

EVALUATING THE UTILITY OF PORTABLE X-RAY FLUORESCENCE TO THE DISCRIMINATION OF
HUMAN REMAINS FROM COMMINGLED CONTEXTS

By

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“It is dangerous, that power. It is most perilous. It must follow knowledge, and serve need. To light a candle is to cast a shadow.”

- Ursula K. Le Guin, A Wizard of Earthsea

INTRODUCTION

This thesis is a response to the rapidly expanding number of biological anthropology and forensic publications (ex. Gonzalez-Rodriguez, 2013; Perrone, 2014; Richards and Jones, 2015; Stevens, 2016; Finlayson, 2016; Winburn, 2017) which attempt to utilize portable X-Ray Fluorescence (pXRF, also known as p-ED-XRF, handheld XRF (HH-XRF), and field-portable XRF (FP-XRF)) spectrometry for the purpose of segregating and analyzing commingled human remains. The impetus for this trend is a byproduct of the rising number of handheld spectrometers owned by museums, universities, and private institutions across the globe. Museums and archaeological sites that lack the expensive high-end spectrometry facilities formerly relegated to top research institutions have been keen to embrace the rapid, non-invasive, and non-destructive technique. The resulting publications and papers have sought to apply this methodology to a variety of materials including ceramics, lithics, metals, paintings, sculptures, soil samples, and organic remains. Within the last decade there has been a magnitudinal increase in the number of Society for American Archaeology Annual Meeting abstracts describing research related to pXRF (Abstract Archives, 2019).

Due to its enhanced accessibility and visibility, pXRF has also recently permeated the field of biological anthropology. The emerging applications have ranged from the detection of heavy metals like Mercury (Hg), Lead (Pb), and Iron (Fe) (Zuckerman, 2010; Little et al. 2014; Killoran, 2017), to the analysis of paleodiets (Gilbertson, 2015; Bergmann, 2018), to the comparisons of trace elements for the purpose of resolving commingling (Gonzalez-Rodriguez, 2013; Perrone, 2014; Richards and Jones, 2015; Stevens, 2016; Finlayson, 2016; Winburn, 2017). While many of these studies have returned findings strongly suggesting that pXRF is a valid and useful technique for resolving anthropological questions, there appears to be a general uncertainty regarding the underlying practical and theoretical limitations and capabilities of pXRF and the ways in which data collection can be optimized so that researchers can attain interpretable and quantitative results (Shugar and Mass, 2013, pg. 20). Indeed, several researchers have been exceptionally vocal about the misuse and misinterpretation of pXRF data (ex. Shackley, 2012; Speakman and Shackley, 2013).

Rather than discourage the use of pXRF in the field of biological anthropology, the purpose of this thesis is to outline the theory, advantages, and realistic limitations of current pXRF equipment and methodologies in the resolution of commingled skeletal remains. Therefore, this discussion will: (1) introduce the underlying theory of pXRF in the context of the commingling of human remains, (2)

summarize and evaluate the past applications of spectrometry to the study of human remains, and (3) outline a collection of successful approaches for future projects as well as research frontiers which are yet unexplored.

In order to achieve these objectives, the thesis will be divided into the following chapters: Chapter I will introduce the concept of commingled remains and describe both the processes of their formation and their use. It will also discuss past, current, and developing methods of re-association such as quantitative, qualitative, and archaeometric techniques. Chapter II will provide a general introduction to pXRF and outline its history, development, theoretical models, and current instrumentation. This chapter will also address common concerns regarding the problematic use of this technique. Chapter III will outline the anatomy and elemental composition of human bone, including the molecular structure, chemical incorporation strategies, and non-pXRF investigations of trace elements in osseous tissues. Chapter IV will describe and analyze previous attempts to resolve commingling with respect to their stated goals, instrumental analysis procedures, statistical procedures, and findings. Chapter V will outline successful techniques and describe directions for future studies to explore.

Throughout the following discussion, the recurring question will be whether pXRF spectrometry is a valid approach to the resolution of commingled human remains. An ostensible affirmative response is complicated by the fact that, just like isotopic analysis, molecular techniques, and advanced quantitative methodologies, pXRF is far from ‘point and shoot’ or ‘plug and chug’. In addition to understanding the underlying principles of X-ray spectrometry, a successful investigator must possess a thorough understanding the trace element composition of the human bone as well as an ability to combine disjoint streams of data into an interpretable form. Therefore, in order to validate the utility of pXRF as a useful technique for individuating (resolving) commingling, the following must be appropriately demonstrated:

1. There exists less intraskeletal variation than interskeletal variation for some set of chemical elements. Such variation can result from any of a number of processes, but should be predicted and explained by the researcher.
2. The technique (pXRF) can be utilized accurately and precisely by investigators in order to determine the elemental composition of specific trace elements in human bones.

3. Statistical analysis of pXRF data can be effectively used to distinguish (n) skeletal remains from (n) distinct individuals.

CHAPTER I: STANDARD & EMERGING COMMINGLING TECHNIQUES:

Commingling of human remains in forensic and bioarchaeological contexts poses significant barriers to the goals of anthropological investigators and is intrinsic to the collection on hand. Because of the diverse deposition, excavation, laboratory, and curational processes through which commingling occurs, the homo- or heterogeneity of individuals in the collection, and the questions asked by investigators, every commingled scenario exhibits unique challenges. Sorting out individual remains to address commingling requires a considerable amount of time, resources, skill, and effort. Thus, researchers must decide whether resolution is necessary to achieving their research goals, and if so, which of several individuation methodologies to follow. In this chapter, commingling and the processes leading to commingling will be defined, motivations for resolutions will be described, and existing and emerging methods of investigating commingled collections will be explored.

Definition of Commingling:

Commingling can be defined as the mixing of skeletal elements belonging to different individuals and is a frequent feature of both archaeological and forensic contexts. This process can occur through human processes (ossuary burials, lack of excavation records, subpar laboratory techniques) and natural processes (mechanical mixing through hydrological or geological agitations, mass disasters, etc). According to Anna Osterholtz (2014), there are three major types of commingled assemblages: long term usage assemblages, episodic assemblages, and commingled lab assemblages (fig. 1). Understanding which of these most closely matches the target collection is fundamental to analysis as each type poses different challenges to be approached with different techniques.

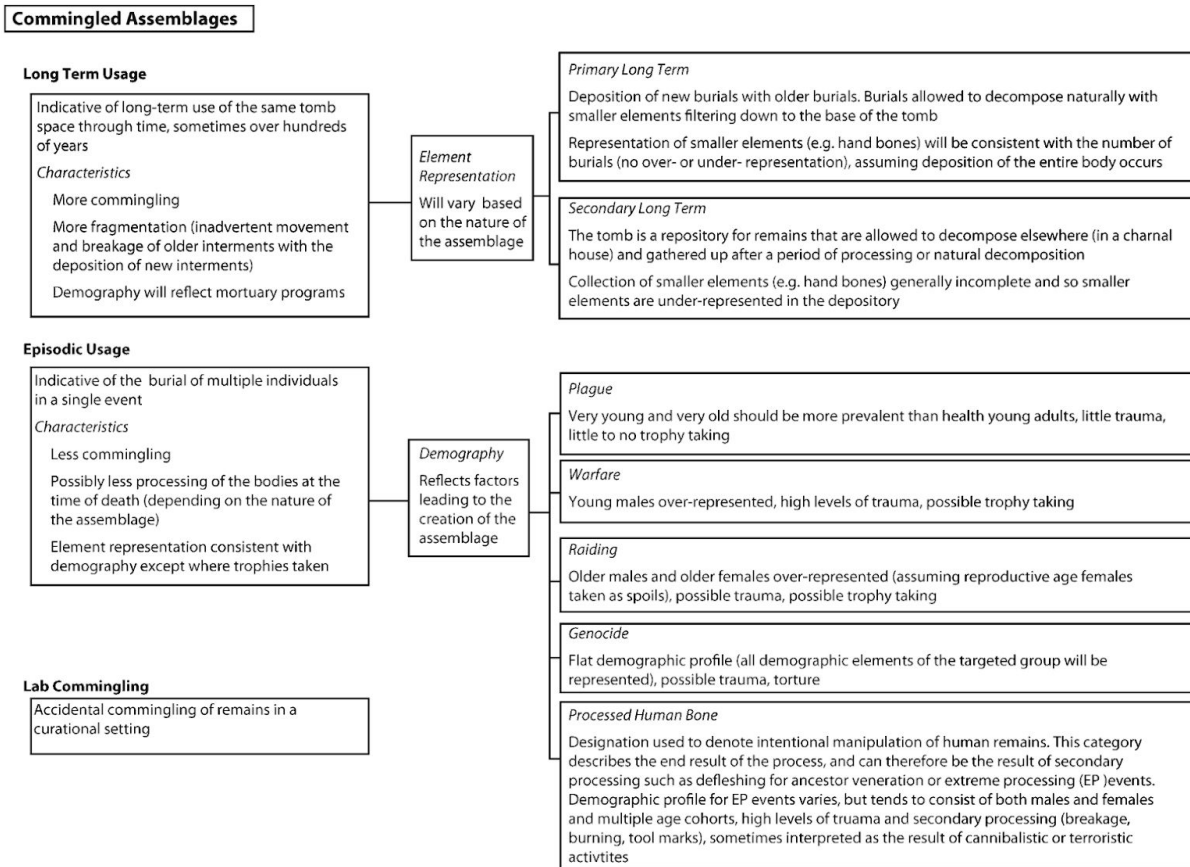


Fig. 1. Types of commingled and fragmentary assemblages (from Osterholtz, 2014, pg. 2)

Long term usage assemblages occur when a depositional location such as a tomb has been utilized over a long period of time, frequently by specific communities for multi-generational deposition. Due to the temporal and spatial nature of such interment patterns, these types of collections frequently exhibit a higher degree of commingling and fragmentation since earlier individuals are frequently disturbed to make space for new depositions. Depending on whether secondary burial is a feature of the site, there may be a lower representation of smaller and more fragile skeletal elements. Because such assemblages are attritional, “demography will reflect mortuary programs” and thus the skeletal element composition of such assemblages will likely be different in age and sex ratios than others (Osterholtz, 2014, pg. 2).

Episodic usage assemblages, are, by contrast, typically formed in far shorter periods of time (single generation, often under a year). Reasons for their formation are often extraordinary events such as warfare, plague or religious activities. Episodic assemblages tend to exhibit a lesser degree of commingling and potentially more representative and complete individuals unless selective removal of

skeletal elements, such as crania, occurred at the time of death (Osterholtz, 2014, pg. 2).

Demographically, these types of collections will not reflect natural populational attrition but might feature subpopulations such as young males (if warfare), the very old, infirm, and young (if plague), or social subgroups (if genocide).

Investigator-commingled assemblages are the third type of commingling and the only completely avoidable type. While modern commingling is generally unintentional and largely occurs due to subpar laboratory practices (lack of labeling of skeletal elements, unorganized notes, collection issues) many 19th and early 20th century legacy collections are commingled because they were assembled when certain skeletal elements (i.e., crania, spines) were valued above others for research purposes. Many long-term museum collections are also commingled due to accession and deaccession, cataloging changes, and administrative agendas (Osterholtz, 2014, pg. 173). Once commingling occurs in a laboratory or museum setting, it is unlikely to ever be resolved with absolute certainty and thus loses value to both modern and future investigators.

