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## Preliminary Investigation into Biological Sex Estimation Using Trace Element Analysis in Human Hair

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PRELIMINARY INVESTIGATION INTO  
BIOLOGICAL SEX ESTIMATION USING  
TRACE ELEMENT ANALYSIS  
IN HUMAN HAIR

by

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A thesis submitted in partial fulfillment of the requirements  
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## **ABSTRACT**

In forensic anthropology the estimation of biological sex of unidentified human remains is critical, as it essentially halves the number of potential identities. Sex estimation is also important in bioarchaeology, because the creation of the biological profile is critical to the interpretation of different sociocultural aspects of past populations. Furthermore, certain aspects of the biological profile are sex specific, so it is important to be able to accurately determine biological sex (France 1998). Typically, biological sex is estimated by assessing sexually dimorphic differences within the pelvis and skull. However, because sexually dimorphic differences arise during puberty it is difficult, and oftentimes impossible, to use these traditional techniques on juveniles. Recently, human hair has gained prominence in anthropological research. This is particularly evident in bioarchaeology, where hair can be used to discern information concerning health, toxicology, culture, and diet of past populations. This study focuses on the relationship between the content of trace elements in hair and biological sex to determine if biological sex can be assessed from the content of trace elements in human head hair. Hair was collected from three human sample groups: modern living individuals, modern cadavers, and archaeological remains. Data on trace elements was collected using laser-induced breakdown spectroscopy (LIBS). The statistical relationship between the content of trace elements and sex was then analyzed using a multivariate analysis of variance (MANOVA), post-hoc analysis of variance (ANOVAs), and stepwise binary logistic regression. The MANOVA revealed a statistically significant multivariate main effect for sample group using mean values ( $p$ -value < 0.0001) and

mean variance values (MVs) ( $p$ -value = 0.018). Given the significant results of the MANOVA test, the univariate main effects were examined with post-hoc ANOVA tests. Significant univariate main effects were obtained for mean values for C/Mg ( $p$ -value < 0.0001), C/Fe ( $p$ -value < 0.0001), C/Ca ( $p$ -value < 0.0001), and C/Sr ( $p$ -value < 0.0001). Significant univariate main effects were obtained for MVs for C/Mg ( $p$ -value = 0.016), C/Fe ( $p$ -value = 0.010), and C/Sr ( $p$ -value = 0.042). These preliminary results demonstrate that biological sex of humans can be accurately estimated through trace elemental analysis approximately 85% of the time in living samples, and 79% of the time overall. The results also demonstrate the viability of this technique for sex estimation in juvenile remains with approximately 83% success in predicting juvenile biological sex. This sets the stage establishing trace elemental analysis of hair as a technique for estimating biological sex which is critical to forensic individuation and identification as well as further contextualization of archaeological remains.

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## LIST OF ABBREVIATIONS

Ag	Silver
Al	Aluminum
ANOVA	Analysis of Variance
Ar	Argon
As	Arsenic
ATP	Adenosine Triphosphate
AU	Arbitrary Unit
Ba	Barium
Bi	Bismuth
C	Carbon
Ca	Calcium
Cd	Cadmium
Co	Cobalt
Cr	Chromium
Cu	Copper
DNA	Deoxyribonucleic Acid
EPA	Environmental Protection Agency
F	Fluorine
Fe	Iron
Ga	Gallium
Hg	Mercury

I	Iodine
IRB	Institutional Review Board
K	Potassium
LA-ICP-MS	Laser Ablation Inductively Coupled Plasma Mass Spectrometry
LIBS	Laser Induced Breakdown Spectroscopy
MANOVA	Multivariate Analysis of Variance
Mg	Magnesium
Mn	Manganese
mtDNA	Mitochondrial Deoxyribonucleic Acid
MV	Mean Variance Values
Na	Sodium
Ni	Nickle
NIJ	National Institute of Justice
P	Phosphorus
Pb	Lead
RNA	Ribonucleic Acid
S	Sulfur
Se	Selenium
Si	Silicon
SPSS	Statistical Package for the Social Sciences
Sr	Strontium
Ti	Titanium

UCF	The University of Central Florida
U.S.	The United States of America
Zn	Zinc

## **LIST OF NOMENCLATURE**

Age Group	Classification based on age. There are three age groups: Juvenile (<18 years old), Adult (18-50 years old), and Older Adult (>50 years old).
Context Group	Classification based on temporal context. There are two context groups: Archaeological Context Group (remains that belong to a past population, regardless of age at death) and Modern Context Group (individuals that are either living or recently deceased, regardless of age at death).
Sample Group	Classification based on sample origin. There are three sample groups: Archaeological Sample Group (remains from the Kellis 2 cemetery archaeological site, Dakhleh Oasis, Egypt), Cadaver Sample Group (remains of recently deceased from the UCF College of Medicine's Willed Body Program), and Living Sample Group (living individuals who reside within the state of Florida).

## **CHAPTER ONE: INTRODUCTION**

In many research contexts it is very important for biological anthropologists to estimate biological sex when creating a biological profile for the individuals being studied. In a forensic context the categorization of biological sex helps to 1) narrow down the potential identity of unknown remains by 50%, 2) estimate stature (Trotter 1970; Trotter and Gleser 1952), and 3) assist in ancestry estimation (O'Connell 2004). In a bioarchaeological context the estimation of biological sex helps to answer important questions concerning sociocultural aspects of life, such as differential status (Munson 2000; Vercellotti et al. 2011), diet (Eerkens and Bartelink 2013; Pearson et al. 2013; Somerville et al. 2015), labor division (Ogilvie and Hilton 2011; Villotte and Knüsel 2014; Waidhofer and Kirchengast 2015) and gender identity (Hanks 2008; Lambert 2001; Perry and Joyce 2001). Biological sex, however, can be difficult to ascertain in highly fragmented, ambiguous, and/or juvenile remains (Tierney and Bird 2015). Nevertheless, this is an important aspect of demography that must be investigated.

The central issue investigated in this research project will be the isolation of trace elements that are dependent on biological sex. The focus of this study is to determine if the level of absorption of certain trace elements is dependent on biological sex and which trace elements can be utilized to estimate the biological sex of an individual. It is expected that Zinc (Zn) and Copper (Cu) will likely be the best for determining differences in biological sex, due to the fact that both of these trace elements are relatively stable and scarcely dependent on the level in the environment (Soroko et al. 2014). In addition, these elements have been shown to be metabolized by males and

females at different rates (Soroko et al. 2014). Similarly, it is expected that Magnesium (Mg) and Strontium (Sr) will be useful in determining differences in biological sex based on previous research into trace element differences between the biological sexes (Ashraf et al. 1995; Chojnacka et al. 2010; Dongarrá et al. 2011; Huang and Beauchemin 2014; Sturaro et al. 1994; Zaichick et al. 2011; Zakrgynska-Fontaine et al. 1997). The results of this investigation may have many applications for important issues within the field of biological anthropology.

Typically, in biological anthropology, sex estimations are made using morphological and metric analysis of the skeletal elements. Due to sexually dimorphic differences that form during puberty, the pelvis, particularly the subpubic region, is the best skeletal indicator of biological sex, followed by the skull (Buikstra and Ubelaker 1994; Phenice 1969). However, because juvenile remains have not fully developed the sexually dimorphic changes associated with puberty may not be relied on as a method of biological sex estimation for juvenile skeletal remains. Furthermore, it is important to remember that populations vary, sometimes markedly, with respect to morphological differences between the sexes (Buikstra and Ubelaker 1994). Generally, the adult female pelvis is larger and exhibits a wider pelvic outlet and a broader greater sciatic notch than the male pelvis (Buikstra and Ubelaker 1994; Phenice 1969). The male skull is generally more robust than the more gracile female skull (Buikstra and Ubelaker 1994). Metric analysis also contributes to the sex estimation techniques employed by biological anthropologists (DiBennardo and Taylor 1983; Van Gerven 1972). However, all of these methods are made more difficult, less accurate, or even impossible if the



skeletal remains are incomplete, fragmented, juvenile, or from different geographic regions. Therefore, new techniques are needed for estimating biological sex when these methods are not possible or unable to provide acceptable results.

The purpose of this study is to assess whether biological sex can be reliably estimated using human hair. Human hair is very resilient to degradation and destruction over time, making it a good tissue for research in bioarchaeological contexts as well as forensic contexts (Wilson et al. 2007a; Wilson and Tobin 2010). The main focus of this research is the use of trace elements in hair to predict biological sex across forensic and bioarchaeological contexts within all age groups. This method would offer anthropologists an opportunity to estimate, or assign probability to, the biological sex of any individual, no matter the age when the typical morphological techniques are not reliable. Furthermore, this method can be applied more quickly and is cost effective when compared with current forensic techniques, such as deoxyribonucleic acid (DNA) analysis (Nelson 2010). In addition, this method would provide another line of evidence for biological sex estimation, in the form of trace elements found in the hair, which may persist for thousands of years under harsh conditions (Wilson et al. 2007a; Wilson and Tobin 2010). The main issue investigated in this research will be whether or not trace element analysis of hair can be used to reliably estimate biological sex for a variety of different populations. Based on background literature it is expected that this technique will result in reliable sex estimations.

