The Puzzle of Commingling: Portable X-ray fluorescence analysis of post-medieval human skeletal remains from Arnhem and Middenbeemster
Karasimou, Christina

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The Puzzle of Commingling:

Portable X-ray fluorescence analysis of post-medieval human skeletal remains from Arnhem and Middenbeemster.

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Master Thesis Archaeological Science (1084VTSY)

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It has been a fun ride. I would like to conclude this chapter of my life with a sentence that has accompanied me since I was 19 years old:

«Το μαθησιακό ταξίδι της ζωής δεν έχει τελειωμό για κείνους που επιθυμούν να είναι μακρύς ο δρόμος για την Ιθάκη.»

Δημήτρης Μπουραντάς, (2008, p. 26). Όλα σου τα 'μαθα, μα ξέχασα μια λέξη.

Translation: "*The lifelong learning journey has no end for those who desire the road to Ithaca to be long.*"

Dimitris Bourantas, (2008, p. 26). I taught you everything, but I forgot one word.

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Chapter 1. Introduction

1.1 Commingled Remains

Analyzing human skeletal remains holds significant importance for physical anthropologists, forensic archaeologists, and osteoarchaeologists. Regarding physical anthropology, skeletal remains serve as a fundamental resource for investigating various subjects, including human adaptation to environmental challenges and the process of human evolution (UC Santa Cruz, 2022, para. 1). In forensic contexts, accurately identifying a substance as human bone or dentition (Zimmerman et al., 2015, p. 131) and successfully determining an individual's identity can notably advance the case-solving process. Furthermore, for archaeologists, the analysis of skeletal remains offers a rich source of data and starting points that can guide diverse research directions. Osteoarchaeologists typically begin by developing biological profiles—estimating sex, age at death, and stature (Viner, 2014, p. 87)—followed by the assessment of pathological lesions and traumas. Subsequently, further analysis is conducted to address various research questions that can relate to social status, health, pathologies, dietary preferences, migration patterns, and ancestry. Given that larger sample sizes enable more robust inferences about different populations, the accurate identification of all unearthed individuals becomes pivotal.

One aspect that often poses a serious challenge in skeletal analysis is the commingling of the remains (Byrd & LeGarde, 2014, p. 167; Perrone et al., 2014, p. 145; Stevens, 2016, p. 3). Commingled human remains (Figure 1) refer to the situation in which skeletal elements from more than one individual are mixed together (Gonzalez-Rodriguez & Fowler, 2013, p. 407.e1). When more individuals are intermingled and fragmentation is extensive, it leads to a heightened challenge for reassociation. Several processes and agents can create such contexts. Among these factors are natural processes, such as floods and erosion, as well as cultural practices (Stevens, 2016, p. 3) related to burial customs. Animal activity, such as scavenging and excavation practices that employ improper techniques, comprise just a few of the contributing factors to disarticulated remains. Proper estimations and assessments of individuals cannot be conducted until the reassociation of the remains (Byrd & LeGarde, 2014, p. 167), making it impossible to draw further inferences when dealing with such contexts. It becomes evident that, particularly for archaeologists, the segregation of commingled remains is of paramount importance (Adams & Byrd, 2014, p. ix).

Figure 1. A case of commingled remains from Çatalhöyük (Haddow et al., 2015, p. 7).

As early as 1948, Charles Snow published a paper discussing the procedure for sorting disarticulated remains (Snow, 1948), a work that eventually served as a pioneering guide that established the fundamental principles and methods for handling commingled remains (Stevens, 2016, pp. 93–94). These methods, which consider factors such as bone size, overall morphological characteristics, and the presence of pathological lesions (Stevens, 2016, p. 94), encompass osteometric sorting (Byrd & LeGarde, 2014, p. 171), the assessment of articulating surface alignment, and the evaluation of taphonomic alterations (Perrone et al., 2014, p. 145) observable to the naked eye, such as discoloration and changes related to preservation. Deviating from these traditional methods employed in commingled contexts, this study will adopt a different approach, using portable

X-ray fluorescence spectrometry (pXRF) to investigate the chemical composition of human skeletal remains, aiming to facilitate reassociation.

1.2 Elemental Analysis and Commingled Remains

When referring to human remains under examination, it includes both the bones and teeth of the individuals. These hard tissues are biological materials known for their resilience, with teeth being particularly noted for their exceptional resistance. This makes them less susceptible to chemical degradation (White & Folkens, 2005c, p. 127). Moreover, bones and teeth share a similar chemical makeup (Zimmerman et al., 2015, p. 132), while the distribution of the chemical elements within the tissues (Brätter et al., 1977, p. 393) and the concentrations (Castro et al., 2010, p. 18) vary. The most abundant elements are calcium (Ca) and phosphorus (P) (Shaik et al., 2021, p. S952), and several other elements that can be found in human remains include strontium (Sr) and zinc (Zn), which have been linked to dietary habits (János et al., 2011, p. 2593), potassium (K), magnesium (Mg), and iron (Fe) (Perrone et al., 2014, p. 151).

Numerous studies have utilized the presence of chemical elements in the skeleton to explore potential correlations and provide insights into various topics such as diet, different diseases, and diagenesis. Recognizing that the challenge of commingling must be addressed before any further examination can yield meaningful results (Byrd & LeGarde, 2014, p. 167), some of these papers have focused on the segregation of commingled remains by exploring the chemical composition within the bones and teeth (see Davis, 2021; Gonzalez-Rodriguez & Fowler, 2013; Perrone et al., 2014; Smith, 2021). These studies are based on the suggestion that intra-skeletal variation, the variation in elemental concentrations in the bones of the same individual (Figure 2), can be less than the inter-skeletal variation, which is the difference in concentrations among individuals (Perrone et al., 2014, p. 154). Fulton et al. (1986, as cited in Stevens, 2016, p. 125), were pioneers in exploring the use of chemical elements for the reassociation of mixed remains. Subsequently, the studies conducted by Finnegan (1988, as cited in Stevens 2016, p. 122), as well as Finnegan and Chaudhuri (1990, as cited in Stevens, 2016, p. 122), continued to build upon this research direction. Twenty years later, Castro et al. (2010, pp. 18, 26) conducted examinations on the bones and teeth of deceased American soldiers, emphasizing the importance of separately considering bones from different anatomical positions, specifically humeri and femora, to achieve proper segregation.

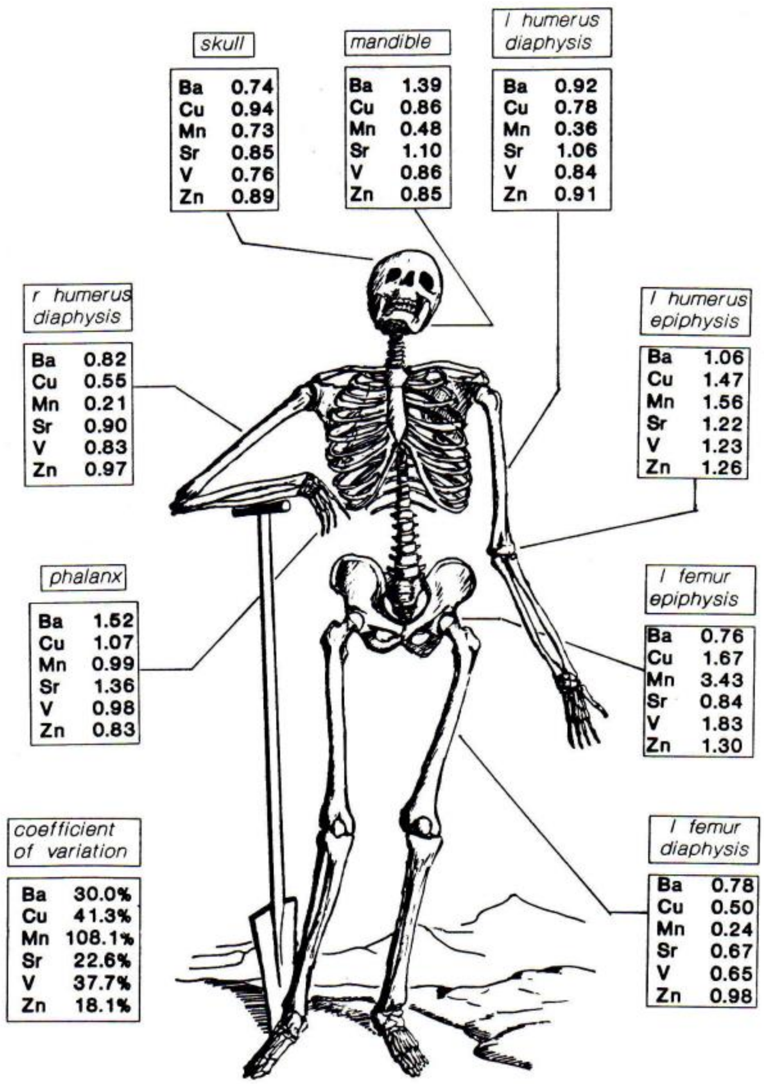


Figure 2. Intra-skeletal variation of trace elements in the individual E64 (Francalacci, 1990, p. 228).

While research involving the examination of elemental profiles in human remains may use methods that result in the partial or complete destruction of the sample, such as the inductively coupled plasma mass spectrometry (ICP-MS), this paper primarily focuses on studies utilizing the non-destructive pXRF method. Although pXRF was not originally designed for bone analysis, it has gained popularity in recent decades among studies exploring skeletal remains due to its cost-effectiveness (compared to methods such as DNA analysis) and its non-destructive properties (Gonzalez-Rodriguez & Fowler, 2013, p. 407.e5). The problem of commingling and the attempt to resolve it by using this technique has been addressed and commented on by several different studies during the last few years (Byrnes et al., 2009, 2010, as cited in Stevens, 2016, p. 116; Davis, 2021; Finlayson et al., 2017; Gancz, 2019; Gonzalez-Rodriguez & Fowler, 2013; Perrone et al., 2014; Smith, 2021; Stevens, 2016; Winburn et al., 2017). The results so far widely agree on the potential for segregating individuals when the pXRF is applied to the analysis of small-scale commingling and skeletal elements with non-overlapping elemental values (Gonzalez-Rodriguez & Fowler, 2013, p. 407.e5; Perrone et al., 2014, p. 161). However, Winburn et al. (2017, p. 31) demonstrated that, due to probable taphonomic factors, even as few as two individuals may not be successfully reassociated. Furthermore, this technique shows more promise when used as a supplementary method (Stevens, 2016, p. 124).

The studies that have been conducted so far serve as a crucial foundation for addressing the challenge of disarticulated human remains. They have discussed potential limitations, offered possible solutions, identified areas for future investigation, and consequently provided valuable insights. However, it is evident that further research is needed, particularly regarding the application of pXRF on commingled remains in archaeological contexts. To date, the published papers are either focused on forensic contexts (Finlayson et al., 2017, p. 493; Perrone et al., 2014, p. 149; Winburn et al., 2017, p. 24), or have examined relatively small sample sizes (two to eight individuals) (Finlayson et al., 2017, p. 493; Gonzalez-Rodriguez & Fowler, 2013, p. 407.e2; Smith, 2021, p. 38; Winburn et al., 2017, p. 24). The duration of time that skeletal remains spend buried underground is significant, as taphonomic factors can impact their preservation state, appearance, and chemical composition. While studies in forensic contexts provide crucial data, they do not precisely mirror the conditions in archaeological settings, where individuals remain buried for extended periods. Furthermore, it has been observed that an increase in the number of individuals under examination may potentially reduce the effectiveness of pXRF in reassociating the remains (Perrone et al., 2014, p. 161). Consequently, further research on larger scales of commingling is necessary to investigate potential causes and solutions.

1.3 Aim of the Study and Research Questions

As already mentioned, papers have previously discussed the topic of segregating commingled remains using a pXRF instrument. However, more emphasis needs to be given to archaeological contexts and large-scale commingling. To my knowledge, no similar study has been conducted in the Netherlands. This thesis examines the effectiveness of the non-destructive pXRF method on a scenario of disarticulated remains. More specifically, the paper analyzes skeletal remains from 40 individuals dating to the post-medieval period. The individuals come from two skeletal collections, Arnhem and Middenbeemster, which are housed at the Laboratory for Human Osteoarcheology at Leiden University. The pXRF equipment used in this study is the Bruker Tracer 5g. Previous research papers have employed either equipment from different brands or older models from the same brand. The use of a more advanced model could potentially enhance the effectiveness of the analysis, particularly in detecting lighter chemical elements. This improvement could, in turn, aid in the segregation process. The aim is to examine whether or not this technique can be successfully used by osteoarchaeologists to sort mixed human remains from a post-medieval context that were buried in the Netherlands. The main research question of this thesis is:

- How effective is the pXRF in aiding the reassociation of commingled archaeological remains from the post-medieval period in the Netherlands?

To answer this question, several sub-questions will be explored:

1) How significant is the intra-skeletal elemental variation, and among which skeletal elements is it observable?

   Some studies have suggested that intra-skeletal variation can exceed inter-skeletal variation (Grupe, 1988, p. 124), while others present results that indicate greater variation among individuals (Perrone et al., 2014, p. 154).
2) How significant are the differences in elemental concentrations among individuals, and how effectively can these variations assist with the sorting of commingled remains?

   For the reassociation to be successfully achieved, the difference in elemental concentrations needs to be greater among individuals than within the bones of the same individual.

3) To what extent does the sex of the individuals affect their elemental profiles, and how does this factor contribute to the reassociation of the skeletal remains?
4) How do different age categories influence the elemental concentrations in individuals, and how does this affect the sorting process?
5) How do the sites impact the elemental profiles of the individuals, and which elements should be prioritized in each case for reassociating the remains?

   Taphonomic factors have the potential to alter the chemical composition of bones. When bones are buried together for a significant amount of time, they may present similar elemental profiles. Winburn et al. (2017, p. 31) experienced the challenge of successfully sorting remains, possibly due to taphonomic reasons. While this poses a problem in a field setting, it could be leveraged in a laboratory setting (e.g., when dealing with mixed individuals from different sites in the laboratory due to improper handling).

1.4 Thesis Structure

Chapter 1 introduced the topic of the thesis and underlined the significance of conducting further research on commingled remains. Moreover, previous work and fundamental concepts that will be discussed in the following chapters were mentioned. Finally, the paper's objectives and research questions were outlined. Chapter 2 presents essential information to enhance the understanding of this topic. It provides foundational knowledge about bones and teeth while exploring their elemental composition. Additionally, this chapter delves into the concepts of commingling and segregation methods, including the fundamental techniques commonly employed. Furthermore, it introduces the pXRF method and provides a brief review of prior research on disarticulated remains employing this technique, focusing on their results. Chapter 3 provides the materials and methodologies used in the paper, along with the statistical analysis conducted on the collected data. Chapter 4 presents the findings and results of this study, which are later discussed and interpreted in Chapter 5. Finally, Chapter 6 serves as a summary of this thesis and provides recommendations for future research.

Chapter 2. Background

2.1 Introduction

The human bones provide a plethora of information for archaeologists, enabling them to draw inferences about entire populations. Equally vital in these studies are teeth, which yield critical insights into the health, potential social status, dietary habits, and the presence of pathological or congenital conditions in past populations. Before exploring the concept of commingling, it is essential to provide a foundational understanding of human remains. Therefore, this chapter commences by introducing key information about bones and teeth, especially regarding their structure and chemical composition, while touching upon different terms that are being used throughout this thesis. Subsequently, it presents various types and classifications of commingling recognized by researchers, as well as the diverse methodologies employed in the analysis of commingled remains. Furthermore, this chapter discusses the pXRF method, focusing on its historical context, how it operates, and the advantages and disadvantages it comes with. Finally, a brief overview of earlier studies on this topic is provided.

2.2 The Human Remains

2.2.1 The Bones

The human bone is a hard living tissue that supports the body and protects the vital organs. It is composed of two components. The first component is collagen, an organic element responsible for the bone's flexibility, while the second constituent is hydroxyapatite ($Ca_5(PO_4)_3OH$), an inorganic substance that endows the bone with its strength (Zimmerman et al., 2015, p. 132). The cell types that are responsible for creating and maintaining the bones are the osteoblasts, the osteoclasts, and the osteocytes (Zimmerman et al., 2015, p. 132; White & Folkens, 2005b, p. 43). Osteoclasts are bone-forming cells that synthesize the organic components of bone, while osteocytes are mature bone cells. On the other hand, the osteoclasts are involved in bone resorption, breaking down and reabsorbing bone tissue (White & Folkens, 2005b, p. 43). This cyclical process of formation and resorption is known as remodeling, or bone turnover. Even after an individual has stopped growing, the remodeling process continues. For adults, the entire skeleton undergoes a complete remodel approximately every 7–10 years (Ezzo, 1994, p. 609). Coyte et al. (2022, pp. 2–3), proposing slightly different ranges, noted that trabecular and cortical bone remodel at different rates, with trabecular bone undergoing a faster process, taking approximately 5–10 years, while cortical bone may take over 20 years. Adult bones are typically classified into two structural categories: cortical/compact and trabecular/spongy bone (Figure 3) based on their porosity. Cortical bone, known for its density and low porosity, is primarily situated on the external surfaces of bones. On the other hand, trabecular bone, which is more porous and lightweight, is typically located in the interior of most bones and at the ends of the arms and legs (White & Folkens, 2005b, p. 40). Based on their morphology, bones can be categorized as flat, long, or irregular. Flat bones are thin and usually consist of two layers of compact bone enclosing a layer of trabecular bone (Stevens, 2016, p. 189). Examples include the ribs and the bones of the skull. Long bones are characterized by their elongated shape, featuring a cylindrical shaft known as the diaphysis and proximal and distal ends (epiphyses) containing trabecular bone. The humerus, the upper arm bone, serves as a good example. Irregular bones, on the other hand, are those that do not fit into the other two categories, displaying unique and irregular shapes, such as the vertebrae. Adults typically have 206 bones in their bodies (Figure 4), although the number of certain skeletal elements, such as the ribs and vertebrae, may vary slightly among individuals.

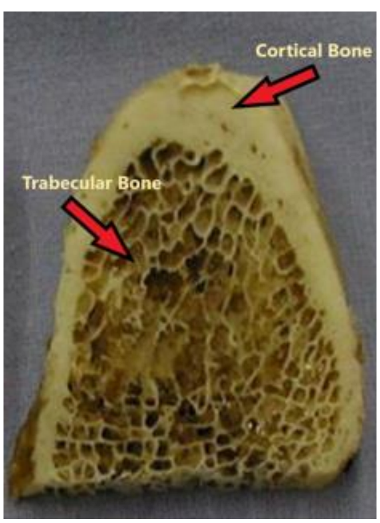


Figure 3. The cortical and trabecular bone at the proximal end of a femur (Coyte et al., 2022, p. 4).

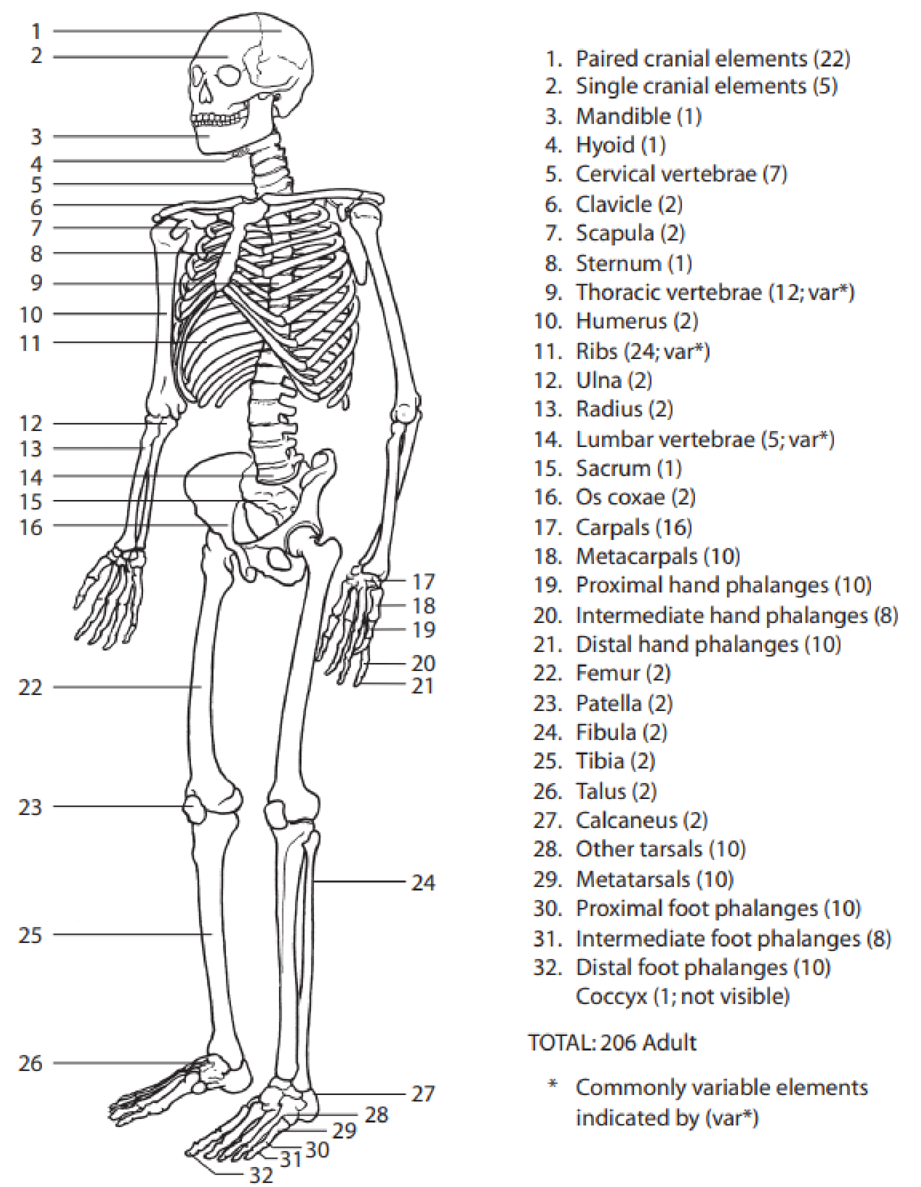


Figure 4. The 206 bones of an adult human skeleton (White & Folkens, 2005a, p. 71).

2.2.2 The Dentition

Teeth are hard, mineralized structures, primarily made of hydroxyapatite (Carvalho et al., 2007, p. 702). They consist of several essential features, including enamel, dentin, and cementum (Figure 5). Enamel, which serves as the outermost layer of the tooth, is recognized as the body's hardest material (Carvalho et al., 2007, p. 702; Zimmerman et al., 2015, p. 132). Directly beneath the enamel lies another layer known as dentin. Although dentin is less hard than enamel, it still maintains greater hardness in comparison to bones (Zimmerman et al., 2015, p. 132). Cementum is a calcified tissue specifically designed to encase the tooth's roots (Zimmerman et al., 2015, p. 132; White & Folkens, 2005c, p. 131). The part of the tooth above the gumline is called crown, while the roots are situated below the gumline, anchoring the tooth in the bone. Adults typically have 32 permanent teeth, in contrast to non-adults, who have 20 deciduous teeth. The teeth from both the maxillae and mandible, the upper and lower jaw respectively, include eight incisors, four canines, eight premolars, and 12 molars (Figure 6). Unlike bones, teeth do not undergo re-modeling (Perrone et al., 2014, p. 148). Once they are fully developed, they do not regenerate. Nevertheless, it has been suggested that, to some extent, cementum and dentin may undergo turnover (Castro et al., 2010, p. 26).

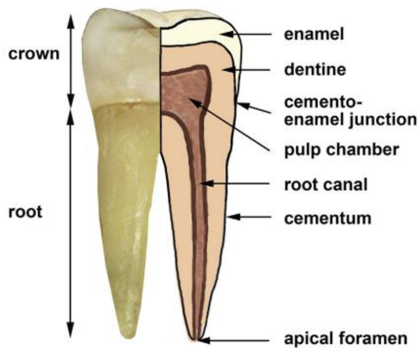


Figure 5. Tooth anatomy (Nikita, 2017a, p. 56).

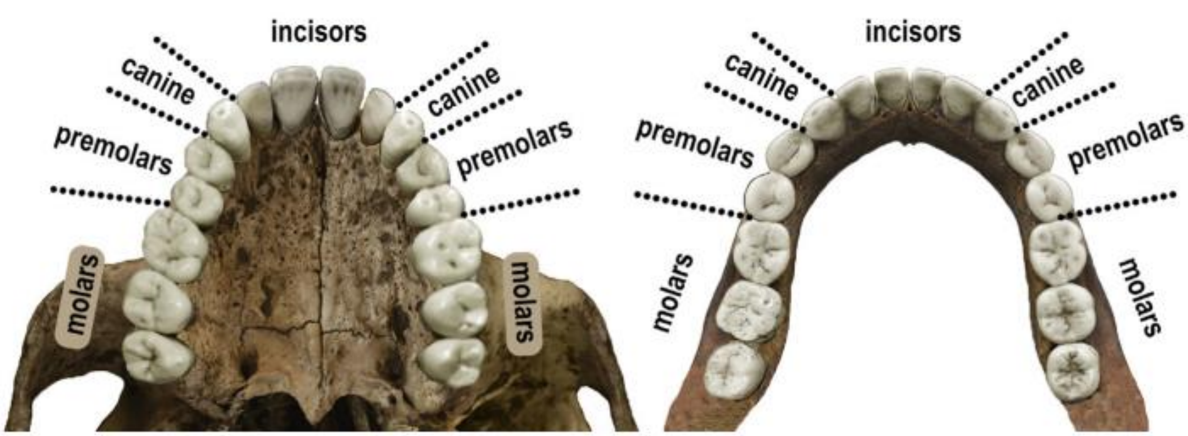


Figure 6. The different adult tooth types and their position. On the left is the inferior view of the maxillae, while on the right is the superior view of the mandible (Nikita, 2017a, p. 54).