In addition to the three commingled assemblage patterns identified by Osterholtz, also worth mention are those that can be attributed to extreme natural and human processes such as construction and development, disturbance of burials by natural processes such as wind or water erosion (gradually or by natural disasters), and irresponsible excavation or collection practices by untrained personnel (Stevens, 2016, pg. 3).

Approaches to Commingled Collections:

Depending on the goals and research questions of the investigators, it may be neither necessary nor useful to resolve commingling in order to analyze a collection. However, in most paleopathological and forensic commingling scenarios, reassociating skeletal elements to form distinct individuals must precede further analysis. This is because estimations of age, sex, stature, health, lifestyle, and experiences generally require skeletal remains to be as complete as possible. However, due to the challenge of re-association and the lack of reliable methodologies, many collections ‘languish’ in museums and repositories and lack demographic and paleopathological investigations as a result (Osterholtz, 2014, pg. 1).

For paleopathological studies, resolving commingling is critical. This is because many infections and nutrient deficiencies will systematically affect multiple skeletal elements. For example, consider a micronutrient deficiency such as scurvy or a condition such as syphilis, both of which lead to diagnostic osseous lesions on multiple skeletal elements. Without examining the morphology and pathology of different osseous tissues belonging to the same individual, the certain presence of a specific condition may be mistaken for a population containing several unhealthy individuals. In addition to gaining the ability to pinpoint nutritional and pathological conditions, resolving commingling is necessary for paleopathological investigation because epidemiological tools such as life-tables are complicated to construct for commingled assemblages, particularly when considering the prerequisites of much-improved emerging aging techniques. While techniques have been suggested to extrapolate information from single skeletal elements to the entire assemblage (Osterholt, 2014, pg. 38), neither precision or accuracy can be reliably achieved.

Forensic workers, driven by the need for positive identification and rapid return of remains to loved ones, value commingling resolution as much as if not more so than archaeological investigators. (Broehl, 2018, pg. 2). Many forensic assemblages caused by natural disasters, terrorist attacks, or every-day violence need to be quickly resolved due to legal, economic, and political pressures. Because of cases such as these, forensic anthropologists require accurate ways of resolving commingling in order to provide accurate demographic data and ultimately return individuals to their families. A major difference between archaeological and forensic applications, however, is that forensic investigators have access to funds that enable them to perform DNA work and permission for destructive analysis. These resources, despite decreasing costs, still remain out of reach for many biological archaeologists.

Analytical Methods Without Resolution:

There are three major approaches to describing commingled human remains without attempting to resolve individuals: the minimum number of individuals (MNI), the Lincoln Index (LI), and the most likely number of individuals (MLNI). Of these, MNI and MLNI are by far the most commonly-applied techniques in almost every forensic and bioarchaeological commingling scenario (Broehl, 2018, pg. 8). All three of these techniques originated from zooarchaeological and biological approaches to population sampling and estimation (Adams and Konigsberg, 2004, pg. 138).

The MNI began being used in the mid 20th century and returns an estimate of the minimum number of individuals necessary to form an assemblage. It is typically determined by using the discrete skeletal element (i.e. proximal tibia) with the highest count with respect to characteristics such as age, sex, pair-matching, and pathologies. While it is extremely simple to calculate, the drawback of MNI is that it has a high probability of returning an underestimate of the original number of individuals. According to Adams and Konigsberg (2004), there are three major ways of calculating MNI where L signifies the number of left skeletal elements, R the number of right skeletal elements, and P the number of known pairs:

1. $Max (L + R)$
2. $\frac{(L+R)}{2}$
3. $L + R - P$

As can be demonstrated using simple models (Adams and Konigsberg, 2004, pg. 139), all three of these methods return gross underestimations of commingled assemblages, (1) and (2) by approximately 30% and (3) by 9% when the probability of recovery is fairly high ($p = 0.7$). However, because the probability of recovery is nearly always unknowable, it is difficult to attain accurate estimations using MNI.

Like MNI, the Lincoln (also called the Peterson) Index was developed for use on living population of animals based on capture-recapture techniques (Adams and Konigsberg, 2004, pg. 139). The advantage of LI over MNI is that it can be used to estimate population sizes even if taphonomic biasing has occurred because it does not assume that all members of a population can or will be observed. Crucial to this calculation is extensive pair-matching, which can be achieved through osteological sorting or other techniques described later in this chapter. Preferably, the skeletal elements used for the P value ought to be those more likely to preserve, to be distinct, and have definitive features (i.e. femur, tibia, humerus, innominates). Analogously to living animal populations, one side of the skeleton (i.e., L) is treated as the initial catch (those animals which are first captured and tagged) while the other (i.e., R) is treated as the re-capture (those which are caught for a second time during an additional round of sampling). A general form of the LI can be calculated as:

4. $LI = \frac{LR}{P}$

Finally, MLNI was also adapted from ecological and zooarchaeological models, specifically from capture-recapture studies estimating faunal populations. When the expected sample size is small, this measure outperforms the LI (Adams and Konigsberg, 2004, pg. 141), although it can have a substantial negative bias if the number of recovered pairs is low (Broehl, 2018, pg. 8).

$$5. \quad MLNI = INT\left[\frac{(L+1)(R+1)}{(P+1)} - 1\right]$$

While LI and MLNI are certainly an improvement over MNI, it is unlikely that MNI will ever be completely replaced due to its simplicity and the fact that calculations for LI and MLNI should be approached somewhat more carefully, with considerations for rates of pair matching and expected population sizes. However, these two measurements are advantageous because they are considerably less prone to bias and allow for consideration of taphonomic processes which are natural to commingled collections.

Analytical Methods for Resolution:

The best way to control commingling is to prevent it in the first place. While the first two commingling processes discussed earlier in this chapter (long term, episodic) are largely unavoidable, field, laboratory, and curatorial commingling can easily be prevented by keeping extensive and detailed excavation and shipping photos and records (both digitally and in print), labeling and tagging every element as soon as possible and with carefully considered identification numbers, bagging carefully and distinctly, keeping laboratories and analysis tables carefully segregated and organized, and limiting analysis to one assemblage and individual at a time. While these steps may seem arduous at the time, once commingling occurs, valuable information is irrevocably lost.

In the case where commingling is unavoidable, resolution of commingling must begin as early as possible, even during excavation. As soon as commingled skeletal elements are discovered, contextual information such as provenience, photographs, and observations ought to be recorded, preferably through precise georeferencing combined with high-quality 3D models achieved with scanners or photogrammetry. Additionally, specialized ArcGIS extensions can also assist in later analysis (Hermann and Devlin, 2008). Following extraction, careful labeling should occur as soon as possible either in the

laboratory or in the field. Once skeletal elements arrive in the lab, more serious re-association can begin through qualitative factors, quantitative methodologies, DNA analysis, and a variety of other techniques.

Qualitative Techniques:

Qualitative techniques are typically the first step towards resolving commingling because they require little to no equipment, are relatively quick, and are generally reliable when performed by experienced osteologists. All begin with an inventory of the remains where information is collected on the identification of each element, its siding, visible pathologies and taphonomies, sutures, and other distinguishing characteristics. Once that information is collected and skeletal elements can be examined in logical groups (i.e., articulating skeletal elements, matching pairs, etc), pair-matching and joint-matching can be conducted.

In pair-matching, all left and right skeletal elements of a certain type are considered as possible pairs from a single individual. These determinations can be made based on a combination of morphology, trauma, taphonomy, and other characteristics. Although highly reliable when conducted by the experienced practitioner, visual pair-matching can be difficult for inexperienced osteologists. Studies have shown that the success of this technique can range between 75.6% to over 90% based on skeletal element types (Broehl, 2018, pg. 10-11). Aside from experience, other factors influencing the utility of this technique are the size of the commingled sample and the general degree of preservation of the skeletal elements. The method can also be more successful if individuals of different sexes are being compared and if the skeletal elements being considered are sexually dimorphic (in that case, both long bones and innominates could be more easily distinguished).

Articulation, or joint, matching, is frequently used in conjunction with pair-matching and can be used to associate mandibles with crania, femurs with innominates, and so on. Through this process, individuals can be gradually re-assembled, although some skeletal elements such as phalanges, carpals, and ribs are resistant to this approach.

Quantitative Techniques:

Since early in the history of biological anthropology, quantitative commingling resolution techniques have been a major goal. Even as early as the 1980's, simple metric evaluations were used to

assess the articulation and association of the hip joint (femur and acetabulum) (Stevens, 1015, 103-104). However, as both statistical and computational techniques improved, increasingly complex and multivariate methods for addressing commingling emerged.

Of these, osteometric sorting is the most common. This technique relies on the assumption that the human skeleton varies in predictable and population-specific ways which allow distinct skeletal elements to have predictable relationships with each other (Stevens, 2016, pg. 104-105). Osteometric sorting utilizes confidence intervals derived from known reference populations to evaluate whether two skeletal elements originate from the same individual. Statistical methods to evaluate similarities and differences include the use of confidence intervals as well as t-tests (Broehl 2018, pg. 15). Unlike qualitative methods, osteometric sorting can be used to consider skeletal elements from different areas of the skeleton. On the other hand, it provides less definitive guarantees of matches and should therefore be used in combination with other methods (Broehl 2018, pg. 11). Due to a combination of research interests and practical limitations of osteological sorting, most studies have focused on long bones, innominates, and sacrums (Broehl, 2018, pg. 3). This technique is most useful when individuals are extremely different (in terms of age, sex, physique, etc) and when there are few individuals. When individuals are similar or numerous, overlapping measurements can complicate the results.

Geometric morphometrics:

Following many of the same basic assumptions as osteological sorting regarding intra-skeletal elemental proportions, geometric morphometrics is a relatively new technique which utilizes the size and shape data of skeletal elements to compare similarity. While some programs are based off of landmarks derived from either 2D or 3D captures, others utilize more advanced computational models such as mesh-to-mesh value comparison (MVC) (Karell, 2016, pg. 1315). Much of the interest involving geometric morphometrics in the United States has been associated with the Department of Justice and been applied less for the resolution of commingling but rather for age, sex, and ancestry estimation. The biggest challenges with this technique are the effort required for data capture, the size of the data, and the computational power required for meaningful analysis. Distinct but related to geometric morphometrics is the use of computed tomography scans which utilize cross-sectional measurements to attempt to resolve commingling (Stevens, 2016, pg. 3).

DNA Sequencing:

By far the most accurate method to resolve commingling, DNA analysis has become widely available over the last decade for anyone with the financial resources to send out samples. Particularly in forensic contexts, DNA analysis is the principal method applied to the resolution of commingling. However, this method is constrained by the large cost of processing samples, the degree of preservation of the bones, and whether legal, ethical, and political contexts permit destructive analysis (Gonzalez-Rodriguez, 2013, pg. 1). This technique is most frequently employed in modern mass disaster events (wars, plane crashes, natural disasters, terrorist attacks) and is used to attain the highest degree of confidence in an identification. It can also be used when deceased individuals need to be associated with living relatives (ex. Cakar, 2018). Typically, this technique requires DNA extraction (and therefore destructive analysis), amplification, and extraction of profiles (usually based off of a discrete number of specified loci). Aside from the necessary destruction of minute portions of skeletal elements, further drawbacks of this commingling resolution technique are the preservation state of the DNA since time, erosion, and extreme climatic conditions such as heat, cold, and moisture can cause degradation. However, the clear advantage is that skeletal elements do not need to be identified in order to be reassociated.