Another issue that will be investigated is the problem of estimating sex from juvenile skeletal remains. Currently there is no satisfactory method for assigning

biological sex to juvenile skeletal remains (Saunders 1992). A small sample of seven juvenile individuals from the archaeological site of Kellis 2 cemetery in the Dakhleh Oasis, Egypt will be used to evaluate whether trace element analysis of hair can be used to reliably estimate sex in juvenile individuals. Based on the metabolic differences between males and females which are present prior to puberty, it is expected that there may be success in reliably estimating biological sex of juveniles using this technique (Ayyavoo et al. 2014).

The chapters that follow will attempt to investigate these issues and examine the applicability of this method using head hair from 77 individuals that were analyzed using laser-induced breakdown spectroscopy (LIBS). This method was chosen because of the ability to obtain numerous data points along the shaft of the hair for use in a variety of different statistic tests. The LIBS results will be used in a stepwise binary logistic regression to determine a potential prediction equation. The results from the stepwise binary logistic regression will be compared to determine the variables that affect the trace element levels within these individuals. Chapter Two will provide a review of the literature pertaining to the current methods of biological sex estimation in biological anthropology, the structure, growth, and preservation of hair, the bioavailability of the 9 trace elements examined, and previous research concerning elemental analysis of hair and sex within a multi-disciplinary context. Chapter Three will outline the methodology and materials used in this study. Chapter Four will present the results of the LIBS analysis for each trace element examined as well as the statistical analysis and the stepwise binary logistic regression results. Chapter Five will examine the differences in

the trace element data between the biological sexes, age groups, sample groups, and context groups, as well as the reliability of the stepwise binary logistic regression analysis and prediction equation within the forensic and bioarchaeological contexts and discuss the significance of these results. Chapter Six will end the study with project conclusions and a discussion of broader application as well as concerns about limitations and opportunities for future research studies.

## **CHAPTER TWO: LITERATURE REVIEW**

The use of trace elemental analysis to estimate biological sex from human head hair is a project that lies at the intersection of many analytical techniques and research interests. In order to make informed conclusions about the results of this research it is important to understand the processes and variables that may factor into it. Therefore, a review of the literature concerning current standard methods of biological sex estimation, the anatomical and physiological properties of human head hair, and elemental analysis itself is presented. This chapter includes an examination of the cosmetological, biological, and anthropological background of hair analysis. A review of the current trace element research is also presented, which focuses on studies that involve hair and consider sex as a variable.

### **Biological Sex Estimation**

Estimation of the biological sex of unidentified skeletal or highly decomposed remains is critical in both bioarchaeology and forensic anthropology. Biological sex can be estimated in a number of ways, such as morphological assessment, metric analysis, and DNA analysis. Morphological analysis is highly accurate in adults and the most commonly used method, particularly when the pelvis and skull are present and in good condition (Byers 2011). Metric analysis is also fairly reliable in adults and is typically used either in conjunction with morphological assessment or when the pelvis or skull is not available (Byers 2011). DNA analysis is quite accurate in adults and juveniles and does not rely as heavily on the experience of the anthropologist as does morphological

assessment. However, DNA is not always preserved satisfactorily for analysis (Keyser-Tracqui and Ludes 2005; Quincey et al. 2013). Despite the advent of DNA testing, skeletal analysis remains the quickest and cheapest method for estimating the biological sex of extremely decomposed or skeletonized remains (Nelson 2010).

### Adults

Sex estimation of adult skeletal remains can be performed in two different ways, with a nonmetric, or morphological assessment, or with a metric assessment. In adult skeletal remains sex is most commonly estimated from the innominate and/or the skull using both metric and morphological techniques. Sexual dimorphism is the difference in size and shape of the body between the different sexes of the same species (Warren et al. 2008). The adult human innominate is the best single skeletal element from which to estimate biological sex because the innominate is morphologically related to childbirth. As a result of this relationship, the innominate is more sexually dimorphic in nature than the other bones in the body (Byers 2011). Other skeletal elements have been shown to have certain morphological characteristics that may be sexually dimorphic in nature, including the cranium (Giles and Elliot 1963), the first rib (İşcan 1985), the mandible (Giles 1964), the sternum (Hunnargi et al. 2008), the humerus (İşcan et al. 1998; Rogers 1999), the femur (King et al. 1998), and the fourth rib (İşcan 1985). Postcranial skeletal elements can be assessed both morphologically and metrically as well, however, the morphological differences are not as obvious and metric analysis may be preferential (Byers 2011).

The more complete the skeletal remains are, the easier it is to provide an assessment of biological sex. However, if one of the main diagnostic skeletal elements, such as the pelvis or cranium, is highly fragmentary or missing all together the process becomes much more difficult. In incomplete, highly fragmentary, or ambiguous skeletal remains, methods of skeletal analysis may not be useful to determine a confident biological sex estimation. In forensic cases that are incomplete, highly fragmented, and/or morphologically ambiguous, DNA may be useful in determining the biological sex of the individual. However, in bioarchaeological cases that are incomplete, highly fragmented, and/or morphologically ambiguous, the DNA may be too degraded to be useful and other methods of sex estimation must be considered. Furthermore, contamination is a significant issue for ancient DNA studies (Keyser-Tracqui and Ludes 2005; Quincey et al. 2013). DNA analyses may also be cost prohibitive. For example, between 2004 and 2009 only 137,753 DNA cases were tested using the over \$330 million in federal funds provided by the National Institute of Justice (NIJ) (Nelson 2010). The cost of DNA analysis, while constantly decreasing, may be particularly prohibitive for academic researchers who are not guaranteed funding.

### Juveniles

Unlike adult skeletal remains, which can be assessed for sex using morphological characteristics and measurements on nearly every skeletal element, juvenile skeletal remains exhibit little to no significant sex-based differences before puberty (Byers 2011; Loth and Henneberg 2001). Because of this, and a lack of juvenile

skeletons of known sex and age as well as ethical research issues surrounding juvenile remains, forensic anthropologists and bioarchaeologists have had trouble developing methods similar to those used with adults that would allow for replicable and accurate sex estimation results in juveniles. Many studies have attempted to remedy this problem with new techniques and criteria, yet none have stood up to scrutiny (Hunt 1990; Schutkowski 1993; Weaver 1980). The absence of a reliable method for sex estimation becomes even more problematic when the juvenile remains are less than ideally preserved. Just like adult skeletal remains, incomplete remains and fragmentation add to the impossibility of estimating the sex of juveniles.

### Hair

Hair, which is characteristic of all members of the class Mammalia at some point in their lives, including humans, serves sexual attractiveness, protective, and sensory functions (Robbins 2012). Hair covers a large percentage of the skin in many mammals and likely evolved from epidermal scales found on reptiles (Franbourg and Leroy 2005; Robbins 2012). The human head, however, differs from the rest of the body in that it has a greater quantity of large hair follicles with associated sebaceous glands. These follicles produce hair fibers that are much longer and coarser than the hair fibers found covering the rest of the body (Franbourg and Leroy 2005). Although it performs no vital function in humans, head hair has cosmetic as well as protective functions, acting as a thermal insulator and a barrier protecting the skin against the sun (Dawber and Van Neste 2004). Despite the pervasiveness of human head hair, it is an extremely

underused biological tissue in biological anthropology. However, head hair can be a very valuable asset to the biological anthropologist not only because it can shed light on diet, toxicology, and status (Kempson and Lombi 2011; Wilson et al. 2010), but also because of its potential to provide reliable sex estimations for juveniles, which is currently not possible outside of DNA analysis.

### Structure and Growth of Human Head Hair

Human head hair is a stable biological tissue that is composed primarily of a class of proteins called keratins. Keratin is typified by a high content of an amino acid known as cysteine, which contains sulfur and is able form disulfide bridges that provide hair with its structure (Franbourg and Leroy 2005). Because of its organized structure and keratinized cellular makeup, hair is incredibly resistant to environmental effects (Franbourg and Leroy 2005). Hair grows from follicles, which are large sacs found in the subcutaneous tissue of the dermis (Figure 1) (Bland 1984; Franbourg and Leroy 2005; Robbins 2012).