2.2.3 Elemental Composition

As mentioned earlier, the inorganic component of both bones and teeth is hydroxyapatite (Carvalho et al., 2007, p. 702; Zimmerman et al., 2015, p. 132). Apart from Ca and phosphate (a compound composed of oxygen (O) and P) which are the major components along with hydrogen (H), carbon (C), and nitrogen (N), (Price et al., 1985, p. 421), a variety of minor and trace elements can be found incorporated in bones (Iyengar & Tandon, 1999, p. 1; Price et al., 1985, p. 419). Minor elements, essential but not found in large quantities, include K and sodium (Na) (Iyengar & Tandon, 1999, p. 3), while trace elements, typically found in much lower concentrations, include Fe, Zn, and copper (Cu) (Iyengar & Tandon, 1999, pp. 10–12). Certain trace elements such as Sr and lead (Pb), have been described as "bone seekers" (Iyengar & Tandon, 1999, p. 1). These elements exhibit an affinity for bone tissue and can become incorporated into bones, even at low concentrations. In relation to teeth, this paper will provide further insights into enamel since it is one of the materials analyzed in this study. Enamel shares similarities with bone in terms of its inorganic composition, predominantly consisting of Ca, P, Mg, and carbonate (a compound composed of carbon and oxygen) (Iyengar & Tandon, 1999, p. 5). Minor and trace elements mentioned in the context of bones can also be found in teeth.

During the turnover process of the bone, minerals are incorporated within the tissue (Price et al., 1985, p. 419). However, the concentration of minerals in different bones of the same skeleton can vary due to different rates of remodeling (Perrone et al., 2014, p. 152). These rates can vary depending on whether the bone is cortical or trabecular, with the latter remodeling at a faster pace (Byrnes & Bush, 2016, p. 1042). Furthermore, the thickness of cortical bone in different skeletal elements can also impact the turnover rate, with thinner bone turning over more rapidly than thicker bone (Perrone et al., 2014, p. 152). With regard to enamel, since it does not remodel, the incorporation is achieved during its formation (Perrone et al., 2014, p. 148).

In addition to the remodeling process, the elemental concentrations in the skeletons can be affected by dietary intake and pathological conditions (Castro et al., 2010, p. 18). Another important factor that can significantly influence the elemental composition of the human skeleton is diagenesis. In archaeological contexts, diagenesis refers to post-mortem alterations in the chemical composition of buried bones (Guimarães et al., 2016, p. 108). Various factors, such as burial conditions, hydrological events, and soil pH, can have a profound effect on the chemical composition of bones (Stevens, 2016, pp. 113–114). Studies have indicated that diagenesis has a lesser impact on enamel (Castro et al., 2010, p. 18). When it comes to bone, cortical bone is generally less susceptible to these effects compared to trabecular bone (Grupe, 1988, p. 124; Rasmussen et al., 2017, p. 91), which is thinner and more porous. When exploring the elemental concentrations in bones and their capacity to offer insights into various subjects, such as diet and pathological conditions, it is important to consider the concept of diagenesis.

2.3 Commingled Remains: Types and Methods

Commingled human remains describe a scenario in which the skeletal elements of more than one individual have become mixed (Gonzalez-Rodriguez & Fowler, 2013, p. 407.e1). As a result, it is not visually discernible which bones belong to each specific individual. This concept poses notable challenges in forensic anthropology and archaeology. In general, commingling can result from various factors including natural disasters and processes (Gonzalez-Rodriguez & Fowler, 2013, p. 407.e1) where burial grounds could potentially be disturbed, cultural practices such as ossuaries (Stevens, 2016, p. 3) where bones of the deceased individuals are being exhumed from their initial burial and placed in a shared repository along with other members of the community, taphonomic factors such as trampling (Nicolosi et al., 2023, p. 1), and inadequate excavation practices. Osterholtz et al., (2013, pp. 2–5) discussed three distinct categories of intermixed assemblages (Figure 7). The first type is long-term usage, where the repeated use of the same burial sites and the repositioning of remains to accommodate new burials can lead to the mixing of skeletal elements. The second type mentioned by the authors is episodic usage, where events like wars or disease outbreaks simultaneously affect multiple individuals, resulting in the formation of mass graves. The final category is laboratory commingling and includes assemblages that originate from the inexperience of personnel and improper handling of remains following their excavation.

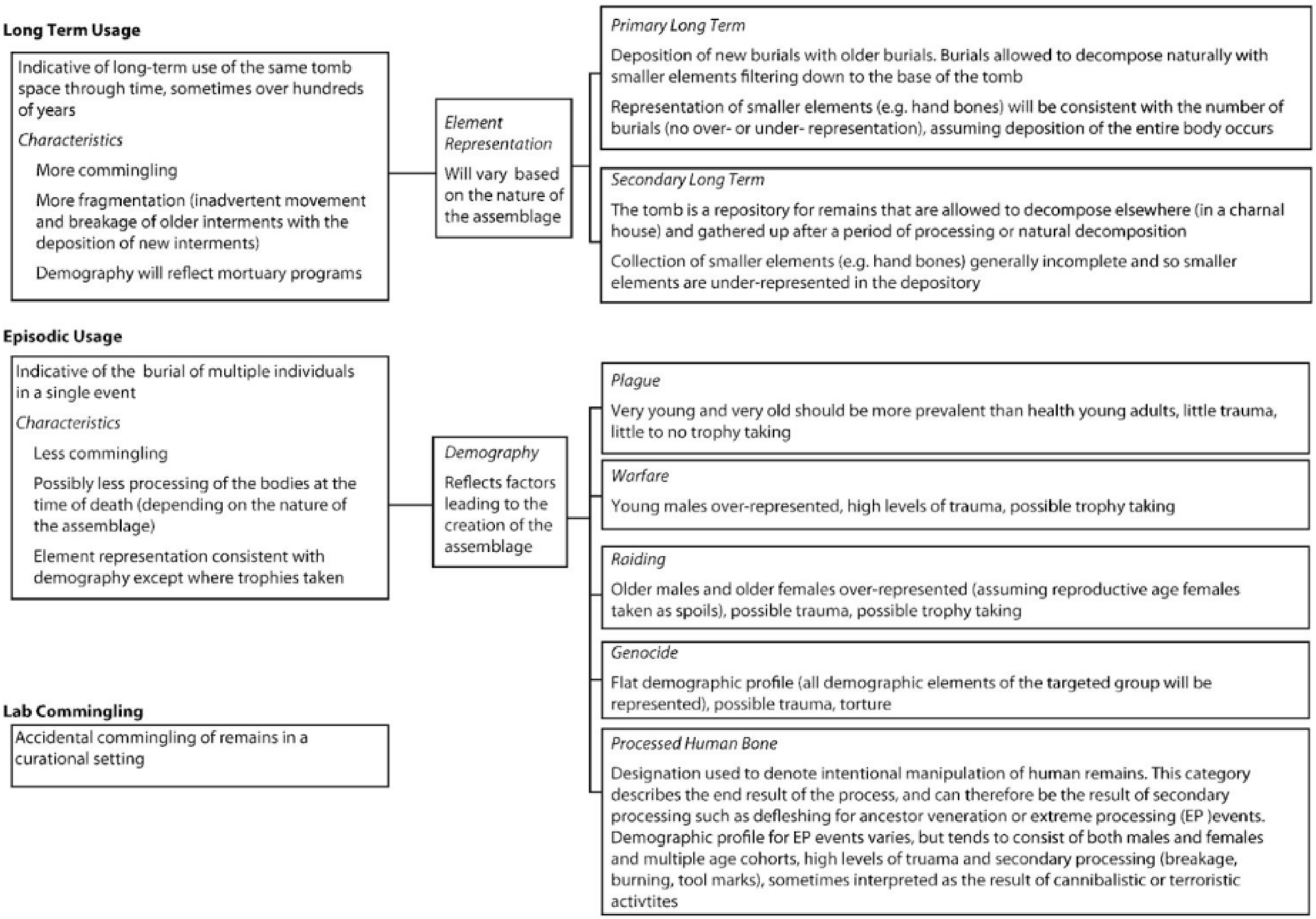


Figure 7. Three categories of commingling (Osterholtz et al., 2013, p. 2).

Regarding sorting methods, in 1948 Snow proposed the procedures that need to be followed in the case of mixed individuals, establishing the primary framework for reassociating commingled remains (Stevens, 2016, pp. 93–94). A decade later, McKern (1958, p. iv) suggested the use of short-wave ultraviolet light to reassociate human remains, taking advantage of the spectrum of colors that substances within the bones exhibit during the procedure. While this method could not produce quantitative data, the possibility for it to be used complimentarily was underlined (McKern, 1958, p. 6). Nowadays, several different methodologies are in use to tackle commingled remains. Two methods that are commonly used together are visual pair-matching and assessment of joint articulation (Stevens, 2016, p. 102). During the visual pair-matching (Figure 8) the observer attempts to match left and right skeletal elements by examining their morphology (Adams & Byrd, 2006, p. 64). The method is relatively fast but heavily reliant on the expertise of the observer. The assessment of joint articulation is a reliable approach that evaluates how well the articular surfaces of bones that form joints fit together. However, its reliability diminishes when there is a lack of close fit in certain joints, such as the glenohumeral (shoulder) joint (Adams & Byrd, 2006, p. 65). The evaluation of the epiphyseal union is a different technique that primarily serves to estimate the age at death for non-adults by determining bone fusion, but it also facilitates the reassociation of skeletal elements (Schaefer, 2014, p. 123). Additional methods that rely on visually assessing bone appearances to match them focus on the alterations due to pathological reasons, age, and taphonomic factors (Perrone et al., 2014, p. 145).

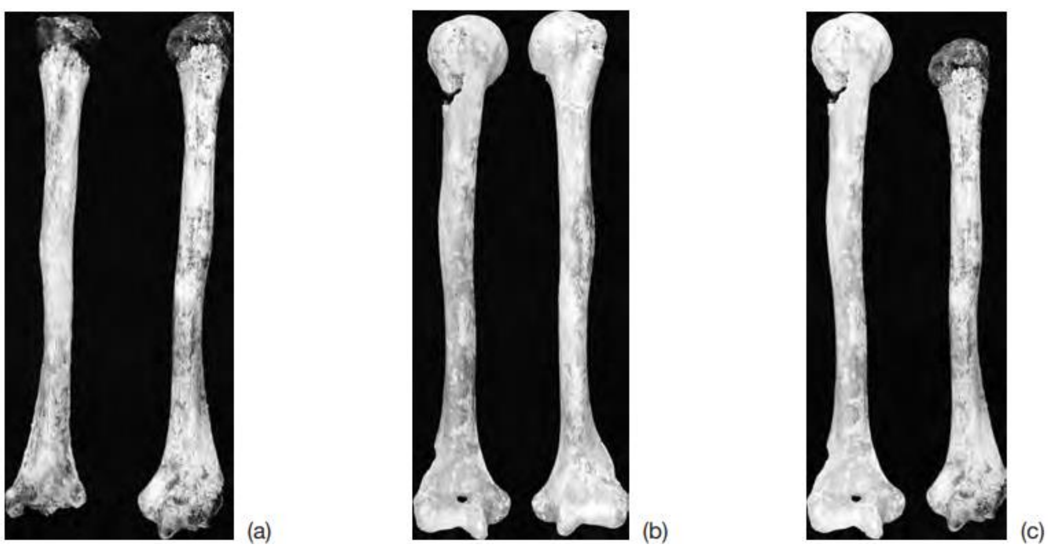


Figure 8. The visual pair-matching method. The humeri in (a) and (b) appear to belong to the same individual based on their morphological characteristics. In contrast, the humeri in (c) do not seem to be from the same individual (Byrd & Adams, 2016, p. 230).

Moving on to quantitative techniques, it is important to highlight the MNI (minimum number of individuals), LI (Lincoln index), and MLNI (most likely number of individuals). The MNI estimates the number of individuals in the sample by counting the most frequently occurring skeletal element. While this method is popular and easy to use, its results may be misleading as it does not consider the impact of taphonomic loss (Konigsberg & Adams, 2014, pp. 193–194; Stevens, 2014, p. 95). The LI, on the other hand, instead of the number of individuals in the sample, estimates the initial population and considers post-mortem loss (Adams & Konigsberg, 2004, pp. 139–140).

Finally, MLNI is a variation of LI, offering a more accurate estimation among the three methods. It aids in mitigating potential underestimates found in the MNI and the biases present in LI (Adams & Konigsberg, 2004, p. 138). Another technique commonly used is osteometric sorting, a method that utilizes statistical analyses as a means to match skeletal elements (Adams & Byrd, 2006, p. 66). In contrast to the visual pair matching mentioned earlier, osteometric sorting does not rely on the observer's experience and observations but rather is a more objective method using bone measurements.

Various other techniques are commonly used for the segregation of commingled remains, such as DNA analysis, recognized for its accuracy but also its high expense (Perrone et al., 2014, p. 145), protein analysis, and bone histology (Zimmerman et al., 2015, p. 131). Additionally, radiology (Viner, 2014, p. 87) and the examination of spatial relationships between disarticulated bones using algorithms (Tuller & Hofmeister, 2014, p. 8), have also been suggested to be of value. Finally, methods that analyze isotopes and chemical elements are commonly used in conjunction to assist in the sorting process (Stevens, 2016, p. 107). One such method, pXRF, will be discussed next.

All the techniques mentioned above have their respective advantages, often including accuracy, user friendliness, and cost-effectiveness. However, they also present drawbacks, including time consumption and high susceptibility to factors like taphonomy and sample size. This emphasizes the necessity for additional research to develop new techniques and enhance the existing ones, especially those that combine non-destructiveness and cost-effectiveness, like pXRF.

2.4 Portable X-ray fluorescence

The pXRF (Figure 9) is an analytical technique that has gained popularity among archaeologists due to its non-destructive nature and its ability to provide rapid, on-site elemental analysis of various materials (Liritzis & Zacharias, 2011, pp. 109–110). By irradiating these materials with X-rays, it can effectively determine their elemental composition. Before examining further details on its methodology, advantages, and disadvantages, a brief discussion of its history will be presented.

Figure 9. The pXRF instrument on its desktop stand, facing upwards, with the lid on (Bruker, n.d.).

In 1901, Wilhelm K. Röntgen received the Nobel Prize for his discovery of X-rays made a few years earlier (Shackley, 2011, p. 7). Subsequent events, such as Charles G. Barkla's discovery in 1909 of the correlation between X-ray emissions from a sample and its atomic weight, followed by Henry G. J. Moseley's observations on the relationship between X-ray spectral lines and the atomic number of elements a few years later, contributed to the realization of the technique's significance (Shackley, 2011, pp. 7–8). While Edward Hall is considered one of the first to have used this method on coins in 1960 (Shackley, 2011, p. 11), the origins of X-ray fluorescence spectrometry, particularly focusing on portable equipment, can generally be traced to the late 1970s (Lemiere, 2018, p. 350). Over the next 20 years, aside from the United States, countries involved in the development of this equipment included China and Russia (Lemiere, 2018, p. 350). Initially, its use was limited, focusing primarily on exploratory purposes. Its early applications encompassed the identification of Pb present in house paints, metal recycling (Lemiere, 2018, pp. 350–351), mining, and applications for environmental purposes (Lemiere, 2018, p. 351; Shackley, 2018, p. 4). Nowadays, this method is extensively employed in various fields. In archaeology and related disciplines, pXRF has emerged as a valuable method for analyzing pigments and metal artifacts, determining the origins of materials such as ceramics, and verifying the authenticity of important artifacts like paintings and ancient texts (Liritzis & Zacharias, 2011, pp. 112–113). Although not as commonly employed as in other archaeological studies, the use of this method in examining human remains is gradually gaining popularity.

As mentioned earlier, pXRF determines the elemental composition of the materials under examination. All materials are composed of atoms, each consisting of a nucleus with protons and neutrons, surrounded by electrons. These electrons orbit the nucleus within specific areas called shells or energy levels (Bruker, 2016, p. 2). The shells are distinguished by letters, beginning with K as the one closer to the nucleus, followed by L, M, N, and beyond for the subsequent layers, which also contain sub-levels (Bruker, 2016, p. 2). The electrons located in the inner shells exhibit stronger bonding than those in the outer levels (Bruker, 2016, p. 2). With pXRF, the materials are bombarded with X-rays, a form of short-wavelength radiation. The electrons within the sample's atoms absorb the X-rays, and when the radiation is sufficiently high, it causes the electrons from the inner shells to be released (Figure 10), creating vacancies and leading to an unstable state of the atoms (Bezur et al., 2020, p. 18; Pollard et all, 2007, p. 101; Shackley, 2011, p. 16). In order for the atoms to return to their stable state, electrons from the outer shells fill the vacant positions (Figure 11), and during this transition they emit a fluorescent X-ray photon (Bezur et al., 2020, p. 18; Shackley, 2011, p. 16) that is unique for each element. Utilizing this emission, pXRF can identify the major, minor, and trace elements present in the materials and their quantities.

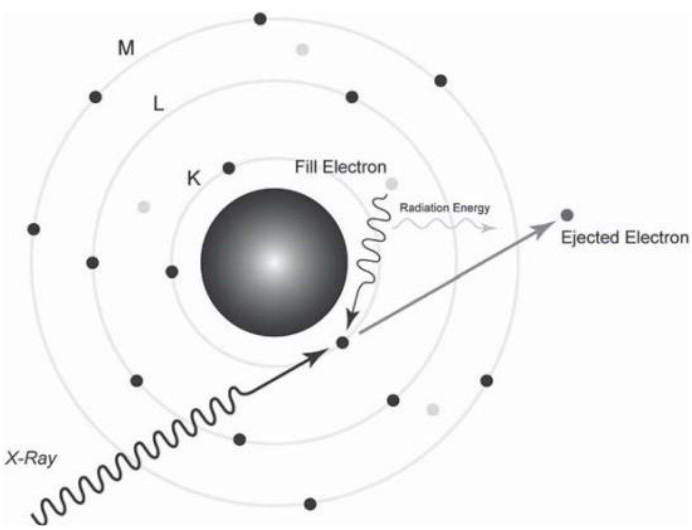


Figure 10. Illustration of the excitation of an atom. X-rays induce the ejection of an electron from the K shell. As an electron from an outer shell fills the vacant position, energy is emitted (Perrone et al., 2014, p. 146).

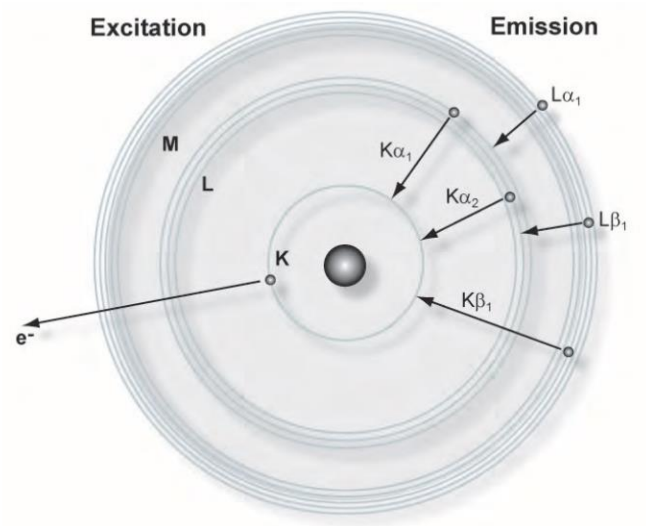


Figure 11. The excitation of an atom, as described in Figure 10. In this illustration, the depiction includes the sub-levels of the shells from which the substitute electrons can originate (Bruker, 2016, p. 2).

The pXRF method offers both advantages and disadvantages. Among its positive aspects, it is non-destructive and portable equipment (Liritzis & Zacharias, 2011, p. 110; Shackley, 2018, p. 2), allowing its use in laboratories, on-site during excavations, and within museum settings. Moreover, it requires minimal sample preparation, such as simple washing or dry brushing, unlike other techniques that may require the sample to be turned into powder. Additionally, it is a cost-effective method compared to approaches such as DNA analysis (Shackley, 2018, pp. 2–3). The equipment is user-friendly, usually accompanied by data management software and manuals that meticulously outline each step. This easy-to-use interface makes it accessible even for first-time users. Finally, its rapidity is also a standout feature, providing results within a few seconds to a few minutes (Shackley, 2018, p. 3). On the other hand, it may not be suitable for every sample, particularly if the samples are too small (less than 10 mm) or too thin (less than 2 mm), as this could yield misleading results (Shackley, 2018, p. 3). Additionally, the samples need to be flat (Liritzis & Zacharias, 2011, p. 132), as irregular shapes prevent the equipment from being in close contact, potentially leading to air gaps that may result in erroneous measurements. Furthermore, the pXRF has limitations in its capability to detect every chemical element on the periodic table (Shackley, 2018, p. 3). It is unable to provide information about the chemical compounds in the materials under examination but rather detects the presence of various chemical elements

(Liritzis & Zacharias, 2011, p. 119). Specifically concerning bone samples, the concentrations determined by the equipment may not reflect real values due to diagenetic factors (Liritzis & Zacharias, 2011, p. 124). Like every other method, pXRF has its assets and drawbacks. Nevertheless, the combination of its advantages, particularly its portability, cost-effectiveness, and non-destructive nature that allow archaeologists to preserve samples for future studies, highlights why this method is increasingly gaining favor and necessitates more attention within the field.

2.5 The Research So Far

A few studies have already attempted to segregate commingled remains using the pXRF method (Table 1). In 2013, Gonzalez-Rodriguez and Fowler (p. 407.e2) created a mock commingling scenario, a deliberate and controlled simulation aimed at emulating the mixing of remains, to experiment with the sorting of five individuals. Using elemental ratios, they successfully segregated the individuals, underscoring the reduction in sorting precision as the number of individuals increased (Gonzalez-Rodriguez & Fowler, 2013, p. 407.e5). A year later, Perrone et al. (2014, pp. 148–149) explored intra-skeletal and inter-skeletal variation to offer insights into the effectiveness of the pXRF method in differentiating mixed remains. This study involved 19 individuals from forensic contexts, and one dating back to the 1800s (Perrone et al. 2014, p. 149). The results supported a variation in elemental concentrations within the bones of the same individual, which was found to be insignificant compared to the greater variation observed between different individuals (Perrone et al. 2014, pp. 152–154). Some elements displayed higher success rates than others, with manganese (Mn) and silicon (Si) achieving the highest rates at 35.5 %. Overall, the segregation rate observed in this study was lower compared to the previous one. Perrone et al. (2014, p. 154) highlighted the method's challenge in accurately segregating skeletal remains when overlapping confidence intervals exist. In 2015, Richards & Jones (2015, personal communication, April 14, 2023) conducted a pilot study, evaluating the application of pXRF in a case involving real commingling. The researchers assessed the technique using a sample comprising two control lots from the cemetery under examination, five sets of commingled remains (displaying varying degrees of commingling), and a reference skeleton previously acquired, which also served as a control lot. While the results from the control lots showed promise, successful sorting was achieved only for the reference skeleton when considering all skeletal elements collectively. The researchers underlined the promising aspects of this technique while also acknowledging the challenges arising from potential diagenetic factors (Richards & Jones, 2015, personal communication, April 14, 2023). In 2016, Stevens (p. 140) examined the pXRF method in conjunction with other sorting techniques on 138 limb bones as part of his research. The results suggested the method's effectiveness as a supplementary tool but revealed reduced efficiency, particularly concerning the reassociation of upper and lower limbs. This reduction in effectiveness was attributed to diagenesis or inconsistencies in instrument settings (Stevens, 2016, p. 151). Finlayson et al. (2017, p. 493) also employed this method to address the issue of the commingling of two individuals in a forensic context. When other methods were ineffective in segregating the remains, pXRF demonstrated its ability to resolve the problem (Finlayson et al., 2017, p. 497). While this case yielded positive results, Winburn et al. (2017, p. 31) presented a forensic case study where the differentiation of two individuals based on elemental concentrations in the bones was unsuccessful, which was assigned to diagenesis. A few years later, Davis (2021, pp. 21–23) conducted a study to determine the applicability of pXRF in segregating commingled remains from modern contexts. The study involved analyzing intra-skeletal and inter-skeletal elemental variation, as well as differences between males and females and buried versus surface remains. Reassociation of the remains was not attempted in this case. Noteworthy findings included the absence of differences between males and females and a disparity in the concentrations of two elements between buried and surface remains (Davis, 2021, pp. 48–49). Finally, Smith (2021, pp. 5–7) attempted to segregate eight individuals from an archaeological context by initially exploring the elemental variation among different locations on the same bones. Additionally, the variation among bones from both the same and different individuals was examined. While some individuals could be grouped together, the overall grouping of individuals was deemed unreliable (Smith, 2021, pp. 72–73).

Table 1. Previous studies on commingled remains that used the pXRF method.

Study	Context	Sample size	pXRF	Commingling Type	Outcome
Gonzalez-Rodriguez & Fowler, 2013	Archaeological (12th to 16th century)	5 individuals	Niton XL3t Spectrometer	Mock commingling	Mostly accurate separation
Perrone et al., 2014	Mostly Forensic/ Modern	20 individuals	Bruker Tracer IV	Mock commingling	Limited success
Richards & Jones, 2015	Archaeological (1882 to 1925)	5 commingled sets of human remains	Bruker Tracer IIIv+	Combination of mock and real commingling	Limited success
Stevens, 2016	Archaeological (Mid 19th century)	138 limb bones	Bruker Tracer III SD	Real commingling	Relatively accurate separation
Finlayson et al., 2017	Forensic/Modern	2 individuals	Bruker Tracer IV	Real commingling	Successful sorting
Winburn et al., 2017	Forensic/Modern	2 individuals	Bruker Tracer III-V+	Real commingling	Unsuccessful sorting
Davis, 2021	Forensic/Modern	40 individuals	Niton XL3t 950 GOLDD+	Mock commingling	Sorting was not attempted
Smith, 2021	Archaeological (18th century)	8 individuals	Olympus Innov-X Delta	Mock commingling	Mostly unsuccessful sorting

2.6 Summary

This chapter provided the reader with essential information for a better understanding of this study's topic. It discussed the structure and elemental composition of bones and teeth, which are the materials under examination in this paper. It presented different classifications for commingled remains and the methods usually applied for their resolution. Additionally, it provided a basic understanding of the operation of pXRF, the method applied in this thesis. Finally, this chapter touched upon previous studies exploring the commingling concept, offering context and outcomes. In the next chapter, the skeletal collections utilized in this study, the pXRF model employed, the procedural steps followed with the samples, and the statistical analysis conducted will be presented.

