consistency of this observation across both embalmed and unembalmed modern bone (present study; Pemmer et al., 2013) and buried and nonburied archaeological bone (present study; Swanston et al., 2012, 2017), this pattern has previously been interpreted as biogenic and is an interpretation that is consistent with these present data. By contrast, it has been observed that diagenetic alteration is often confined to the periosteal and endosteal surfaces of bone (Wittmers et al., 2008; Rasmussen et al., 2019) and it has been hypothesized that diagenetic elements would more-or-less homogeneously accumulate in the osteons within a given area (Swanston et al., 2012, 2015). However, it is possible that diagenetic Pb could mimic the “biogenic pattern” by entering through central canals in bone and binding to the hypermineralized structures and non-collagenous proteins, or diagenetic Pb is taken up differentially into osteons with differing mineral densities (i.e. different tissue ages).

This study supports the hypothesis that diagenetic and biogenic Pb uptake result in different spatial patterns of accumulation. Through comparison of unaltered modern bone sections with corresponding diagenetically-altered modern bone sections, this study has demonstrated that in both modern and archaeological bone, diagenetic Pb is typically confined to the periosteal surface

of bone and small pores close to the periosteal surface. In only one case did the diagenetic Pb enter the modern cadaveric bone through large internal pores close to the endosteal surface (Sample 1565; Figure 4.5). Another possibility for the high lead concentration in these zones is that these “pores” contain soft tissue or unmineralized osteoid to which the diagenetic Pb could bind. Archaeologically speaking, the Lithuania sample exhibited signs of diagenetic Pb pervading into bone via post-mortem cracks; by contrast, the Antigua sample, despite having poorer histological preservation, did not have Pb present in post-mortem cracks. The variation in histological preservation between the two archaeological samples was likely caused by the differences in depositional environment; the Antigua individual was interred in a coffin while the Lithuania individual was in an above-ground coffin in stone mausoleum. It is presumed that the primary pathway of diagenesis for the Lithuanian remains could be through leaded paint on the coffin, increasingly mobilized by moist sea air.

These findings are promising for the field of bioarchaeological trace element analysis, as SR-XFI can be used to identify regions biogenic and diagenetic Pb accumulation, and potentially act as a visual guide for recovering biogenic information from bone using additional techniques. SR-XFI can be coupled with synchrotron radiation X-ray Absorption Spectroscopy (XAS) techniques to further confirm the nature of Pb in regions suspected as being biogenic or diagenetic. While not employed in this particular study, XAS has been previously used to speciate elements into their different elemental, inorganic, or organic forms at specific loci in archaeological bone and in doing so, infer their presumed biogenic or diagenetic origin (Swanston et al., 2015; Choudhury et al., 2017). SR-XFI could also be used to inform sampling regions for LA-ICP-MS, in which microsamples of a bone or teeth can be extracted to assess element concentrations or isotopic analysis. For example, Scharlotta and colleagues (2013) used LA-ICP-MS to microsample

from specific osteons that they hypothesized to be unaffected by diagenesis in order to recover biogenic strontium isotope data.

4.4.2 Lifetime Pb Exposure in Modern Saskatchewan

As this is the first analysis of Pb in human bone in Saskatchewan, the second aim of this study was to characterize and situate biogenic Pb exposure experienced by this modern Saskatchewanian sample. Because the Saskatchewan population sampled in this study consists of mature and senior adults and because bone can sequester trace elements for up to several decades (Hedges et al., 2007), it was hypothesized that the majority of each individual's lifetime Pb exposure would have occurred earlier on in life, when anthropogenic Pb was used far more pervasively in industrial endeavours and consumer goods.

Tetraethyl lead was a common additive to gasoline beginning in the 1920s. It began to be phased out in the 1980s but was only prohibited for on-road vehicles in Canada in 1990 (Nriagu, 1990; Health Canada, 2013, p. 20). This had extremely toxic health implications for the general public in the twentieth century (Boeckx et al., 1977; Zhang et al., 1994) though its prohibition has drastically reduced atmospheric Pb levels (Shotyk et al., 2016). Pb was also widely used in plumbing infrastructure and paint during this time. Pb plumbing, fittings, and solders can gradually contaminate residential drinking water, particularly when systems are older and corroded, when water chemistry conditions, such as low pH, mobilizes Pb, or when the tap water sits stagnant (Health Canada, 2013; Lytle & Schock, 1996; Safruk et al., 2017). Degraded Pb paint and airborne Pb-contaminated soil particles would have contributed to household dust and likely been inhaled (Lanphear & Roghmann, 1997; Health Canada, 2013).