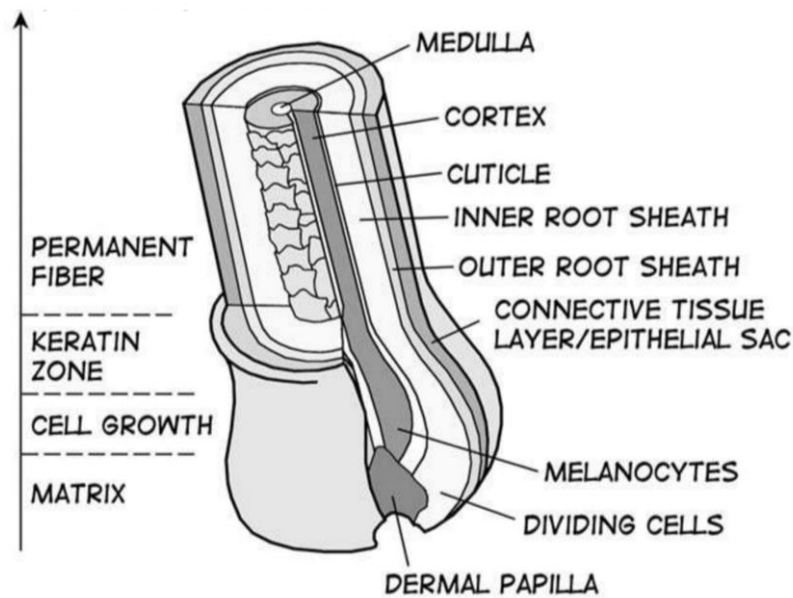


Figure 1: Image showing the cross section of a hair follicle demonstrating the typical structure of human hair. Image courtesy of L. Williams.

The hair shaft is arranged into three separate layers. These layers are called the cuticle, the cortex, and the medulla (Figure 1). The cuticle is the thick protective sheath on the outer layer of the hair that is comprised of one or more superimposed layers of scale-like cells (Hicks 1977; Ogle and Fox 1999; Robbins 2012). The cuticle is originally laid down as a single cuboid layer, the cells of which begin to flatten and overlap like roof shingles as growth continues and they are moved farther from the follicle. The free external edge of the cell is pointed towards the end of the hair shaft, while the trapped internal edge is closer to the follicle (Dawber and Van Neste 2004; Franbourg and Leroy 2005). The cortex is surrounded by the cuticle and makes up the majority of the hair shaft. The cortex is made up of long thin cells that run parallel to the axis of the hair shaft. These cells consist of large quantities of the fibrous keratin proteins that form the

bulk of the hair mass (Robbins 2012). These proteins are very similar to the ones that form tooth enamel and fingernails (Robbins 2012). This inflexible internal structure of hair starts out as irregularly shaped fluid filled cells, which dries and strengthens as the hair grows (Bland 1984). The innermost layer is known as the medulla. The medulla consists of loosely packed cells with large air filled spaces between them. Arginine and tricholyalin, both proteins, as well as citrulline, an amino acid, can be found in the medulla (Harding and Rogers 1999). The medulla may or may not continue through the entire length of the hair shaft and may even be absent entirely in human hair. In most mammalian hair, however, the medulla forms the main component of the fiber that can be used diagnostically in species determination (Moore et al. 1974; Harding and Rogers 1999; Hicks 1977).

Human head hair, like all hair fibers regardless of species or location, grow in a cycle that consists of three separate and distinct stages: 1) anagen (active growing stage), 2) catagen (transitional stage), and 3) telogen (resting stage) (Robbins 2012). Van Neste et al. (2007) also discuss the possibility of a fourth stage, exogen (shedding stage) (Figure 2). While all human hair goes through this same cycle, it has been documented that human head hair has a much longer cycle than human body hair, with the full replacement of human head hair occurring every 3 to 5 years (Courtois et al. 1996; Kligman 1959; Saitoh et al. 1970). During the anagen stage active hair growth is occurring at an average rate of 0.35 to 0.5 mm per day, which equates to approximately 1 centimeter per month (Barth 1986; Harding and Rogers 1999; Hayashi et al. 1991; Valkovic 1977). The formation of hair takes place in the follicle. At the center of the

follicle lies the dermal papilla, which is involved with growth during the anagen stage. Surrounding the follicle is the basal layer, which produces the cells that make up the hair fibers. Pigment producing cells, known as melanocytes, surround the dermal papilla and blood vessels nourish the hair fiber as it grows (Robbins 2012).

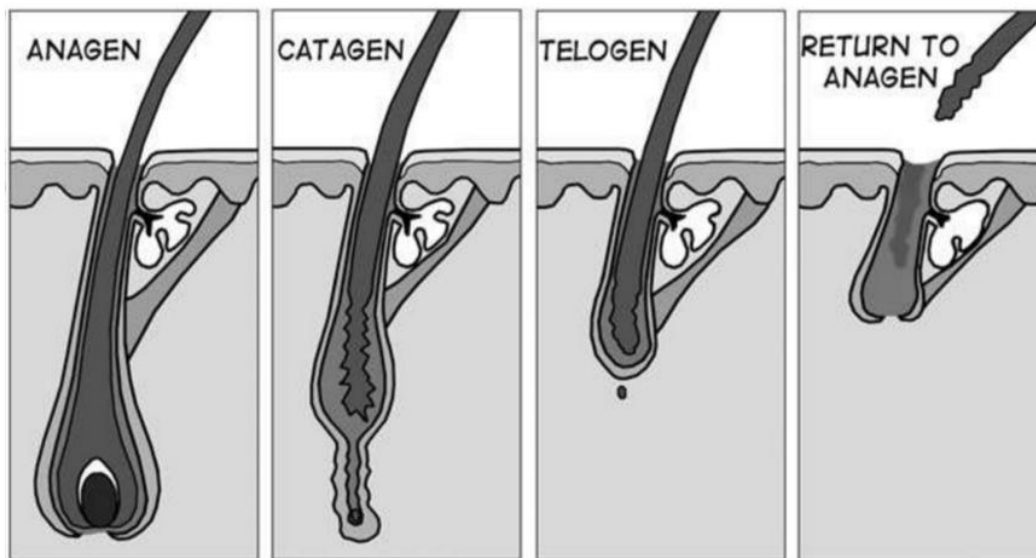


Figure 2: Image showing the different stages of hair growth within the hair cycle (Image courtesy of L. Williams).

The portion of the hair fiber that resides below the surface of the skin can be divided into multiple zones (Figure 1). The first zone, which is located at the same level as the follicle is known as the zone of protein and cell synthesis. Above this is the zone of cellular differentiation (Robbins 2012). In the next zone cysteine bridges are formed, which lends stability and structure to the hair fiber (Barnett and Seligman 1952). This zone is called the zone of keratinization. The final zone begins just below the skin surface and extends up the shaft of the hair. This is the region of the permanent hair fiber, which is made up of the fully formed hair shaft (Robbins 2012).

While approximately 85-90% of human hair is actively growing at any one time, approximately 10-15% are inactive, and about 1% of hair is in transition (Dawber and Van Neste 2004; Lenihan 1988). This means about 10,000 of the approximately 100,000 human head hairs are inactive at any one time. As the production of the hair fiber ceases, the catagen stage begins. The catagen stage is defined as the transitional stage in which the hair fiber shifts from production to growth arrest. This stage is marked by the regression of the hair follicle. During this time a “club” hair is formed by the constriction and keratinization of the base of the hair fibers (Williams et al. 2011). At the same time pigment production ceases and the outer root sheath degenerates and keratinizes, as the now colorless base of the hair moves upward toward the surface of the skin in preparation for the shedding of the hair fiber. Once the “club” hair is formed the telogen stage begins (Williams 2008). During this resting stage is when the majority of hair is shed. The average person sheds approximately 100 hairs per day (Turner-Pearson 2007). However, inactive hair fibers may be retained in the skin until as late as the following cycle in the anagen stage (Williams 2008).

All hair growth on the human body is dependent on hormones at different levels. Head hair, along with eyebrow and eyelash hair, are less dependent on hormones than pubic, mustache, auxiliary and beard hair. There are approximately 100,000 hairs on the average human head. However, this varies by hair color, with blonde individuals having more, red headed individuals having slightly less, and brunettes falling around average (Turner-Pearson 2007).

There are multiple types of hair fibers that grow and are shed at predictable times within the human lifetime. The first hair fiber type to appear, known as laguno hair, are fine hair fibers that form in follicles but lack a medulla (Pecoraro et al. 1964). Laguno hair are originally shed around eight gestational months and a second cycle of laguno hair is shed approximately three months after birth (Pecoraro eta l. 1964). Postnatal hair growth can be categorized as either vellus or terminal hair (Saitoh et al. 1970). Vellus hairs, similar to laguno hair, are fine and lack a medulla, they also are short and lightly pigmented. This is in contrast to terminal hairs which are thick, long, pigmented hairs that often contain a medulla. Terminal hairs are present in children, in they eyebrows, eyelashes, and scalp, while vellus hairs cover the rest of the body. Both of these hair fiber types are grown and shed less predictably than laguno hair (Saitoh et al. 1970). Vellus hair will typically give way to terminal hair at or around puberty.