Archaeometric Techniques:

Archaeometry (here defined as chemical or physical techniques) emerged as early as 1958 through McKern's analysis of a mixed skeletal assemblage with short-wave ultraviolet light (McKern, 1958). The purpose of McKern's work was to capture differences in the taphonomy of skeletons and was specifically geared towards collections with vastly different postmortem treatments. Other techniques such as isotopic analysis, mass spectrometry, and XRF also emerged and were improved upon throughout the 20th century. The driving theory underlying these techniques was that the intra-skeletal elemental variation (chemical composition) of individual remains was smaller than the inter-skeletal elemental variation. While all three of these methodologies have proved to be fairly reliable and useful for the resolution of commingling, the drawbacks of all three are that they require destructive analysis as well as expensive and often non transportable laboratory equipment.

Thus, when pXRF (portable X-Ray spectrometry) was developed, it was considered in the context of an extremely well established XRF method which had been in use for anthropology since the 1960's, when Edward Olson utilized XRF to evaluate selenium (Se) and Hg in organic compounds (Olson, 1960). Unlike XRF, however, pXRF had the advantage of being nondestructive, portable, and (seemingly) simple to operate and interpret, in part due to the ways in which companies marketed the device to academic consumers. The portability of the devices was especially desirable for archaeologists because it allowed for *in situ* analysis of monumental buildings, artifacts, and fragile objects (Shugar and Mass, 2013, pg. 17). Finally, the cost of the devices was negligible in comparison to the cost of setting up a full-scale spectrometry or DNA laboratory, and instruments could often be acquired by anthropologists in cooperation with geology and chemistry departments.

The temptation to seize upon pXRF as a means of resolving commingling has thus been considerable. However, use of pXRF spectrometers for this purpose is still in a wild-west phase without well-defined standards or methodologies. Therefore, in the following chapters this thesis will focus on describing the methodology of pXRF, the biological principles upon which its utility for commingling is based, and the state of current literature.

CHAPTER II: REVIEW OF PORTABLE X-RAY FLUORESCENCE (PXRF):

X-Ray Fluorescence (XRF) analysis has evolved over the last century into a generally accepted and extremely versatile technique with direct and meaningful applications to disciplines as diverse as archaeology, environmental science, museum studies, conservation, and geochemistry. Advances in physical theory, software capability, and instrumentation have led to direct benefits in the form of a new generation of portable instruments that can perform relatively high-resolution multi-elemental analysis. Currently, of the various analytic techniques for the determination of a material's elemental composition (XRF, ICP-MS, etc), pXRF holds major advantages as an effective, quick, and non-destructive methodology. However, the same features which make pXRF such an enticing tool for researchers also make it problematic because if the underlying theories of the technique are not properly understood and accounted for by investigators pXRF will return useless or misleading data. Therefore, the following sections in this chapter will describe the history and development of modern instruments, the underlying physical theories of the method, differences between portable and stationary instruments, the applications of pXRF, approaches to data analysis, and concerns raised by pXRF experts regarding inappropriate use of the technique.

History and Development:

On November 8th, 1895, the German physicist Wilhelm Conrad Röntgen discovered the form of radiation which came to be known as X-rays (Panchbhai, 2015). Prior to this date, X-rays were simply counted among other types of unidentified radiation which emanated from gas-filled discharge tubes. Not unlike the fortuitous discovery of penicillin by Alexander Fleming, the story goes that Röntgen happened to place an electric tube on top of a book resting upon a photographic plate. Upon removing the book, Röntgen was amazed to discover the outline of a key which was hidden within the book's pages. Clearly, "the strange light from the glass tube had penetrated the pages of the book", and thus X-rays were discovered (Panchbhai, 2015, pg. 91). Because Röntgen ordered in his will that all his laboratory notes be destroyed, the truth of how X-rays were discovered is unknown. Despite the aura of mystery, it is clear that his research did not evolve in an academic vacuum. Indeed, the work of several other researchers studying cathode rays (streams of electrons observed in vacuum tubes when a voltage is applied) influenced Röntgen's research. Alongside Röntgen, other major contributors included Julius Plucker,

Johann Wilhelm Hittorf, Heinrich Hertz, Eugene Goldstein, Wilhelm Conrad Guericke, and William Crookes (Panchbhai, 2015).

In the months subsequent to his discovery, Röntgen continued to test his new form of radiation and by December 22, 1895, had photographed his spouse's hand using X-rays, thus creating the first radiograph of a human being (fig. 2) (Panchbhai, 2015)¹. Röntgen also named the new rays as 'X-rays' because of the variable 'x's mathematical attribute of denoting an unknown quality, although in many non-English languages they are known as Röntgen rays, after their discoverer. Soon after the first publications, the uses of the new imaging technology became starkly evident and were embraced by diverse medical professionals, especially dentists (Panchbhai, 2015². Indeed, in modern times, dentistry is one of the foremost applications of the technology.



Fig. 2. *Hand mit Ringen* (Hand with Rings). This is a 1896 print of Wilhelm Röntgen's first radiograph, depicting his wife's hand (Jaya, 2009).

Following the initial excitement about the utility of Röntgen's rays, the next major step was taken in 1904 by Sir John Fleming, an English physicist and electrical engineer. By inventing the thermionic

¹ Upon seeing her skeleton fingers on a radiograph, Bertha, Röntgen's wife, is rumored to have exclaimed, "I have seen my death!" (Panchbhai, 2015, pg. 92).

² Ironically, many of these early adopters were also the first to suffer from the medical consequences of overexposure to X-rays, although happily the detrimental effects were discovered before X-rays became standard components of treatments (Feldman, 1989).

diode, a vacuum tube which utilized a hot cathode by running a second electric current through it and thus emitting a significantly higher number of electrons, Fleming allowed the replacement of problematic cold-cathode tubes in X-ray work. Another advancement took place in 1906 when Charles Barkla, a Nobel-winning British physicist, discovered that each element had a characteristic X-ray spectrum when scattered by gases. Specifically, by observing the secondary X-rays which were emitted from a sample, Barkla found that “the polarization of X-rays, the gaps in atomic absorption, and the distinction between continuous and characteristic X-rays, which consisted of several series of X-rays, named K, L, M series” (Beckhoff, 2006). In other words, he discovered that the pattern of X-rays was related to the electron patterning of an atom. Advancements continued on through work by researchers such as Max von Laue, who discovered the diffraction of X-rays by Crystals, Sir William Bragg, who established Bragg’s law of X-ray diffraction, and William Coolidge, who invented the Coolidge X-ray tube still in use today. Eventually, the working principle of modern XRF emerged: it is possible to measure the energy of characteristic photons emitted by a sample and thus determine which chemical elements the sample is composed of, and even their concentration.

During World War I, X-rays were turned to military applications by researchers such as Marie Curie, who developed mobile X-ray units used for imaging soldiers in the field (Jorgensen, 2017). Concurrently, X-rays became ever-more integrated into medical, chemical, and physical research and practice. Perhaps the most well-known application of X-rays was the X-ray diffraction pattern captured by Rosalind Franklin in 1952 at King’s College in London. Using that tool, the three dimensional structure of DNA as a double helix composed of paired nucleotide bases was discovered.

As can be expected, World War II resulted in even further developments in X-ray technologies. Specifically, great strides were taken in the construction of x-ray fluorescence spectrometers. In 1948, the first successful commercial X-ray fluorescence spectrometer was constructed (Beckhoff, 2006). In 1958, an X-ray spectrometer was developed specifically for industrial applications. Once XRF was made available for public and commercial consumption, there began a rapid development and miniaturization of the various components which resulted in the eventual creation of portable instruments such as are available today.

Underlying Physical Theory:

To begin describing the theory underlying X-ray fluorescence, it is important to first define what an X-ray actually is: a form of electromagnetic radiation generated by electrons slowed down in the outer field of an atomic nucleus or by a change of bound states of electrons in an electronic shell of an atom with wavelengths ranging from .01 to 10 nanometers and energies ranging from 100 eV to 100 keV (Beckhoff, 2006). Meanwhile, electromagnetic radiation above one MeV generated by nuclear processes is usually called γ -radiation. The divisions between these two types of radiations are to “to some extent artificial and misleading” (Beckhoff, 2006).

The process of XRF spectrometry has three major operational stages: primary radiation, ionization, and detection. Primary radiation occurs in the unit, which emits photons. The process of ionization takes place when samples are excited by X-rays which cause the atoms to eject electrons. When an electron is removed, it makes the electronic structure of the atom unstable and causes other electrons still in orbit to fill the vacancy left by the excited electron. When the outer orbital electron shifts to the open lower position, energy is released as a photon of a predictable energy- in fact, the energy difference between the orbitals. As described by Charles Barkla when he won the Nobel prize, characteristic X-rays are given off when outer orbital electrons drop into a vacancy in an inner shell of an atom and a photon with the energy difference of the two orbitals is emitted. For those who work with X-rays, there exists a traditional notation used to describe transitions between different electron orbitals. For example, a transition from the L shell to the K shell results in a $K\alpha$ X-ray, while a transition from the M shell to the K shell results in a $K\beta$ X-ray. Although there are other transitions, these are the two major ones used in XRF analysis. The basic function of an XRF is therefore to sort through the energies of the detected photons and to determine the relative quantity of each element in the material. The term fluorescence describes the emission of photons when a material absorbs radiation. The process of detection again occurs in the unit, and describes the conversion of characteristic radiation into electrical signals which can be converted to interpretable data.

Beginning with the process of primary radiation, in order to excite electrons in the sample material an original source of radiation is needed. Therefore, XRF spectrometers will have X-ray generators which can emit either gamma or X-rays, with gamma rays being preferred for portable applications due to their lower energy requirements. The first X-ray generator used by Röntgen was an ion tube, specifically a Hittorf-Crooks tube filled with air at a reduced temperature. In this set up, electrons were produced by an ion bombardment of a cold cathode in a high voltage gas discharge, which caused electrons to accelerate onto the anode, thus generating X-rays. Unfortunately, this type of ion tube

had a relatively short lifetime, was unstable, and difficult to control (Beckhoff, 2006). Therefore, when Coolidge introduced the schema for the hot filament electron emitter in a high vacuum X-ray tube, the stability and efficiency allowed it to remain the dominant equipment choice into the modern era (Beckhoff, 2006). With a hot filament set up, the heated cathode is usually a tungsten (W) wire and the anode typically an element such as chromium (Cr), copper (Cu), molybdenum (Mo) or W held at many kilovolts of potential difference relative to the cathode (Guthrie, 2012). Some manufacturers will use rhodium (Rh) as the standard anode material because its energies are suitable for exciting chemical elements both light and heavy. Thermal electrons accelerate to the anode from the cathode, causing bremsstrahlung radiation to be emitted³. This radiation then leaves the unit through a beryllium window in the side of the tube.

When it leaves the tube, the bremsstrahlung radiation then excites the characteristic radiation of the chemical elements in the sample material. As discussed, each element emits photons of characteristic energies. One important aspect of the excitement stage is that the chemical elements which can be detected are limited by the maximum energy emitted from the tube. For example, if the maximum energy emitted is 20 keV, then it is impossible to excite chemical elements with an atomic number exceeding 43 as the K binding energy would surpass the maximum. Other factors of importance during this stage are the penetration of the radiation, matrix effects, thickness of the material, and scattering patterns.