Studies on Canadian individuals dating to or immediately postdating this era higher Pb use (pre-1990s) found that modern bone Pb concentrations ranged from 8.47 to 29.8 $\mu\text{g/g}$ (Kowal et

al., 1989, 1991; Samuels et al., 1989; Gamblin et al., 1994) while among British and Canadian occupationally exposed individuals, this bone Pb concentration rose to between 16.4 and 80 $\mu\text{g/g}$ (Barry, 1975; Green et al., 1978; Gamblin et al., 1994). This was not the case globally since bone Pb concentrations of 2.93, 3.94, and 4.47 $\mu\text{g/g}$ were observed in modern individuals from this time period in Croatia, Bavaria, and Japan, respectively (Drasch, 1982; Kniewald et al., 1994; Hisanaga et al., 1988). Comparatively then, the mean bone Pb concentration of 4.28 $\mu\text{g/g}$ (± 1.95) observed in this modern Saskatchewan sample marks a relative temporal decrease in Pb exposure in Canada. Given the phasing out of Pb in gasoline and consumer goods, this decrease is to be expected, and mirrors trends observed in blood Pb levels of Canadians. Between 2007 and 2009, blood Pb levels in Canadians were found to be, on average, 1.34 $\mu\text{g/dL}$, and in 2012, blood Pb levels in rural Saskatchewan children were 1.41 $\mu\text{g/dL}$, which altogether is an approximately 70% decrease from blood levels observed in Canadians between 1978–1979 (Bushnik et al., 2010; Health Canada, 2013; Safruk et al., 2017). Because bone represents a record of long-term trace element exposure, the bone Pb concentrations in this Saskatchewan sample potentially encompasses both time periods.

The question then remains regarding the extent of current Pb exposure. While anthropogenic Pb use has certainly been phased out to a large extent and Pb exposure has decreased, it remains a significant area of concern to modern health. Soil is still enriched with Pb from leaded gasoline, meaning that Pb can be taken up into plants, become deposited on plant surfaces, and contaminate natural water supplies (Nagajyoti et al., 2010; Foley et al., 2011; Rasmussen et al., 2011; Health Canada, 2013). Within the province of Saskatchewan, there is a continued reliance on leaded plumbing in some urban areas, causing some of the highest reported levels of Pb-contaminated water in Canada, and exceeding the recommended limit of 5 ppb

(Wilson & Ackerman, 2019). Between 2005 and 2010, the province of Saskatchewan reported Pb concentrations in tap water ranging between 0.1 and 60 µg/L, with a median concentration of 6.7 µg/L, though the median was <2 µg/L in the city of Saskatoon (Health Canada, 2013). Pb can continue to cycle through the local ecosystem on a seasonal basis; the large weather variability experienced by the Canadian prairies means that Pb from urban sewage infrastructure and industrial waste is released into the surrounding environment through rapid seasonal events such as spring snowmelt and summer storms (Codling et al., 2020). Today, older houses still containing degraded Pb paint may also present a risk of Pb exposure through inhalation of contaminated house dust (Lanphear & Roghmann, 1997; Health Canada, 2013).

Pairing SR-XFI maps with ICP-MS data can further shed a unique light on the life history, time sequence, and relative extent of Pb exposure for each individual. The SR-XFI maps of the cadaveric bone samples indicate overall low Pb exposure, with Pb enrichment mostly occurring in mature osteons and interstitial osteonal fragments structures that correspond to earlier periods in life. Newly formed or young osteons (superimposed on older existing structures) do not contain visible signs of fluorescence. This confirms the hypothesis that Pb exposure was higher in the past for these individuals. Unaltered maps from Individuals 1366 and 1628 show evidence of higher enrichment within single structures, possibly reflecting specific events of elevated Pb exposure. It is difficult to determine, however, whether these events stem from extrinsic sources of Pb exposure, or the re-integration of Pb into actively mineralizing sites that was mobilized from osteoclastic resorption of bone. The relatively higher intensity of fluorescence in these microstructures potentially suggests the former. In the map from Individual 1628 (Figure 4.4), these enriched areas correspond to interstitial bone structures and mature osteons, suggesting that these events of Pb exposure occurred a relatively long time ago.