### Hair Analysis in Anthropology

In the field of anthropology, the potential informational value of keratin based tissues is well documented. This is particularly true in forensic, archaeological, and paleontological research. Keratin proteins provide the basis for many biological materials, such as hooves, feathers, horns, claws, baleen, nails, skin stratum corneum, reptilian carapaces, and mammalian hairs (Bertrand et al. 2014). For example, keratinous fibers such as wool, which were used in the past to create textiles, can provide anthropologists with valuable information on the use and process of production of these textiles (Bertrand et al. 2014).

Human hair has been an area of scientific interest since the late 19<sup>th</sup> century. This early research ranged from average diameters of hair from Peruvian mummies to more advanced microscopic examination (Bertrand et al. 2014; Hrdy 1978; Trotter 1943; Woodbury and Woodbury 1932). In the late 20<sup>th</sup> century a large portion of the literature focused on the structural analysis of hair found preserved in dry environments (Bertrand et al. 2003; Brothwell and Spearman 1963; Macko et al. 1999). Recently, human hair has gained even greater anthropological interest. This is particularly evident in the sub-discipline bioarchaeology, where hair can be used to discern information concerning health, toxicology, culture, and diet of past populations (Kempson and Lombi 2011; Wilson et al. 2010). Hair is an excellent material for these types of studies due to the permanence of the record of keratinized hair. Furthermore, the specific morphology of particular keratin based biological tissues found in the fossil record can inform anthropologists about the development and evolution of past species (Edwards et al. 2011). Research using hair has also provided important information and insights concerning psychotropic substance usage in past populations (Ogalde et al. 2009; Springfield et al. 1993).

#### Hair in Forensic Anthropology

Hair is of extreme value in forensic contexts including the process of human identification (Wilson and Gilbert 2007). It is the physical properties of hair that contribute to its forensic value. For example, humans have a great quantity of hair on their heads and bodies and the nature of the human hair cycle means each person

loses many hair fibers throughout every day. In addition, the morphology allows for species distinction, and the characteristics of the cuticle allow hair fibers to easily cling to clothing for extended periods (Pfeiffer et al. 2004; Wetton et al. 2003; Wilson and Tobin 2010). Furthermore, hair may be the most prevalent, and in some cases only, variety of forensic evidence available at crime scenes (Oien 2009).

Due to the development of DNA testing and its importance and prevalence in forensic contexts, it is not surprising that much of the use of hair in forensic applications is in relation to DNA techniques. Genetic developments in hair research include the extraction of mitochondrial DNA from human hair shafts and low copy DNA analysis of telogen hair (Hedman and Jangblad 2003; Wilson et al. 1995). Our current understanding of genetics, however, has limits which extend to hair analysis (Grzybowski et al. 2003; Wilson and Gilbert 2007). Furthermore, DNA testing in hair has its own physical limitations. The growth stage, water exposure, and the presence or absence of the root of the hair fiber can all affect genetic testing (McNevin et al. 2005; Williams et al. 2011). In fact, approximately 95% of all hairs that are recovered from crime scenes are in the telogen stage and lack a follicle and associated cellular material necessary for DNA analysis (Edson et al. 2013; McNevin et al. 2005).

Hair is particularly useful in forensic cases when exploring usage of controlled and toxic substances. In forensic cases hair analysis can provide answers concerning alcohol usage, suspect administration of legal prescription drugs, and other chronic substance abuse (Nakahara 1999; Politi et al. 2007; Wilson and Tobin 2010). Because of the excretory nature of hair fibers, hair is the final depository for many toxic materials.

Therefore, hair is also used in biomonitoring and exploring occupational and environmental exposure to toxic heavy metals (Kales 2005). Wilson and Tobin (2010) noted that laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is ideal for this type of research, due to its ability to produce viable measurements with a single hair. Furthermore, hair can effectively trap environmental and anthropogenic evidence due to the unique structural characteristics of the hair cuticle and the presence of various biological secretions (Wilson and Tobin 2010). For example, hair can trap evidence such as pollen, soil, and even gun-shot residue (MacCrehan et al. 2003; Wiltshire 2006).

Another important analytical technique for hair is stable isotope analysis. Stable isotope analysis can provide a variety of useful information including diet (Dupras 1999; McCullagh et al. 2005; Santamaria-Fernandez et al. 2009), geographical movement (Dupras and Schwarcz 2001; Wilson and Tobin 2010), and health (Wheeler et al. 2013; Williams and Murphy 2013). Stable isotope data from a forensic context can be utilized to provide information concerning place of origin and movement of unidentified individuals through analysis of hydrogen and oxygen isotopes (Fraser and Meier-Augenstein 2007; Meier-Augenstein and Fraser 2008; Rauch et al. 2007). It has also been suggested that this same technique could be used on living individuals who were suspected of terrorism (Wilson and Tobin 2010). Similar to radiocarbon dating bone using measurements of carbon-14 activity, or what is known as the bomb-curve, hair can be radiocarbon dated relative to the levels of artificial carbon-14 in the atmosphere, which can help date remains to before or after 1955 (Nakamura et al. 2007; Ubelaker



2001). Both of these analytical techniques can be used on hair from bioarchaeological contexts as well.

### Hair in Bioarchaeology

Hair as a biomonitor is well documented in the bioarchaeological literature. Of particular interest is its ability to provide information on diet and health, as well as drug use and social status. When viewed incrementally, stable isotopic data can help bioarchaeologists examine mortality patterns, trade patterns, social status, diet, physiological status, seasonality, and geographic movement (Wheeler et al. 2013; Williams et al. 2008; Wilson 2005). Recent developments in stable isotope analysis of hair include the development of LA-ICP-MS, which allows for detailed isotopic variation along the length of a single hair fiber and the ability to analyze the direct relationship between individual amino acids and diet (McCullagh et al. 2005; Santamaria-Fernandez et al. 2009).

Genetic testing and analysis can be used on hair from bioarchaeological contexts to establish familial relationships among past populations. A notable example is one presented by Gilbert et al. (2007) concerning the relationship of eight paleo-Eskimo individuals uncovered at Qilakitsog in 1978 in Greenland. Originally researchers concluded that the individuals were representative of two separate familial groups based on age estimations, grave location, and tissue typing. However, mitochondrial DNA from hair and nails was later used to determine that these individuals in fact formed three distinct mitochondrial DNA (mtDNA) haplogroups (Gilbert et al. 2007). The

noninvasive nature of using hair fiber allowed the researchers to run DNA tests on the youngest infant, approximately 6 months old, which had not been previously possible (Wilson and Tobin 2010).

Hair analysis for drug use and toxic elements is not limited to forensic anthropology. Bioarchaeological studies have used hair data to determine frequency of coca ingestion in ancient Chile (Cartmell et al. 1991). Similar studies have been conducted on alcohol and hallucinogen usage in past populations (Cartmell et al. 2006; Ogalde et al. 2009). Toxic element concentration in bioarchaeological hair samples can provide researchers with important comparative data that can be used for assessing exposure differences between current and past populations of specific geographic regions (Wilson and Tobin 2010).

Social importance of hair exists at some level in most modern and ancient cultures. Some cultures maintain specific rituals concerning hair cutting, which often are associated with a specific age, a change in status, or rites of separation, and many believe hair may have magical properties (Wilson et al. 2007b; Wilson and Tobin 2010). For example, Inca capacocha ceremonies included human child sacrifices, some of whose mummified remains have been excavated. Oftentimes these remains were found with small bags containing cut hair. Stable isotopic and mtDNA analysis was carried out on this hair and showed that the hair was from either the child it was found with or a close maternal relative (Wilson et al. 2007b). Hair styles are also heavily dictated by culture. However physical evidence of these styles in the form of actual preserved hair is rare (Wilson et al. 2007b; Wade and Nelson Wilson and Tobin 2010). Hair treatments,

however, can be examined through hair analysis. Evidence of lead being used to dye hair has been discovered, as has evidence for different varieties of hair gel throughout the world (Walter et al. 2006). For example, hair samples from individuals recovered from the Kellis 1 cemetery, Dakhleh Oasis, Egypt, the same location where the archaeological hair samples used in this study originate, were found to contain a fat-based hair gel (McCreesh et al. 2011).

### Preservation of Hair

Hair is a valuable resource to bioarchaeologists and forensic anthropologists because of its ability to resist destruction and degradation over time. Along with dentition and bone, hair and other keratin based fibers are able to repel the effects of the environment and time and survive for millennia while other biological tissues are subject to rapid degradation by bacteria and physico-chemical activity (Bertrand et al. 2014; Brothwell and Spearman 1963; Macko et al. 1999). Hair is most likely to be well preserved in an environment, whether natural or artificial, that impairs microbial decay. This would include extremely cold, dry, or anoxic environments, such as deserts or bogs. This type of environment may also be created, intentionally or accidentally, through the application of different physico-chemical treatments (Hofreiter et al. 2012; Kempson et al. 2010; Wilson 2008). Some of the oldest hair samples that have been studied anthropologically were collected from 30,000 year old woolly mammoths that had been preserved in extremely cold icy environments (Farrand 1961).