Chapter 3. Materials and Methods

3.1 Introduction

Individuals from two different collections were utilized for this study. While field commingling of individuals from various sites is not feasible, it becomes possible in a laboratory commingling scenario. Chapter 3 introduces these two skeletal collections, outlining the reason behind their selection. Additionally, it presents the sample strategy employed and details the sample preparation procedures. The pXRF instrument utilized in this thesis is a newer model compared to those used in prior studies, potentially improving the efficiency of the analysis. This chapter provides information on this instrument and outlines the corresponding procedures. Finally, it discusses the statistical analysis applied to the data derived from the pXRF method.

3.2 The Skeletal Collections

One of this study's questions examines potential elemental differences in skeletons between sites, which could also arise due to diagenetic factors. While similarities among skeletons within the same site might hinder differentiation during comparisons, in cases involving individuals from distinct sites in laboratory commingling scenarios, these differences could potentially aid the analysis. This assumption relies on the premise that the elemental composition of soil in various geographic locations in the Netherlands varies significantly. Such variations could prove valuable in segregating individuals affected by taphonomic changes that alter their chemical profiles. It is important to note that not all soil types are uniformly distributed across different regions of the Netherlands (Figure 12a). Additionally, the “background" values, representing the naturally occurring metal concentrations in soils, seem to vary according to Spijker (2012, p. 3). The four main soil types in the Netherlands include peat, sand, marine clay, and fluvial clay (Spijker, 2012, p. 16). Figure 12b illustrates these major soil types and the chromium (Cr) concentrations across the country. To explore potential elemental differences in skeletons between sites, this thesis examines two sites from different geographic regions, Arnhem and Middenbeemster (Figure 13).

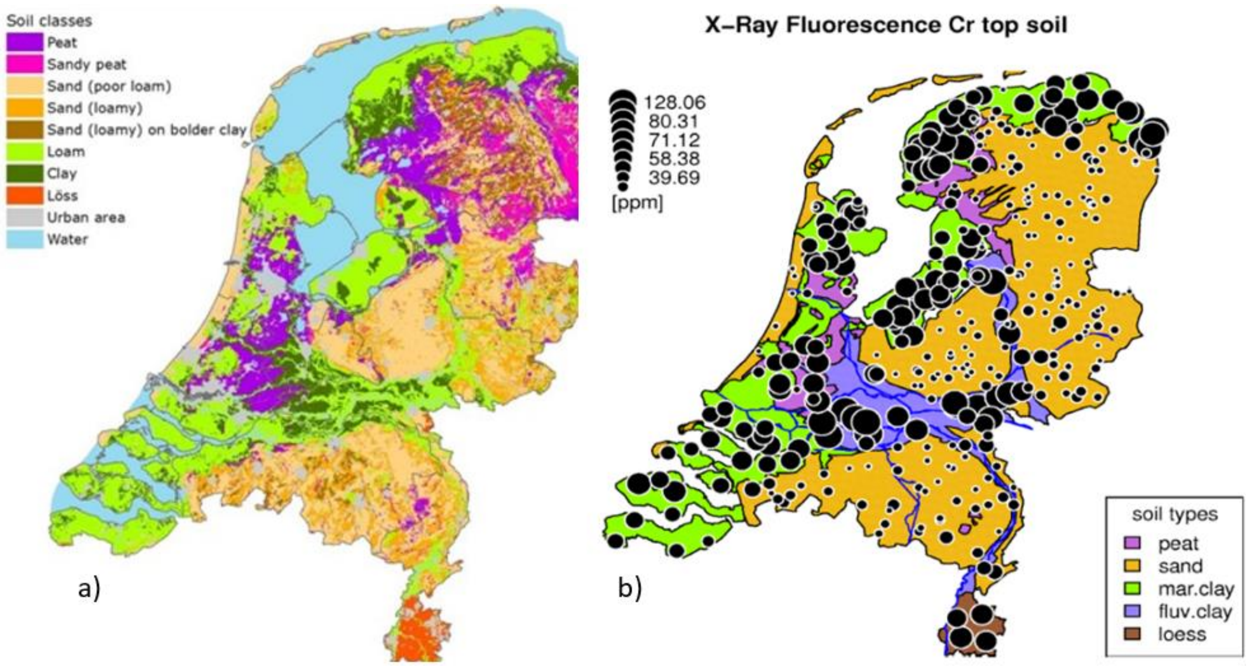


Figure 12. The map of the Netherlands. a) The distribution of different soil types across the country (van den Berg et al., 2017, p. 12). b) The major soil types in the Netherlands, along with the different concentrations of Cr, as an example (Spijker, 2012, p. 18).

Figure 13. The map of the Netherlands with the location of the two sites relevant to this study, Arnhem and Middenbeemster (adapted from Casna et al., 2021, p. 894).

3.2.1 Arnhem

Arnhem, located in the province of Gelderland in the eastern part of the Netherlands, served as an urban settlement during the post-medieval period. Its inhabitants were engaged in various occupations such as the tobacco industry, typography, and shoemaking (Casna & Schrader, 2022, p. 221). The living conditions were notably challenging, characterized by poor hygiene and limited access to fresh air and clean water. The excavation conducted by RAAP in 2017 in the city center, which was part of the project “Jansbeek” started in 2011, focused among other areas on the “Oude Kerkhof” or “Old cemetery” located on the north side of the Eusebius church (Zielman & Baetsen, 2020, p. 361). This project, encompassing approximately 800 track numbers containing human skeletal remains, unearthed 659 skeletons from the cemetery, which was in use from 1444 to 1829 CE (Zielman & Baetsen, 2020, p. 381–382). The burial of individuals on the north side likely correlates with their lower socioeconomic status. The “Old cemetery” encompasses two distinct periods, namely 1350–1650 and 1650–1829 CE. The twenty individuals chosen for this study, sourced from Arnhem's skeletal collection, specifically belong to the latter period of this cemetery.

3.2.2 Middenbeemster

Middenbeemster, situated within the Beemster municipality in North Holland, is a rural site (Li-agre et al., 2022, p. 770). The village traces its origins back to the year 1613 CE (Palmer, 2019, p. 17). Throughout the post-medieval period, its economy relied on agriculture and dairy farming (Casna et al., 2021, p. 894). Osteological research indicates a division of labor between men and women, both facing physically demanding tasks, yet women are primarily engaged in household activities. Despite coexistence among various social classes, the middle class made up the majority of the population (Piso, 2020, p. 18). Twenty out of the 40 individuals included in this study were buried at the village’s cemetery, which was actively used from 1615 to 1866 CE, when a new cemetery was established. An excavation in 2011, conducted by Leiden University (Palmer, 2019, p. 17) and the archaeological company Hollandia, unearthed around 450 skeletons. Most of the individuals that were brought to light were associated with the period from 1829 to 1866 CE (Lemmers et al., 2013, p. 35).

3.3 Sample Strategy

For this study, 40 individuals—20 from Arnhem and 20 from Middenbeemster—were selected. None of the individuals were intermixed initially; instead, mock commingling scenarios were created during the later stages of data exploration. All individuals in the sample were adults, spanning various age categories (Table 2). Typically, adult categories are defined as “18–25”, “26–35”, “36–49”, and “50+”. However, if individuals during the analysis do not fit into one of these categories, broader groups are selected. For example, there is an individual estimated to be “18+” (Table 2a). The sample included both males and females. When estimating the sex of individuals based on their biological traits, they are typically categorized into five groups: probable male (PM), male (M), probable female (PF), female (F), and indeterminate (I) for those who cannot be placed in any of the other four categories. The distribution of individuals in each sex category is presented in Table 2b. For the purposes of this study and for subsequent comparisons, individuals classified as M and PM were considered males, while those classified as PF and F were considered females. This categorization resulted in 23 males and 17 females.

Table 2. a) Distribution of age categories in the sample. b) Distribution of sex in the sample.

Age category	Number of individuals
18+	1
18–25	8
26–35	7
36–49	19
36–50+	1
50+	4
Total	40

a)

Sex	Number of individuals
PM	5
M	18
PF	7
F	10
Total	40

b)

Each individual was scanned using the pXRF instrument, targeting eleven bones and one tooth. The selected bones, sourced from various anatomical regions, were chosen to ensure a representation of the entire skeleton. Moreover, the skeletal elements selected belong to different bone categories (flat, long, or irregular), based on their morphology. In the category of flat bones, the parietal and the first rib were chosen. Regarding the long bones, the humerus, radius, femur, and tibia were included in the scan. Irregular bones encompassed the 3rd lumbar vertebra, the pubic bone of the pelvis, the capitate from the carpals, the calcaneus from the tarsals, and the mandible from the skull. The dental element chosen for scanning was a maxillary incisor.

With regard to the bones, priority was given to the left side. In cases where the left bones were missing or too fragmented to be deemed suitable, those from the right side were selected. Each sample underwent a single scan at a specific location. To minimize the air gap between the instrument and the samples, which could lead to erroneous measurements, the flattest surface of each sample was scanned. Specifically, for the parietal bone, the measurement was taken in the vicinity of the coronal and sagittal sutures. The 3rd lumbar vertebra (L3) was scanned on the superior surface of its body unless depressions present due to pathological reasons prevented it, in which case the inferior aspect was chosen. In a few cases of absence, the 2nd, 4th, or 5th lumbar vertebrae were scanned. The 1st rib was measured on its flattest area, and all long bones were scanned at the midshaft, which stands out with the highest bone mineral density (Stevens, 2016, p. 141). The pubic bone was scanned on the posterior aspect of its body, and the capitate on the medial aspect, to avoid irregularities present on the surface. The measurement of the calcaneus was taken on its lateral aspect unless it was fragmented, in which case the medial aspect was chosen. The calcaneus was the bone that was quite often damaged in some aspects. Finally, the mandible was scanned on the lateral aspect of the ramus. Regarding the tooth, the enamel of the maxillary incisor was targeted. In cases where no incisors were present, a canine, premolar, or molar was selected. Only on three occasions was a mandibular tooth scanned instead of one from the maxillae. Figure 14 shows the exact locations of the samples that were scanned.

In this study, only the cortical bone was sampled. This bone type is less susceptible to diagenetic factors (Grupe, 1988, p. 124; Rasmussen et al., 2017, p. 91) and is often favored in studies involving chemical concentrations compared to trabecular bone (Castro et al., 2010, p. 18). In addition to its increased susceptibility, trabecular bone, with its distinct surfaces and air gaps, would not be optimal for pXRF analysis, where the presence of extra air between the instrument and the sample could potentially alter the results.

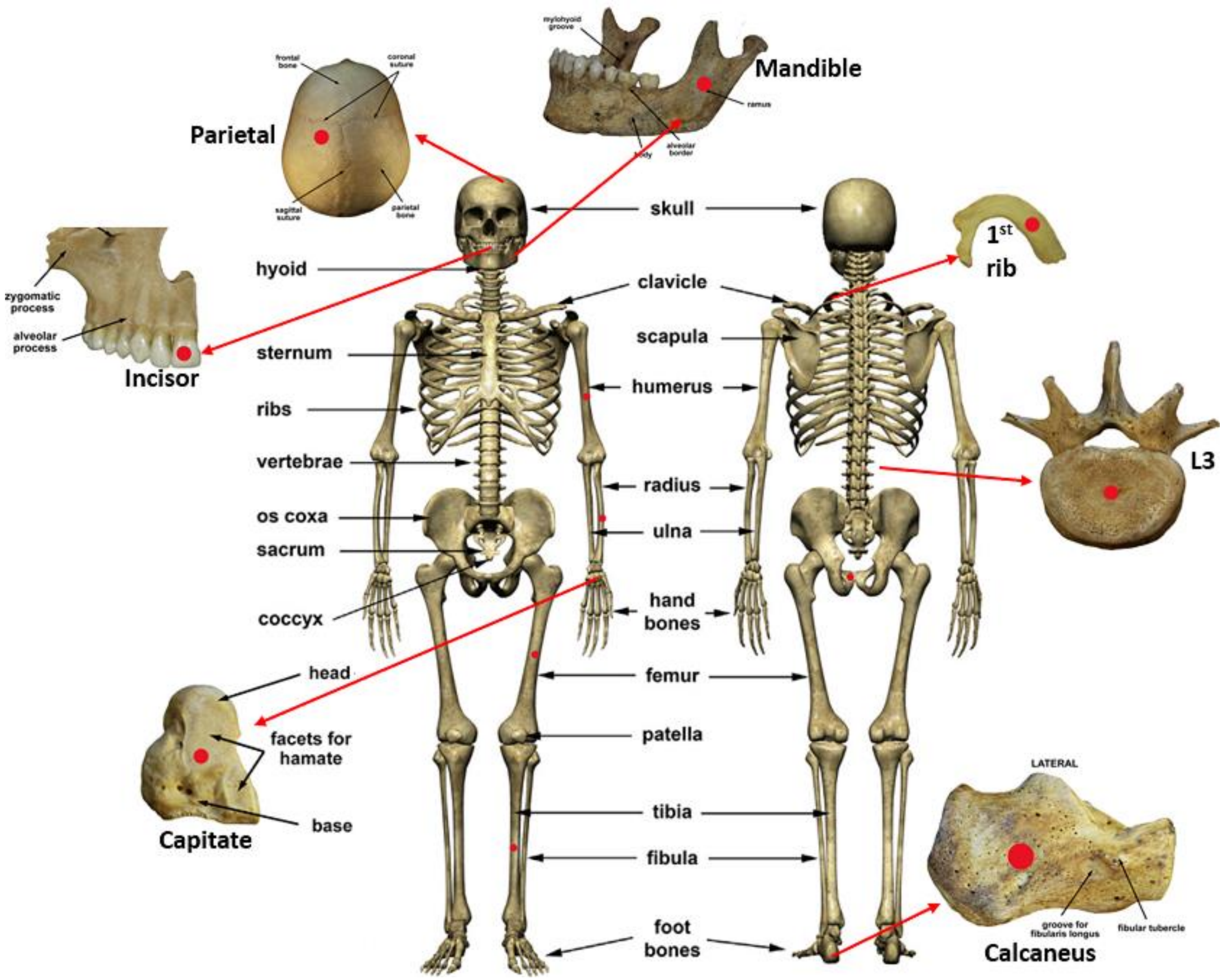


Figure 14. The red dots mark the scanned locations on each skeleton (adapted from Nikita, 2017a, pp. 2, 9, 11, 12, 23, 28, 45, 49).

Regarding the measurements taken, eleven bones and one tooth from each skeleton were scanned once with the pXRF instrument. Out of the 40 individuals, three were missing one skeletal element. For two of them, no capitate was present, while for the third one, there were no teeth. This brings the total number of measurements taken to 477.

3.4 Sample Preparation and pXRF Procedure

Since the skeletons were previously washed, dried, and stored at the Laboratory for Human Osteoarchaeology of Leiden University, there was no need for extra cleaning. Nevertheless, every sample was dry-brushed before each scan to ensure a smooth and debris-free surface. This precaution aimed to prevent any potential crumples or fragments of bone that might be adherent to the surface of the remains from affecting the delicate detector window of the pXRF instrument during the scanning process.

For the majority of scans, the pXRF was secured on its tripod, while the samples were placed on foam rings or their position was secured with the aid of sponges (Figure 15) so they would lay flat and the instrument could be as close to them as possible to minimize the air between them.

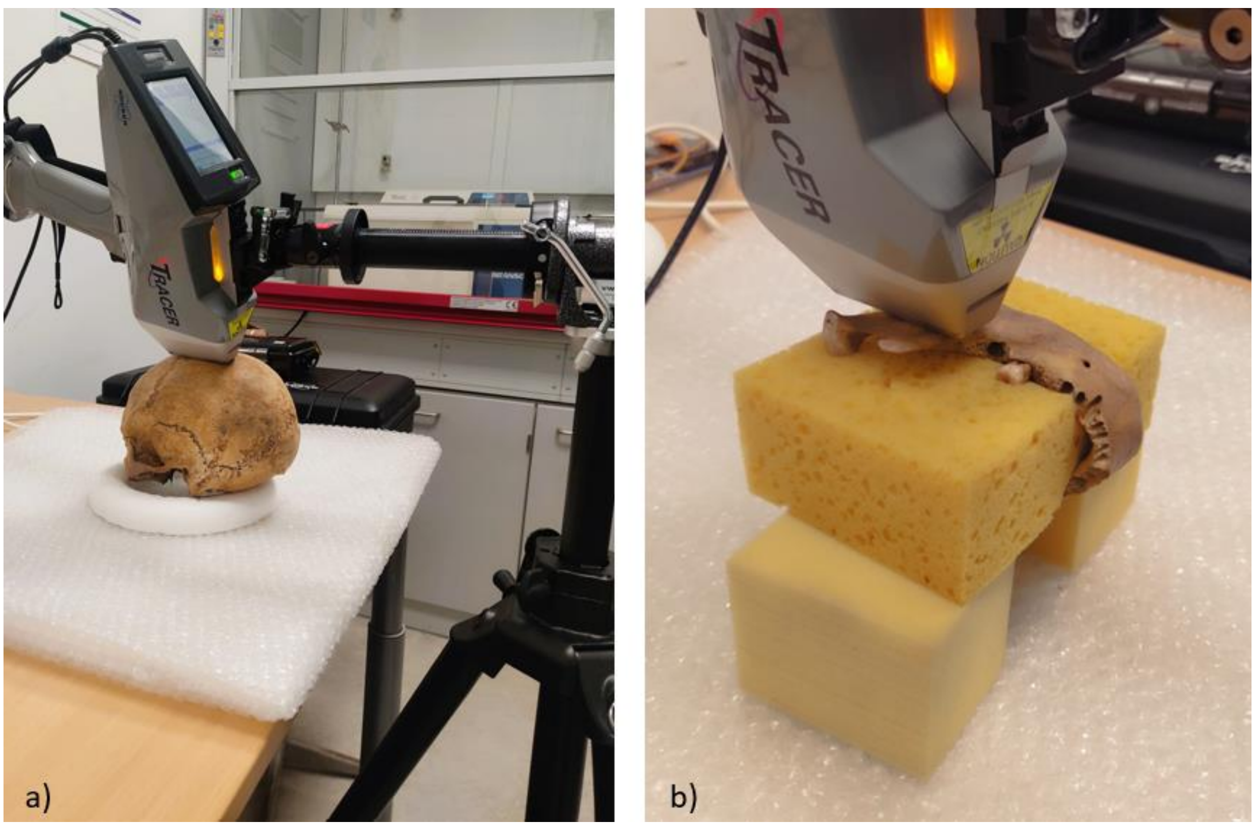


Figure 15. The Bruker Tracer 5g pXRF on the tripod. a) The skull was placed on a foam ring for protection and position fixation. b) Sponges were used to maintain the stability and flat position of the mandible for better measurements (Photographs by Karasimou).

Every day during data collection, following the completion of most scans using the tripod, the instrument was placed on the desktop stand, and the capitates and teeth were measured under the lid (Figure 16). The small surface area of these samples could allow x-rays to escape, potentially resulting in inaccurate readings. The lid was used to prevent that scenario. A dosimeter/radiation detector was consistently present, signaling if a predetermined level of radiation exposure had been reached or exceeded.

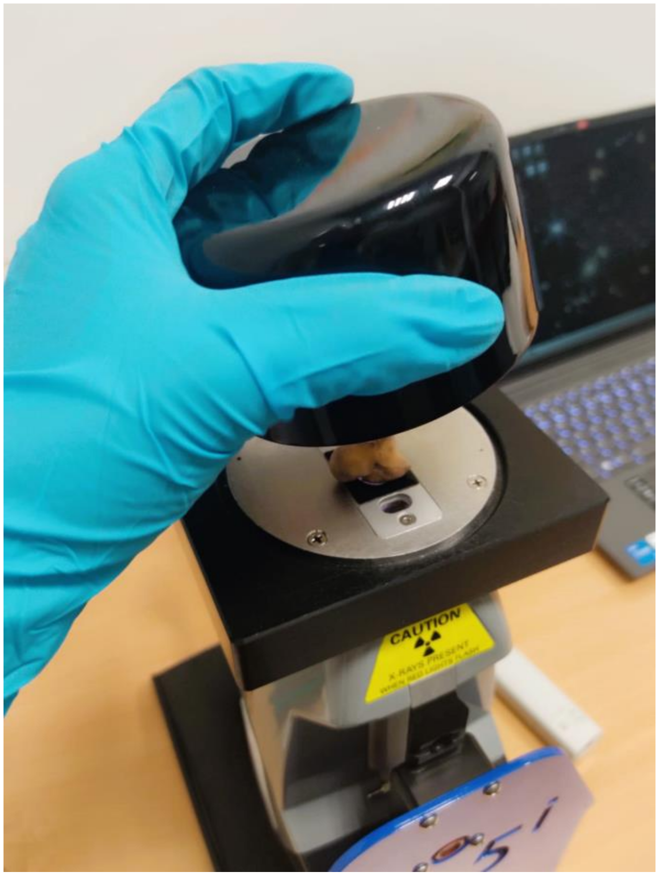


Figure 16. The Bruker Tracer 5g pXRF on the 5i desktop stand. A capitate is placed under the lid (Photograph by Karasimou).

The model used is the Bruker Tracer 5g pXRF. This instrument has an elemental range from sodium (Na) to uranium (U) when operating in an air atmosphere, as was the case in this study. No helium or vacuum settings were utilized, mimicking field conditions. Vacuum settings typically eliminate air between the sample and the detector, whereas helium flush settings replace air and are beneficial when analyzing lighter elements (Bruker Nano Analytics, n.d., D.J.G. Braekmans, personal communication, January 27, 2023). A collimator with a spot size of 8 mm was used, coupled with a 1 µm graphene detector window. The concentrations of chemical elements were determined using the Mudrock calibration file, typically recommended for bone material analysis (Finlayson et al., 2017, p. 496; Perrone et al., 2014, p. 150; Winburn et al., 2017, p. 28). Each sample was analyzed for 90 seconds in total, and the process was divided into two different phases. During phase 1, the samples were scanned for 30 s with a Ti 25µm:Al 300µm filter to measure the heavy elements, while the instrument was operating at 50 kV and 17.7 µA. During phase 2, the scanning would last 60 s with no filter for the lighter elements, employing different voltage and current settings this time, 15 kV and 22.2 µA, respectively. The results can be in the form of spectra for a qualitative analysis, which can be handled with the Artax software that accompanies the Bruker Tracer 5g pXRF. Apart from the spectra, the results can be displayed in percentages or parts per million (ppm) for quantitative analysis. In this thesis, the measurements are in percentages.

The measurements obtained from the scans were automatically stored on the device's USB stick and subsequently exported to a Microsoft Excel spreadsheet. Thirty chemical elements were detected with concentrations above the limit of detection (LOD) in at least one sample. Only U consistently displayed “< LOD” for all the measurements and was therefore removed from the dataset. The percentage of measurements below the LOD varied from 0 to 99 %. Table 3 presents these percentages for every detected chemical element. In order to maintain the sample size, it was decided not to eliminate those values. Instead, the "< LOD" entries were substituted with half the limit of detection value. During the initial data processing and before any statistical analysis, it was discovered that the pXRF did not properly measure phase 2 for one tibia from an individual from Middenbeemster and one humerus from an individual from Arnhem. This resulted in NaN (Not a Number) values for 11 elements.

Table 3. The percentage of measurements that were below the LOD for every detected chemical element.

Percentage	Elements
0–7 %	Zinc (Zn), Potassium (K), Calcium (Ca), Lead (Pb), Sulfur (S), Iron (Fe), Phosphorus (P), Arsenic (As), Strontium (Sr), Magnesium (Mg), Sodium (Na), Titanium (Ti), Manganese (Mn), Silicon (Si), Copper (Cu)
20–54 %	Nickel (Ni), Aluminum (Al), Gallium (Ga)
72–87 %	Vanadium (V), Yttrium (Y), Chlorine (Cl), Cobalt (Co), Rubidium (Rb), Zirconium (Zr), Molybdenum (Mo)
94–99 %	Selenium (Se), Thorium (Th), Barium (Ba), Niobium (Nb), Chromium (Cr)

3.5 Statistical Analysis

Statistical analysis was primarily performed using the IBM SPSS (v. 29) software, supplemented by additional analysis that was conducted in the Python programming language. First, descriptive statistics, particularly summary statistics, were generated. Subsequently, the Shapiro-Wilk test was employed to assess the normality of the data. This step determined the choice of tests used in the analysis. Based on the results of the normality test, if the *p*-value was greater than the alpha significance level ($\alpha = .05$), parametric tests were utilized; conversely, if the *p*-value was smaller, non-parametric tests were conducted.

To explore intra-skeletal variation, differences in elemental concentrations among different bones were assessed using repeated measures ANOVA (analysis of variance) accompanied by Mauchly's test of sphericity to verify the assumption of sphericity within the repeated measures ANOVA. Post hoc tests, and more specifically, pairwise comparisons with the Bonferroni correction, served as the next step. This correction was employed to control for Type I errors (false positives) that might lead to the rejection of the null hypothesis when it was true (Abdi, 2007, p. 1). Additionally, intra-skeletal comparisons were also conducted using the non-parametric Friedman test, followed by post hoc analysis using the Wilcoxon signed-rank test.

For inter-skeletal comparisons, a one-way ANOVA was employed to detect potential differences among individuals when the assumption of normal distribution held true. This was accompanied by a test for Homogeneity of Variances, which is an assumption of ANOVA. Following this, multiple comparisons using the Bonferroni correction were conducted. In cases where the data deviated from normal distribution, ANOVA was substituted with the Kruskal-Wallis test, followed by post hoc pairwise comparisons. Similar tests were utilized when analyzing the factor "age". However, when comparing males and females or when attempting to observe differences between the two sites, the Mann-Whitney U test was employed.