As the energy leaves the sample material, some of it will return to the unit. For detection of incoming photons, proportional counters or other detectors are used. These components work because X-ray photons from the sample material will ionize with detector atoms in a manner such that the amount of charge created is proportional to the energy of the photon. These charges create pulses which are proportional to the energy of each incoming photon. By recording each of these pulses, an energy spectrum with frequencies can be mapped and counts of photons can be used to describe which chemical elements are present and, if the machine is calibrated, in what quantities. Common ways of analyzing these data are by employing computer software to create spectra overlay or to create elemental concentration charts which can be used for quantitative analysis (Perrone, 2014, pg. 146).

The final step of analysis is to account for the errors inherent in the analysis and with each specific machine. As an X-ray moves through a material, its energy is modified by the matrix effects,

³ Bremsstrahlung radiation is generated when electrons are accelerated by the anode and release X-rays, and results in a continuous spectrum.

secondary excitation, absorption of emitted X-rays, and interference with characteristic X-rays (Beckhoff, 2006). Some of these factors can be controlled for and predicted using derivations of mathematical correction formulae. The formulae allow the raw readings, or counts, to be converted to actual numeric quantities which allow direct comparisons of elemental compositions.

Instrumentation:

Since the early 2000s, advancement of X-ray generation and detection technology has allowed for the miniaturization of bulky laboratory-based units into portable and lightweight handheld pXRF devices which can be easily packed, transported, and utilized in non-traditional settings (Shugar and Mass, 2013, pg. 17). This has permitted studies involving the analysis of large, bulky, or fragile objects which cannot be investigated in laboratories. Furthermore, pXRF does not require the destructive preparation procedures required for a stationary XRF unit such as powdering or cutting (Perrone, 2014, pg. 147). The drawback of pXRF is that it has a significantly more limited detection range than XRF and is typically (though not always) limited to the accurate detection of element numbers 22 (titanium, Ti) to 41 (niobium, Nb) whereas a desktop XRF can precisely capture element numbers 11 (sodium, Na) to 92 (uranium, U) (Shackley, 2012). If detection of lower atomic number chemical elements is desirable, a vacuum can be used with a pXRF to improve readings. Another major limitation of pXRF is that since it is nondestructive, the thickness and homogeneity of the material must be accounted for. In comparison, most XRF samples are powdered and can thus be reliably replicated.

Applications:

Historically, XRF has been used for a variety of purposes mainly related to measuring chemical elements found in natural or industrial settings. The prime consumers of pXRF units are steel, manufacturing, and cement industries that regularly use XRF for material development and quality control (Guthrie, 2012). Another major user of XRF is the National Institute of Standards and Technology (NIST), which uses XRF as one way to quantitatively analyze and test its standard reference materials (SRMs). Some medical applications have also emerged for pXRF as a measure to trace elements within living systems (Specht, 2014)). For anthropological applications of XRF, it is vital to remember that the disciplines of art, archaeology, geology, and forensics are at best marginal applications for the

manufacturers of commercially available equipment as they compose an extremely minute share of the market. Therefore, most of the calibrations and factory-settings are not optimized for the uses researchers envision.

In the last three decades, archaeologists and museum curators have made extensive use of the archaeometric applications of XRF for the analysis of materials such as metals, ceramics, lithics, and textiles. Many of these studies have been geared towards either sourcing materials or determining whether they contained harmful or dangerous substances. The tool has also been used by forensic investigators at crime scenes to detect gunshot residues and body fluids, and thus it was a natural transition for biological anthropologists to utilize pXRF for projects ranging from the detection of heavy metals like Hg, Pb, and Fe (Zuckerman, 2010; Little et al. 2014; Killoran, 2017) to analysis of paleodiets (Gilbertson, 2015; Bergmann, 2018) and comparisons of trace elements for the purpose of resolving commingling (Gonzalez-Rodriguez, 2013; Perrone, 2014; Richards and Jones, 2015; Stevens, 2016; Finlayson, 2016; Winburn, 2017).

Data Analysis:

Analysis of pXRF data in archaeometry is generally considered to be a qualitative or, at best, a semiquantitative endeavour (Holmqvist, 2016). Data examined for presence/absence is generally straightforward, but determining real compositions is challenging because comparative studies of pXRF and higher-sensitivity techniques such as inductively coupled plasma mass spectrometry (ICP-MS) have demonstrated that elemental concentrations from pXRF are inaccurate (despite generally following real patterns and trends) (Holmqvist, 2016). Such results are subpar because they are hard to validate and less precise than destructive methods. Additionally, such semi-quantitative values are specific to each pXRF unit and cannot be compared between different machines. For example, the same material analyzed with two different handheld units might return statistically different results.

As a result, a major hurdle of pXRF usage is the collection of comparable, quantifiable data. Achieving such data requires both precision and accuracy of detection as well as matrix-matched calibration. For archaeological artifacts with non homogeneous matrices (i.e., ceramics, bones), the gold standard is to powder and prepare samples as pellets in order to ensure that particle size, mineralogy, and porosity are consistent (Holmqvist, 2016). This allows for control of matrix effects which include

inter-elemental interactions, variations in absorption and emittance, and surface effects (i.e., the ‘shielding effect’) (Holmqvist, 2016). In addition, a vacuum can help mitigate some of the issues of matrix heterogeneity, as can helium flow.

In addition to optimized sample detection, if a researcher wants to convert pXRF count data into mass units, calibrations must be used which match the matrix of the sample (Holmqvist, 2016). The general recommendation offered by Speakman et al. (2011) is that investigators produce specific calibrations for their materials since manufacturer settings will neither be accurate nor precise for archaeological materials (Speakman et al., 2011). As described by Drake (2018), there are multiple assumptions which must hold true for a calibration to function as intended. First, the matrix should be homogenous; second, the calibration reference should be of the same material as the sample; third, every element in the sample should be present in the reference; fourth, the reference should have both minimum and maximum values of each element; and fifth, readings should be taken with the same parameters (unit, energy, current, filter, vacuum, etc) (Drake, 2018). The process of creating a calibration generally involves using both a pXRF unit and a trusted method of detection (ex. ICP-MS) to analyze a sample. Therefore, the process of creating an appropriate calibration for archaeological materials is a destructive one.

Limitations:

There are multiple commonly expressed concerns regarding the application of pXRF to archaeological studies. These include issues originating from non-ideal surfaces and matrices. Bones are a fantastic example of non ideal pXRF samples because they have very few flat surfaces and exhibit extreme elemental and structural variation from one location to another and by depth. Furthermore, many archaeological materials do not account for surface level treatments common to many museum collections. As demonstrated by Shugar (2013), a treatment such as a slip on pottery can dramatically alter the peaks and concentrations of chemical elements detected by pXRF (Shugar, 2013). Therefore, investigators must be either willing to accept inaccurate readings or remove the outer surface of analytes.

In addition, there are investigators who are concerned that many users of pXRF lack the understanding of the principles of X-ray spectrometer which are crucial to appropriately utilizing these devices (Shackley, 2012). Many institutions and individuals purchase pXRF devices as ‘point and shoot’ approaches to material analysis projects but lack an understanding of the physics required to account for

and interpret photon interactions. Even in the most straightforward analysis, the photons used by pXRF will exhibit at least four different phenomena in addition to X-ray fluorescence: incident beam absorption: elastic scattering (Rayleigh), inelastic scattering (Compton), and diffraction (Bragg) (Shugar and Mass, 2013, pg. 18). When archaeological objects with complex morphologies and heterogeneous matrices are involved, the resulting data will be problematic and potentially meaningless.

Despite these challenges associated with the use of pXRF, it is certainly possible to utilize this technique to answer specific anthropological questions so long as the investigator approaches the design, execution, and interpretation of the project with appropriate care. In the following chapters, the understanding of pXRF described above will be turned to the question of whether this technique is useful for the resolution of commingled human remains.

CHAPTER III: STRUCTURE & ELEMENTAL COMPOSITION OF HUMAN BONE:

In order for pXRF to be considered a useful technique for the resolution of commingled human remains, the first criterion that must be demonstrated, as described in the introduction, is that there exists a lower degree of intraskeletal variation within the skeletal elements of a single individual than interskeletal variation within a commingled population with respect to a subset of chemical elements. The purpose of this chapter is to demonstrate multiple lines of evidence why this might hold true for some, but not all, skeletal populations. In the process of achieving that objective, the physical structure and elemental composition of osseous tissue will be described, elemental incorporation pathways will be outlined, and the history of elemental human bone studies will be discussed.

Skeletal Composition:

Human bone, synonymously called osseous tissue, is a dynamic feature of the body which is responsible for several vital functional roles such as red and white blood cell production, service as a mineral depository, and support of other anatomical structures. An adult human being has approximately 206 bones (excluding sesamoid bones) [see Appendix 2 with image of skeleton] while juveniles boast substantially more centers of ossification until their skeletal elements fuse into final forms by early adulthood. Osseous tissue is composed of multiple types of cells organized in a matrix-like structure that allows bones to be both strong and lightweight. These cells include osteoblasts and osteocytes which form and mineralize bone and osteoclasts which reabsorb osseous materials throughout the course of the human life.

According to Clarke (2008), there are four major categories of human bone: long bones, short bones, flat bones, and irregular bones. The major differences between these categories are the forms of the skeletal elements as well as the types of bone structure present. The two major types of bone structure present throughout the categories are cortical and trabecular bone. Cortical bone comprises the outer, much denser layer of osseous tissue. This type is identifiable by its hard and smooth appearance. Trabecular bone, on the other hand, is significantly more “spongy” in appearance and has a porous structure. Although trabecular bone takes up more area than cortical bone, it is much less dense. In the adult human skeleton, 80% of the bone mass is cortical and 20% is trabecular (Clarke, 2008, pg. S131). Both cortical bone and trabecular bone form in lamellar patterns of collagen fibrils laid down in opposing

orientations (Clarke, 2008, pg. S132). Because of this, newly deposited bone is on the periosteal (external surface) while older bone will be underneath. This feature of lamellar bone is an important consideration for studies proposing to utilize pXRF. In contrast to lamellar bone is woven bone, which is comprised of a more haphazard structure of collagen.

On a molecular level, bone is a complex amalgam of both organic (30% dry weight) and inorganic components (70% dry weight) (Gilbertson, 2015, pg. 79). Its composition is mainly water, phosphate, calcium (Ca), and a variety of trace elements (Stevens, 2016, pg. 108). The organic component is made up of cells (osteoblasts, osteoclasts, and osteocytes) as well as the osteoid, the organic portion of bone matrix which consists of collagen, proteoglycans, and glycoproteins (Zimmerman, 2015, pg. 132). This organic portion of bone is what provides the tissue with the tensile strength and flexibility that allows it to undergo physical stress. The part of the bone that gives its structure is the inorganic bony matrix which is composed mainly of hydroxyapatites, or mineral calcium phosphates, which can be described with the formula $Ca_5(PO_4)_3OH$ (Zimmerman, 2015, pg. 132)

Bone Growth and Remodeling:

Although bone growth is rapid during childhood, all skeletal elements continue to grow, develop, model, and remodel throughout the lifetime. That early childhood bone growth is referred to as longitudinal and radial, whereas remodeling is a more gradual process by which the skeleton adjusts to physiological conditions, mechanical stress, and environmental factors. During the process of remodeling, human bone composition continually shifts based on nutrition, environment, and metabolic demand. Although bone is actively remodeled in humans of all ages, the rates are not constant, nor are the rates constant across different skeletal elements. There are several sequential phases to the remodeling process: (1) resorption, when osteoclasts digest old bone; (2) reversal, when cells appear on the bone surface; and (3) formation, when osteoblasts deposit new bone until replacement or growth occur. While the process nominally maintains existing structure, remodeling also serves to help the skeletal system adapt to new mechanical needs and to repair damage caused by stress. During these steps, the basic components of bone are removed and added on both a cellular level and molecular level. These processes are controlled by complex signaling pathways, especially during early life when bones are still undergoing development.