Comparing the modern Saskatchewan SR-XFI data against historical examples helps to further contextualize the extent of Pb exposure for all populations. The mean Pb concentration of the modern Saskatchewan sample is approximately 60 times lower than that of the Antigua individual analyzed (253.94 $\mu\text{g/g}$). While the Saskatchewan samples show evidence of low Pb exposure that peaked earlier in life, both newly formed and mature structures from the Antigua sample are enriched with Pb, suggesting that this individual sustained a high level of Pb exposure over his lifetime. During the eighteenth and nineteenth centuries, Pb was widely exploited industrially and used in ceramics, food containers, utensils, ammunition, pigments, plumbing, water catchments, and other equipment (Warren, 2001; Montes-Santiago, 2013; Vuorinen et al., 2013; Jonasson & Afshari, 2017). In this Caribbean British Royal Navy context, high levels of Pb exposure likely also included the consumption of rum distilled from sugar cane juice which were processed using leaded equipment (Handler et al., 1986; Varney et al., 2012). As such, evidence of high, sustained Pb exposure in this area has been discovered in both historical documentation (Buckley, 1978) and skeletal remains (Handler et al., 1986; Corruccini et al., 1987; Schroeder et al., 2013; Giffin et al., 2017). In the Lithuania sample, while the outer periosteal surface is heavily enriched with diagenetic Pb, there is evidence of comparatively lower biogenic Pb enrichment of internal bone microstructures. This is consistent with sediment core data from the Baltic region suggesting that anthropogenic Pb use would not have been as excessive in this region during this time period (Zaborska, 2014), though regional environmental data does not necessarily correspond to anthropogenic exposure. Apart from occupational exposure to Pb, common cultural pathways of Pb exposure in Europe at this time were through Pb in paints, pigments, glazes, solders, spirits and wines, and plumbing (Lessler, 1988; Warren, 2000; Riva et al., 2012; Montes-Santiago, 2013).

4.4.3 Limitations

This study has some limitations. Absolute quantification of Pb concentrations within regions of interest is not currently possible with SR-XFI when working with thick three-dimensional samples (e.g. bone), as self-attenuation of X-ray photons may occur, and as a result, the underestimation of absolute Pb counts. Additionally, it is important to note that SR-XFI is a highly specialized technique with limited user access and availability.

When drawing comparisons of bulk Pb concentrations across samples, it is also important to consider the different facilities, procedures, and spectrometers used to analyze the archaeological vs. modern bone samples.

It could be argued that by exposing the bones to a Pb solution for such a short period of time, the artificial diagenetic treatment in this study would not adequately mimic natural diagenesis that occurs over a relatively lengthy period of time. However, SR-XFI maps from experimentally altered cadaveric bone and naturally diagenetically altered archaeological bone are extremely similar, both containing a consistent pattern of periosteal diagenetic enrichment, and in some cases, entry via large pores or cracks. That being said, skeletal samples that have undergone vast physical, chemical, microbial diagenetic alteration and recrystallization are likely unsuitable for chemical analysis. Another difference between the experimental conditions of this study compared with natural diagenesis is that this study used cut bone sections, rather than intact whole bones, which could alter pathways of Pb accumulation and diffusion.

As an exploratory, proof-of-principle study, this study was restricted in the range of diagenetic treatments tested, both in terms of Pb concentration and duration of exposure. Experimental studies (e.g. Von Endt & Ortner, 1984; Nicholson, 1996; Person et al., 1996; Nielsen-Marsh & Hedges, 1999; McNulty et al., 2002; Trueman et al., 2004; Harbeck & Grupe, 2009; Fisk et al., 2019; Krajcarz, 2019) and analyses from multiple archaeological contexts (e.g.

Elliott & Grime, 1993; Hedges et al., 1995; Nielsen-Marsh & Hedges, 2000; Berna et al., 2004; López-Costas et al., 2016) have revealed that diagenesis is affected by numerous factors including temperature, pH, site hydrology, soil type, bone type, and bone porosity. However, the impacts of these factors on the diagenetic compositional alteration of bone by Pb specifically is still an under-researched area. López-Costas and colleagues (2016) attempted to address this gap in the research with their study assessing the effect of differences in soil conditions and bone type on the extent of diagenetic chemical alteration in bone from various archaeological sites, but they were unable to determine whether the Pb was biogenically or diagenetically derived. Future experimental studies on modern and archaeological bone could implement a more longitudinal perspective controlling for these other factors on the diagenesis of Pb in bone.