Finally, to assess the potential for sorting these skeletons in cases of commingling, Principal Component Analysis (PCA) was performed. This resulted in the reduction of the dimensions (Nikita, 2017b, p. 418) and allowed the observation of potential clusters. Along with the PCA, the *k′*-means clustering was employed. This algorithm groups data points into clusters, with the number of clusters needing pre-determination (Zalik, 2008, pp. 1385–1386). In conjunction with this algorithm, the adjusted Rand index (ARI) was used in order to evaluate the accuracy of the clustering by comparing the results from the *k′*-means with the true labels of the data. The ARI value ranges from -1 to 1. When the segregation aligns with the true grouping, the value is "1". A value of "0" would suggest a random clustering, while a "–1" would indicate total disagreement (OECD AI Policy Observatory, 2023, para. 2)

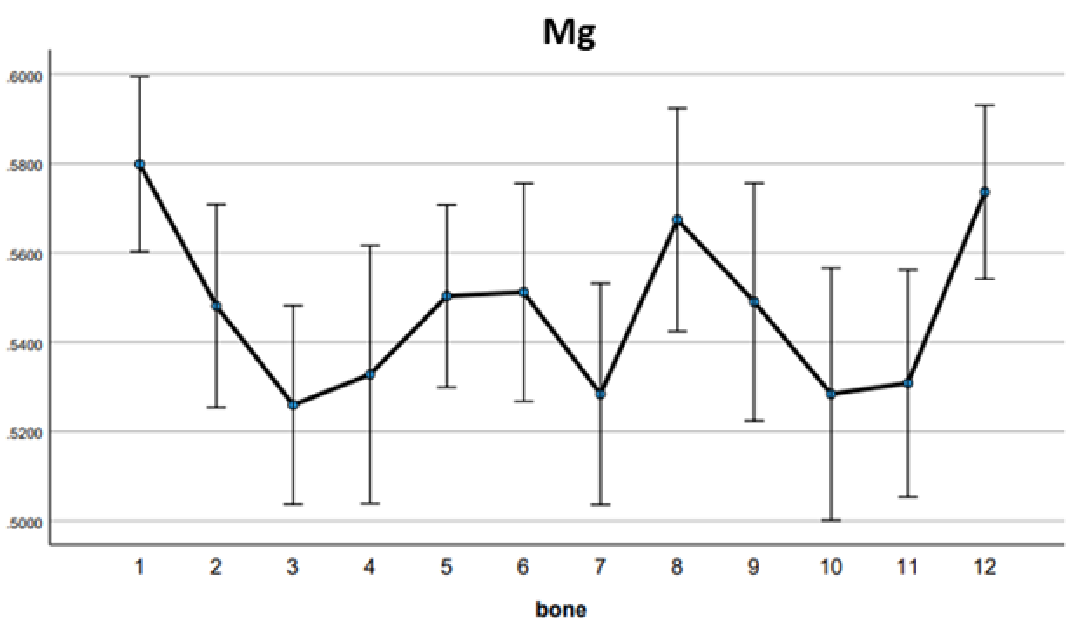
3.6 Summary

This chapter outlined the rationale for selecting individuals from two distinct sites and provided essential background information about these locations. It also detailed the sample strategy, sample preparation method, and procedural steps followed. Additionally, it covered information about the equipment settings, a critical aspect for future comparisons with studies utilizing the same methodology. Finally, the chapter concluded with a presentation of the statistical analysis applied to the data.

Chapter 4. Results

4.1 Intra-skeletal Variation

The pXRF equipment detected 30 elements, as shown in Table 3. Initially, descriptive statistics were conducted, focusing on the mean and standard deviation values for each element, categorized by skeletal element, sex, and site. Subsequently, the normality test was performed, indicating a normal distribution only for Mg (p = .361). To explore variations in Mg concentrations among different bones in the human skeleton, repeated measures ANOVA was conducted. The results showed statistically significant variation (p = .001), and pairwise comparisons were performed using the Bonferroni correction as a post hoc test. Out of the 66 possible pairs tested, a significant difference was observed only between the calcaneus and the femur (p < .001). Figure (17) illustrates the differences in Mg means among the 11 bones and one tooth that were examined.



bone	Variable
1	Mg.calcaneus
2	Mg.capitate
3	Mg.femur
4	Mg.humerus
5	Mg.lumbar
6	Mg.mandible
7	Mg.parietal
8	Mg.pubis
9	Mg.radius
10	Mg.rib
11	Mg.tibia
12	Mg.tooth

Figure 17. The variance in Mg concentrations among the 12 skeletal elements tested. Although variation is evident, only the calcaneus and femur exhibited a statistically significant difference.

For the other 29 elements that were non-normally distributed, the Friedman test was used to compare the elemental concentrations. No significant differences were observed for Th (p = .063), Ba (p = .082), Mo (p = .101), Nb (p = .799), Cr (p = .151), and Se (p = .274). The element V showed a *p-value* of .003, while for the rest of the elements, a *p*-value of < .001 was indicated. Subsequently, the Wilcoxon signed rank test showed which of all the 66 possible pairs of skeletal elements exhibited differences in elemental concentrations. Four pairs, humerus–femur, tibia–parietal, tibia–rib, and rib–mandible, demonstrated no differences in any of the 30 elements (including Mg). Six pairs showed varying concentrations for only one element, which was Zn in almost all of the cases. For pairs exhibiting differences in two elements, the variation was primarily detected in Ca and P. With an increasing number of elements displaying variation across different pairs, no specific pattern emerged. The teeth were in those pairs where concentrations varied for 11 to 17 elements simultaneously (for all 66 pairs and the number of elements for which differences were detected, see Appendix A, Table 7). Overall, chemical elements with a higher frequency of pairs showing intra-skeletal variation included Ca, P, S, Zn, and Pb (Table 4). Regarding which elements displayed a tendency to have higher values in each bone, the results indicated that the capitates, on average, exhibited higher values for nine elements: Pb, Si, K, Ti, As, Ga, Y, Th, and Mo. In contrast, the calcanei and femora showed higher values for Sr, Se, Rb, Mg, Na, and Mn, Fe, Al, respectively. The parietals also displayed higher values for four elements: P, S, Ca, and Ni. The teeth examined exhibited on average lower values for Si, S, K, Ca, Ti, Mn, Fe, Sr, Ni, Al, V, Co, and Rb, while demonstrating the highest values in terms of Cl.

Table 4. The 23 out of 29 non-normally distributed elements that exhibited significant differences between pairs of skeletal elements. For instance, Na varied in 20 out of the 66 possible pairs. Those with a higher frequency of pairs showing variation, are outlined in red.

Chemical element	Number of pairs exhibiting variation
Na	20/66
Al	27/66
Si	14/66
P	42/66
S	35/66
Cl	11/66
K	12/66
Ca	51/66

Chemical element	Number of pairs exhibiting variation
Zn	35/66
Ga	13/66
As	28/66
Rb	17/66
Sr	17/66
Y	5/66
Zr	17/66
Pb	31/66

Chemical element	Number of pairs exhibiting variation
Ti	13/66
V	2/66
Mn	14/66
Fe	21/66
Co	10/66
Ni	11/66
Cu	11/66

4.2 Inter-skeletal Variation

After completing the tests to detect possible variations between different skeletal elements, additional analysis was conducted to observe differences in elemental concentrations among individuals. Separate analysis was carried out for normally and non-normally distributed data. To explore potential differences in Mg concentrations among the 40 individuals, one-way ANOVA was performed. The results provided evidence that there were differences between means ($p < .001$). Subsequently, post hoc multiple comparisons using the Bonferroni correction were conducted to precisely identify which of the 780 possible pairs of individuals exhibited differences. Seven pairs showed statistically significant results (with a p-value ranging from .004 to .040), while for the majority of the pairs, the p-value was 1.000. One individual appeared in six different pairs, and a second one in two. For these seven pairs, the individuals came from a different site. Figure 18 illustrates the variation in Mg concentrations among all 40 skeletons.

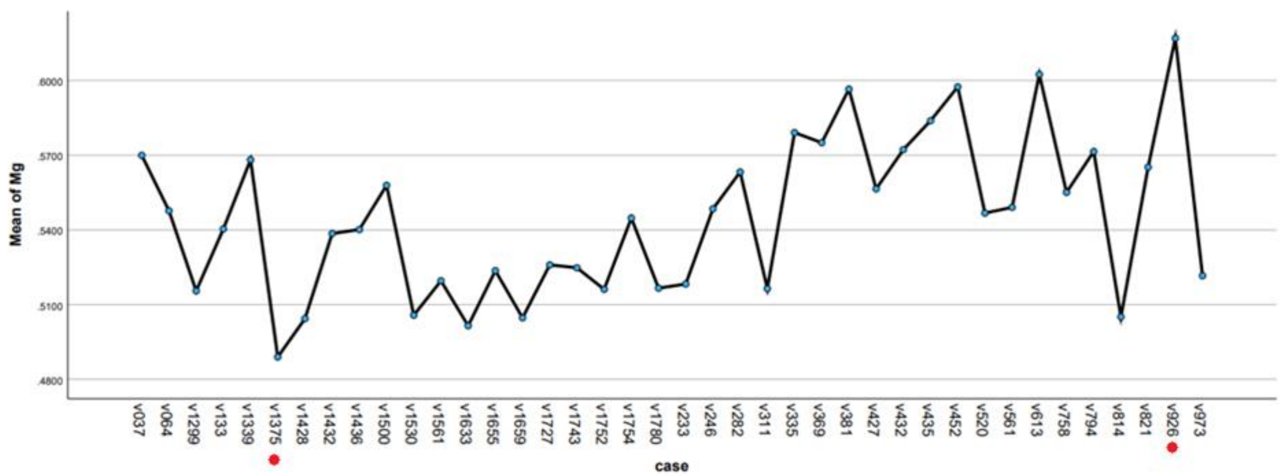


Figure 18. The variance in Mg among the 40 individuals. The red dots mark the two skeletons that appeared in more than one pair. The individual v1375 appeared in two pairs, while v926 was involved in six.

The same process was followed for the rest of the elements, using the Kruskal-Wallis test. The results indicated that there was no significant difference for the elements Co ($p = .057$), Zn ($p = .176$), Se ($p = .227$), Zr ($p = .083$), Nb ($p = .651$), Mo ($p = .052$), Ba ($p = .420$). For the elements with a p-value less than .05, pairwise comparisons followed. After the Bonferroni correction, it was observed that the elements As and Pb displayed differences across a larger number of pairs compared to the other elements. More specifically, As exhibited variation among 123 pairs out of 780, while Pb showed differences among 117 pairs. While variation was initially indicated, the post hoc test failed to detect differences in some elements at the level of pairs. For instance, Na, which displayed a *p*-value of .028 after the Kruskal-Wallis test, and Ca with a *p*-value of .043, exhibited differences in zero pairs after the pairwise comparisons. Specifically for Na, although there were initially 86 pairs of skeletons showing significance according to the raw *p*-value, no significant differences were observed after the adjustment. Figures 19 to 22 depict the distribution of As, Na, Pb, and Ca concentrations within that sample, respectively.

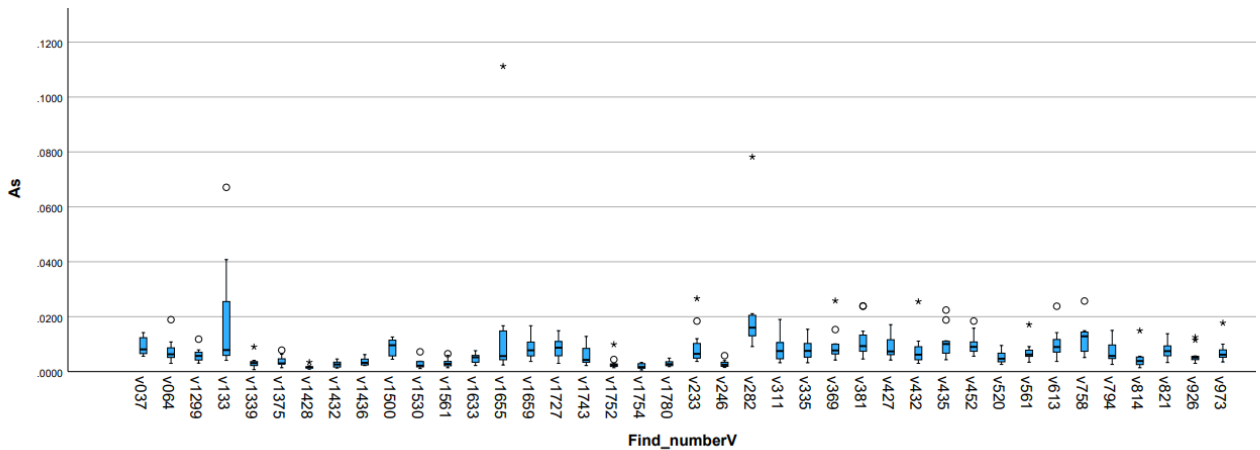


Figure 19. The distribution of As concentrations among the 40 individuals. The circles indicate the outliers, while the asterisks indicate the extreme outliers. This applies to all box plots presented in this paper.

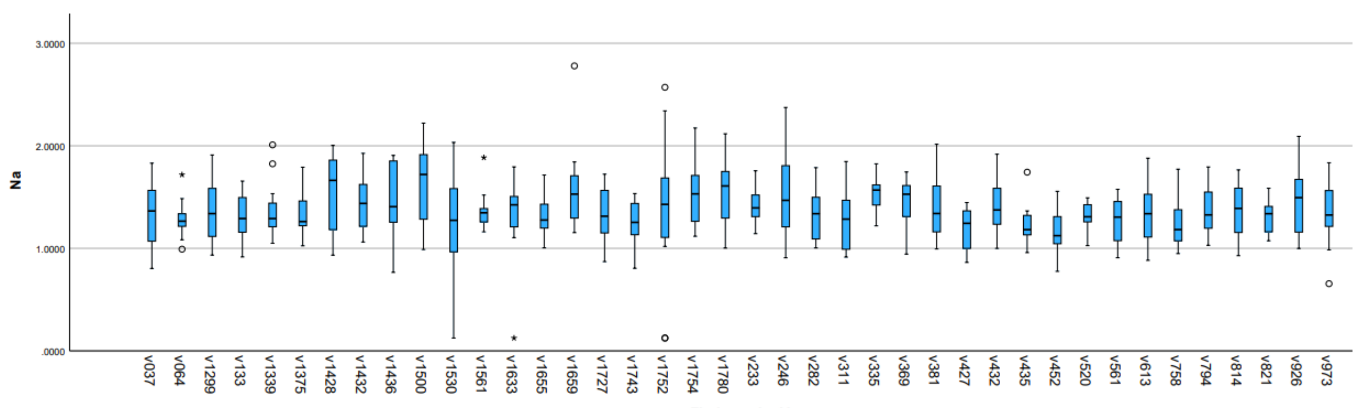


Figure 20. The distribution of Na concentrations among the 40 individuals.

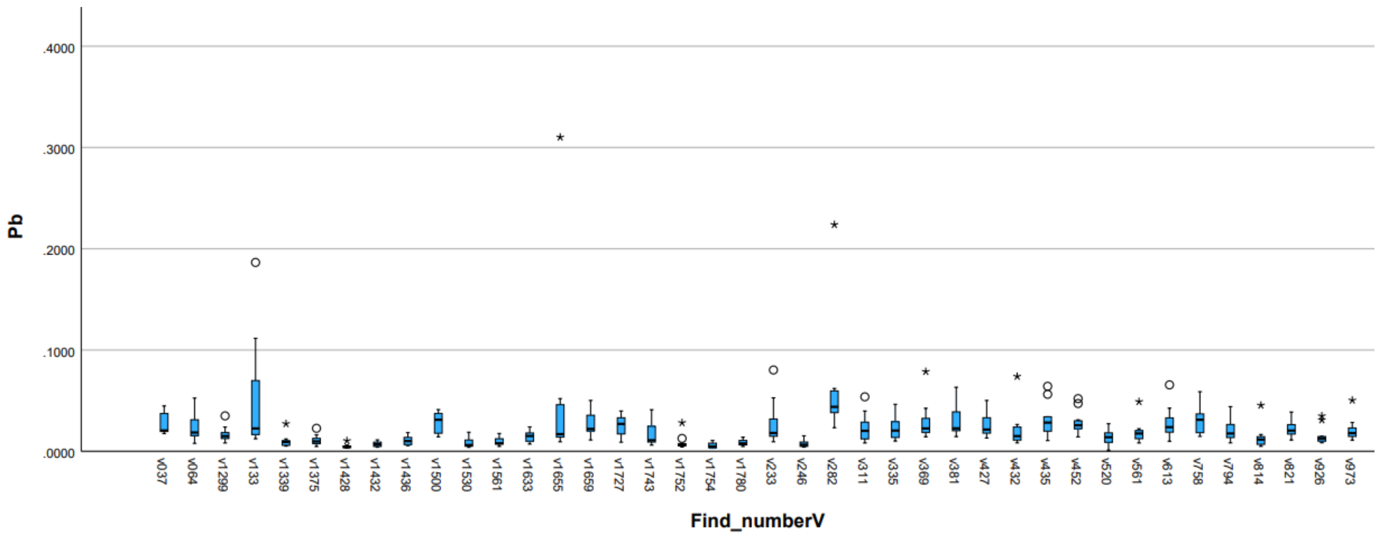


Figure 21. The distribution of Pb concentrations among the 40 individuals.

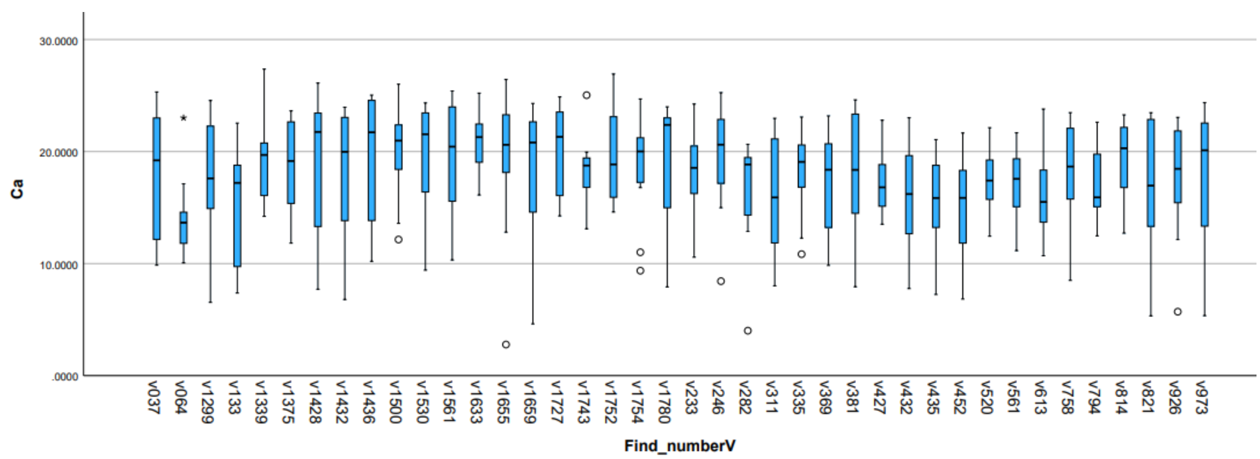


Figure 22. The distribution of Ca concentrations among the 40 individuals.

Apart from the raw values of the chemical elements that were detected, it was decided to also explore specific ratios. Summary statistics and normality tests were performed for Zn/Fe, K/Fe, Sr/Ca, Pb/Ca, Sr/Pb, Ca/P, Zn/Ca, Mn/S, Mn/K, Ba/Cl, Ba/Sr, S/Sr, Mn/Fe, Zn/Cl, a total of 14 ratios. Since none of them followed a normal distribution, the next step was to perform the Kruskal-

Wallis test with post hoc analysis. The ratio Zn/Ca was the only one that did not exhibit statistical differences (p = .274). The ratios that varied significantly across a larger number of two-skeleton groups were Sr/Pb with 83, and Pb/Ca with 81 out of 780 pairs. In terms of the fewest pairs, Ba/Cl and Zn/Cl stood out, with seven and four pairs respectively, displaying statistical differences. The same trend was observed for the ratios as for the raw chemical elements, wherein the count of adjusted p-values less than .05 was notably smaller compared to the initial p-values. For instance, initially, 350 pairs showed significant variation in Sr/Pb. However, after the correction, this number was reduced to 83, as previously mentioned. Figures 23 and 24 show the distribution of Sr/Pb and Zn/Ca in the sample, respectively.

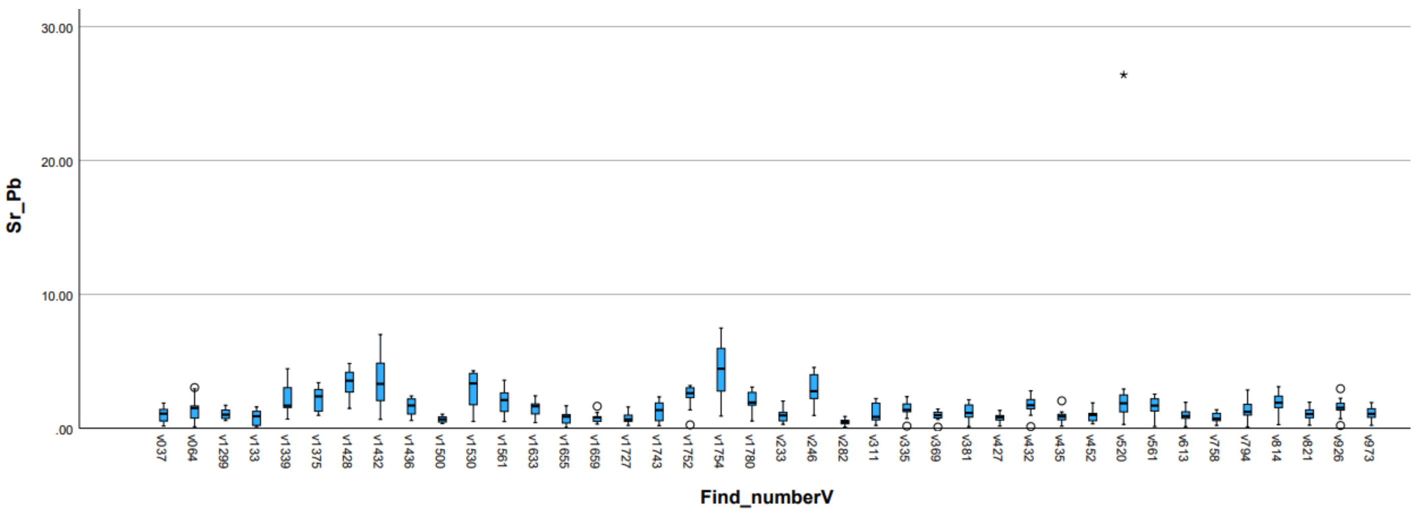


Figure 23. The variation in Sr/Pb observed among the 40 individuals. This ratio exhibited significant variance across a larger number of pairs of skeletons.

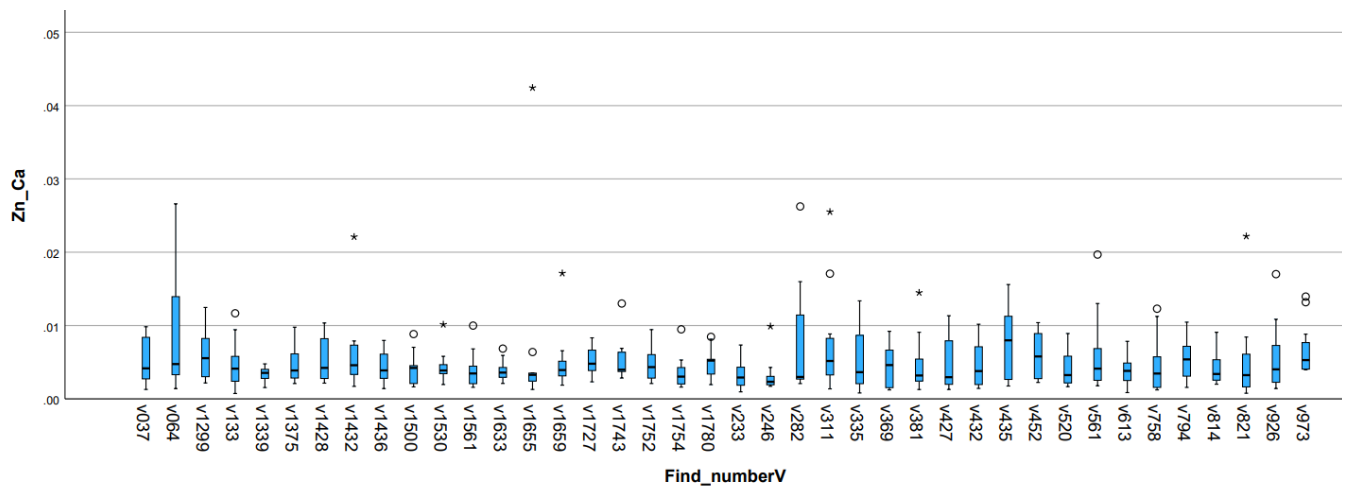


Figure 24. The variation in Zn/Ca observed among the 40 individuals. This ratio did not exhibit significant differences among individuals.

4.2.1 Inter-skeletal Variation with Sex, Age, and Site Factors

After exploring the variation in elemental concentrations among different individuals, additional analyses were carried out, incorporating factors such as sex, age, and site. Initially, potential differences in ratios between males and females were investigated using the Mann-Whitney U test for the 14 previously mentioned ratios. The null hypothesis suggested that the distribution of each ratio was the same across sex categories. For Sr/Ca (p = .024) and Ca/P (p = .040) (Figure 25), the null hypothesis was rejected. These two ratios exhibited statistically significant variation between males and females. Regardless of the p-values, and whether or not the ratios showed significant difference, the mean ranks indicated which ratios tended to be higher in males and females. On average, Zn/Fe, Sr/Ca, Pb/Ca, Ca/P, Zn/Ca, Mn/K, S/Sr, and Mn/Fe showed higher values in males, while K/Fe, Sr/Pb, Mn/S, Ba/Cl, Ba/Sr, and Zn/Cl displayed a tendency to be higher in females.