Generally, cortical bone remodels over much greater time periods than trabecular bone (Hill, 1998). As a whole, remodeling of bone in the human body is responsible to 5-10% turnover of skeletal

mass each year (Kini and Nandeesh, 2012). During this turnover, the chemical composition of the bone is transformed based on accessibility of different skeletal elements within the body. Therefore, different skeletal elements are informative of different time periods in an individual's life (e.g. teeth are useful indicators of childhood because they form during early life and are not remodeled, while skeletal elements such as ribs reflect more recent chemical environments).

Elemental Incorporation Strategies:

The major chemical elements found in bone are Ca and phosphorus (P) in addition to minor chemical elements such as magnesium (Mg), sulphur (S), iron (Fe), Zn, manganese (Mn) and cadmium (Cd). While all human bone includes a similar basic structure, variation between individuals of bone composition can be caused by a variety of factors including: diverse developments, exposure to dissimilar environments (i.e., heavy metals contaminants), different pathological experiences, consumption of chemically dissimilar food or water, effects of postmortem contamination (diagenesis), and human processing (post mortem). The chemical elements which are commonly detected using XRF include potassium (K), Ca, Fe, Mg, P, Zn, strontium (Sr), and Pb (Perrone, 2014, pg. 148). Both the distribution and the concentration of these chemical elements can vary considerably within the skeleton based on the type of bone, the speed of its turnover, and the chemical environment of the body at the time at which the tissue was formed. In the following section, the mechanisms by which these chemical elements are incorporated into the human body will be discussed in more depth.

Excluding diagenesis and processing, the other factors leading to elemental variation occur during life, typically during the process of deposition. During that time, the composition of the inorganic portion of bone can change due to ionic substitution of bone which causes the calcium phosphate matrix to be replaced by trace elements (Zimmerman, 2015, pg. 132). For example, the Ca ions can be replaced by a variety of chemical elements (Zimmerman, 2015, pg. 132). Meanwhile, the phosphate group (PO_4) can be exchanged for citrate, phosphate esters, diphosphates, pyrophosphates, and amino acids (Zimmerman, 2015, pg. 132). Lastly, the hydroxyl group (OH) can be switched for fluorine (F) or chlorine (Cl) (Zimmerman, 2015, pg. 132).

The ratios of these substitutions are dependent upon the availability of these chemical elements in the body, bone turnover rates, and other conditions, and thus there is considerable flux in the quantities of

these chemical elements within individuals and even skeletal elements. For example, the variation in elemental concentrations by skeletal element can be seen through the fact that Zn, vanadium (V), nickel (Ni), Cr, Pb, Mn, and Co are more concentrated in epiphyseal bone regions while Ca, Sr, Na, and K tend to collect near the diaphysis (Zimmerman, 2015, pg. 132). Indeed, knowledge of the intra-skeletal variability in elemental signatures has been established for several decades and is a recurring issue investigators seeking to analyze elemental data (Stevens, 2016, pg. 11). Therefore, investigators should carefully consider the known distributions of the chemical elements relevant to their study and choose skeletal elements in ways that suit their research questions.

Developmental and Environmental Effects:

The chemical environment of both children and adults has a significant effect on which chemical elements are incorporated into bone. Thus, the elemental composition of bone contains information relevant to geographical origin and residence, diet, and environmental exposures (Byrnes, 2016, pg. 1042). The trend to use skeletal elemental composition to answer questions about origin is most evident in isotopic studies which frequently utilize oxygen and Sr comparisons between teeth and bone to determine whether an individual migrated from one area to another. This technique is effective because oxygen and Sr isotopic ratios vary based on the region where an individual is living (Gilbertson, 2015, pg. 88-99).

Major chemical elements relevant to environmental exposure are Pb, Cd, mercury (Hg), Cu, and aluminium (Al). Exposure to significant concentrations of these chemicals can lead to serious health outcomes, although it is important to note that paths to exposure to these chemical elements are not the same today as they were in the past (ex. Ericson, 1991). Of these, Pb is probably the most well known and investigated because it is a toxic poison which, when ingested by children, can result in higher mortality and delayed mental development rates (Flora, 2012). Pb bioaccumulates and the quantity of Pb in a bone can account for up to 70-90% of a person's total lifetime exposure (Little et al. 2014, pg. 19). In the past, populations were likely exposed to Pb through the consumption of grains and the drinking of water (Gilbertson, 2015, pg. 95). The majority of Pb uptake therefore occurs in the gastrointestinal tract, and the element is incorporated into the hydroxyapatites matrix through exchange with Ca (Stevens, 2016, pg. 110). However, the consideration of Pb should not occur in isolation because its uptake is negatively associated with Ca, Cu, Fe, potassium (K), and zinc (Zn) (Gilbertson, 2015, pg. 95).

Pathological Effects:

The pathology of an individual can also influence which chemical elements make up osseous tissue. This is both because the pathogenesis of certain conditions can influence the chemical balance of the body and because certain cultures attempt to treat illness with chemical elements that are then incorporated into the matrix. The first mechanism is the relationship between elemental composition and pathology, a frequent subject of research that has been used to understand the physiological response to disease (ex. Karaaslan, 2014). Other examples include studies utilizing micro-XRF to investigate the distribution of trace elements within specific bone histological structures (e.g.. Pemmer et al. 2013).

The second mechanism through which the elemental composition of bone can be informative about pathology is through evidence of treatment. The best examples of such cases are those involving the use of Hg to treat syphilis in the 17th-19th centuries⁴. For example, Zuckerman (2010) utilized pXRF on English skeletal samples in order to explore the utilization of Hg for this medicinal purpose. Aside from syphilis, a high Hg concentration in an individual could also be associated with leprosy. The mechanism through which Hg is incorporated into osseous tissue is through the substitution of Hg for Ca in bone carbonate (Rasmussen, 2008).

Nutritional Effects:

Perhaps the most impactful factor influencing the incorporation of chemical elements into osseous tissue is the diet of an individual. Of course, diet is closely related both to pathological lesions as well as to the chemical environment during life (i.e., environmental contaminants such as Pb) and growth since differing physiological requirements will impact which chemical elements are kept in the body's reserves. Some of the chemical elements most commonly associated with diet are Zn, Cu, Mo, Se, Sr, Mg, Mn, cobalt (Co), and Ni. Of these, the first four are predominantly associated with animal proteins, while the latter are linked to plant resources (Stevens, 2016, pg. 109). The composition of human bone, depending on the element, can generally be used as a composite of the last 10 to 15 years of a person's life (Gilbertson, 2015, pg. 87). Therefore, any dietary changes which have taken place prior to that time period are undetectable (unless dental elements are available in order to compare adult to childhood nutrition).

⁴ Hence the origin of the popular saying: "A Night with Venus, a Lifetime with Mercury" (Asfiya, 2018).

Chemical elements that recur frequently in investigations of ancient diets are barium (Ba), Ca, and Sr, all alkaline earth metals which are incorporated into human tissue through the consumption of plants and animals (ex. Gilbert, 1994; Bergmann, 2018). The utility of these chemical elements to investigations of nutrition is derived from the fact that there is a reduction in an organism's Ba/Ca and Sr/Ca ratios in relation to the diet that it consumes (Burton, 199, pg. 609). So, for example, a herbivore would have lower ratios than plants and carnivores would have lower ratios than omnivores. Therefore, reductions in Ba/Ca and Sr/Ca relate to ascensions in trophic level. While there are several issues associated with matching these ratios directly to plant versus meat diets (for example, unusually high Ca content in plants can skew ratios and thus diets with the same amount of meat can appear identical), “experimental results overwhelmingly demonstrate that bone Ba/Ca and Sr/Ca faithfully track dietary Ba/Ca and Sr/Ca” (Burton, 1999, pg. 609).

Diagenetic Effects:

Of all of the elemental incorporation strategies, diagenetic effects are considered the most problematic because they cause the elemental composition of retrieved osseous tissues to reflect not only the composition of the bones during life, but the chemical composition of a depositional environment following death. Diagenesis is the post-mortem alteration in the makeup of bone caused by contamination by surrounding soils (Price, 1992). This is a frequent issue in archaeological investigations seeking to study the composition of ancient remains (Burton, 1999, pg. 609). Although the general effects of diagenesis are known, there is no single universal trajectory for the process. Rather, a complex array of factors specific to the depositional environment and ranging from soil composition, breakdown and leaching of collagen, microbial attacks, hydrological and geological events, physical erosion, acidity, and more interact to alter the composition of bone (Stevens, 2016, pg. 112-113).

While diagenesis is largely unavoidable, there are several possible indicators that allow investigators to know their osseous samples can return reliable data. These include elevated Ca/P ratios which suggest that the bones were enriched by Ca in the soil as well as elevated Sr, Pb, Al, Mn, and Fe (Stevens, 2016, pg. 114). In addition, certain chemical elements are more likely to withstand diagenesis than others based on their density and surface features. For example, teeth are generally more resistant to diagenesis than other tissue due to their lack of porosity (Byrnes, 2016, pg. 1041). Even within a skeletal element, not all of the components are altered at the same rates. For example, bone collagen is frequently

the first component of osseous tissue to be altered, while bone hydroxyapatite is typically altered at slower rates (Gilbertson, 2015, pg. 1).

When diagenetic effects are suspected, the prudent anthropologist should collect samples of soil from the area of deposition in order to compare the elemental composition with recovered skeletal elements. Another good practice is to note the positioning of skeletal elements during their recovery since the positioning of a bone can influence the concentration of chemical elements (Lambert et al. 1985, pg. 480).

Processing Effects:

The final mechanism that can influence the elemental composition of skeletal elements is post-recovery processing. Admittedly, this mechanism is less relevant to forensic applications but can be extremely important in the analysis of museum collections recovered prior to the development of high tech analytical methodologies. Materials that were historically used to preserve and treat bones include paraffin wax, animal glue shellac, polyvinyl acetate emulsions, acrylic emulsions, and acrylic resins in solvent (Wills, 2014). While these may be visible in some scenarios as shiny or discoloured patches on the bone, at times it is difficult to determine if processing has occurred. However, application of foreign materials to skeletal materials is important to establish prior to analysis so that, if necessary, the external surface of skeletal elements can be removed and uncontaminated tissue can be analyzed.

History of Elemental Human Bone Studies:

As is clearly evident by the chemical element incorporation mechanisms described above, the composition of osseous tissue has the potential to be extremely informative both about the lives of deceased individuals as well as what happened to their remains after death. While it can be difficult to disentangle the various factors which influence the presences, absences, concentrations, and ratios of various chemical elements and form concrete conclusions about deceased individuals, many investigators have made the effort using various techniques ranging from mass spectrometers to pXRF units.

Among the earliest individuals to attempt such analysis was Robert Gilbert, who in 1975 conducted trace element analyses of three skeletal Amerindian populations at Dickson Mounds. Since that time, other researchers have utilized bone chemistry to investigate diets, occupational exposures to toxins,

and migrations (Stevens, 2016, pg. 108-109). Many of these researchers struggled against the same issues posed by anthropologists interested in the resolution of commingling: interskeletal vs intra skeletal variation in elemental incorporation and diagenesis.