Given the sensitivity of using samples from human cadavers and the time restrictions of synchrotron beamtime, the sample size of this study (n=14 individuals; n=9 SR-XFI scans with high quality spatial resolution) was smaller than ideal. Caution should be exercised when considering these results in the larger population context. Lastly, while these samples are from individuals who likely lived in Saskatchewan toward the end of their lives, long-term residential information was not available.

4.5 Conclusions

Using ICP-MS and SR-XFI on modern cadaveric and archaeological bone samples, this study sought to (1) assess whether there are different spatial patterns of uptake for biogenic and diagenetic Pb exposure, and (2) characterize lifetime biogenic Pb exposure experienced by the modern individuals in this sample. Comparing unaltered and experimentally altered bone samples with archaeological bone samples supports the initial hypothesis that biogenic uptake results in a heterogenous spatial distribution concentrated on osteon canal surfaces and cement lines, while

diagenetic contamination primarily enriches the periosteal surfaces, subperiosteal pores, endosteal pores, and cracks. ICP-MS results show that the modern individuals analyzed in this study experienced comparatively lower Pb exposure relative to individuals from decades and centuries ago. SR-XFI maps confirm that Pb exposure was higher in the past.

Chapter 5—Conclusion

In sum, this thesis has evaluated biogenic and diagenetic Pb exposure in bone from cadaveric individuals sampled from a modern Saskatchewan population, and compared the modern and experimental data with historical archaeological bone samples. The research study of this thesis has been situated within the context of SR-XFI research on Pb in bioarchaeology (Chapter 3) and against the larger backdrop of bioarchaeological trace element analysis (Chapter 2) by reviewing relevant research and technological developments in these areas.

The main aims of this thesis were to (1) determine whether there are spatial differences of uptake for biogenic and diagenetic Pb exposure in experimentally-modified modern human bone, and (2) characterize Pb exposure experienced by individuals from a modern Saskatchewan population. To answer these research questions, diagenesis was experimentally simulated in bone samples belonging to cadaveric individuals from Saskatoon, SK, and differences in biogenic and diagenetic Pb-derived distribution patterns among cadaveric and archaeological bone were evaluated using ICP-MS and SR-XFI.

In doing so, this study found that that biogenic and diagenetic Pb exposure resulted in different spatial patterns of uptake among the unaltered vs. experimentally altered modern bone. These patterns mirror what was observed in archaeological bone samples from Antigua and Lithuania representing probable biogenic and diagenetic Pb exposure, respectively. In SR-XFI scans, biogenic Pb was localized to bone microstructures that formed during the time of Pb exposure, with disproportional enrichment occurring along osteonal central canal surfaces and cement interfaces. By contrast, the scans demonstrated that diagenetic Pb was mainly confined to the periosteal surface of bone samples, with some permeation in sub-periosteal canals, as well as in some large endosteal canals or resorption spaces (Sample 1565) and post-mortem cracks

(Lithuania sample). Diagenetic Pb uptake is an under-researched topic in the body of literature on diagenesis and to date, all existing Pb diagenesis research is based on archaeological observations. The experimental simulation of diagenesis, as carried out in this thesis, exerts controls over otherwise unpredictable variables, lending further support to previous archaeological inferences. These experimental research results have promising implications for the bioarchaeological study of Pb exposure by supporting the postulation that biogenic and diagenetic Pb exposure result in different spatial patterns of uptake—patterns which can be observed using SR-XFI.

In the scans from the Saskatchewan individuals, Pb was mostly confined to older bone microstructures including mature osteons, osteonal fragments, and interstitial fragments of primary lamellae. By contrast, Pb is relatively absent in newly formed osteons. This demonstrates that Pb exposure occurred earlier on in life, such as during the era of leaded gasoline (1920s–1980s)—a time in which leaded plumbing, paint, and other everyday objects were far more commonplace. ICP-MS can help further contextualize this SR-XFI data within a larger human history of Pb exposure. While the individual from Antigua had a bone Pb level of 253.94 $\mu\text{g/g}$, the modern Saskatchewan individuals had bone Pb levels of 1.2 to 7.1 $\mu\text{g/g}$. As bone represents a far longer-term record of Pb exposure than blood lead levels, the data from this study contributes insights into the long-term life history of Pb exposure experienced by these individuals.