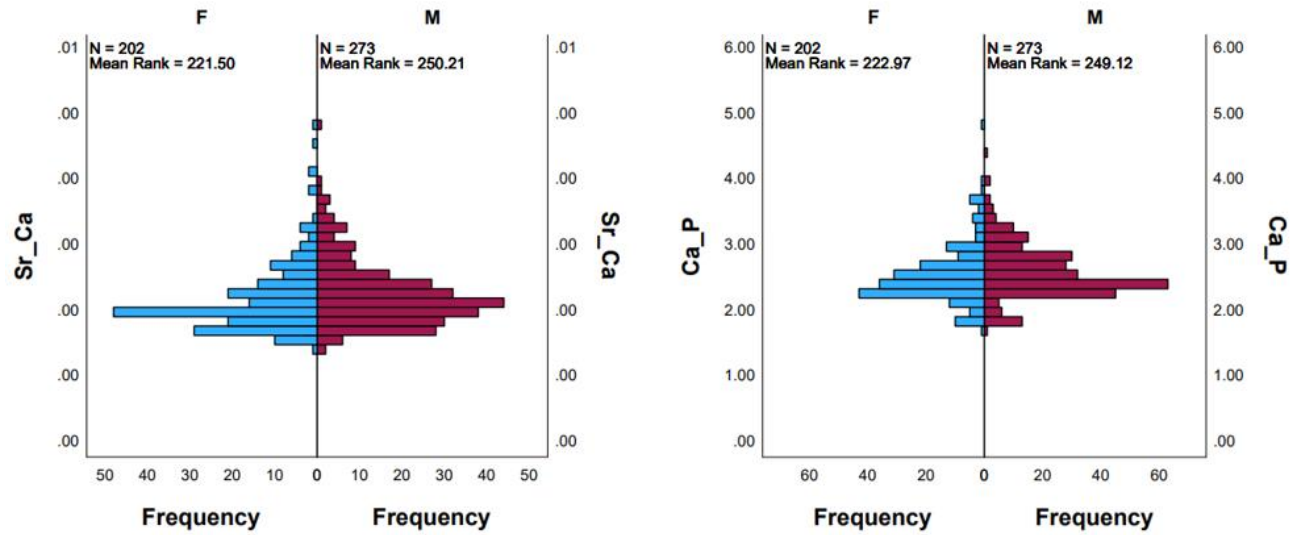


Figure 25. Ratios demonstrating statistically significant variation between males and females. Both ratios exhibit higher average values in males compared to females within this sample (see mean rank).

In the analysis that followed, different age categories were targeted. Although all individuals in this study were adults, they fell into different sub-groups (see Table 2). To detect potential differences in ratios among these age groups, the Kruskal-Wallis test was conducted. Since there were six adult groups, the possible two-group combinations were 15. The results indicated that for three out of the 14 ratios examined, Zn/Ca (p = .384), Ba/Cl (p = .992), and Zn/Cl (p = .549), the null hypothesis was not rejected. Among the ratios showing variance, Ba/Sr displayed differences in 8 out of 15 pairs, whereas the majority of other ratios exhibited variations within a range of one to five pairs. In the case of Zn/Fe (Figure 26), while according to the Kruskal-Wallis test the null hypothesis was rejected (p = .024), differences in zero pairs were detected after the post hoc test with the Bonferroni adjustment. Although the majority of ratios exhibited no clear patterns when examining the age group pairs, three ratios differed in this regard. Specifically, in the case of Mn/S, K/Fe, Mn/K, and Mn/Fe, all combinations displaying variation featured the 50+ group. Figures 27 and 28 depict the distribution of K/Fe and Mn/Fe across the age groups and illustrate the specific groups exhibiting variation. Regarding which age category had on average higher values, the "18+" group showed greater values for half of the ratios, including Zn/Fe, Zn/Ca, Ba/Cl, Ba/Sr, S/Sr, Mn/Fe, and Zn/Cl.

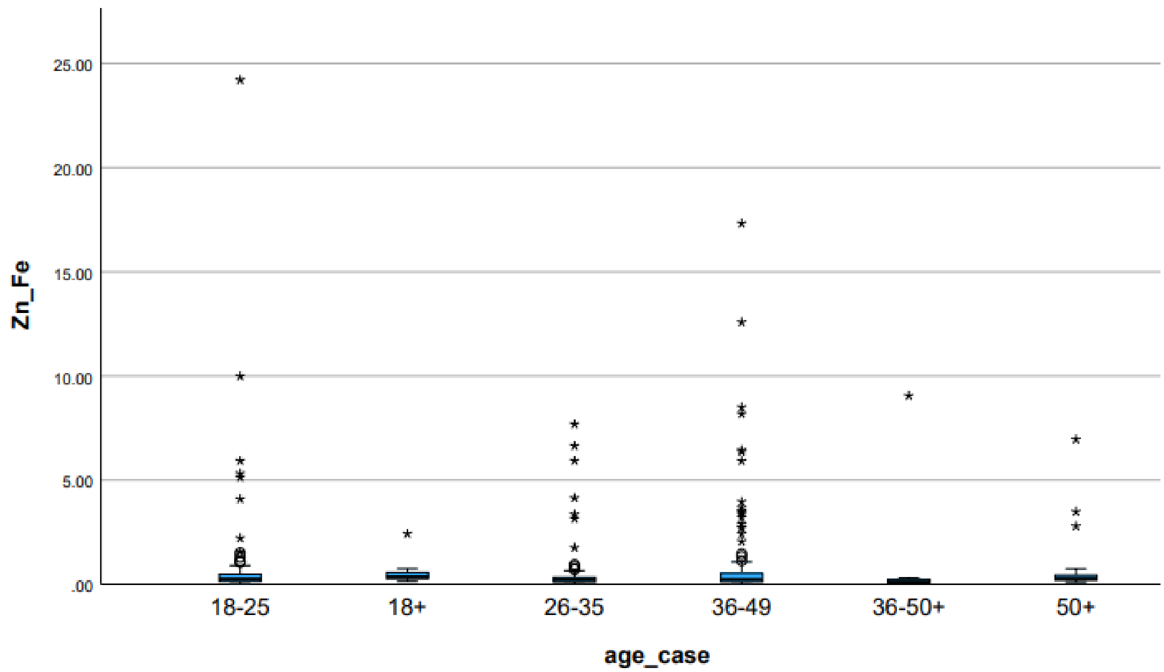


Figure 26. The distribution of Zn/Fe among different age categories. A great number of extreme outliers is observed.

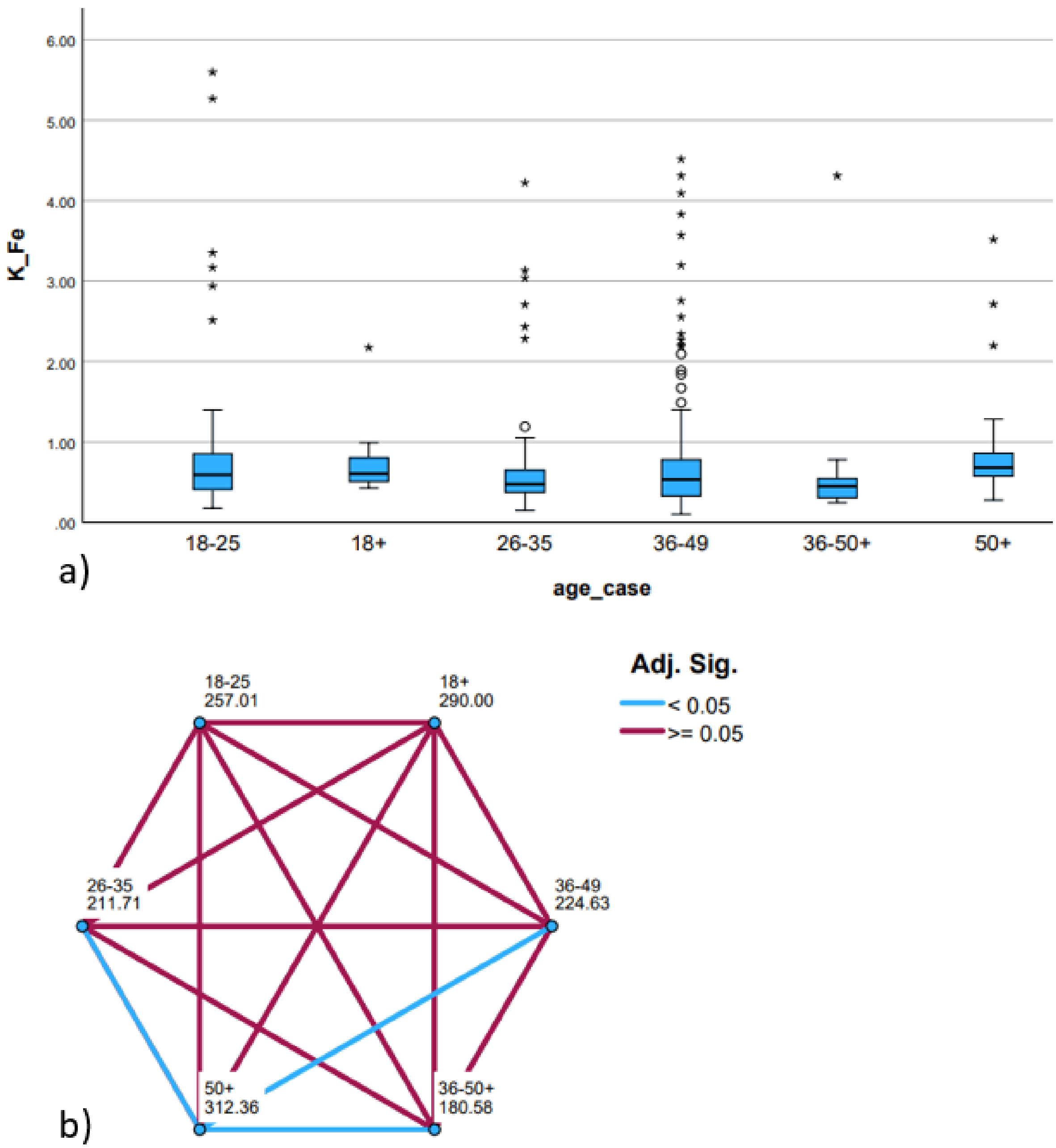


Figure 27. a) The distribution of K/Fe across the age groups. b) The blue lines indicate the specific groups that exhibited variation (where the *p*-value was < .05). The mean ranks are also indicated below each age category. The 50+ group exhibited on average higher K/Fe values.

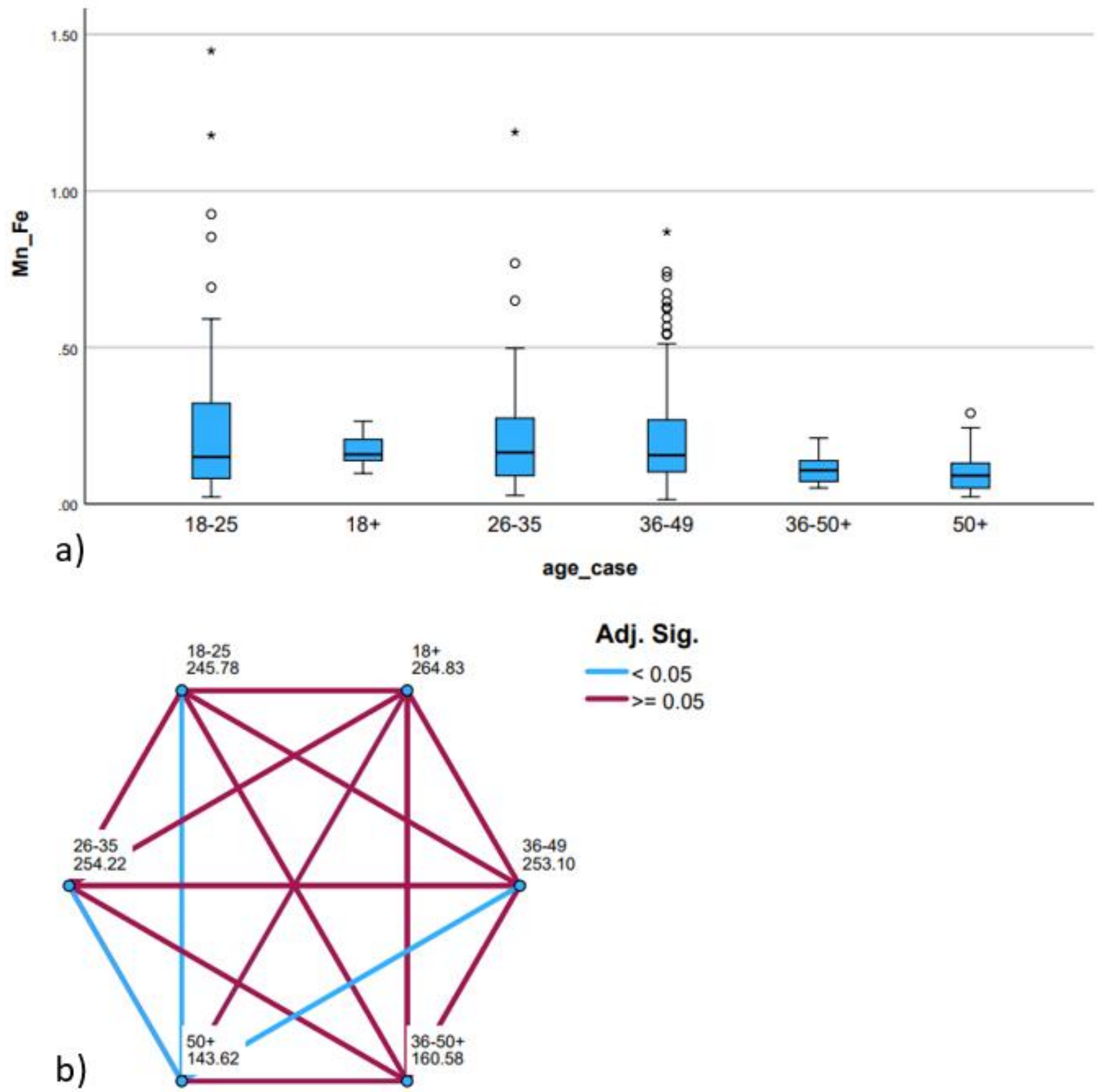


Figure 28. a) The distribution of Mn/Fe across the age groups. b) The blue lines show the groups that exhibited variation. The mean ranks are also depicted below each age category. On average, the 18+ group exhibited higher Mn/Fe values.

The final analysis centered on inter-skeletal variation, specifically within Arnhem and Middenbeemster. A comparison between these sites was conducted using the Mann-Whitney U test across 14 ratios. The results revealed statistically significant variation in 11 ratios. K/Fe (p = .835), Zn/Ca (p = .861), and S/Sr (p =.091) were the three ratios that did not show variation. Figure 29 depicts K/Fe, and Ca/P which exhibited statistically significant difference ($p < .001$). Furthermore, nine out of 14 ratios (i.e., Zn/Fe, Sr/Pb, Zn/Ca, Mn/S, Mn/K, Ba/Cl, Ba/Sr, Mn/Fe, and Zn/Cl) showed a tendency to be higher in individuals from Arnhem, and five (i.e., K/Fe, Sr/Ca, Pb/Ca, Ca/P, and S/Sr) exhibited higher values in those from Middenbeemster.

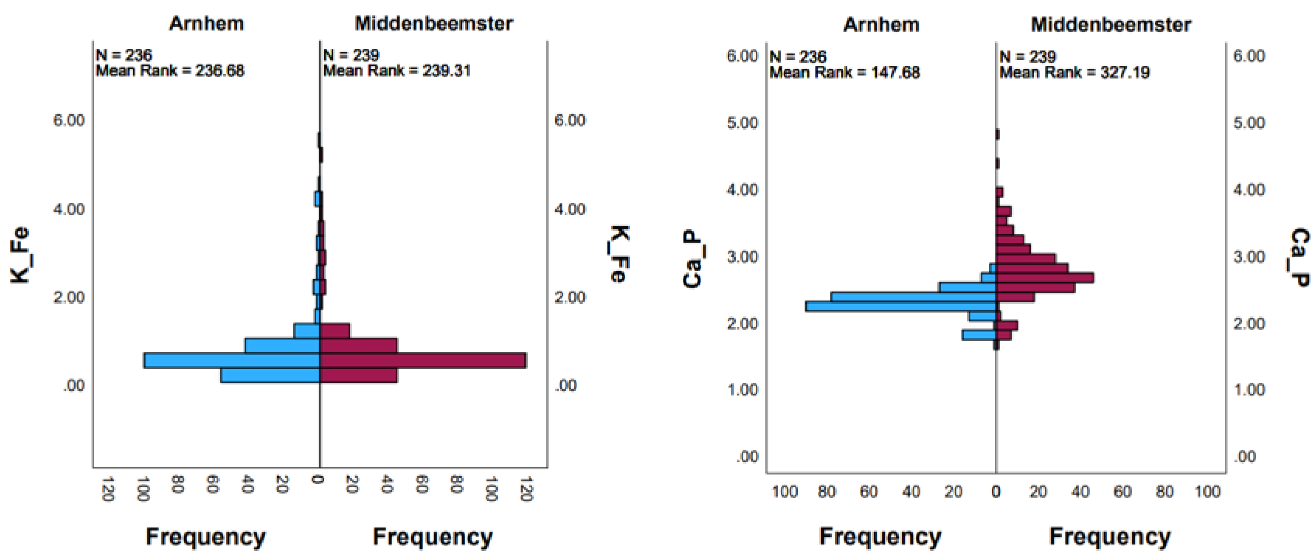


Figure 29. The differences in K/Fe and Ca/P between the two sites that were examined. K/Fe showed no variation, while Ca/P exhibited differences among sites.

Apart from the ratios, for this comparison involving the two sites, it was decided to also use the raw values of the elements. To investigate potential variation, the independent sample t-test (for Mg) and the Mann-Whitney U test (for the rest of the elements) were performed. The elements that did not display statistically significant difference included Al, Ti, Mn, Co, Rb, Nb, and Ba. For the remaining 23 elements, the null hypothesis was rejected. Regarding the quantities of the elements in the skeletons, individuals from Arnhem, on average, showed higher values in Na, P, Ca, Mn, Co, Cu, Zn, Zr, and Mo, while those from Middenbeemster exhibited greater values in the other 21 elements. Figure 30 shows Al, which did not vary between the two sites ($p = .315$), and P, which exhibited variation ($p < .001$).

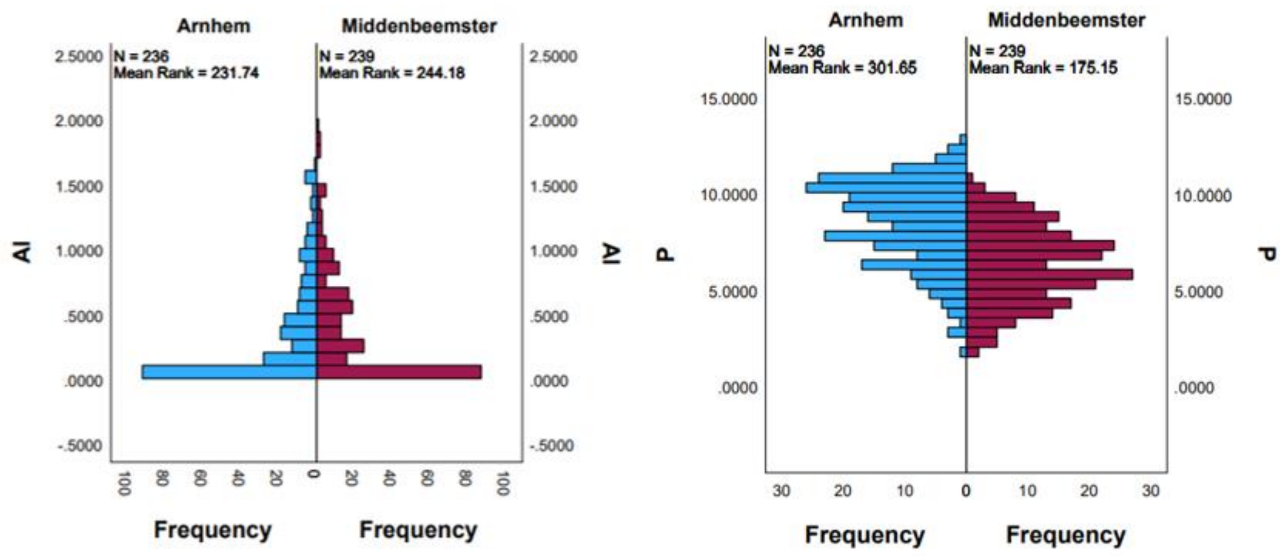


Figure 30. The differences between two elements across sites, showcasing the absence of variation in Al and presence of variation in P.

4.3 Principal Component Analysis

After exploring the intra-skeletal and inter-skeletal elemental variation, the following step involved testing whether individuals could be sorted in a mock commingling scenario based on their elemental profiles. Initially, to reduce the number of variables, PCA was conducted using all 30 elements detected with the pXRF. The analysis provided 30 components. An eigenvalue that is larger than one suggests that the principal components capture a greater amount of variance compared to a single original variable (Kassambara, 2017, Eigenvalues/Variances section). In this case, nine components had eigenvalues equal to or greater than one and explained 71.44 % of the variation. The elements that contributed the most to the first principal (PC1) were As, Ga, Pb, Th, Y, S, and Se, while those that contributed to the second were K, Ti, Si, Al, Mg, and Fe (for further details, refer to Appendix B, Table 8).

Subsequently, the initial sorting attempt involved the segregation of two individuals. Employing the k'-means clustering algorithm using the calculated scores of these nine components, all possible two-individual combinations were assessed. Out of the 780 potential pairs, four pairs exhibited the highest value (ARI = .68). As previously mentioned, an ARI value of "0" indicates random clustering, “1” suggests total agreement, and “–1” signifies total disagreement. Since the visual examination did not result in the partitioning of the clusters, and the ARI mean value was low (.05), it was decided to exclude all elements that initially had measurements below the LOD from 20 to 99 %, and therefore focus solely on those with LOD entries less than 7 %. This was done to assess if the results could be improved.

After removing the aforementioned elements, the principal components were recalculated, resulting in 15 components. Among these, five had values exceeding one and collectively accounted for 72.24 % of the variation. The primary contributing elements to PC1 were P, Ca, Mg, Na, S, and Mn, while those influencing PC2 included Mg, K, Ti, Fe, and Sr (for more information see Appendix B, Table 9). Attempting to segregate two individuals by testing all possible combinations was performed again. The results indicated a mean ARI value of .06, with the best cases revealing a pair achieving the highest value (ARI = .83), and six more pairs with values around .68. Figure 31 illustrates the best case employing PC1 and PC2, which collectively accounted for 41.07 % of the variance. As the results showed only a slight improvement, further analyses focused on ratios.

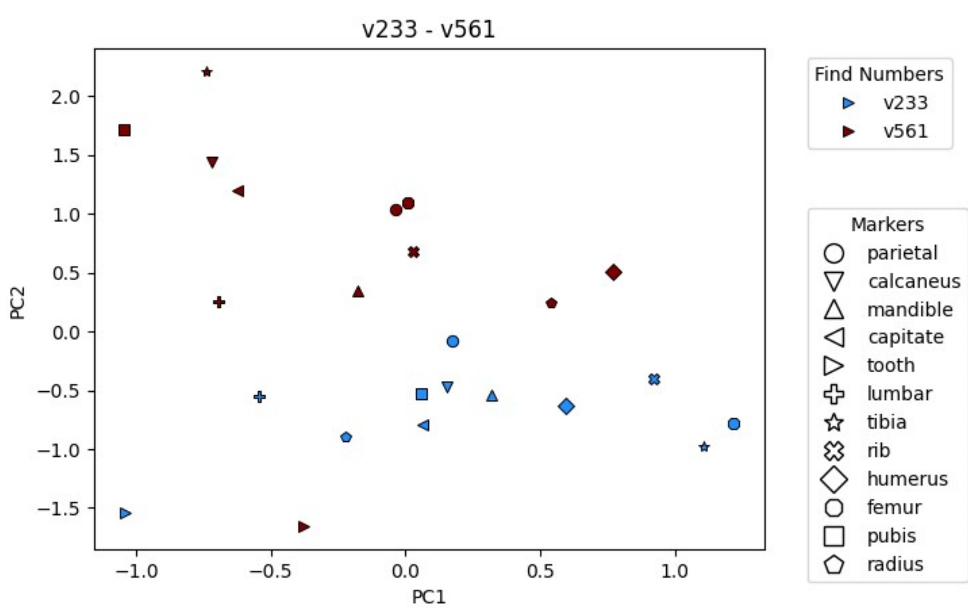


Figure 31. PCA plot featuring components derived from elements with LOD entries < 7 %. The depicted scenario represents the best case (ARI = .83). No distinct clustering is evident. Note: For the “Find Numbers” box, the colors indicate the individuals; the shapes are randomly assigned by the program. To examine the location of specific bones, see the “Markers” box. This applies to all PCA visualizations in this study.

In this stage, the objective was to investigate whether utilizing ratios could enhance the effectiveness of the sorting process. Out of the 14 previously explored ratios, three were excluded as they were comprised of elements with a high percentage of entries below the LOD. This resulted in using 11 ratios (Zn/Fe, K/Fe, Sr/Ca, Pb/Ca, Sr/Pb, Ca/P, Zn/Ca, Mn/S, Mn/K, S/Sr, and Mn/Fe) to calculate the new principal components. The PCA generated 11 principal components, four of which demonstrated eigenvalues greater than one. These four components explained 78.52 % of the variation. The predominant contributors to PC1 were Mn/S, Mn/K, and Mn/Fe, and to PC2 were Zn/Fe, K/Fe, Ca/P, and Zn/Ca (for more details see Appendix B, Table 10). In this attempt to segregate pairs of individuals, among the 780 possible pairs, six showed an ARI value of 1, seven had a value of .83, and one displayed a value of .82. The number of best cases increased from seven to 14, while the mean ARI value rose from .06 to .09. All pairs with an ARI value of 1 were printed, and combinations of principal components contributing to the visual sorting of individuals were evaluated. The visualizations indicated that with a combination of PC2 and PC4, the clustering of the individuals was clear compared to other combinations. Figure 32 shows the difference of employing two different principal component combinations on the same pair, while Figures 33 depicts two more cases where the ARI value was 1.