Probably among the most versatile, accurate, and precise tool for such investigations has been the inductively coupled plasma mass spectrometer, or ICP-MS. While more time-intensive, expensive, and destructive than XRF, ICP-MS offers an extremely successful and reliable method to analyze the elemental composition of most substances. Indeed, prior to the development of pXRF, ICP-MS was a popular methodology for the attempts at using chemical elements to resolve skeletal commingling.

In 1986, Fulton et al. was published. This study utilized trace metal ratios to attempt to resolve commingling among a small number of individuals from an air crash scenario using ICP. Their results indicated that Mg/Zn ratios were the most reliable and that Zn/Na, Mg/Na, and Cr/Na could be used as supplements. However, they also found that single chemical elements (excluding arsenic) were not useful in distinguishing between individuals.

In 1988, Grupe set out to compare trace element content in bone specimens from different anatomical sites. Using individuals excavated in northern Germany from the 11-12th century CE, the researcher utilized neutron activation analysis and plasma emission spectrometry to investigate Ca, P, Sr, Mg, Ba, Zn, Fe, Co, Cr, Se, Sc, Antimony (Sb), Cs, and silver (Ag). What Grupe found was that there were trace element differences between trabecular and cortical bone and that trabecular bone was more susceptible to diagenetic impacts.

In 1990, Francalacci utilized ICP to study the Ba, Cu, Mn, Sr, V, and Zn from 32 bones belonging to 11 individuals from 3 archaeological sites (see example in Figure 4). The results obtained suggested a high intra-individual variation and concluded that a single bone could not be considered to be representative of the entire skeleton (Francalacci, 1990, pg. 227). More work on commingling was conducted by Finnegan and Chaudhuri in 1990 when researchers attempted to utilize the isotopic ratio of Sr ($\text{Sr}^{87}/\text{Sr}^{86}$) and found that it could be used to effectively segregate individuals if teeth and bones were considered separately (Stevens, 2016, pg. 123).

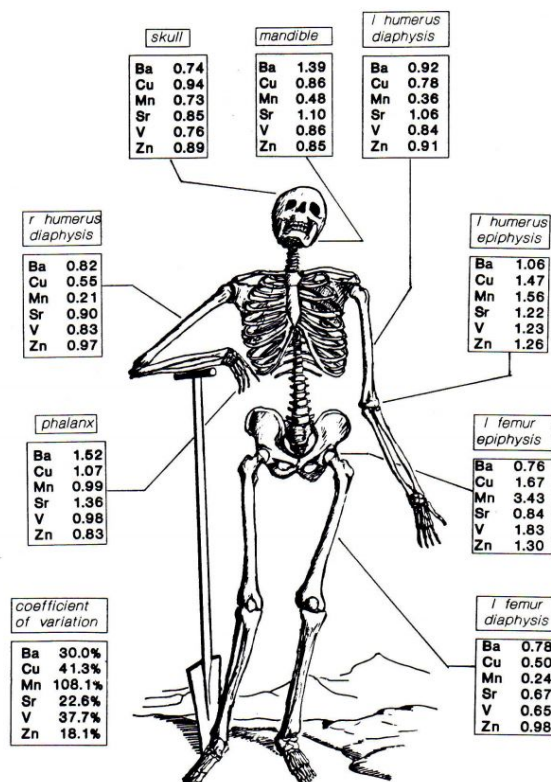


Fig. 3. Ratios of the trace element content in different bones in individual E64 (Francalacci, 1990, pg. 228).

More recent studies, such as Castro et al. (2010), have continued to explore possibilities for resolving commingling using trace element analysis. In their investigation, the researchers conducted elemental analysis of bone samples via ICP-MS from 12 individuals and determined that although full discrimination was not achieved, elemental analysis was a promising tool for separation of commingled remains.

Ultimately, while most of the trace element commingling studies have been unable to achieve full discrimination of individuals, the overall consensus has been that in conjunction with other approaches to the resolution of commingling, trace element analysis can be a useful technique. In the following chapter the new generation of trace element commingling studies utilizing pXRF will be explored.

CHAPTER IV: REVIEW OF APPLICATIONS OF PXRF TO BIOLOGICAL ANTHROPOLOGY:

This chapter will provide synopses of recent publications characterized by the application of pXRF to biological anthropology and specifically human bone. These studies are roughly sorted into four categories according to the focus of their research questions, as detection, diet, commingling, and methodology. Detection studies are those which address questions pertaining to the presence or absence of particular chemical elements such as Pb or Hg. Diet studies are those which attempt to associate spectrometry data to the nutritional patterns of ancient populations. Commingling studies are those which seek to resolve mixed skeletal elements into discrete individuals. Finally, methodology studies are those which attempt to delimit the applications and interpretations of pXRF through formal experimentation on specific aspects of the technique.

Although determining the effectiveness of pXRF to the resolution of commingling is the primary objective of this discussion, studies within the other three categories have been included in this chapter because of their relevance to the topic at hand. Both detection and diet studies attempt to determine information about skeletal remains that could be used to differentiate individuals in certain commingling scenarios (i.e., social differentiations in diet, workplace heavy metal exposure, etc.) Similarly, the methodology studies have been incorporated because they are necessary to determining the applicability, precision, and accuracy of the technique.

Detection Studies:

An early study which applied pXRF to the detection of chemical elements was published by J. Rebocho in 2006. Comparing the Pb content of two archaeological skeletal collections, one from a Pb polluted burial context and the other from a church cemetery turned parking lot, using both a ^{109}Cd -based X-ray Fluorescence technique and EDXRF, the researchers determined that the non-destructive Cd technique was suitable for Pb analysis (Rebocho et al. 2006, pg. 961). By comparing spongy and compact bone, Rebocho et al. also found that there was differential post-mortem alteration based on skeletal element and structure. They also clearly demonstrated the effects of burial environments on the chemical elemental concentrations of osseous tissues.

In 2010, Molly Zuckerman applied pXRF to her research on syphilis in late medieval and early modern England (Zuckerman, 2010). Employing trace element analysis via pXRF, Zuckerman ascertained differences in Hg levels in skeletal remains from several archaeological sites (Zuckerman, 2010, pg. 14). Multiple skeletal elements and locations were systematically analyzed and comparable Hg values were obtained. Overall, despite the heterogeneity of intraskeletal Hg distribution, Zuckerman found pXRF to be a valid way to study Hg exposure in archaeological populations.

Like Zuckerman, in 2018 Peter Killoran utilized pXRF to detect the heavy metals Arsenic, Lead, Cr, Cd, Ba, and Cs in 107 individuals from Kentucky in an effort to isolate individuals who received heavy-metal laden medications. Unlike previous investigations, Killoran found that the pXRF output was not sensitive enough to unequivocally determine the presence of mercury.

Diet Studies:

Dietary studies seeking a nondestructive substitution to ICP-MS and isotopic analysis techniques have appeared more recently than detection studies. An early iteration was the doctoral dissertation composed by Theresa Gilbertson from the University of South Florida in 2015. Focusing on ancient Peruvian coastal diets, Gilbertson sought to compare pXRF and stable isotope analysis. To do so, she tested 209 skulls via pXRF and compared those results to a subsampling of individuals for whom she used isotope ratio mass spectrometry (IRMS) on bone collagen and apatite (Gilbertson, 2015, pg. ix). The bivariate correlations and linear regressions that resulted indicated that pXRF was a valid way to quantify Sr, barium, and Fe for the purpose of dietary analysis (Gilbertson, 2015, pg. ix). Overall, Gilbertson concluded that pXRF could not yet replace destructive analysis in dietary studies.

In 2018, Christine L. Bergmann, also of the University of South Florida, utilized both pXRF and ICP-MS in her MA thesis to investigate Andean paleodiets. This work was particularly notable because Bergmann sought to create a calibration for her skeletal materials by plotting linear regressions of 19 human and animal bones which were analyzed via both techniques. Her calibrations for both Ca and Sr proved effective while Ba and Fe could be improved. Overall, Bergmann concluded that pXRF was a valid technique for studies seeking to investigate paleodiets, although calibrations need to be improved if the accuracy of pXRF is to allow comparison of results across studies (Bergmann, 2018, pg. 66).

Commingling Studies:

As discussed in chapter III, the use of trace element analysis to distinguish between commingled human remains is a tradition dating back to the mid 20th century. Therefore, several attempts have been made to utilize pXRF to discern differences between skeletons. One of the earliest and therefore most-cited of such investigations was that by J. Gonzalez-Rodriguez and G. Fowler in 2013. Using a set of 5 mostly complete archaeological skeletons (four males, one female) excavated from the city of Lincoln (United Kingdom), Gonzalez-Rodriguez and Fowler tested trace element concentrations of Pb, Sr, Zn, Fe, Ca, and K in 23 skeletal elements ranging from cranium to long bones (Gonzalez-Rodriguez, 2013, pg. 5). Using principal component analysis (PCA), the researchers found that skeletal elements could be separated with a high degree of accuracy when two to four individuals were examined, although accuracy dropped when the number of individuals increased (Gonzalez-Rodriguez, 2013, pg. 1). The chemical elements found to be the most important for separation were Pb, Sr, Fe, Ca, and K and the ratios Pb/Ca, Zn/Fe, and Sr/Pb were found to be the most influential (Gonzalez-Rodriguez, 2013, pg. 5). Overall, the findings of this research indicated that pXRF seemed to be effective but quickly lost strength in large contexts due to overlapping chemical element readings.

The Gonzalez-Rodriguez and Fowler study was followed by one by Alexandra Perrone et al. in 2014 which likewise investigated the utility of pXRF for the resolution of commingled human remains. The researchers focused on measuring intra- and inter- skeletal chemical element variation by using pXRF to analyze 20 complete adult skeletons, almost all from forensic anthropology cases. Using ANOVA tests, the researchers found minimal variation between skeletal elements (intraskkeletal variation) except for Mg. To simulate commingling, the researchers used 95% confidence intervals to determine whether individuals could be distinguished. The chemical elements which Perrone et al. found to be most useful for the resolution of commingling were Si, P, K, Ca, Mn, Fe, and Co. Like Gonzalez-Rodriguez and Fowler, Perrone et al. found that segregation rates dropped with increasing number of individuals. However, unlike Gonzalez-Rodriguez and Fowler, the pXRF used in this study was unable to detect Sr and Pb.

In 2015, John D. Richards and Catherine R. Jones from the University of Wisconsin-Milwaukee utilized pXRF both for detection and commingling and presented their work in a Society for American Archaeology (SAA) symposium. In part, this study was a response to the drawbacks of the Gonzalez-Rodriguez and Fowler (2013) and the Perrone et al. (2014) studies to address issues of

diagenesis. The Milwaukee County Institution Grounds Cemetery focused on in this study was suspected to have significant diagenetic alterations due to groundwater infiltration, the deposition of industrial and medical wastes, and other natural and anthropogenic activities (Richards and Jones, 2015, pg. 5). The investigators focused on several commingled burial lots and investigated cranial, scapular, humeral, ulnar, innominate, femoral, and tubular elements. The chemical elements detected at 'useful' levels were arsenic, Ca, copper (Cu), Fe, Mg, Mn, Ni, P, Rh, Sn, Sr, Zn, and zirconium (Zr). In this study, the pXRF elemental readings were not especially effective at resolving commingling, although Sr/Ca and Zn/Fe ratios did lead to some separation. Ultimately, the investigators concluded that pXRF might prove to be useful in combination with archaeological and osteological analyses, but that more careful care should be taken with how measurements are taken and that results should be calibrated to specific reference materials (Richards and Jones, 2015, pg. 10).