During the next steps of this research, I will analyze diagenetically-altered samples with ICP-MS to ascertain how bulk bone Pb concentrations were influenced by the pseudo-diagenetic treatment. The pseudo-diagenetic conditions used in this project produced a post-experimental Pb bone concentration of 200 $\mu\text{g/g}$ in bovine bone (Appendix 1). As human cortical bone is less dense than bovine cortical bone, and particularly because the sample demographic mainly consisted of bone samples from mature adults which may vary with respect to bone porosity, it is anticipated

that these post-experimental Pb concentrations will vary slightly from the bovine bone results and between individual samples. The next steps will also involve investigating variability in the pseudo-diagenetic experimental conditions. Some of the cadaveric bone samples have now been pseudo-diagenetically exposed to a 10 µg/g Pb aqueous solution for 2.5 months. Future steps will involve analyzing these samples with ICP-MS and potentially imaging them with SR-XFI to ascertain the diagenetic impacts on bone of lower Pb concentrations over longer periods of time.

Future directions for research could entail similar experimental diagenesis studies using elements beyond Pb and/or incorporating a larger breadth of environmental variables, such as soil type, pH, temperature, and moisture, and intrinsic skeletal variables, such as bone type, bone porosity, and bone mineralization. A similar experimental project simulating diagenetic Pb contamination in teeth would also be valuable to confirm spatial differences in biogenic vs. diagenetic Pb in tooth structures. Continued research using SR-XFI to investigate trace element exposure in past and present human populations will be enormously valuable to the disciplines of archaeology and public health, respectively, though improvements in the ability to quantify elements or estimate element quantities using this technique would be useful. Future research in this area should perhaps more commonly use SR-XFI in conjunction with SR-XAS to speciate elements and/or LA-ICP-MS determine element concentrations or isotopic ratios localized in bone and dental microstructures. Doing so would help with confirming the biogenic or diagenetic nature of localized elements, provenance element sources, and gain greater temporally specific insights into element exposure.

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Appendix—Pseudo-Diagenesis Experiments

Different pseudo-diagenetic Pb treatments were tested on bovine bone samples in order to determine an adequate Pb concentration and treatment time to mimic diagenesis. An accelerated treatment that would produce bone Pb levels comparable with the archaeological sample from Antigua (253.94 $\mu\text{g/g}$ Pb) was required. I hypothesized that at relatively high concentrations, diagenetic Pb uptake would occur quickly, due to the lack of seasonal groundwater changes and Pb's strong affinity for bone. Bone samples were submerged in these various treatments and a small portion of each sample was ground with a Spex liquid nitrogen freezer mill (Lakehead University) and analyzed via ICP-MS by the Saskatchewan Research Council Environmental Analytical Labs. The results are presented in Table S1.

TABLE A.1—*Results of experimental pseudo-diagenetic Pb treatments using bovine bone.*

Treatment	Lead (Pb) Concentration ($\mu\text{g/g}$)
Untreated	5.3
50 $\mu\text{g/g}$ Pb x 5 minutes	3.6
50 $\mu\text{g/g}$ Pb x 1 hour	21
50 $\mu\text{g/g}$ Pb x 1 day	29
50 $\mu\text{g/g}$ Pb x 5 days	36
50 $\mu\text{g/g}$ Pb x 1 week	48
200 $\mu\text{g/g}$ Pb x 5 min	120
200 $\mu\text{g/g}$ Pb x 1 hour	34
200 $\mu\text{g/g}$ Pb x 1 day	88
200 $\mu\text{g/g}$ Pb x 5 days	34
200 $\mu\text{g/g}$ Pb x 1 week	200

A Pb concentration of 200 $\mu\text{g/g}$ for 1 week produced a bone Pb concentration of 200 $\mu\text{g/g}$, which was closest to the bone Pb concentration of the individual from Antigua (Giffin et al., 2017)

and consistent with other archaeological populations of interest from this time period (Kowal et al., 1989, 1991). Consequently, these experimental conditions were selected to simulate diagenesis.

A few of the data do not align with expectations. The sample submerged in 200 $\mu\text{g/g}$ for 5 minutes resulted in a bone Pb concentration of 120 $\mu\text{g/g}$ while a sample submerged in 200 $\mu\text{g/g}$ for 5 days produced a bone Pb concentration of 34 $\mu\text{g/g}$. One explanation for this discrepancy is that these two samples were switched. Re-analysis of these samples would be required for this supplemental experimental data to be publishable.