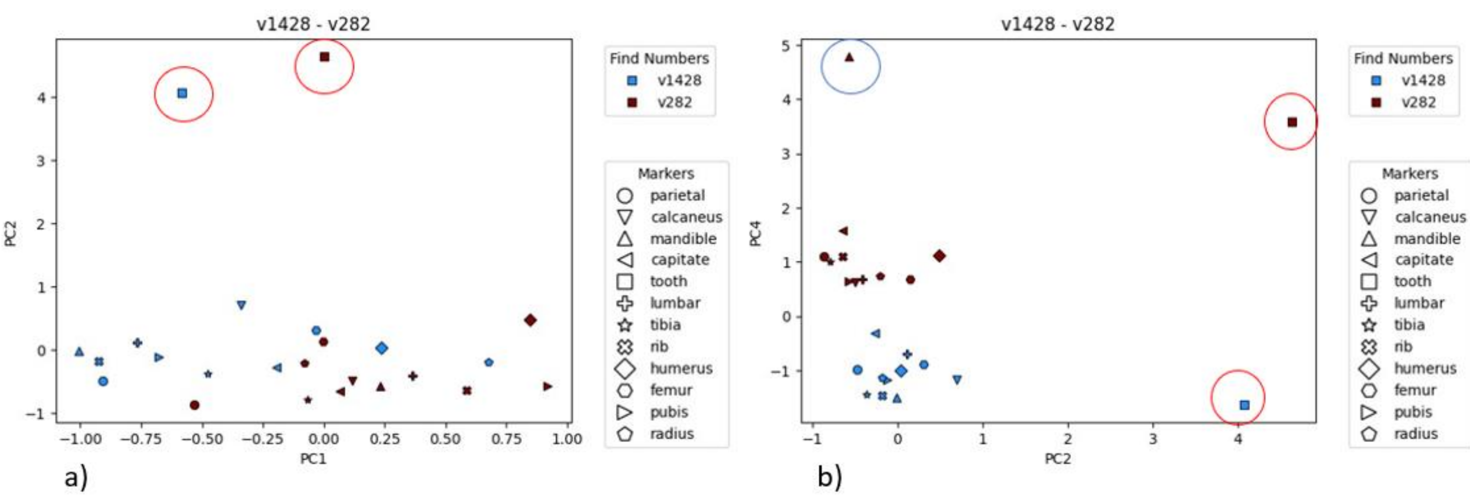


Figure 32. Two principal component combinations for the same case. a) When PC1 and PC2 were used, no distinct clustering was observed. The red circles enclose the tooth measurements. b) When PC2 with PC4 were used on the same pair of skeletons, the clustering was evident. The red circles enclose the tooth measurements, while the blue encloses the one from the mandible.

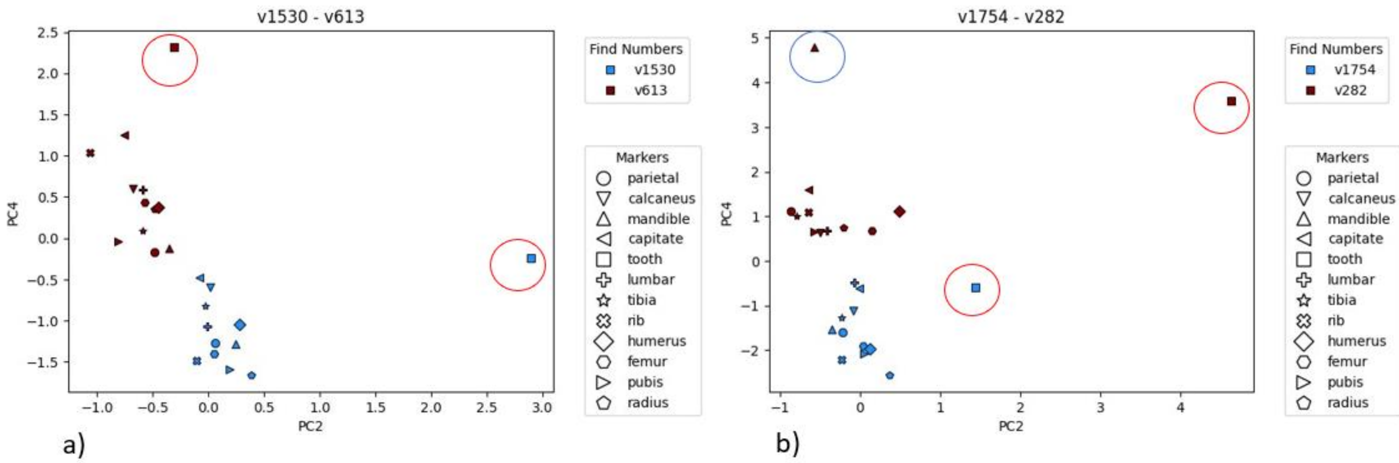


Figure 33. Two different pairs with an ARI value of 1. The combination of PC2 and PC4 revealed clear clustering patterns. The red circles enclose the tooth measurements, while the blue encloses the one from the mandible.

Before attempting to sort three individuals, the decision was made to exclude the tooth measurements. This choice stemmed from their consistent positioning on the outskirts, away from the bones, and aimed at evaluating if the results could be improved. A new PCA was performed, generating 11 principal components, with four having eigenvalues greater than one. Although this was also the case before the tooth measurements were removed, in this scenario, the eigenvalues were smaller, and the four components accounted for 74.37 % of the variation. The major influencers of PC1 were Mn/K, Mn/S, Mn/Fe, and K/Fe, while for PC2 they were Sr/Ca, Ca/P, Pb/Ca, and S/Sr (for more information see Appendix B, Table 11). The sorting attempt reintroduced combinations of two skeletons to allow comparison with the previous results. This time, the best cases increased from 14 to 52. Nineteen pairs displayed an ARI value of 1, while 33 pairs had .81. The mean ARI value rose from .09 to .19. Figure 34a depicts a case with an ARI value of 1, using PC2 and PC4. Although this combination of components predominantly clustered the skeletons, there were instances where individuals could not be distinctly separated. Notably, in some of the best cases, a more effective sorting was observed using PC1 and PC4 (Figure 34b). Even for those pairs that were clearly separated, there were bones that were positioned at a distance from the clusters.

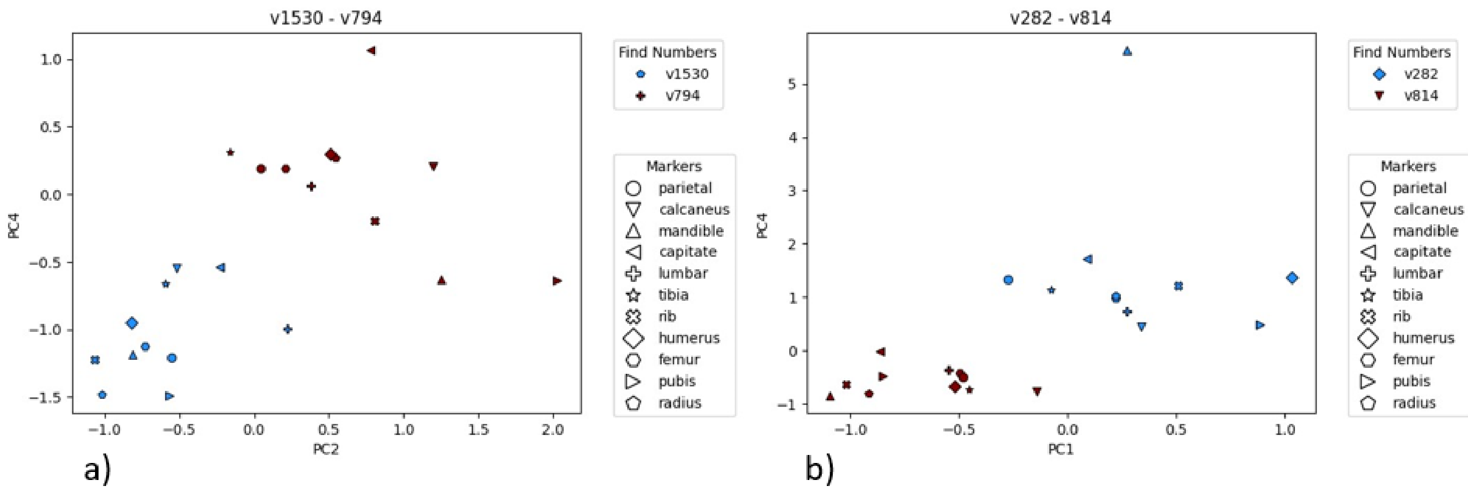


Figure 34. Two different pairs of skeletons with an ARI value of 1. a) The combination of PC2 and PC4 revealed a clustering pattern, albeit sparse. b) For this pair, the most effective sorting was achieved using PC1 and PC4.

For the next step, the sorting of three individuals was attempted. Every possible grouping with three skeletons was tested, resulting in 9,880 groups. Among these groups, one exhibited an ARI value of .91, two showed .82, and six had a value of .81. The mean ARI dropped from .19 to .18. Additionally, the combination of principal components revealing the most optimal clustering was inconsistent. Although clustering patterns were observable in some cases, misplacement of bones into incorrect groups was evident (Figure 35).

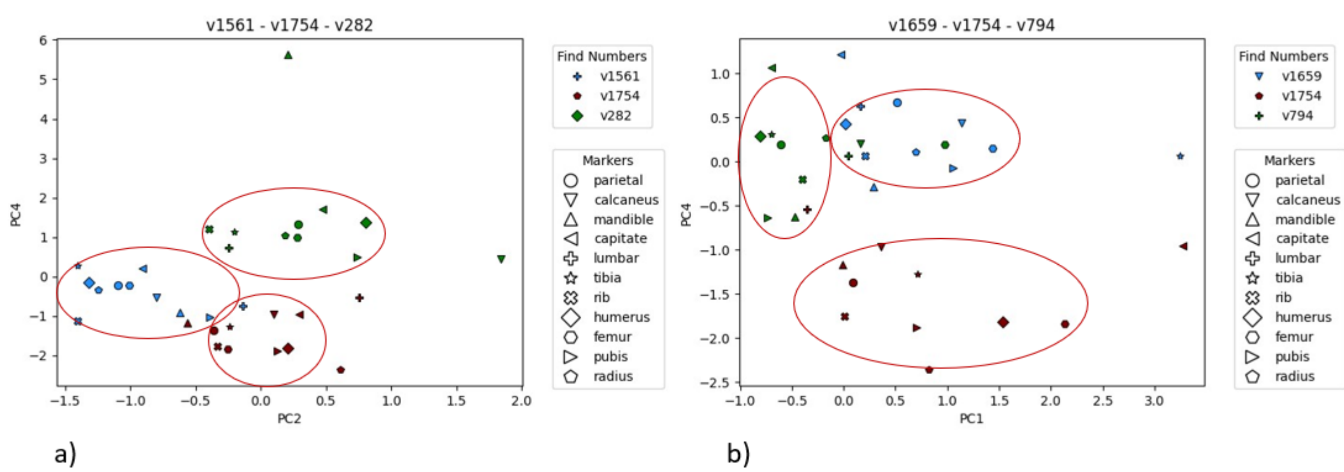


Figure 35. Two of the best cases. a) A three-skeleton group with an ARI value of .82 Some bones are positioned on the outskirts, and there are instances of misplacement. b) In this case (ARI = .81) a sparse clustering is observed, accompanied by misplaced or distantly positioned bones.

In addition to two-dimensional plots, three-dimensional visualizations were employed to better explore the potential for visual segregation (Figure 36). Subsequently, the clustering of four individuals was examined, encompassing a total of 91,390 possible combinations. The results indicated a decrease in the ARI mean value to .17, while the best sorting cases amounted to two, achieving an ARI value of .73 and .70. The individuals from the best case could be partially sorted using a combination of 2D and 3D plots (Figure 37). Although the accuracy of clustering dropped, an attempt was made to segregate five individuals. Testing 658,008 possible combinations of five-skeleton groups, the results showed a mean ARI value of .15, while the best case attained a value of .60. In the plots for the best case, only the bones of one specific individual clustered distinctly from the other four skeletons (Figure 38). As the mean ARI value this time was even lower than the initial sorting attempt in this study, and there were no cases above .60, no further attempts were made to cluster more than five individuals.

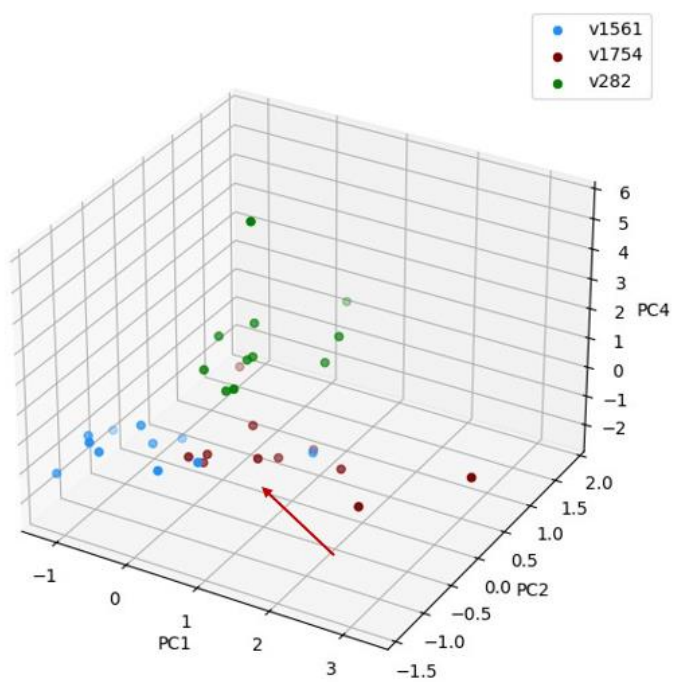


Figure 36. Three-dimensional PCA visualization employing PC1, PC2, and PC4. This visualization corresponds to the group depicted in Figure 35a. In this representation, some of the misplaced bones seem to appear at a lower level (indicated by the red arrow).

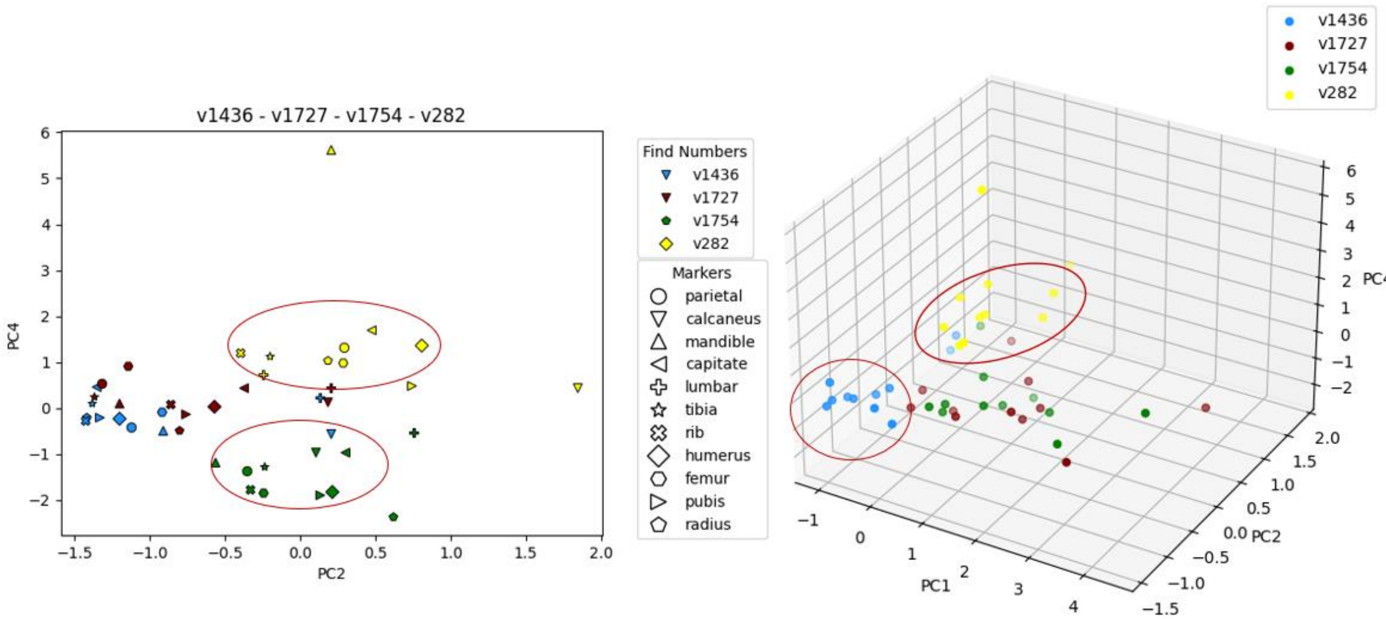


Figure 37. The best four-skeleton sorting case (ARI = .73). In the 2D plot, the individuals v1754 and v282 displayed a clustering pattern (though not tightly), while the other two formed a single group. In the 3D plot, the individual v1436 forms a tight cluster, but now v1754 and v1727 cannot be distinguished.

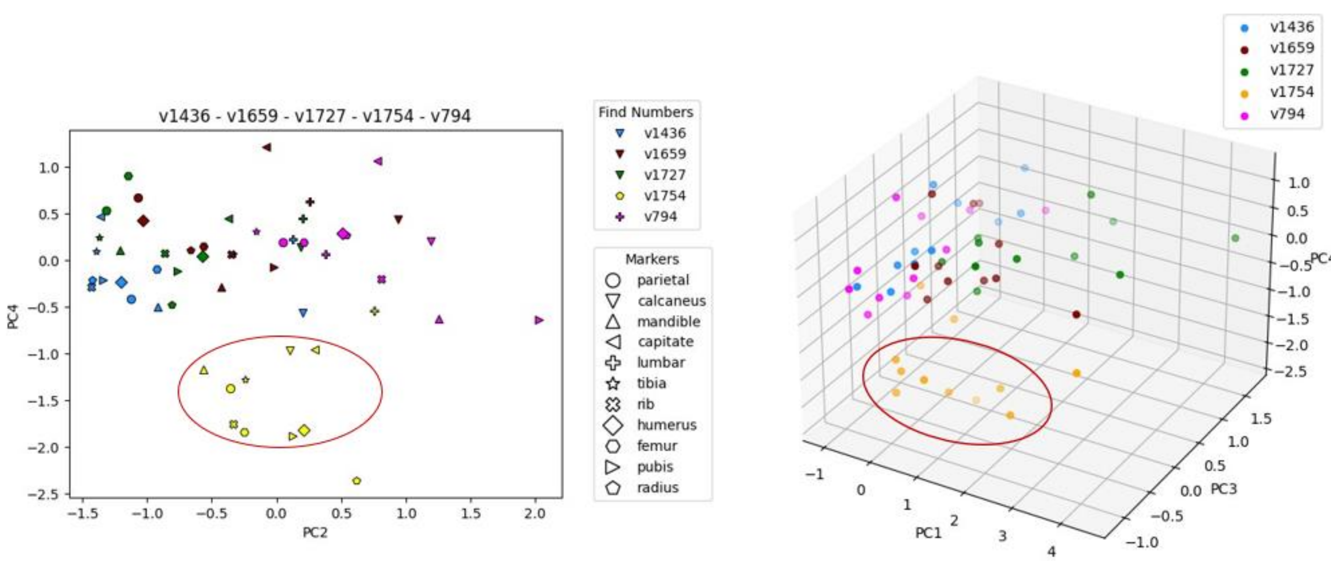


Figure 38. The best case from an attempt to sort five individuals. Despite testing various combinations of principal components, none resulted in a plot where all individuals were successfully separated. Only one individual (v1754) consistently clustered distinctly from the others across multiple groupings.

As a final test, it was attempted to reassociate the tooth measurements that were previously excluded from the analysis with bones. During the exploration of the intra-skeletal variation, the results indicated that the first pair in which the tooth appeared was the tooth–capitate pair (Appendix A, Table 7). Since the tooth measurements varied in elemental concentrations from the capitate measurements in 11 out of the 30 elements tested, compared to the rest of the pairs encompassing teeth that varied in 13 to 17 out of 30, the tooth–capitate pair was selected for analysis. For the elements for which no statistically significant differences were observed (Na, Mg, P, V, Cr, Mn, Co, Cu, Zn, Ga, As, Se, Rb, Y, Zr, Nb, Mo, Ba, Pb), only those with LOD entries less than 7 % were chosen. These elements were Na, Mg, P, Mn, Cu, Zn, Pb, and As. The PCA generated eight components, four of which exhibited eigenvalues above one and explained 66.60 % of the variation. As, Pb, and Cu were the key contributing elements to PC1, while P, Mg, and Mn were the predominant contributors to PC2 (for more information, see Appendix B, Table 12).

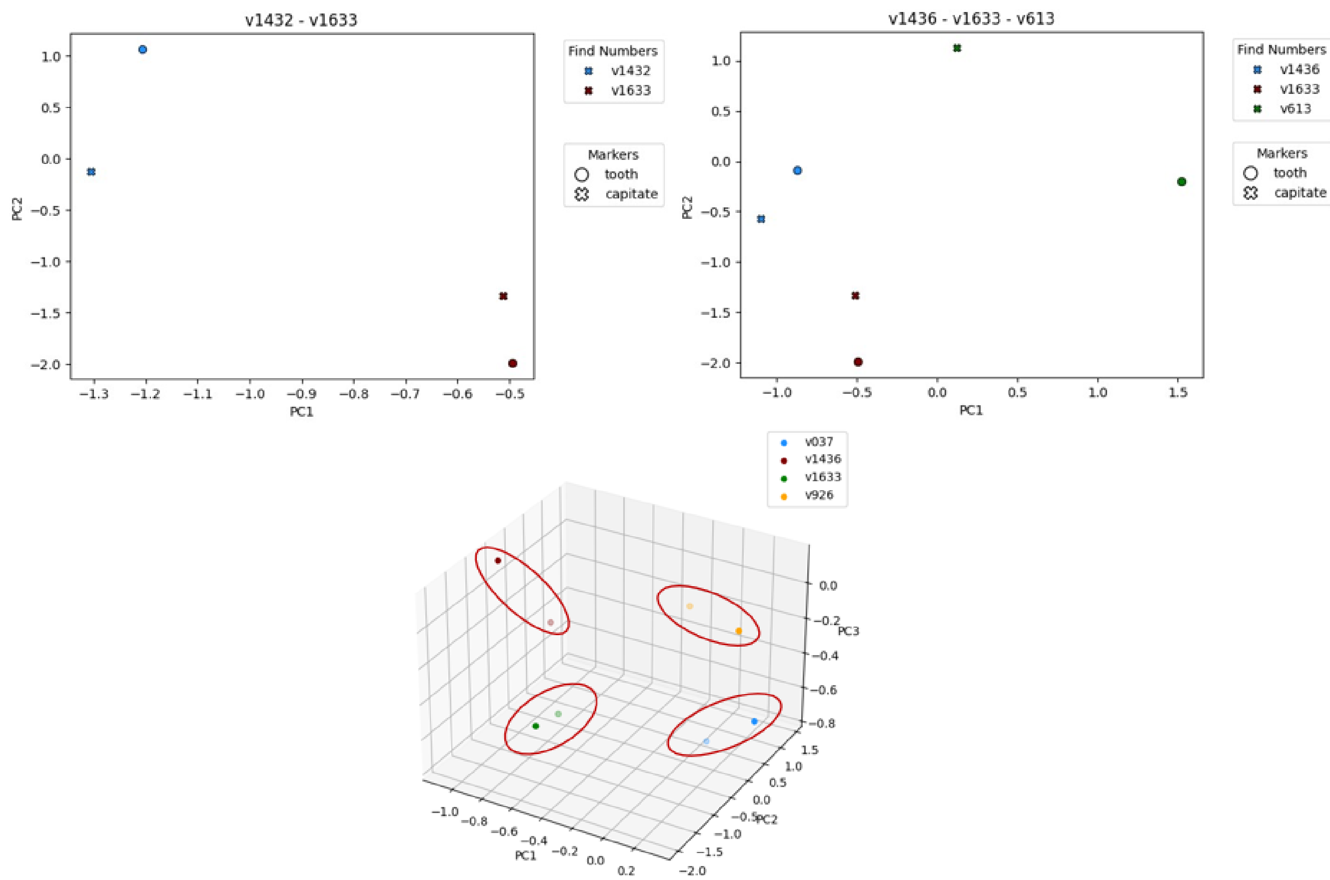


Figure 39. Some of the best cases of the attempts to reassociate teeth with capitates, testing groups of two, three, and four individuals.

The attempts at reassociation involved testing all possible combinations of two, three, and four skeletons, utilizing only the teeth and capitates. The results indicated ARI mean values of –0.01, .01, and .02 respectively. Despite the generally low mean values, some instances aligned with the true groupings. More specifically, 78 pairs, 49 cases of three-skeleton groups, and 9 cases of four-skeleton groups demonstrated ARI mean values of 1. Figure 39 illustrates three such instances. The visualizations from these best cases, depending on the principal component combinations, would allow the reassociation of the teeth with the capitates for most of the individuals. However, a considerable number of cases indicated random clustering or total disagreement with the true groupings, resulting in extremely low mean values. Consequently, no further analysis was pursued.

Chapter 5. Discussion

5.1 Introduction

The sorting of commingled remains serves as the first and crucial step in the examination and interpretation process of human skeletal remains. It has been previously stated that a proper analysis of the remains is unfeasible unless reassociation is performed (Byrd & LeGarde, 2014, p. 167). Since the 1940s, academics have recognized this and focused on developing and enhancing sorting methods. As these methods usually cannot stand alone and work better when combined with other sorting techniques, alongside the fact that each method, despite its assets, also comes with drawbacks, it is clear that further research is necessary. Over the last two decades, a few studies have focused on portable XRF devices and their application to this “puzzle” of commingling. Since this equipment was not originally designed for this purpose, further investigation is required to determine if and how it should be applied in this context. This study utilized one of the newest pXRF devices available to test its applicability on a sample of 40 skeletons from the post-medieval period buried in the Netherlands. The variation in elemental concentrations among different bones and the differences in elemental profiles among individuals were initially explored, followed by sorting attempts at mock commingling scenarios, aiming to address the primary question of this thesis. In this chapter, the previously presented findings will be discussed.