Despite being incredibly resilient, hair is still susceptible to biodegradation via keratinolytic microorganisms, which consists primarily of fungus but also includes certain insects and bacteria (Wilson et al. 2007a; Wilson and Tobin 2010). The keratin based structure of hair protects against proteolytic enzymes and contributes to its endurance. However, this is only true of intact hair fibers. After hair has suffered initial damage it is much less resilient to destruction from either microbial or other physical means (Tsoucaris et al. 2003; Dent et al. 2004). The rate at which degradation occurs following initial damage is dependent mainly on temperature and humidity, and it can take anywhere from a couple of years to hundreds of years to reach advanced stages of degradation (Janaway et al. 2009; Wilson et al. 2007a; Wilson et al. 2010). With the inhibition of keratinolytic activity, however, hair can persist for much longer in ideal environmental conditions (Janaway et al. 2009).

### Trace Elements in Hair

Human head hair contains inorganic elements at various, but typically low, levels. These elements are known as trace elements, and the overall content never exceeds 1%. The trace elements that are encountered most frequently in human hair are: 1) alkaline earth metals, such as calcium (Ca), Mg, and Sr, 2) alkaline metals, including potassium (K) and sodium (Na), 3) other metals, such as arsenic (As) cadmium (Cd), iron (Fe), mercury (Hg), manganese (Mn), lead (Pb), selenium (Se), and Zn, and 4) metalloids, such as phosphorus (P) and silicon (Si) (Franbourg and Leroy 2005). Trace elements can be deposited into the hair either exogenously, from the outside

environment, or endogenously, internally from the body. Endogenous trace elements are deposited into the structure of the hair during its synthesis, which occurs in the follicle. Exogenous trace elements are deposited onto the hair as a result of continuous contact with factors such as hygiene products, ions from water, and dust (Valkovic 1988). In fact, a large amount of the trace elements found on human hair are exogenous and are a result of sweat deposits (Bate et al. 1966). Accumulation of trace elements in hair can also be indicative of certain disorders, toxic exposure, and drug use. For example, individuals diagnosed with cystic fibrosis have hair that contains higher levels of Na and lower levels of Ca (Maugh 1978). Furthermore, increases in the level of heavy metals in water or air leads to an increase in the hair of those individuals who have been exposed (Baumgartner 1989; Gibson et al. 1983; Phelps et al. 1980).

Multiple studies have suggested that Cu and Zn are reliable indicators of endogenous consumption (Kempson et al. 2007; Robbins 2012; Trunova et al. 2003). This is supported by the identical Cu and Zn concentrations noted between modern human hair and hair from 2,500 year old mummies from South America (Du et al. 1996). Trunova et al. (2003) further suggest that Cobalt (Co), Gallium (Ga), Sulfur (S), Se, and Titanium (Ti) could also be potentially reliable indicators of endogenous consumption. However, both Cu and Zn were found to be altered significantly in individuals with certain diseases and disorders such as breast cancer, esophageal cancer, and manic depression (Altaf et al. 2004; Azin et al. 1998; Kilic et al. 2004). Increased levels of Pb and Cd from exogenous sources were also found to impact metabolism and influence concentrations of Cu and Zn (Srivastava et al. 1997; Wasiak et al. 1996).

Keratin, the main component of hair, is a protein that has significant levels of the sulfhydryl amino acid cysteine. This has the ability to bind heavy metals effectively in an ionic state because sulfhydryl is a strong chelator (Bland 1984; Cone and Joseph 1996). Due to the composition of hair, the levels of trace elements are significantly higher in hair than in urine and blood (Hanson and Asmund 2002; Obrušnil et al. 1973). Hair is preferable over blood or urine for examining changes in environment or nutrition due to its sensitivity to elemental fluctuations in the body and the metabolic inactivity that occurs once the hair structure has been synthesized (Gordus et al. 1975; Grupe and Dörner 1989; Williams et al. 2011). Furthermore, hair is extremely stable over long periods of time, making it ideal for any analyses concerning ancient populations (Brothwell and Grimes 2002).

Hair follicles contain many blood vessels that deliver both essential and toxic trace elements into the hair shaft where it is laid down into the protein that is being formed (Robbins 2012). However, the amount and rate at which trace elements are deposited into the hair is dependent on growth rate as well as sulfhydryl content (Bland 1984). Similarly, chemicals and hormones can influence the rate of deposition of trace elements as well as the rate of growth of the hair itself, as can changes in an individual's nutritive status (Bland 1984; Tuner-Pearson 2007).

The color of an individual's hair is derived from the presence of a brownish pigment called eumelanin. Eumelanin is a type of melanin, similar to the pigment that is responsible for the determination of skin color. Eumelanin is converted into its active form for incorporation into the shaft of the hair by the enzyme tyrosinase (Bland 1984;

Ogle and Fox 1999). Individuals with red hair have an additional pigment called phaeomelanin which contains Fe (Ogle and Fox 1999). Phaeomelanin is produced by the enzyme trichosilverin, which is responsible for the red pigmentation. Because of the iron content of this pigment individuals with red hair will typically have a higher level of iron present compared to blonde individuals or brunette individuals. This is important to consider when comparing trace element contents of individuals who have different colors of hair and when comparing populations with higher rates of one hair color (Bland 1984). Frompovich (1982) found that individuals with blonde hair typically have higher levels of Cu and lower levels of Zn, while individuals with black hair have higher levels of Chromium (Cr), Pb, and Zn. The color of an individual's hair is also affected by other factors such as reflectivity and surface transparency (Ogle and Fox 1999).

There have been approximately 60 different elements that have been identified in human hair in various concentrations that range from 0.01 to 100 micrograms per gram (ppm) (Bland 1984; Lenihan 1988). Because of the unique structure and growth pattern of hair, it can be used to study metabolic shifts of these 60 elements over long periods of time, which is extremely useful in the research of nutrition in the past (Bland 1984). Analysis of trace elements in human hair measures the intracellular level of that particular element (Turner-Pearson 2007). High intracellular levels of an element may be indicative of disproportionate deposition of that element in the hair tissue, which can cause disproportionately low levels of that element in other biological tissues. Bland (1984) argues that high levels of certain trace elements are just as, if not more, indicative of tissue imbalance than are low levels of certain trace elements. However,

the levels of trace elements that are found in human head hair are not just related to nutritive status but also to the ability of that particular individual to absorb any given trace element (Bland 1984). Trace elements that are typically found in the highest quantities are K, Na, P, and S, all of which are associated with physiological functions inside the human body (Goulding 1999).

### Elemental Analysis of Hair and Sex Estimation

Trace element analysis of hair was developed in the United States (U.S) in the second half of the twentieth century and since that time has been applied in toxicological, medical, forensic, environmental, and anthropological research (Chojnacka et al. 2010). Trace element content in hair has been used primarily to explore contact with toxic elements (Sera et al. 2002), pollution levels (Bencko 1995; Miekeley et al. 1998), relationship with diseases (Chojnacka et al. 2006; Man et al. 1996), and nutrition (Apostoli 2002). Even in anthropological research trace elements are mainly seen as a way to evaluate exposure to toxic elements in populations and individuals (Sen and Das Chaudhuri 2001). This technique is rarely used in anthropology outside of environmental contexts despite the potential it has for answering questions about nutrition, growth, and development within past and present populations. Hair is an excellent tissue for these kinds of analyses because it acts as a receptacle for excreted trace elements, and the trace element content of hair is stable over long periods of time (Lee et al. 2000; Sera et al. 2002; Zakrgynska-Fontaine et al. 1998). Furthermore, hair samples can be collected quickly and painlessly as opposed to



other human body tissues. Hair samples are also small and easy to store (Chojnacka et al. 2005). These same qualities that make hair a great tissue for use in these studies also make it suitable for studying many other features of humans (Valkovic 1988).

The elements that accumulate in hair over a person's lifetime can be split into two different groups: macro-elements and micro-elements, also known as trace elements (Chojnacka et al. 2005). Trace elements are further divided into essential and toxic categories, although these categories are somewhat debated. Additionally, essential trace elements are categorized as either major or minor. The major essential trace elements include Cu, Fe, and Zn. The minor essential trace elements include Co, Cr, fluorine (F), iodine (I), Mn, nickel (Ni), Se, and Si (Rahil-Khazen et al. 2002). The toxic trace elements include silver (Ag), aluminum (Al), Cd, Ni, and Pb (Bermejo-Barrera et al. 2002). The content of these different trace elements in hair is dependent on many factors: age, hair color (Sturaro et al. 1994), the presence of other elements, seasonal variation, geography (Chojnacka et al. 2006), diet (Miekeley et al. 1998), and sex.