Following the Richards and Jones study, in 2016 William D. Stevens of the University of South Carolina utilized pXRF to address commingling as part of his doctoral dissertation. In addition to utilizing classic approaches to commingling, Stevens also tested the efficacy of pXRF in separating skeletal remains of enslaved African American tidal rice field laborers from South Carolina from the 19th century. One interesting finding of Steven's study was that differences existed in the distribution of chemical elements between upper and lower limbs (Stevens, 2016, pg. 158). However, pXRF generally worked well in conjunction with other approaches to commingling to create possible associations and support pair-matches (Stevens, 2016, pg. 159). As in earlier studies, difficulties arose as larger sets of commingled remains were analyzed. Overall, Sr and Pb were found to be the best for sorting remains. Stevens conjectured that this was because these chemical elements reflect individual dietary differences and the possible origins of the enslaved (Stevens, 2016, pg. 245). Metals such as Cu and Fe, however, displayed high degrees of both variation and overlap, probably due to burial practices, and were thus not useful for the resolution of commingling (Stevens, 2016, pg. 245).

In 2016 Janet E. Finlayson and colleagues utilized pXRF in conjunction with physical matching of fragmented skeletal elements, articulation to evaluate joint congruence, visual pair-matching of bilateral skeletal elements, osteometric pair-matching, evaluation of taphonomic patterns, and DNA analysis to segregate two individuals who were found in a marijuana field in rural California in shallow graves. Using methods and a pXRF unit identical to those utilized by Perrone in 2014, Finlayson et al. detected the chemical elements Si, P, K, Ca, Mn, Fe, and Co and ran 95% confidence intervals. The

results indicated that P had the greatest potential for resolving commingling and that pXRF could be used to segregate skeletal elements when other techniques are ineffective (Finlayson et al. 2016, pg. 497).

In 2017, Winburn et al. published a similar investigation which utilized numerous commingling techniques to attempt to segregate two sets of commingled remains. Unlike the Finlayson 2016 study, the skeletal remains from this investigation originated from a freshwater context and, likely due to the resulting diagenetic alteration, pXRF was unable to resolve commingling (Winburn, 2017, pg. 24).

Methodological Studies:

The final category of study related to this discussion are methodological studies seeking to test the practical and theoretical limitations of the technique. An early example of this is the 2014 study conducted by Nicole C. Little and her colleagues. The goal of the investigation was to compare data collected from an ICP-MS to that derived from a pXRF with respect to Pb content in bone for 25 individuals. However, the researchers found that there were extreme differences between pXRF readings taken for unburred and burred bones (Little et al. 2014, pg. 21). This was attributed not only to differing Pb concentrations within and between skeletal elements but also to the differences in what ICP-MS and pXRF are measuring- average bulk composition versus analytical surfaces. The take away from this investigation is that researchers should consider what, if any, treatments skeletal elements should endure prior to being analyzed.

In 2016, another methodological study was published by Jennifer F. Byrnes and Peter J. Bush that considered the practical limitations of bone surface and analysis depth for accurate trace element detection. The researchers focused on Sr, Cu, Pb, Zr, and Sn (Byrnes, 2016, pg. 1042). The study concluded that diagenesis was a vital factor for pXRF studies because surface effects created detectable differences in readings. Moreover, bone density and thickness must be considered in order to account for the expected penetration distances of specific chemical elements.

Also in 2016, Aaron Kuzel and colleagues conducted a study where benchtop XRF and pXRF were compared as tools for distinguishing between skeletal and non-skeletal remains. The researchers found that atmosphere can effect the detection levels of Ca and P, and thus analysis by pXRF must be conducted under similar settings in order for results to be comparable.

Summary:

As demonstrated by this chapter, over the last decade pXRF has been utilized frequently and enthusiastically by biological anthropologists seeking to add another technique to their commingling toolbox. While many of these researchers were aware of the studies that preceded their own, there have been limited systematic efforts to define and refine the methodology despite the promise and potential it seems to hold. Instead, there has been significant variation in sample preparation, analytical procedures, and adherence to accepted pXRF protocols as have been established for ceramic and lithic studies.

Thus, the purpose of the next chapter will be to synthesize these existing studies, address their similarities, differences, successes, and failures, and ultimately propose an acceptable methodology for researchers seeking to resolve commingling of human remains via pXRF in the future.

CHAPTER V: ANALYSIS OF EXISTING STUDIES:

While a significant amount of work has been conducted on the resolution of commingled human remains via pXRF units, there is substantial variation among published studies with respect to which chemical and skeletal elements are selected for investigation, the ways in which samples are cleaned and prepared, and how the resulting data are analyzed. The purpose of the following section is to analyze these differences and synthesize the most successful features of the studies summarized above into a set of general guidelines for future investigators to employ as they set out to plan their analyses.

Comparisons and Contrasts of pXRF Studies:

The skeletal collections which have served as subjects for pXRF commingling studies have emerged from a varied array of temporal and spatial contexts. As is appropriate for a technique that holds great potential for both bioarchaeologists and forensic investigators, the studies published thus far vary from medieval internments to recent forensic remains recovered from marijuana fields. In Table 1, the dates and contexts of the studies discussed in Chapter IV are provided. The number of individuals analyzed by investigators ranges from as low as two to as high as 20 in the Perrone et al. 2014 investigation. While some of the mock commingling investigations (those which artificially create commingling scenarios out of known remains) have analyzed pXRF data for complete skeletons, with as many as 23 skeletal elements analyzed per individual in order to compared inter- and intra-skeletal variation, others such as Finlayson's 2016 investigation compared a few fragmentary skeletal elements to whole individuals.

Table 1. Dates, Contexts, and Sample Sizes of Commingling pXRF Studies.

Study	Dates	n	Type	Context
Gonzalez-Rodriguez and Fowler, 2013	12th - 16th C.	5	Archaeological	The articulated adult skeletons were inhumed in stone lined cists in St. Katherine's Priory, a site with inhumations dating from the 12-16th century.
Perrone et al. 2014	Mostly Modern	20	Mixed (mostly forensic)	The twenty adult skeletons came from the collection of CSU, Chico and were mostly derived from closed forensic cases except one pioneer burial.
Richards and Jones, 2015	1882-1925	NA	Archaeological	The individuals analyzed in this study were adults and sub adults excavated from the Milwaukee County Institution Grounds Cemetery.

Stevens, 2016	Mid 19th C.	NA	Archaeological	The individuals were enslaved African American tidal rice field laborers from South Carolina in the mid-19th C.
Finlayson, 2016	Modern	2	Forensic	The remains were recovered from a marijuana field in rural California. The individuals were initially buried in shallow graves and then scattered by large carnivores over a large area.
Winburn, 2017	Modern	2	Forensic	The two individuals, one male and one female, were recovered from a freshwater context by law enforcement.

Due to the variety of skeletal contexts, the studies have had to account for different rates of diagenesis and chemical trace element variation. Additionally, depending on the questions posed by each specific study, the differences between the pXRF trace element signatures may have needed to vary to lesser or greater degrees in order to prove effective for quantitative resolution. For example, in Finlayson's study, pXRF proved useful because fewer tests were required to test the similarity of the two mandibular fragments to the other, more complete, individual. On the other hand, studies such as Gonzalez-Rodriguez and Fowler's 2013 investigation or Perrone et al.'s 2014 study had to account for a substantially higher number of skeletal elements and therefore a greater overlap in similarity. Particularly for Perrone et al.'s confidence interval method, the introduction of more skeletal elements would cause a significant reduction in the ability of pXRF to effectively distinguish between individuals.

The chemical elements of interest to pXRF commingling studies have also been fairly diverse. In Table 2, the chemical elements under investigation by each are compared. Although some chemical elements feature in nearly every study (ex. P, Ca, Fe), others are analyzed less frequently likely due to detection issues with the instrument as mentioned in Chapter II or the suspicion of researchers that certain chemical elements such as heavy metals may aid in the resolution of commingling in specific contexts.

Table 2. Chemical Elements Utilized by Commingling pXRF Studies.

	Gonzalez-Rodriguez and Fowler, 2013	Richards and Jones, 2015	Stevens, 2016	Finlayson, 2016	Winburn, 2017	Perrone et al. 2014
Na						X
Mg		X				X

Si				X	X	X
P		X	X	X	X	X
K	X			X	X	X
Ca	X	X	X	X	X	X
Ti						X
V						X
Cr						X
Mn		X		X	X	X
Fe	X	X	X	X	X	X
Co				X	X	X
Ni		X				X
Cu		X	X			X
Zn	X	X	X			X
As		X	X			
Rb		X				
Sr	X		X			
Zr		X	X			
Cd						
Sn		X				
Cs						
Ba			X			
Pb	X					

Additionally, the element and elemental ratios found to be most effective for the resolution of commingling differed from study to study. These can be seen in Table 3. Some, like Sr/Ca, Sr/Pb, and

Zn/Fe, proved to be effective in multiple investigations. Others only had singular demonstrated instances of success. The differences in the utility of different chemical elements and elemental ratios can likely be attributed to the differences in skeletal collection contexts as discussed previously as well as the different analytical methodologies employed by the different investigators.

Table 3: Chemical Elements and Ratios Considered Effective by Study (Note: This does not include detection studies).

Study	Si	P	K	Ca	Mn	Fe	Co	Pb/Ca	Zn/Fe	Sr/Pb	Sr/Ca
Gonzalez-Rodriguez and Fowler, 2013								X	X	X	
Perrone et al. 2014	X	X	X	X	X	X	X				
Richards and Jones, 2015									X		X
Stevens, 2016										X	X
Finlayson, 2016		X									
Winburn, 2017											

The differences in analytical procedures utilized by each of the commingling studies are described in Table 4. The majority of the studies utilized the confidence intervals technique pioneered by Perrone et al. in 2014. However, both Gonzalez-Rodriguez and Fowler (2013) and Richards and Jones (2015) utilized Principal Component Analysis to attempt to resolve commingling. Additionally, the skeletal element preparation procedures employed by investigators differed between studies and may well have influenced results as described by the Little et al. study in 2014. The most common form of preparation was brushing or other non-invasive cleaning usually using H₂O or dH₂O. Some studies also employed a secondary cleaning with alcohol before air drying the bones. Due to the burial contexts from

which some of the skeletal collections originated, the lack of more thorough surface cleaning may well have influenced how well the pXRF chemical elemental data truly reflected the composition of the bones.

Table 4: Analysis Details of pXRF Commingling Studies.