5.2 Elemental Differences Among Bones

To evaluate the effectiveness of pXRF in reassociating commingled archaeological remains, which serves as the central investigation in this thesis, the discussion needs to begin with the sub-questions. The first sub-question concerns the significance of intra-skeletal variation and the specific bones that could potentially exhibit differences. The importance of this question lies in the fact that for a successful sorting of intermixed remains, the differences in elemental concentrations in the bones of the same individual need to be smaller than those among individuals. While there are studies that have detected minimal intra-skeletal variation (see Perrone et al., 2014, p. 162), particularly in comparison to inter-skeletal variation, others have presented contradictory findings. More specifically, in 1988, Grupe (p. 124) commented on this topic, noting higher intra-skeletal variability when analyzing cremated human remains. Two years later, Francalacci (1990, pp. 226–227, 229) presented his study's results, highlighting the substantial variability within the bones of the same individual, and justified the notion that studies trying to make inferences about diet by analyzing single bones, cannot be reliable because of that.

In this study, significant variation was found within bones for 24 out of the 30 elements detected by the pXRF. Nine of these elements (Ni, Al, Ga, V, Y, Co, Rb, Zr, Cl) had "< LOD" entries in 20–87% of the total measurements. Particularly for elements like Cl, Co, Rb, and Zr, where the "< LOD" percentages exceeded 80%, the results should be interpreted with caution. As previously mentioned, to examine all chemical elements without reducing the sample size, a common practice was performed, involving the substitution of "< LOD" entries with half the limit of detection value. Consequently, certain elements displayed identical values across the majority of their entries. Inevitably, comparisons between pairs of skeletal elements sometimes revealed no variation. However, in cases where differences were observed, these discrepancies could be attributed either to significantly higher pXRF measurements compared to those after substitution or to genuine variation among the raw values. For instance, in the case of Cl (< LOD entries 81%), the highest detected values (0.3203–0.6519 %) significantly exceeded the substituted values (0.0032), potentially leading to statistically significant results. However, even without considering the substituted values, a notable difference persisted. Although the highest value originated from a pubic bone, overall, higher values were predominantly associated with tooth measurements, supporting a probable "true" variation. A similar trend was noticed with Zr and Rb, with the highest values mostly observed in measurements from the calcanei and lumbar vertebrae. Nevertheless, for this study, the results obtained from elements having zero to up to 7% of their values replaced are considered more reliable.

To further investigate intra-skeletal variation, all 66 possible pairs of skeletal elements were examined. As indicated by the results, only certain pairs did not show statistically significant variation: humerus–femur, rib–mandible, tibia–parietal, and tibia–rib. Among the six pairs that displayed variation in only one element, in five of those, Zn was the differing element. In pairs with differences in two or three elements, Ca and P were frequently involved. A noticeable pattern emerged among pairs, showing variation in 11–17 out of the 30 elements, as most of them involved teeth. Pairs that varied in 15–17 elements exclusively included teeth. This observation aligns with the understanding that while bones and teeth share a similar chemical composition (Zimmerman et al., 2015, p. 132), they are composed of different materials. Therefore, even though they contain the same elements, their concentrations may differ (Castro et al., 2010, p. 18). Moreover, the enamel does not undergo remodeling. Thus, the elemental composition found in teeth mirrors the period of their formation (Perrone et al., 2014, p. 148), whereas bones provide insight into an individual's life during the past decade. This discrepancy in timelines might also contribute to the observed differences.

Regarding which pairs of bones exhibited differences systematically, no specific pattern was observed. One hypothesis could be that differences based on the bone's location in the skeleton—such as between upper or lower limb bones—could manifest. However, the findings revealed that pairs involving for example the humeri, radii, and capitates displayed significant variations in multiple elements, similar to pairs involving the tibias, femora, and calcanei. The pair of bones that exhibited variation across the most chemical elements was the lumbar–femur pair. This could be interpreted not by the location of bones in the skeleton, but by the type and the way these bones are structured. The femoral measurements were taken on the midshaft, which is characterized by dense cortical bone. In contrast, the lumbar vertebrae were scanned on the superior aspect of their body, where the cortical bone is notably thinner and encloses trabecular bone. As the pXRF equipment penetrates a few millimeters into the material under analysis, it is possible that the scan of the lumbar vertebrae captured the trabecular bone instead of the cortical. These bone types have a different turnover rate. Since the incorporation of elements is achieved during the remodeling, and the trabecular bone remodels faster (Coyte et al., 2022, pp. 2–3), the concentrations of the chemical elements could vary depending on the bone type. Noteworthy is the fact that concentration variances are not only observed between different bone types but also within the same type due to differences in thickness. Skeletal elements characterized by thin cortical bone experience a faster turnover than those with denser bone of a similar type (Perrone et al., 2014, p. 153). In 2004, Carvalho et al. (p. 1256), while exploring the distribution of specific elements in human bones, observed that the concentrations in the same bone were different, depending on whether the measurements were taken from the trabecular, the inner, or outer layer of the cortical bone. Figure 40 depicts intra-skeletal variation as presented by Rasmussen et al. (2013, p. 9), exhibiting not only differences among bones, but also within the same bone depending on the location. Additionally, skeletal elements with a thicker layer of compact bone are less influenced by external factors (Allmäe et al., 2012, p. 31), compared to those with a thin layer or consisting mostly of spongy bone which are more susceptible to diagenesis. While these observations could partially explain the variation in elemental concentration between the lumbar and femur, or the absence of variation in pairs such as the rib–mandible—both composed of thin layers of cortical bone enclosing trabecular bone—they could not apply to every pair. For example, in the case of the tibia–rib pair, where the cortical bone scanned on the tibias is thicker than that on the ribs, no differences were observed.

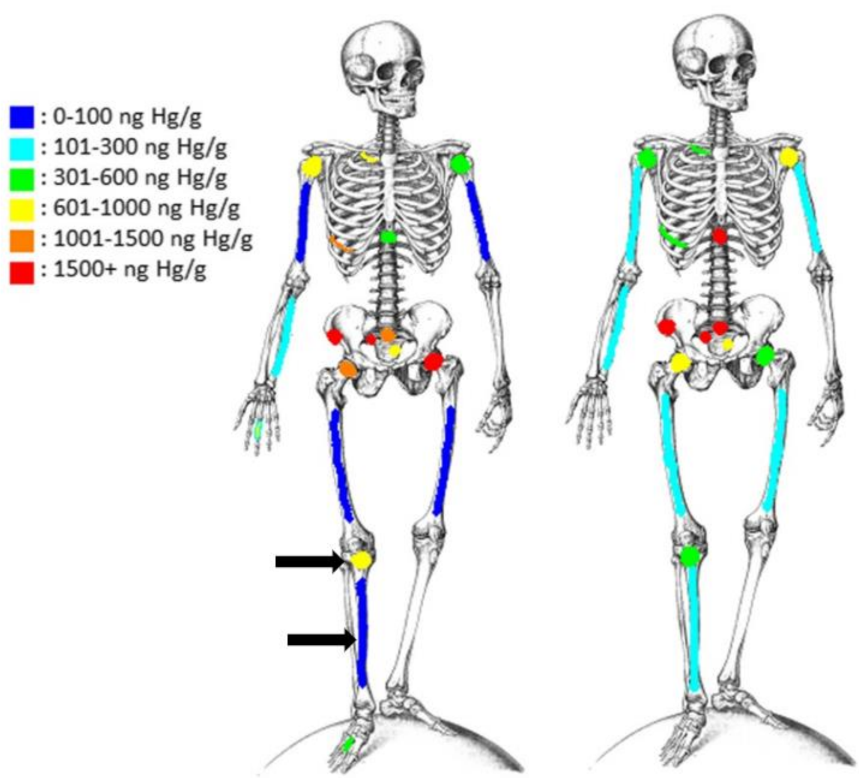


Figure 40. Intra-skeletal variation of mercury (Hg) concentrations. Although Hg was not detected by the pXRF used in this thesis, the figure illustrates the variations in element concentrations not only within the same skeleton but also within the same bone. The black arrows indicate higher Hg values at the proximal end of the tibia and lower values at the midshaft. Additionally, inter-skeletal variation between the two skeletons from medieval Denmark is observed (adapted from Rasmussen et al., 2013, p. 9).

According to the results, certain chemical elements (Ca, P, S, Zn, and Pb) were frequently associated with pairs demonstrating intra-skeletal variation. Specifically, Ca and P, which are main components of bones, were expected to exhibit minimal variation. However, the discrepancies observed in their concentrations could be partly attributed to elemental substitution. For instance, elements like Sr, Pb, and F, often referred to as “bone seekers” (Iyengar & Tandon, 1999, p. 1), are known to substitute for Ca (Rasmussen et al., 2020a, p. 3; Swanston et al., 2012, p. 2411). Additionally, certain skeletal elements displayed higher values for specific chemical elements. The capitates consistently showed elevated values across numerous elements. While elemental concentrations can vary due to several factors, the capitate's higher values might also be influenced by the scanning process. X-rays commonly escape during scanning, especially if the material is thin, irregularly shaped, or not in close contact with the equipment. As the capitates were measured under a lid, preventing the escape of radiation, these measurements might more accurately reflect the “true” concentrations. In contrast, the skeletal elements consistently displayed lower values across multiple elements, were the teeth. Previous research revealed that teeth showed lower values for specific elements (Al, Fe, Sr, S, Ba, Zn) and higher values only for Cl (Kyle, 1986, p. 403). This thesis aligns with part of Kyle’s findings, as it indeed detected lower values for the first four elements, alongside Si, K, Ca, Ti, Mn, Ni, V, Co, and Rb in teeth. Consistently, both studies concluded that teeth exhibited the highest concentration of Cl.

Overall, the findings on intra-skeletal variation revealed statistically significant differences among bones for numerous elements. The distribution of chemical elements in human skeletons discovered in archaeological contexts could be influenced by various factors. According to Zimmerman et al. (2015, p. 132) these factors include the location of the bones within the skeleton, the exact areas on the bones that are being examined, the bone type, the dietary habits of the individuals, and diagenesis. Thus, to better understand intra-skeletal variation, additional information on these factors is needed.

5.3 Elemental Differences Among Individuals

The subsequent sub-question tackled the significance of inter-skeletal variation following the exploration of intra-skeletal differences. Out of the 30 detected elements, 23 exhibited statistical differences across individuals. Notably, among the seven elements showing no variation, four (Se, Nb, Mo, and Ba) also lacked intra-skeletal differences. However, the remaining three elements (Co, Zn, and Zr), while not differing among individuals, had previously shown variation among bones. An element that did not exhibit variation and is related to diet, is Zn. Zn concentrations in the bones are associated with meat consumption, including fish (Allmäe et al., 2012, p. 28). Increased consumption of these foods leads to a higher Zn content in the skeleton. The fact that the results showed no variation, might imply consistent meat-related dietary habits within the 780 pairs of individuals examined.

Among the elements showing differences, Mg varied only in seven cases involving nine individuals. One individual was involved in six pairs, while another appeared in two. Both were males and categorized in the "36–49" age group. Noteworthy is that in all pairs with variation, one individual was from Arnhem and the second from Middenbeemster. Mg is abundant in the human body and has an important role in over 300 metabolic processes (Volpe, 2013, p. 378S). For this reason, the body usually maintains its concentration stable and within a specific range (de Baaij, 2012, p. i15). Since it is homeostatically regulated, no significant differences among individuals would be expected. However, variations in Mg concentrations within skeletons might still occur due to several factors. Two of these are variations in dietary intake and diseases, where low concentrations have been linked to several conditions such as diabetes mellitus (de Baaij, 2012, p. i15, Volpe, 2013, p. 378S). Two other elements that are homeostatically regulated and have a crucial role in the human body are Na and K (Pohl et al., 2013, p. 31). No significant variation would also be expected here. The results after the Bonferroni correction indicated no differences within the pairs for Na, while K exhibited variation. Perrone et al. (2014, p. 154) similarly found no statistically significant variation among individuals in Na levels. Despite being regulated by the body, abnormal Na and K levels can occur, often linked to diet or pathological conditions like hyponatremia and hyperkalemia (Pohl et al., 2013, pp. 38, 43).

The elements that displayed variation across a larger number of pairs compared to others were As and Pb. As, an omnipresent and toxic element, can contaminate soil and water, accumulating in plants and animals, and consequently, in humans. It can also enter the body through inhaling metal-laden air, and by using utensils or tools containing this metal (Özdemir et al., 2010, p. 1033). Previous studies have indicated that the concentration of As in modern bones, which have not been buried, averages around 2 µg g^{-1} (0.0002 %) (Rasmussen et al., 2020b, p. 14). Additionally, variations between sexes have been observed, with males exhibiting higher values than females (Özdemir et al., 2010, p. 1037). The findings of this thesis revealed variation within individuals for 123 out of 780 pairs. The measured values across skeletal elements ranged from 0.0006 to 0.1112 %. Among these, the 75 measurements with the lowest values (0.0006–0.0026 %) were primarily from Arnhem, followed by 69 measurements (mostly from Arnhem) in the range of 0.0026–0.0038 %. Furthermore, 299 measurements (predominantly from Middenbeemster) fell within the range of 0.0038–0.0149 %. The highest values, ranging from 0.0150–0.0782 %, were observed in 33 measurements, mainly from skeletal elements buried in Middenbeemster (only four were from Arnhem) and were found in males. However, one exception was a tibia from a female individual (v1655) buried in Arnhem, exhibiting the highest value (0.1112 %). No specific pattern was observed across different age categories for As. Overall, higher-value measurements for As were primarily derived from skeletal elements buried in Middenbeemster, primarily in males, except for one instance involving a female (v1655). The measurements obtained by the pXRF often exceeded those mentioned by Rasmussen et al. (2020b, p. 14). This discrepancy could be attributed to As exposure during life or to diagenesis.

The second element showing variation for a substantial number of pairs was Pb. Pb, like As, is not an essential element for the human body and is, in fact, harmful. It has been suggested that this element accumulates over time when exposed to it, resulting in older individuals exhibiting higher concentrations compared to younger ones (Allmäe et al., 2012, p. 30; Rasmussen et al., 2015, p. 366). During the medieval period, Pb was omnipresent, and the accumulation of the element in people’s bodies was not uncommon. It could be found in water due to leaching from the pipelines (Allmäe et al., 2012, p. 30), and in a variety of objects including coins, and glazed culinary vessels (Rasmussen et al., 2015, p. 366). Considering that unglazed vessels were typically more affordable and accessible to individuals of lower socioeconomic status, coupled with higher pollution levels in medieval urban areas, it is inferred that those with higher socioeconomic status residing in urban areas had greater exposure to lead compared to individuals of lower classes or those living in rural settings. The results of this thesis revealed inter-skeletal variation for 117 out of 780 pairs, while the Pb concentrations in the individuals examined ranged from 0.0010 to 0.3101 %. Notably, the tibia of the aforementioned individual, v1655, exhibited the highest value. Apart from that, the other 20 skeletal elements showing higher values (0.0525–0.2238 %) belonged to individuals buried in Middenbeemster, a rural site. Within the skeletons exhibiting these values, no specific patterns were observed regarding different sex and age categories.

Apart from the elements previously discussed, which showed variation in a larger number of pairs, several other elements displayed inter-skeletal variation, but within a smaller subset (1–59 out of 780 pairs). It is important to note that the cautious interpretation of results, particularly concerning elements with numerous "< LOD" entries that were replaced, as discussed earlier in the context of intra-skeletal variation, is also relevant here. In inter-skeletal variation, this applies specifically to Rb, Th, Cr, and Cl, all of which demonstrated differences.

After examining the elements detected by the pXRF, the inter-skeletal variation in specific ratios was explored. The first ratios examined were Sr/Ca, Sr/Pb, Pb/Ca, K/Fe, and Zn/Fe. These ratios have been used in previous studies to sort commingled remains (see Gonzalez-Rodriguez, & Fowler, 2013, p. 407.e3). Similar to Zn, which was discussed earlier, Sr is frequently analyzed to understand the dietary habits of individuals and populations, offering valuable insights into plant and marine resource consumption (Allmäe et al., 2012, p. 29). The Sr/Ca ratio, commonly used to assess dietary patterns (Gonzalez-Rodriguez & Fowler, 2013, p. 407.e3), could also vary among individuals if differences existed in their dietary behaviors. As previously mentioned, Sr and Pb have both been described as "bone seekers" (Iyengar & Tandon, 1999, p. 1), since they can incorporate into the bones easily, and can potentially replace Ca. Therefore, Sr/Pb could vary in individuals depending on the environment they were living in. Regarding Pb/Ca, it is considered to reflect the impact of lead integration into the bones. Finally, Gonzalez-Rodriguez & Fowler (2013, p. 407.e3) highlighted the potential significance of Zn/Fe and K/Fe, relating to metabolic processes and health status, respectively, suggesting the possibility of variation among individuals.

Other ratios explored in this thesis were Mn/S, Ba/Cl, Mn/K, previously associated with diseases, specifically syphilis, and Zn/Cl, Ba/Sr, Mn/Fe, S/Sr, which have been linked to neoplasms (Kilburn et al., 2021, p. 1). Regarding Ba/Sr, studies also suggest its link to diet (Allmäe et al., 2012, p. 30). Finally, two more ratios explored were Zn/Ca, which has been tied to dietary habits (Al-Shorman, 2010, p. 202), and Ca/P, which is considered a tool for assessing bone deterioration induced by medical conditions (López-Costas et al., 2016, p. 45). Noteworthy is that the aforementioned ratios could also result from diagenesis. For the reasons mentioned so far, these 14 ratios were explored in the context of inter-skeletal variation.

The results indicated that from the 14 ratios, only Zn/Ca did not exhibit statistically significant differences. Sr/Pb varied in 83 out of 780 two-skeleton groups, and Pb/Ca in 81. The remaining ratios displayed differences among four to 64 pairs of individuals. Generally, Sr/Pb values ranged between 0.05 and 7.49, with the highest value of 26.40 observed in a calcaneus from a female individual that was buried in Middenbeemster. Apart from this extreme case, the next 20 measurements with high values originated from skeletal elements unearthed in Arnhem. Regarding Pb/Ca, while no variation was visually apparent in the dataset (values ranged between 0.00 and 0.02), statistical analysis revealed significant variation (values ranged between 0.000080 and 0.015187). The variation among pairs of skeletons concerning these two ratios could be interpreted as environmental differences affecting the individuals and their exposure to Pb.

5.3.1 Sex, Age Categories, and Sites

To address the final sub-questions concerning factors potentially influencing inter-skeletal variation, the analysis further explored disparities between sex categories, among different age groups, and between sites . A previous study, exploring elements such as Ca, Fe, K, Mn, and S, found no discernible differences between males and females (Davis, 2021, pp. 38–42). In contrast, this thesis concentrated on ratios for these comparisons, revealing statistically significant variations between sex categories for two out of the 14 ratios: Sr/Ca and Ca/P. The variance in Sr/Ca, linked to diet, might signify differences in food resources and the distinct incorporation of these elements in males and females. On the other hand, Ca/P variations could indicate specific pathological conditions affecting one sex category more than the other. Despite only two ratios displaying variation, trends emerged regarding which sex category exhibited higher or lower values. More specifically, males tended to demonstrate higher values across eight ratios, while females exhibited higher values in six. Notably, for Sr/Ca and Ca/P, which showed variation, both ratios were higher in males.

When examining various age groups, the results revealed statistically significant variations in 11 out of the 14 ratios. The Ba/Sr ratio exhibited differences in more than half of the pairs. However, one crucial aspect that requires discussion is the distribution within each category. As previously presented, the age groups "18+" and "36–50" consisted of only one individual each, whereas the "50+" category encompassed four individuals (Table 2a). Consequently, caution is necessary in interpreting the results, as generalizations might not be valid in this context. Despite this limitation, the findings did indicate that individuals in the "50+" category were involved in 15 pairs across four ratios. Notably, in all these pairs, the "50+" group consistently exhibited lower values for each ratio, except in the case of K/Fe. Variances in bone concentrations among older individuals could potentially be attributed to the tendency of some elements to accumulate over time, but also to the presence of more porous skeletal elements in these individuals, affected by diseases. As bones age, they tend to develop increased porosity. When compared to younger individuals, differences in concentrations could be expected.

Some variation had already been observed in the comparisons between pairs of skeletons from both sites. To delve deeper, initially, the ratios and the raw values of the chemical elements were examined. Eleven out of the 14 ratios exhibited statistically significant differences. On average, Arnhem tended to show higher values in more ratios than Middenbeemster. However, this trend shifted when analyzing the chemical elements directly. Out of the 30 detected elements, 21 displayed higher concentrations in individuals buried in Middenbeemster. Notably, one of those elements was Pb. Generally, there is a notion that individuals residing in rural areas had lower exposure to Pb (Rasmussen et al., 2015, p. 366). A study in 1987 (Drasch et al., 1987, p. 310) reported no significant variation in Pb concentrations between rural and urban sites, attributing this to Pb levels in bones reflecting dietary intake rather than environmental pollution. However, the findings in this thesis revealed higher Pb concentrations in skeletons from the rural site, Middenbeemster. This might be explained by exposure through handling everyday objects like glazed vessels, occupational handling of tools, or, as mentioned by Drasch et al. (1987, p. 310), through differences in food resources. Overall, the results comparing different sites provide useful information. While scenarios involving the mixing of skeletons from different locations on-site are improbable, the information presented here could be useful in laboratory commingling. The findings show varying concentrations that could either reflect intake during the individuals' lives or diagenetic processes.

5.4 Reassociation Attempts

The final step in order to answer the main question of this thesis concerning the effectiveness of the pXRF method in sorting commingled archaeological remains was to attempt the reassociation of the individuals. Table 5 presents all the sorting attempts, including the necessary information for each one. The first attempt involved the sorting of two individuals using all the elements detected by the pXRF, by examining every possible two-individual combination. The results were not optimal with an ARI mean value of .05, and four pairs with a relatively high value. In these four cases, two individuals, v233 and v794 appeared twice, while in all cases except one, the individuals originated from different sites. To optimize the situation, it was decided for all the elements with "< LOD" greater than 7 % to be removed. The purpose of this was to eliminate the possibility of overlapping values due to substitution. One of these elements was Se, having "< LOD" entries for 95 % of the total measurements. Interestingly, while this element did not exhibit statistically significant inter-skeletal variation (p = .227) in a direct comparison, it appeared as a contributor to the PC1. This could be due to the fact that, while in direct comparisons, Se concentrations were not strong enough to reach statistical significance, in the multivariate space they were influential in capturing the variance present among individuals across multiple elements. Nevertheless, all the elements with "< LOD" greater than 7 % were excluded.

Table 5. All the sorting attempts conducted in this study. The table includes the number of individuals in each attempt, the source creating the principal components, the mean ARI value for each attempt, the maximum ARI values along with the number of cases exhibiting it, and lastly, the total number of possible combinations tested for each attempt.

Number of Individuals	Principal Component Sources	ARI Mean	ARI of Best Cases	Number of Cases with the Highest Value	Number of All Possible Combinations Tested
2	All 30 elements that were detected	.05	.68	4	780
2	Filtered elements (15)	.06	.83	1	780
2	Filtered ratios (11)	.09	1	6	780
2	Filtered ratios, excluding tooth measurements	.19	1	19	780
3	Filtered ratios, excluding tooth measurements	.18	.91	1	9,880
4	Filtered ratios, excluding tooth measurements	.17	.73	1	91,390
5	Filtered ratios, excluding tooth measurements	.15	.60	1	658,008

Following this removal, the results were improved. Six pairs achieved an ARI value of .68, previously considered the best-case value, while one pair reached .83. Among these seven pairs, three individuals (v233, v561, and v926) appeared more than once. Moreover, even though the best case involved individuals from the same site (Middenbeemster), five out of the seven pairs included individuals from different sites (Table 6). Additionally, five of the pairs consisted of one male and one female. Regarding the visualizations, even for the best case, the skeletal elements were scattered, and no distinct clustering could be observed.

Table 6. The best cases from the second sorting attempt, using filtered elements. The individuals are indicated by a unique identifier (their find number). The table provides details on the location, age, and sex category of the two individuals involved in each pair, along with the mean ARI value for each case. Individuals appearing more than once, are highlighted.

ARI	Find Number 1	Location 1	Age 1	Sex 1	Find Number 2	Location 2	Age 2	Sex 2
0,8330704	v233	Middenbeemster	36-49	F	v561	Middenbeemster	26-35	M
0,6809584	v233	Middenbeemster	36-49	F	v926	Middenbeemster	36-49	M
0,6805556	v1299	Arnhem	36-49	M	v926	Middenbeemster	36-49	M
0,6805556	v1436	Arnhem	36-49	F	v561	Middenbeemster	26-35	M
0,6805556	v1530	Arnhem	18-25	F	v794	Middenbeemster	26-35	M
0,6805556	v1633	Arnhem	36-49	F	v452	Middenbeemster	50+	M
0,6805556	v1561	Arnhem	36-49	M	v561	Middenbeemster	26-35	M

To further optimize the sorting process, 11 out of the 14 ratios were utilized. Gonzalez-Rodriguez & Fowler (2013, p. 407.e3) had previously highlighted the importance of using ratios over raw chemical values as they reduce background noise and normalize the data. The ratios that were excluded, were those comprised of elements with "< LOD" entries above 7 % (i.e., Ba/Cl, Ba/Sr, Zn/Cl). This adjustment resulted in an overall improvement, indicated by an increased mean, and maximum ARI value, and a higher number of segregated cases. All 14 pairs that exhibited values above .82, indicating a relatively successful segregation, involved individuals from different sites. For these cases, no specific pattern was observed regarding different age and sex categories. Notably, one specific individual, a young adult (18–25) from Arnhem (v1530), appeared in five of these pairs. An evident correlation emerged where more successful segregations coincided with an increase in pairs featuring the same individuals. While observing the visualizations of these cases, most of the time the combination of PC2 and PC4, or PC1 and PC4 would result in distinct clusters. Nevertheless, skeletal elements such as the mandibles or the calcanei, were sometimes positioned farther away from the clusters, while tooth measurements consistently appeared on the outskirts. As previously observed, teeth exhibited notable differences compared to various skeletal elements, likely due to being composed of a different material. Upon the removal of these data points, the results showed further improvement. Out of the 780 possible combinations, 52 resulted in successful clustering, with 19 achieving an ARI value of 1, marking these attempts as the best sorting cases. The ratios contributing the most to the principal components whose combination usually resulted in more visually distinguishable clusters, were Mn/K, Mn/S, Mn/Fe, and

K/Fe (for PC1), Sr/Ca, Ca/P, Pb/Ca, and S/Sr (for PC2), and again Pb/Ca with S/Sr (for PC4), along with Sr/Pb. Once again, in almost all pairs, the individuals originated from different sites. However, with an increasing number of cases, no distinct patterns emerged concerning different sex and age categories. Many individuals appeared in multiple pairs, notably v1754, appearing in 10 different cases. After this point, instead of improvement, a gradual deterioration in results was observed (see Table 5).