Many studies that use trace element analysis of hair have generated information concerning the difference in the content of trace elements in the hair of males and females (Ashraf et al. 1995; Chojnacka et al. 2010; Sturaro et al. 1994; Zaitseva et al. 2015; Zakrgynska-Fontaine et al. 1997) (Table 1). Furthermore, most of these studies report that there is a statistically significant difference in the content of multiple trace elements between the sexes. For example, Rodushkin and Axelsson (2000) found that there was 98.5% more barium (Ba), 200% more Ca, 81% more Mg, 90% more Si, and 84% more Sr in female hair. However, while these studies note that there is a difference

between the sexes, the purpose of these studies is to highlight the effect that sex might have on other varieties of trace elemental analysis. For example, Zaitseva et al. (2015) determined that bismuth (Bi), Co, Fe, Hg, and Mg concentrations were higher in female hair than in male hair, as is seen in Table 1. The purpose of their study, however, was to determine the influence that physical activity had on trace element content in hair, not biological sex. The research into biological sex and trace element analysis has always ended with the acknowledgement of an existing relationship; however, none of these studies have sought to apply this information any further. Also many of these studies have pointed to differences in trace element content between the sexes that can be both exogenously and endogenously deposited, which is not necessarily an accurate representation of biological sex. Nevertheless, these studies provide a very useful basis of knowledge for the expansion of research into the relationship between biological sex and trace element analysis. However, it is important to be aware that some of these studies have differing methodologies, for example, different sample preparation techniques or use of different equipment such as neutron activation analysis, and therefore they may not be comparable in all ways.

Table 1: Significant Differences in Trace Element Content between Sexes in Literature Reviewed

Source	Higher in Females	Higher in Males
Dongarrá et al. 2011	Al, Ba, Sr, Zn	Cr, Li, Rb, Sb, U
Zaitseva et al. 2015	Bi, Co, Fe, Hg, Mg	
Chojnacka et al. 2010	Ba, Ca, Cu, K, La, Mg, Si, Sr	
Rodushkin and Axelsson 2000	Ba, Ca, Mg, Si, Sr	
Heinersdorff and Taylor 1979	Zn	
McKenzie 1979	Zn	
Caroli et al. 1994		Zn
Contiero and Folin 1994		Zn
Zakrgynska-Fontaine et al. 1997	Ca, Co, Cu, Mg, Ni, Zn	Fe, Pb
Sturaro et al. 1994	Cu, Mn, Ni, Zn	
Huang and Beauchemin 2014	Ag, Mg, S, Sr, Zn	K, Mn, Mo, Pb, Sn
Nowak 1998	Ca, Cr, Cu, Fe, Mn	Cd, Na, Pb, Zn
Ashraf et al. 1995	Cu, Zn	

Although there is some conflicting information in the literature about which trace elements are dependent on biological sex, there is also good deal of agreement. Cu, Mg, Zn, and Sr have been found to be present in higher concentrations in females in multiple studies (Ashraf et al. 1995; Chojnacka et al. 2010; Dongarrá et al. 2011; Huang and Beauchemin 2014; Sturaro et al. 1994; Zaichick et al. 2011; Zakrgynska-Fontaine et al. 1997). The reason for this is likely due to the differences in metabolism and biological development associated with puberty (Ashraf et al. 1995; Dongarrá et al. 2011; Heinersdorff and Taylor 1979; Khalique et al. 2005). It is important to note that differences in metabolism are present prior to puberty (Ayyavoo et al. 2014). However, it has been noted that Sr differences may be caused by differences in diet due to the fact that the main source of Sr to the human body is ingested plant matter (Zaichick et al. 2011) Some studies, however, have found Zn concentrations to be higher in males

(Caroli et al. 1994; Contiero and Folin 1994). Ba, Si, Co, Ca, Ni, and Mn have also been found in higher concentrations in females in multiple studies (Chojnacka et al. 2010; Dongarrá et al. 2011; Nowak 1998; Rodushkin and Axelsson 2000; Sturaro et al. 1994; Zaitseva et al. 2015; Zakrgynska-Fontaine et al. 1997). However, Mn has also been found in higher concentrations in males (Huang and Beauchemin 2014). It is important to note that samples in these studies were collected in diverse geographical locations, from individuals of varying ages, cultural backgrounds, nutritional status, and health. Despite these differences, much of the data is in agreement as to which trace element concentrations are dependent on biological sex.

Because the metabolic difference between the biological sexes and the metabolic processes of trace elements are neither well understood nor well documented, it is difficult to determine why trace element absorption differs between the sexes. Nevertheless, the proof of these metabolic differences is noted in the literature. A few articles cite differential dietary habits or environmental exposure as the reason behind these differences; this is unlikely to be the main reason (Wilhelm et al. 2002; Zaichick et al. 2011). While diet and environment likely play a part, the literature shows similar trends across geography and time that invalidates this as the sole or main reason for the trend that is seen. Metabolic differences that are present prior to puberty and those that occur as a result of puberty are the most likely explanation for differing levels of trace elements between the sexes (Dongarrá 2011; Rivai 2001). However, the specifics of these metabolic changes or differences are not directly discussed.

Cu and Zn are represented the most in literature available concerning metabolic differences in trace element absorption between the biological sexes. There has been considerable research conducted on the differences in Cu metabolism between animals of opposite sexes, particularly rats (Uchino et al. 1990). Johnson et al. (1992b) found that the metabolic processes for Cu absorption in rats are completely comparable to that of humans, although in both cases female absorption was found to be significantly higher. It should be noted that this difference was not found in men and women above the age of 60, which suggested that Cu absorption may be related to estrogen levels. However, the authors found that exogenous estrogen such as oral contraceptives did not affect Cu absorption in women, which contradicts this theory (Johnson et al. 1992b). It should also be noted that Cd, which is increased by pregnancy, can interfere with the absorption of both Cu and Zn in women due to shared binding sites (Bhattacharya et al. 2000; Satarug et al. 2004). Despite the complex, incomplete, and contradictory metabolic evidence it seems evident that differential absorption of trace elements still exists between males and females.

#### Bioavailability of Trace Elements

The number and content of trace elements in human head hair is determined based on the bioavailability of those trace elements. The bioavailability of a trace element is the amount of that element that becomes available for use within the human body via inhalation, ingestion, or direct contact (Tchounwou et al. 2012). Bioavailability

is dependent upon multiple different factors, such as geographic location, element solubility, individual diet, and metabolic differences (Freeland-Graves et al. 2015).

### Bioavailability of Mg

Mg is an essential trace element that is involved in numerous metabolic reactions within the human body (Food and Nutrition Board 1997). Mg can affect the contraction of muscles, cardiac rhythms, and nerve impulse conduction through its involvement in the transportation of ions across cell membranes (Rude and Shils 2006). Mg is also heavily involved in energy production, as the mitochondria require Mg for adenosine triphosphate (ATP) synthesis. Mg also is needed for the synthesis of DNA, carbohydrates, antioxidants, lipids, and ribonucleic acid (RNA) (Rude and Shils 2006). Cell migration, cell signaling, and cell membrane structure are also dependent on Mg (Rude and Shils 2006).

Mg levels and absorption are affected by its relationship with other trace elements such as Zn and Ca. Increased rates of Zn have been found to interfere with Mg absorption, while decreased Ca levels have been found to correspond with decreased Mg level (Food and Nutrition Board 1997; Rude and Shils 2006; Spencer et al. 1994).

Mg deficiency is rare, due to an abundance of Mg in food as well as the renal system's ability to moderate excretion, but can occur, especially in individuals who suffer from renal disorders and alcoholism (Rude and Shils 2006; Shils 1997). Older individuals have also been found to be more susceptible to Mg deficiency due to

decreased absorption capabilities of the intestines along with lowered dietary Mg consumption (Food and Nutrition Board 1997; Moshfegh et al. 2009; Sebastian et al. 2007). Furthermore, women suffering from osteoporosis have been found to have lower levels of Mg than women without osteoporosis. It should be noted that studies have found that most Americans do not receive the daily recommended dietary allowance for Mg. This is particularly true of Americans over the age of 50 (Moshfegh et al. 2009).

Dietary sources of Mg include nuts, whole grains, green leafy vegetables, meats and milk (Table 2). Mg can also be ingested via drinking water. However, hard or alkaline water has been found to be high in Mg, unlike soft water (Food and Nutrition Board 1997; Marx and Neutra 1997). Furthermore, Mg is an ingredient in many antacids and can also be taken in supplement form (Hendler and Rorvik 2001).