Year	Study	Unit	Machine Settings	Sample Prep	Calibrations	Analysis Type
2013	Gonzalez-Rodriguez and Fowler, 2013	Niton XL3t Spectrometer	-	-	-	PCA
2014	Perrone et al. 2014	Bruker Tracer IV Series	No filter, 180 seconds, 15kV, high current, vacuum	Cleaned (100% ethanol), air-dried	Mudrock	Confidence Intervals
2015	Richards and Jones, 2015	Bruker Tracer IIIv+	No filter, 180 seconds, 15Kv, 28 μ A, vacuum	Cleaned (water), air-dried, 2nd cleaning with (100% denatured alcohol)	-	PCA
2016	Stevens, 2016	Bruker Tracer III SD	300 seconds, 40kV, 15 μ A, vacuum	dry brushed, rinsed with dH2O	-	Confidence Intervals
2016	Finlayson, 2016	Bruker Tracer IV Series pXRF	180 seconds, 1-15kV, 40 μ A, vacuum	-	Mudrock	Confidence Intervals
2017	Winburn, 2017	Bruker Tracer III-V+	no filter, 12-15 kV, 35 μ A, vacuum	-	Mudrock	Confidence Intervals

Discussion:

Nearly all of the studies (except for Finlayson 2016) showed some potential for distinguishing remains via pXRF. Gonzalez-Rodriguez and Fowler in 2013 and Perrone's 2014 were particularly promising. With small skeletal collections expected to originate from vastly different contexts such as geographically different interment locations, different post-mortem processing scenarios, or from individuals with vastly different occupational, health or dietary experiences during life, pXRF is likely to

prove to be the most useful tool. However, with large numbers of individuals consisting of skeletal elements which underwent similar diagenetic processes and thus became more similar in their chemical element composition over time, pXRF is likely to be a less effective technique for resolution. Even if the skeletal collection is suitable, however, investigators must still utilize pXRF settings recommended for skeletal tissues, measure the flattest part of skeletal elements, collect multiple chemical elemental readings in order to better characterize the skeletal material, and use other methods to attempt the resolution of commingling for pXRF to be the most effective⁵.

More so than the successes, however, the limitations of commingling pXRF studies are evident. First and foremost, none of the researchers applying this methodology to commingling resolution have attempted to formulate calibrations such as Bergmann established for her dietary investigation in 2018. Because of this, the validity, accuracy, and comparability of the chemical elemental composition readings is a significant unknown. Therefore, researchers seeking to embark upon a commingling investigation in the future should take the time to create calibrations suitable to their specific skeletal collection and skeletal elements of interest and thus determine which chemical elements might prove most reliable.

⁵ When considering whether to spend time collecting pXRF data, researchers should also consider the 10-30 minutes required to collect readings from each locus on a skeletal element. Especially with large commingling scenarios, a pXRF investigation may require a serious temporal investment.

CONCLUSIONS:

The purpose of this discussion has been to analyze whether pXRF should be considered a valid tool for the resolution of commingled human skeletal remains. In Chapter I, the benefits and drawbacks of existing techniques to address intermingled remains were explored. In Chapter II, the theory, instrumentation, and applicability of pXRF was discussed. In Chapter III, the structure and elemental composition of human bone was explored in order to demonstrate why chemical elements would differ between individuals. Additionally, previous bone elemental composition studies were described. In Chapter IV, existing pXRF studies were reviewed and categorized into four categories: detection, diet, commingling, and methodology. Finally, in Chapter V, the strengths and limitations of existing commingling studies were compared and guidelines were offered for future researchers seeking to employ pXRF in their commingling investigations.

The three major considerations for such researchers are as follows: First, the success of pXRF commingling studies hinges on the scale, context, and diagenetic history of the skeletal assemblage. Second, researchers must understand the mechanics of pXRF and thus the limitations of detection, accuracy, and validity should proper measures (such as matrix-matched calibration) not be utilized. Finally, pXRF is unlikely to be useful or trustworthy by itself and should be used in conjunction with other methods to address commingling.

Despite these limitations, the conclusion of this analysis is that pXRF should be considered a useful, if limited, tool for resolving intermingled remains. The studies discussed in chapters III and IV demonstrated that certain skeletal collections do exhibit sufficient intraskeletal variation to offset interskeletal variation in chemical elements. Chapter II demonstrated that if used properly, pXRF is capable of detecting those chemical elements. Finally, Chapters IV and V demonstrated that so long as the skeletal assemblage is not large, statistical analyses such as PCA or confidence intervals can distinguish between individuals with some degree of success.

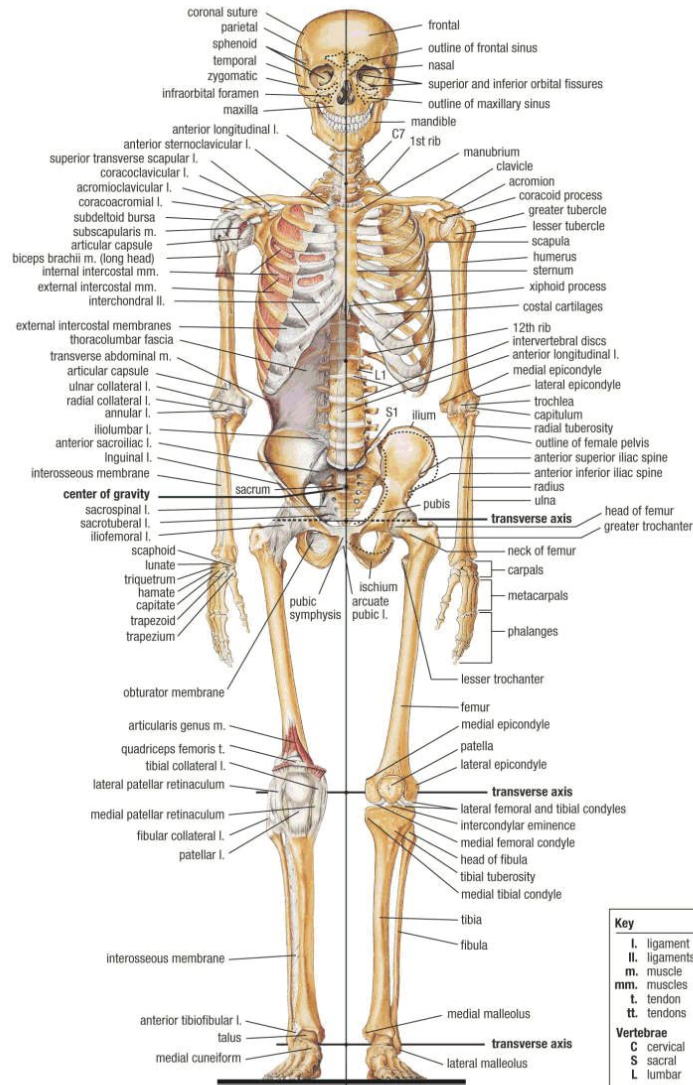
APPENDIX 1: PERIODIC TABLE

PERIODIC TABLE OF THE ELEMENTS

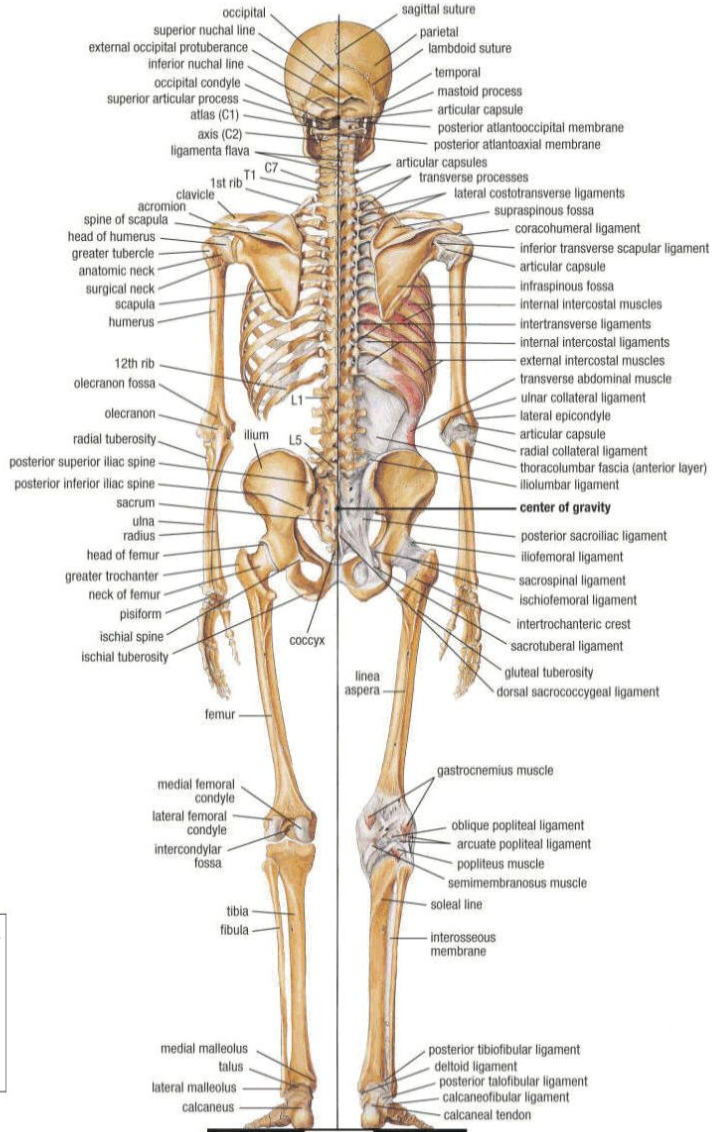
GROUP		PERIOD															
1	2															17	18
1 IA	2 IIA															VIIA	VIIIA
1 H	2 He															2 He	2 He
		</															

APPENDIX 2: HUMAN SKELETAL ELEMENTS

SKELETAL ANATOMY (ANTERIOR VIEW)



SKELETAL ANATOMY (POSTERIOR VIEW)



GLOSSARY:

Analysis of Variance (ANOVA)

This term describes a set of statistical procedures developed by Ronald Fisher to concurrently compare more than two groups at a time.

Atomic Absorption Spectrophotometry (AAS)

An analytical procedure that quantitatively determines the chemical composition of a sample by testing the absorption of light by atoms in a gaseous state.

Bremsstrahlung Radiation

In German, 'Brems' is 'decelerate' and 'strahlung' is radiation. Thus, bremsstrahlung radiation is generated when electrons are accelerated by the anode and release X-rays and results in a continuous spectrum. Also known as the background.

Characteristic X-rays

These X-rays are emitted when an outer-shell electron jumps to a lower energy shell and emits X-rays in a pattern characteristic to a specific element.

Compton Scatter

Also called incoherent or inelastic scatter, a Compton scatter occurs when a photon collides with outer electron and causes a loss of energy corresponding to the electron's movement.

Diagenesis

Alterations to skeletal remains caused by physical, chemical, or biological processes which modify the structure, chemical composition, or other characteristics of organic materials post-deposition.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

This is a type of mass spectrometry which can be precisely used for detecting most chemical elements with a high degree of precision. This analytical tool is frequently used as a standard to which XRF is compared.

Isotope ratio mass spectrometry (IRMS)

A specialized type of mass spectrometry which can measure the relative abundance of isotopes in a sample.

K-alpha (K α 1) Peak

This peak represents the photons produced from the transition of electrons from the L-shell to the K-shell.

K-beta Peak

This peak represents the photons produced from the transition of electrons from the M-shell to the K-shell.

Matrix Effects

This term is used to describe variations in photon behaviors caused by the composition and structure of samples. These can (and should) be minimized by using matrix-matched standards.

Matrix Matched Standards

Standards which attempt to account for variations in grain size, homogeneity or heterogeneity of samples, and the mineralogical component of samples by utilizing standards composed of the same material as samples.

Photoelectric Effect

When X-Ray energy is absorbed by an atom by the transfer of energy to an inner electron, this is called the photoelectric effect. The electrons can then be emitted, creating a vacancy which then leads to characteristic radiation.

Principal Component Analysis (PCA)

Principal component analysis is a statistical procedure, mainly used for exploratory data analysis, which reduces the dimensions of data-sets with large numbers of variables by searching for correlations.

Rayleigh Scatter

This type of scatter occurs as a result of X-rays from the tube bouncing off of atoms without producing fluorescence. Also known as elastic or coherent scatter.

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