When attempts with three individuals were performed, the ARI mean value slightly decreased. Out of the 9,880 possible three-skeleton combinations, nine cases exhibited results indicating relatively successful segregation. With the addition of one more individual to the two-skeleton groups, the overlapping values increased, resulting in less distinct clusters. Nevertheless, among the groups showing clustering patterns, individuals were mostly distinguishable, although some skeletal elements appeared misplaced or positioned at a distance from the clusters. Specifically, the capitates, mandibles, and calcanei were frequently positioned away from the clusters. In instances with higher ARI values, a common combination involved the individuals v1754 and v282, along with a third skeleton. When attempting to sort four individuals, a further decrease in the ARI mean value, as well as the maximum value, was observed. In the best cases, only two out of the four individuals could be distinguished. The segregation attempts concluded with five individuals due to decreased sorting accuracy compared to the initial attempts with raw elements. In the best cases at this stage, only one skeleton could be distinguished.

Regarding the tooth measurements, differences were observed during the intra-skeletal comparisons with several bones, and for numerous chemical elements. Among the bones examined, the capitate exhibited the least variation. As expected during the reassociation attempts of these two skeletal elements, the mean ARI values were notably low due to attempting to cluster different materials. However, several groups, especially those involving measurements of two or three individuals, showed high rates of successful clustering, providing positive results when a small number of skeletons were involved. This finding could prove valuable in studies where, after segregating and assigning the capitates to a skeleton, loose teeth, which could not otherwise be allocated to an individual (e.g., in cases where the maxillae and mandible are missing), could be sorted by forming groups with these capitates.

Previous studies using the pXRF method on commingled remains have yielded varied results. Gonzalez-Rodriguez & Fowler (2013, p. 407.e5) presented findings that indicated accurate sorting for scenarios including up to four individuals. A year later, Perrone et al. (2014, p. 161), using 20 individuals, noted the method's potential while underscoring the problem of dealing with skeletal remains with overlapping values. It has been suggested that as the number of individuals increases, segregation becomes less accurate or feasible (Perrone et al., 2014, p. 161). However, this statement does not imply that scenarios involving a small number of individuals guarantee successful sorting. This notion was supported by Winburn et al. (2017, p. 31), who experienced the unsuccessful sorting of two individuals.

The findings of this thesis revealed that the most accurate sorting occurred when dealing with pairs of individuals, utilizing ratios instead of raw chemical element values, and excluding tooth measurements. Testing all possible pairs provided substantial evidence that a small number of individuals does not guarantee successful reassociation. Out of 780 possible pairs, 52 were accurately sorted. These accurately reassociated cases often involved individuals from different sites and sex categories, although noteworthy instances included skeletons from the same site. As already mentioned, the combinations of principal components that exhibited more discrimination power were PC1 with PC2, and PC2 with PC4. The ratios that contributed the most to the PC3, which was not ideal for accurate visual discrimination, were Zn/Ca and Zn/Fe. When exploring the inter-skeletal variation, Zn/Ca exhibited no differences between pairs of individuals, age groups, sex groups, or sites. Similarly, Zn/Fe displayed no variation among different age groups after the Bonferroni correction. Despite their contribution to PC3, these ratios did not serve as effective markers for distinguishing individuals, and thus rendered this component unsuitable for accurate sorting.

Throughout the sorting attempts, certain individuals consistently appeared among the most accurately segregated cases. These individuals were v233, v282, and v613 from Middenbeemster, and v1530, v1754, v1561, v1659, and v1633 from Arnhem. Five of these skeletons were female individuals. Almost all of them fell into the age category “36–49” and were diagnosed with osteoarthritis and conditions affecting the vertebrae, such as Schmorl’s nodes. Notably, these conditions were commonly observed in individuals from these sites dating back to the post-medieval period. While these conditions were prevalent, if they indeed influenced the chemical profile of these individuals to the extent that it rendered them distinct from other skeletons, further research would be necessary to uncover the underlying reasons for their differences.

5.5 Limitations and Considerations

In this section, the factors potentially influencing the measurements and results of this study when employing the pXRF method on skeletal remains will be addressed. The first aspect relates to the equipment used on the bones. As aforementioned, optimal measurements by the pXRF require the samples to be flat (Liritzis & Zacharias, 2011, p. 132), and to have a thickness of a few millimeters (Shackley, 2018, p. 3). Failure to meet these conditions can result in escaped X-ray radiation, leading to inaccurate measurements. Inconsistencies in measurements could have an impact on both intra-skeletal and inter-skeletal comparisons, affecting the accurate detection of differences and clustering attempts. In such cases, referring to previous studies that explored chemical variation in human skeletal remains using diverse methods would be beneficial.

Throughout this study, a radiation detector was consistently present during scanning. Given that several skeletal elements are irregular and relatively thin, despite correct bone positioning and distance from the equipment, instances occurred where the detector registered X-ray escape. This occurrence was observed sporadically on specific bones such as the rib, lumbar vertebra, calcaneus, and mandible. These bones, primarily composed of thin cortical bone, exhibited variability in the flatness of their measured surface, particularly the ramus of the mandible and the body of the L3, which could be slightly compressed or curved. While previous studies have observed intra-skeletal and inter-skeletal chemical variation, the precision of measurements from certain skeletal elements cannot be definitively confirmed.

Another important consideration pertains to interpreting the results following statistical analysis. Most of the analysis was performed using the IBM SPSS software, which often includes correction choices like the Bonferroni correction for post hoc multiple comparisons. When a considerable number of tests run simultaneously, this can lead to Type I errors, detecting differences and rejecting the null hypotheses, when in fact there is no variation (Abdi, 2007, p. 1). While the

Bonferroni correction controls Type I errors, it tends to be conservative, potentially increasing the Type II error rate by failing to identify true differences (Lee & Lee, 2018, p. 357). In general, it is recommended to rely on the adjusted *p*-values, but in some cases, consideration of both raw and adjusted *p*-values is advised. The results from post hoc tests in this study revealed such discrepancies. During inter-skeletal comparisons, instances occurred where the Kruskal-Wallis test detected variation, yet the post hoc test with the Bonferroni correction showed no differences within the tested pairs (e.g., for Na). In other cases, both the raw and adjusted *p*-values indicated differences, but the number of pairs exhibiting variation differed (e.g., for Al, the raw *p*-values showed differences in 264 pairs, while the adjusted in 9). Similar patterns were observed across various elements and ratios. The differences indicated by the raw *p*-values might arise due to chance or the detected variation by Kruskal-Wallis not being significant enough after the adjustment. Nevertheless, in such cases, the possibility of a Type II error after the correction cannot be ruled out, and for this reason such discrepancies should be considered.

The final factor for discussion is diagenesis, a phenomenon frequently mentioned in this thesis during discussions of previous studies and result interpretations. Diagenesis characterizes the changes in chemical composition that occur post-mortem within buried bones (Guimarães et al., 2016, p. 108), and it can be influenced by several conditions. Even though only the cortical bone of the skeletal elements was scanned, which is less susceptible to diagenesis (Grupe, 1988, p. 124; Rasmussen et al., 2017, p. 91), potential post-mortem alterations might have affected the observed values. In archaeological bones, skeletal elements with greater porosity and thinner cortical bone might undergo more pronounced effects compared to those with denser cortical bone, leading to varying elemental concentrations and consequently displaying increased intra-skeletal variation. Studies have indicated chemical elements like Mn and Zr as potential indicators of diagenesis (Gonzalez-Rodriguez & Fowler, 2013, p. 407.e3), while others highlighted Al, Si, Rb, and Zr as elements typically found in soils rather than human bones (López-Costas et al., 2016, p. 48). In this thesis, the lumbar vertebrae and calcanei, consisting of thin cortical bone surrounding trabecular bone, exhibited the highest Zr (0.0018–0.0068 %) and Rb (0.0010–0.0020 %) values. However, the extreme values that were detected in the study conducted by Gonzalez-Rodriguez & Fowler (2013, p. 407.e3) were not observed here.

In terms of inter-skeletal variation, diagenesis can lead individuals buried in the same location for an extended period to showcase similar elemental concentrations. This might explain why distinguishing a significant number of individuals from the rest during the sorting attempts was challenging, or why the most accurately reassociated cases mainly involved individuals from different sites. However, there are cases where diagenesis can be advantageous. For example, in laboratory commingling scenarios where skeletons from various sites intermix, differences in elemental concentrations in the soil that affected the bones could help attribute skeletons to specific sites. Additionally, the presence of grave goods affecting bone concentrations could be beneficial. Metal objects near certain individuals might alter the elemental concentrations in their bones, making them distinguishable from others. Prior studies have highlighted the notable influence of diagenesis on elements such as Fe, Mn, Al, and As (Rasmussen et al., 2020b, p. 12). Pb is considered highly mobile in the geochemical environment, rendering it also susceptible to diagenesis (Rasmussen et al., 2015, p. 367).

Apart from the raw values of the chemical elements, several studies have employed specific ratios, such as Ca/P, to assess the presence of diagenetic factors. When this ratio is deviating from the reference value for uncontaminated bone, the skeletal remains are considered to have been subjected to diagenetic alterations. The ratio from an unchanged bone has been proposed to be 2.07–2.46 (Hancock et al., 1993, as cited in López-Costas et al., 2016, p. 48), 2.15 (Al-Shorman, 2010, p. 203), and 1.8–2.7 (Szostek et al., 2011, p. 101). In this thesis, Ca/P values for 20 skeletal elements fell within the range of 2.10–2.15, while 39 were lower, ranging from 1.69 to 2.05. Additionally, 416 measurements exceeded the reference value, ranging between 2.16 and 4.73. The highest 92 measurements were from individuals buried in Middenbeemster. Upon examining the Ca/P for each individual, the one closest to the reference value was found in an individual from Arnhem, who had 2.22. The rest of the skeletons had a value between 2.24 and 2.95, while the highest was 3.20. The Ca/P observations imply that most of the individuals have been subjected to some level of diagenesis, with those from Middenbeemster being affected the most. In turn, this could partially explain why the sorting attempts were challenging.

While the Ca/P ratios hinted at diagenetic changes, a more thorough examination would require comparisons with soil samples. When analyzing elemental concentrations in archaeological bones and drawing conclusions about intra-skeletal and inter-skeletal variations related to diet, diseases, and environmental conditions, it is essential to consider the concept of diagenesis in the interpretation.

Chapter 6. Conclusion

6.1 Addressing the Research Questions

The main purpose of this thesis was to assess the effectiveness of the pXRF method in sorting commingled remains, and more specifically, human skeletal remains from the post-medieval period in the Netherlands. To achieve this goal, the intra-skeletal and inter-skeletal elemental variation was explored, and sorting attempts were performed. In this section, the research questions will be answered starting from the sub-questions and concluding with the main question of this study.

Sub-question 1: *How significant is the intra-skeletal elemental variation, and among which skeletal elements is it observable?*

The analysis identified statistically significant variation in 24 out of the 30 elements detected by the pXRF equipment. These elements included Zn, K, Ca, Pb, S, Fe, P, As, Sr, Mg, Na, Ti, Mn, Si, Cu, Ni, Al, Ga, V, Y, Co, Rb, Zr, Cl. The remaining elements, Th, Ba, Mo, Nb, Cr, and Se, did not exhibit differences. Among the 66 possible pairs of skeletal elements, only the humerus–femur, rib–mandible, tibia–parietal, and tibia–rib did not demonstrate variation. Notably, the lumbar–femur pair exhibited variation across the most chemical elements. Additionally, the chemical elements that showed variation across a considerable number of pairs were Ca, P, S, Zn, and Pb. These findings revealed significant intra-skeletal differences, which can pose challenges when this variation surpasses that observed inter-skeletally.

Sub-question 2: *How significant are the differences in elemental concentrations among individuals, and how effectively can these variations assist with the sorting of commingled remains?*

The exploration of the inter-skeletal variation utilizing archaeological skeletal remains indicated statistically significant variation in 23 out of the 30 elements detected, and more specifically in K, Ca, Pb, S, Fe, P, As, Sr, Mg, Na, Ti, Mn, Si, Cu, Ni, Al, Ga, V, Y, Rb, Th, Cr, and Cl. The elements that did not exhibit variation were Co, Zn, Se, Zr, Nb, Mo, and Ba. The differences observed among individuals encompassed one less chemical element compared to the intra-skeletal differences.

As and Pb varied across a larger number of pairs, with Pb also demonstrating substantial intra-skeletal variability. Regarding the examined ratios, variations were evident in Zn/Fe, K/Fe, Sr/Ca, Pb/Ca, Sr/Pb, Ca/P, Zn/Ca, Mn/S, Mn/K, Ba/Cl, Ba/Sr, S/Sr, Mn/Fe, and Zn/Cl, except for Zn/Ca. The ratios that showed substantial variation across numerous pairs were Sr/Pb and Pb/Ca. Especially Pb/Ca was a predominant contributor during the principal component analysis, aiding in the sorting attempts. Overall, a notable inter-skeletal variation was observed, potentially influenced by dietary habits, possible pathological conditions affecting the individuals, environmental factors, or diagenetic processes resulting in variation between individuals from different sites.

Sub-question 3: *To what extent does the sex of the individuals affect their elemental profiles, and how does this factor contribute to the reassociation of the skeletal remains?*

Compared to previous studies that detected no variation between males and females (see Davis, 2021, pp. 38–42), the results from this thesis revealed significant differences in Sr/Ca and Ca/P. Worth mentioning is that Davis' study focused on skeletons from modern contexts, where the possibility of diagenetic alterations is lower compared to archaeological contexts. Even if the observed variation in this thesis is due to diagenesis, it remains valuable for the sorting procedure. Any distinct influence of diagenesis on the skeletal remains of males and females should be further investigated and utilized for reassociation purposes. In general, while variation was detected, the differences between males and females during the segregation process were useful only when two individuals were involved. During the sorting attempts encompassing all possible pairs, and utilizing principal components that derived from filtered ratios where the tooth measurements had been excluded, 59 % of the most accurately sorted cases involved pairs with individuals from different sex categories.

Sub-question 4: *How do different age categories influence the elemental concentrations in individuals, and how does this affect the sorting process?*

While exploring differences among age categories, no variation was observed only for Zn/Ca, Ba/Cl, and Zn/Cl ratios. It has been suggested that some elements tend to accumulate over time (Allmäe et al., 2012, p. 30), and the results of this thesis revealed lower values for specific ratios within the individuals of the “50+” age category. However, due to inadequate representation across all age groups, it is crucial not to generalize these findings. Further investigation is required to gain insights into the influence of age on inter-skeletal variation.

Sub-question 5: *How do the sites impact the elemental profiles of the individuals, and which elements should be prioritized in each case for reassociating the remains?*

No elements unique to one site over the other were identified. However, 23 out of the 30 elements detected, and 11 out of the 14 ratios, showed statistically significant differences between individuals from Arnhem and Middenbeemster. Interestingly, Middenbeemster, a rural area, demonstrated higher Pb concentrations. Overall, the variation in elemental concentrations between sites supported the reassociation process, with the most accurately sorted cases involving individuals from different locations. Nevertheless, the fact that some of the best cases also involved individuals from the same site, indicates that even though diagenesis may have affected the skeletal elements, the levels differ, enabling sorting within the same site.

Main research question: *How effective is the pXRF in aiding the reassociation of commingled archaeological remains from the post-medieval period in the Netherlands?*

Numerous reassociation attempts were performed concerning groups of two to five individuals, and all the possible combinations. The results revealed that the elemental concentrations detected by the pXRF can be used for the sorting of a number of individuals. The most accurate sorting attempts occurred when two individuals from different sites were involved, utilizing principal components derived from ratios, while the tooth measurements were excluded. Key ratios influencing the sorting process included Mn/K, Mn/S, Mn/Fe, K/Fe, Sr/Ca, Ca/P, Pb/Ca, S/Sr, and Sr/Pb, while the most useful principal component combinations were PC2 with PC4, and PC1 with PC4. However, the method encountered limitations when more than three individuals were involved, primarily due to overlapping values. Consequently, while demonstrating potential when a small number of individuals are involved, the method's outcomes lacked consistency. Unless refined, it should complement other sorting methodologies rather than stand alone.

By addressing these questions, this thesis adds to the topic of commingled remains, focusing specifically on the application of the pXRF method as a sorting technique. Regarding the intra-skeletal variation, this study not only identified the chemical elements exhibiting differences, but also pinpointed the exact pairs of skeletal elements involved. Concerning the inter-skeletal differences, it delved deeper, revealing specific influencing factors like sex and site. Moreover, by performing all possible combinations involving up to five skeletons, and examining at the same time different parameters, it uncovered the most successful sorting scenario. This study showed that under certain circumstances, the reassociation of skeletons from an archaeological context, and more specifically from the post-medieval period in the Netherlands, can be successful. However, more research is required in order to find the key behind the variations observed so the method can be optimized, and until then, it should only be used in complementary ways.

6.2 Future Research Recommendations

The findings presented in this paper offer a steppingstone for future investigations within the realm of commingled archaeological remains, particularly focusing on the pXRF method. While the technique displays promise, further studies should focus on its optimization for analyzing skeletal remains. Although Mudrock calibration, utilized in this thesis, is recommended for bone material analysis (Perrone et al., 2014, p. 150), it is not specifically tailored for bones. To build upon this work, future studies could aim to replicate the most successful sorting scenario using a bone-specific calibration matrix, while exploring all available features of the equipment, such as employing a vacuum to eliminate the air between the pXRF and the samples.

Further exploration into the influence of diagenetic alterations on elemental profiles stands as a crucial step forward. The ability to discern between the effects of diagenesis and inherent variations arising from factors like sex and age, could significantly enhance the understanding of elemental intra-skeletal and inter-skeletal variation. This would not only refine the interpretation of these differences but also hold the promise of refining the sorting process.

Given the substantial impact of intra-skeletal variation on sorting attempts, further research should prioritize understanding and controlling for this variation. In this thesis, the skeletal elements were only scanned once, at a location suitable for such analysis based on the equipment used. However, taking multiple measurements on the same bone, across various locations, and using an average value could prove beneficial in mitigating potential inaccuracies in measurements.

Finally, an interesting possible research direction could be the reassociation of teeth. Despite being composed of different materials, this study highlighted a close association between teeth and the capitates. Subsequent research could focus on reassociation attempts including capitates and more teeth from the same individual. Moreover, instead of solely measuring enamel, taking measurements also from the roots and then averaging values from the entire tooth could be a potential investigative approach.

The pXRF method holds great potential for osteoarchaeologists to segregate commingled remains. However, significant optimization is required before achieving full autonomy. Tailoring the method to suit the specific needs of skeletal remains and persisting in research on intra-skeletal and inter-skeletal variation, we move a step closer to solving the puzzle of commingled remains.

Abstract

The commingling of human skeletal remains poses a recurrent challenge for osteoarchaeologists. Prior to any comprehensive analysis, the reassociation of these remains is required. Over the past seven decades, numerous methodologies have emerged to address this challenge. However, each method exhibits limitations, prompting the necessity for the advancement of current techniques and the introduction of novel approaches. Among these evolving methods, the portable X-ray fluorescence technique stands out. Originally not designed for application on human remains, its appeal lies in its non-destructive nature and cost-effectiveness, drawing keen interest from osteoarchaeologists and forensic anthropologists.

This study aims to evaluate the effectiveness of portable X-ray fluorescence in sorting commingled archaeological remains buried in the Netherlands. It involves the examination of 40 adults, 20 unearthed from Arnhem and 20 from Middenbeemster, dating back to the post-medieval period. The research initiates by investigating both intra-skeletal and inter-skeletal chemical variation, further exploring the potential factors contributing to these differences. Throughout the analysis, multiple sorting attempts are conducted to ensure a thorough exploration of the technique's capabilities. The results indicate statistically significant chemical variation within the same skeleton and among different individuals. In terms of intra-skeletal variation, from the 12 skeletal elements tested in each individual, only four pairs of bones (humerus–femur, rib–mandible, tibia–parietal, and tibia–rib) did not exhibit variation in the concentrations of chemical elements. With regard to inter-skeletal variation, 23 out of the 30 elements detected by the pXRF, and 14 out of the 15 ratios explored, demonstrated significant differences. Variation was also observed between males and females, as well as between sites. For the differences among the adult sub-groups that were noted, no generalizations can be made unless further research is conducted. Finally, the reassociation attempts reveal that the most successful sorting cases involved two individuals from different sites, utilizing principal components derived from ratios, while the tooth measurements were excluded. Key ratios influencing the sorting process included Mn/K, Mn/S, Mn/Fe, K/Fe, Sr/Ca, Ca/P, Pb/Ca, S/Sr, and Sr/Pb. While the method showed promise when a small number of individuals were involved, it lacked consistency. Therefore, it should be used in conjunction with other methods until further research is conducted.

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Appendix A

Table 7. Intra-skeletal variation. All 66 possible pairs of skeletal elements, and the number of chemical elements for which statistically significant variation was detected. For example, the pairs rib–humerus and radius–parietal showed variation for four out of the 30 elements detected by the pXRF.

Skeletal element pairs	**Occurrences of chemical variations**
humerus–femur, tibia–parietal, tibia–rib, rib–mandible	0/30
radius–mandible, pubis–mandible, tibia–humerus, tibia–radius, tibia–femur, parietal–femur, parietal–humerus, rib–radius, lumbar–calcaneus	1/30
rib–parietal, radius–femur, radius–humerus	2/30
tibia–mandible, rib–pubis	3/30
rib–humerus, radius–parietal	4/30
rib–femur, mandible–humerus, radius–pubis, pubis–capitate, humerus–capitate	5/30
radius–capitate, mandible–calcaneus, mandible–capitate, rib–calcaneus, tibia–capitate, tibia–pubis, parietal–mandible, parietal–capitate	6/30

rib–capitate, mandible–femur, mandible–lumbar, pubis–calcaneus, pubis–parietal, pubis–lumbar, lumbar–capitate	7/30
humerus–calcaneus, parietal–calcaneus, radius–calcaneus, tibia–lumbar, tibia–calcaneus, femur–capitate	8/30
capitate–calcaneus, pubis–humerus	9/30
femur–calcaneus	10/30
lumbar–humerus, rib–lumbar, parietal–lumbar, radius–lumbar, tooth–capitate	11/30
pubis–femur	12/30
tooth–mandible, tooth–lumbar	13/30
tooth–calcaneus, lumbar–femur	14/30
tooth–rib, tooth–radius, tooth–pubis	15/30
tooth–tibia, tooth–femur	16/30
tooth–humerus, tooth–parietal	17/30

Appendix B

Principal components

Table 8. The nine principal components that had eigenvalues equal to or greater than one, derived from all 30 elements detected by the pXRF. Note: Coefficients below .300 were suppressed for clarity purposes and are not displayed. This applies to all tables presenting the principal components.

	Component								
	1	2	3	4	5	6	7	8	9
As	.964								
Ga	.963								
Pb	.960								
Th	.878								
Y	.861								
S	.563		.368						
Se	.351								
K		.958							
Ti		.951							
Si		.950							
Al		.930							
P			.864						
Ca			.824						
V			.656						
Mg		.349	-.645						
Na			.459	.403					
Mn				.801					
Ni				.593					
Fe		.512		.565					
Co				-.482					
Rb					.880				
Zr					.862				
Sr						.706			
Cl						-.680			
Cu							.782		
Zn							.694		

Table 9. The five principal components derived from the filtered chemical elements.

	Component				
	1	2	3	4	5
P	.933				
Ca	.926				
Mg	-.641	.304			
Na	.594			.407	
K		.946			
Ti		.940			
Fe		.713		.419	
Sr		.495		.389	
As			.951		
Pb			.948		
S	.419		.669		
Se				.653	
Mn	.301			.606	
Cu					.799
Zn					.771

Table 10. The four principal components derived from the filtered ratios.

	Component			
	1	2	3	4
Zn_Fe		.931		
K_Fe		.835		
Sr_Ca			.928	
Pb_Ca				.793
Sr_Pb				-.754
Ca_P		-.435	.710	
Zn_Ca		.740		
Mn_S	.892			
Mn_K	.942			
S_Sr			-.672	.544
Mn_Fe	.874			

Table 11. The four principal components derived from the filtered ratios, excluding the teeth measurements.

	Component			
	1	2	3	4
Mn_K	.949			
Mn_S	.894			
Mn_Fe	.788			
K_Fe	-.604			
Sr_Ca		.920		
Ca_P		.794		
Zn_Fe			.911	
Zn_Ca			.868	
Pb_Ca		.313		.819
Sr_Pb				-.683
S_Sr		-.557		.643

Table 12. The three principal components derived from filtered elements, using only the measurements from the teeth and capitates.

	Component		
	1	2	3
As	.956		
Pb	.946		
Cu	.547		.416
P		-.891	
Mg		.860	
Mn		-.313	.802
Zn			-.604
Na			.435