Table 2: Rich food sources of magnesium, showing amount of magnesium per serving, derived from USDA 2015

Source	Serving	Magnesium (mg)	Percent Daily Value
Bran Cereal	½ cup	112	28
Brown Rice	1 cup	86	22
Fish (Mackerel)	3 ounces	82	20
Spinach	½ cup	78	19
Almonds	1 ounce	77	19
Swiss Chard	½ cup	75	18
Milk	8 fl. ounces	34	8.5

### Bioavailability of Cu

Cu is a major essential trace element in the human body that serves multiple functions. Cu is involved in cellular energy production via a Cu dependent enzyme, which assists the mitochondria with ATP production by producing an electrical gradient

(Uauy et al. 1998). Cu also plays a role in the transportation and metabolism of Fe. Studies have shown that Cu based enzymes oxidize Fe so that it may be transported to red blood cell formation sites. This is supported by reports of abnormal Fe accumulation in individuals who lack these Cu based enzymes and the fact that Fe transportation can be inhibited by Cu deficiency (Kono 2012; Thackeray et al. 2011). Cu based enzymes are also involved in bone and connective tissue formation and many reactions that are imperative to healthy central nervous systems are dependent on Cu based enzymes (Harris 1997; Turnlund 2006). Tyrosinase is a Cu based enzyme that converts eumelanin into its active form for incorporation into hair shafts (Bland 1984; Ogle and Fox 1999; Turnlund 2006). Cu is also shown to have antioxidant functions and assist in the regulation of gene expression (Johnson et al. 1992a; Mattie et al. 2008; Turnlund 2006).

Cu interacts with multiple other trace elements such as Fe and Zn. Sufficient levels of Cu are required for Fe metabolism and the formation of red blood cells. In infants, however, high Fe intake can lead to Cu deficiency as increased Fe can inhibit Cu absorption (Food and Nutrition Board 1997). Similarly, high Zn intake can also lead to Cu deficiency. Higher levels of Zn increase the synthesis of certain proteins, which may bind to Cu and prevent intestinal absorption. However, heightened levels of Cu do not affect Zn levels (Food and Nutrition Board 1997; Turnlund 2006).

Cu deficiency is seen in infants that are fed formula made from cow's milk as opposed to breast feeding. This is because milk from cows has relatively little Cu (Shaw 1988). Individuals with celiac disease and cystic fibrosis have also demonstrated



increased Cu deficiency (Best et al. 2004). Cu deficiency can also be inherited as part of Menkes disease (Kodama et al. 2012; Tumer 2013).

Many foods contain Cu, such as nuts, seeds, shellfish, organ meat, and whole grains (Table 3) (Fraga, 2005; Sadhra et al. 2007). Cu supplements are another source of Cu (Hendler and Rorvik 2001). Cu can also be ingested via drinking water that has been stored in Cu containers or has been routed through Cu plumbing (Bremner 1998; Brewer 2009). Another source of Cu may be exposure to industrial operations and use of Cu piping (Herman et al. 2013).

Table 3: Rich food sources of copper, showing amount of copper per serving, derived from USDA 2015

Source	Serving	Copper (mg)	Percent Daily Value
Beef Liver	3 ounces	8.29	414
Oysters	3 ounces	4.85	242
Crab Meat (Alaskan King)	3 ounces	0.78	39
Cashews	1 ounce	0.62	31
Lentils	1 cup	0.50	25
Pumpkin Seeds	1 cup	0.44	22

### Bioavailability of Zn

Zn is a major essential trace element responsible for multiple aspects of cellular metabolism. There are more than 300 Zn dependent enzymes in the human body (McCall et al. 2000). Furthermore, Zn provides stability to many protein structures and to cell membranes (Food and Nutrition Board 2001; King and Cousins 2006; O'Dell 2000). Zn is also involved in gene expression by attaching to DNA and manipulating the transcription of certain genes. Hormone release and apoptosis, or cellular death, are

also regulated by Zn which means that Zn is very important in overall cellular growth and death (Truong-Tran et al. 2000).

Zn has relationships with multiple other trace elements, such as Fe, Ca, and Cu, which influence its bioavailability. For example, high Ca levels may inhibit the absorption of Zn (Wood and Zheng 1997). Zn absorption may also be impaired by extremely high Fe levels, which is of particular concern for pregnant or lactating women who may be taking Fe supplements (Fung et al. 1997; O'Brien et al. 2000; Sandström 2001). Alternatively, high levels of Zn can inhibit the absorption of Cu by synthesizing a protein which traps Cu within the cells of the intestines (King and Cousins 2006).

Children and adolescents, particularly those in developing countries, are at increased risk for Zn deficiency, as are pregnant and lactating women, individuals over 65 years of age, and vegetarians (Brown et al. 2004; Fischer Walker et al. 2009; King and Cousins 2006; Krebs 2013; Prasad 2012). Zn deficiency can also be inherited in the form of acrodermatitis enteropathica, which restricts the amount of Zn that is absorbed (Hambidge 2000; King and Cousins 2006).

There are many foods which contain Zn, including red meats, such as beef, and shellfish, such as oysters. Nuts and legumes are also good sources of Zn (Table 4). The Zn in meat and shellfish, however, is more bioavailable than the Zn in plants, whole grains, and nuts because of the presence of phytic acid in plants and nuts which impairs the absorption of Zn (King and Cousins 2006). Yeast neutralizes the effects of phytic acid on Zn absorption; therefore, leavened bread has more bioavailable Zn than unleavened bread (Food and Nutrition Board 2001). Other sources of Zn include Zn

supplements and the inhalation of Zn oxide fumes created during welding or smelting (Food and Nutrition Board 2001; King and Cousins 2006). Also foods and or liquids that have been stored in galvanized storage containers can become contaminated with elevated levels of Zn (King and Cousins 2006).

Table 4: Rich food sources of zinc, showing amount of zinc per serving, derived from USDA 2015

Source	Serving	Zinc (mg)	Percent Daily Value
Oysters	6 medium	27 – 50	256
Beef	3 ounces	3.7 – 5.8	35
Beans	½ cup	0.9 – 2.9	19
Cashews	1 ounce	1.6	11
Chickpeas	½ cup	0.5 – 1.3	8

#### Bioavailability of Fe

Fe is a major essential trace element that is required for important metabolic functions for the human body. Fe is involved in the storage and mobilization of oxygen through the protein hemoglobin. Hemoglobin is comprised of heme, which contains iron and is a large component of red blood cells (Brody 1999; Yip and Dallman 1996). Myoglobin also contains heme and, similar to hemoglobin, is responsible for transporting oxygen to the cells found in the muscles (Brody 1999). Fe is also required for DNA synthesis and important immunological responses as well as physiological compensation for low oxygen (Beard and Dawson 1997; Brody 1999; Fairbanks 1999; Ivan et al. 2001; Jaakkola et al. 2001; Yip and Dallman 1996). Fe helps to synthesize ATP for cellular energy through heme based compounds which serve as electron transport for the mitochondria (Yip and Dallman 1996).

Fe bioavailability is dependent on the relationship between Fe and other trace elements. A positive correlation between vitamin A and Fe has been noted; where Fe levels increase as vitamin A levels increase (Suharno et al. 1993). As discussed previously Cu deficiency and Fe deficiency are related (Turnlund 2006). Furthermore, high levels of Fe can impair Zn absorption while Ca intake has been found to decrease the absorption of both heme and nonheme Fe (Food and Nutrition Board 2001).

Fe toxicity can occur when Fe builds up intracellularly. However, the body has processes in place for regulating Fe homeostasis (Anderson et al. 2007). Conversely, Fe deficiency is a much bigger problem as it is the most common nutritional deficiency throughout the world. Fe deficiency is typically separated into three levels of severity: storage depletion, early functional deficiency, and Fe deficiency anemia (Yip and Dallman 1996). Storage depletion is the least severe and occurs when the body's stores of Fe have been depleted but the levels of Fe needed to carry out daily functions are not impaired. Early functional Fe deficiency occurs when the functional Fe supply is affected and red blood cell formation may be affected at this stage. Fe deficiency anemia occurs when red blood cell formation is severely affected and hemoglobin levels have decreased (Yip and Dallman 1996). Fe is extremely important and necessary during periods of rapid growth; therefore, infants and adolescents are extremely susceptible to Fe deficiency (Brody 1999). Conversely, older adults are more likely to have high Fe stores as opposed to Fe deficiency (Fleming et al. 2001). Pregnant women are also at increased risk for Fe deficiency due to blood loss and the development of the fetus. Menstruating women are at similar risk due to blood loss (Brody 1999). Vegetarians

also have a higher rate of Fe deficiency because of the lower bioavailability of Fe from plants. Fe from plant matter is not absorbed as easily as Fe from meat (Food and Nutrition Board 2001).

Foods that are good sources of Fe include red meat, fish, legumes, and poultry, as well as grain products which are typically fortified with Fe in the U.S. (Table 5). Fe found in meat and fish is in the form of heme, whereas Fe found in plants and dairy is not (Lynch 1997). This affects the amount of Fe that is available for absorption because nonheme Fe is not as easily absorbed by the body (Lynch 1997; Yip and Dallman 1996). However, there are certain factors that may increase the absorption of nonheme Fe, such as vitamin C, citric acid, meat, and fish (Food and Nutrition Board 2001; Lynch 1997; Yip and Dallman 1996). Similarly, there are factors that may decrease the absorption of nonheme Fe, such as phytic acid, found in legumes and rice, polyphenol, found in vegetables, tea, and wine, and soy (Fairbank 1999; Food and Nutrition Board 2001). Antacids may also inhibit the absorption of Fe, while Fe supplements provide another source of Fe (Hendler and Rorvick 2008).

Table 5: Rich food sources of iron, showing amount of iron per serving, derived from USDA 2015

Source	Serving	Iron (mg)	Percent Daily Value
Oysters	6 medium	5.04	28
Lentils	½ cup	3.30	17
Beef	3 ounces	2.32	11
Prune Juice	6 fl. ounces	2.28	11
Tofu	⅓ cup	2.15	11
Tuna	3 ounces	1.30	6

### Bioavailability of Ca

Ca is a trace element that is found predominantly within the skeletal structures of the body. Ca is required for many physiological functions within the body. Ca is so important for healthy bodily function that the body will begin to resorb bones if Ca levels in the blood dip too low (Weaver 2012). In this way the body maintains Ca levels in the blood by releasing the Ca that is trapped in the bone. For this reason, acquiring appropriate amounts of Ca is important for maintaining healthy bone density.

Hydroxyapatite, the component of bone that gives it its rigidity, is Ca based; therefore, Ca plays a large role in the structure of bone (Weaver 2012). Ca is also involved in muscle contraction, transmission of nerve impulses, vasodilation, and insulin secretion (Weaver 2012).

Ca interacts with multiple other trace elements, including Fe. Nonheme Fe absorption is decreased by Ca (Weaver 2012). Furthermore, Ca intake in high levels, commonly seen with supplement ingestion, could decrease the absorption of Zn by up to 50% if ingested simultaneously (Wood and Zheng 1997).

Ca deficiency can result from low levels of Mg or vitamin D or from kidney failure. Ca deficiency most often results from abnormal function of the parathyroid, which can be exacerbated by Mg deficiency (Weaver 2012). Ca deficiency typically leads to osteoporosis in older adults, particularly in women over 50 (Kaufman et al. 2013). Lower estrogen levels caused by menopause can decrease the absorption of Ca while increasing the resorption of bone (Breslau 1994; Gallagher et al. 1980; Heaney et al. 1989). As such postmenopausal women are at particular risk for Ca deficiency as well

as osteoporosis. Individuals who are lactose intolerant are also at increased risk for Ca deficiency, due to decreased ingestion of dairy products (Food and Nutrition Board 2010; Johnson et al. 1993; Suchy et al. 2010). Vegans, and to a lesser extent, vegetarians, are also at increased risk because they do not ingest dairy products and ingest higher amounts of plant matter which contain oxalic acid, an inhibitor of Ca absorption (American Dietetic Association 2003; Food and Nutrition Board 2010; Janelle and Barr 1995).

Dairy products, such as milk, cheese, and yogurt, are good sources of Ca, while some grains and vegetables also contain Ca (Table 6). However, the Ca found in dairy is more easily absorbed than that found in vegetables or grains (Food and Nutrition Board 2010). The bioavailability of the plants in the kale family, such as cabbage and broccoli, is similar to that of milk. However, oxalic acid, found in spinach and sweet potatoes, can significantly impair the absorption of Ca (Zhu and Prince 2012). Similarly, phytic acid, found in beans, whole grains and soy, will inhibit Ca absorption at a lower level (Food and Nutrition Board 2010). Ca supplements also constitute a large amount of Ca intake (Bailey et al. 2010).

Table 6: Rich food sources of calcium, showing amount of calcium per serving, derived from USDA 2015

Source	Serving	Calcium (mg)	Percent Daily Value
Yogurt (Plain)	8 ounces	415	42
Cheddar Cheese	1.5 ounces	307	31
Milk (Nonfat)	8 ounces	299	30
Bok Choy	½ cup	74	7
Kale	½ cup	47	4.5

### Bioavailability of Mn

Mn is a minor essential trace element that is involved in multiple functions within the human body. Mn containing enzymes are essential to the metabolism of cholesterol and amino acids (Albrecht et al. 2007; Wedler 1994). Furthermore, Mn based enzymes have important antioxidant roles within the mitochondria which are susceptible to oxidative stress (Leach and Harris 1997). Enzymes containing Mn are also required for the formation of bone and cartilage within the body (Keen and Zidenberg-Cherr 1996). Mn also plays a role in healing of wounds due to its involvement in collagen formation within the skin (Muszynska et al. 2000).

Mn has been shown to have relationships with other trace elements. Although, the exact mechanisms of Mn absorption are not understood, there is evidence that the process is similar to that of Fe absorption (Fitsanakis et al. 2009). Absorption of Fe and Mn have an inverse correlation and Fe deficiency results in heightened levels of Mn (Aschner and Dorman 2006; Keen and Zidenberg-Cherr 1996). Extremely high levels of Mg or Ca, such as that from supplements, will decrease the bioavailability of Mn as well (Kies 1994).

Mn deficiency is not well understood or common, while Mn toxicity is much more frequently documented. Pregnant and lactating women in particular require more Mn than other women; however, in general women require significantly lower levels of Mn (Food and Nutrition Board 2001). Older women with osteoporosis have been found to have decreased Mn levels (Freeland-Graves and Llanes 1994; Reginster et al. 1988). The specific reasons for this, however, are not fully understood. Vegetarians tend to



have higher Mn intakes (Food and Nutrition Board 2001). Mn toxicity is more commonly seen in individuals with Fe deficiency due to their inverse relationship (Aschner and Dorman 2006). Individuals with chronic liver disease are also at risk because Mn is eliminated from the body in bile, and impaired liver function causes less Mn to leave the body (Hendler and Rorvick 2001; Keen et al. 1999). Infants and children are also more susceptible to Mn toxicity because of higher absorption rates and lower excretion rates caused by immature liver elimination systems (Food and Nutrition Board 2001; Ljung and Vanter 2007).

Foods that contain Mn include nuts, teas, whole grains, and leafy vegetables (Table 7). Similar to Fe foods that contain phytic or oxalic acid will inhibit the absorption of Mn. These foods include beans, soy, spinach, sweet potatoes, and nuts. Teas, which are a good source of Mn, contain tannins which can also inhibit the absorption of Mn slightly (Kies 1994). Mn is also present in drinking water at various levels, in the US the Environmental Protection Agency (EPA) recommends water levels of Mn be under 0.05 mg (United States Environmental Protection Agency 1986). Mn supplements are another source of Mn available (Hendler and Rorvick 2001). In infants, breast milk and/or formula provides their main source of Mn. In general, Mn levels are lower in breast milk than either soy or cow milk based formulas, however, the Mn in breast milk is more bioavailable. Nevertheless, the Mn in cow milk formulas is ten times that of breast milk and the Mn in soy based formulas is 100 times that of breast milk (Aschner and Aschner 2005). Mn can also be inhaled in the form of dust created by smelting or welding (Food and Nutrition Board 2001; Keen et al. 1999). Mn can also be inhaled as a byproduct of

gasoline and airborne Mn is higher in urban areas than rural areas (Aschner 2000; Bolte et al. 2004). Mn is also found in many antacids and laxatives (Hendler and Rorvick 2001).

Table 7: Rich food sources of manganese, showing amount of manganese per serving, derived from USDA 2015

Source	Serving	Manganese (mg)	Percent Daily Value
Pecans	1 ounce	1.28	25.6
Pineapple	½ cup	0.77	15.4
Whole Wheat Bread	1 slice	0.60	12
Tea (Black)	1 cup	0.18 – 1.58	3.5 – 31.6
Lima Beans	½ cup	0.49	9.8

#### Bioavailability of Pb

Pb is toxic trace element that does not serve any physiological function within the human body. There is no recommended daily intake for Pb as it is not considered necessary for maintaining healthy living. Pb is not typically found in food sources; however, it can contaminate food. Soil and water contamination are common types of Pb contamination, however, unhealthy levels of Pb can also be taken in by contact with paint, smoking, vehicle emissions, and smelting or mining activities (Rieuwerts et al. 2000).

#### Bioavailability of Sr

Sr is a trace element that is similar to Ca in that they both have similar chemical properties and similar pathways within the human body. However, Ca is preferentially absorbed (Comar et al. 1957). Sr levels are also transmitted from pregnant mothers to their fetuses and from lactating mother's milk to their infants (Comar et al. 1957). Sr is

typically absorbed through drinking water and also through consumption of certain foods (Comar et al. 1957; Rosenthal et al. 1972). A major source of dietary Sr is from green leafy vegetable such as spinach and kale, as well as grain and dairy products (Agency for Toxic Substances and Disease Registry 2004). However, another huge source of Sr is atmospheric strontium from the fallout of nuclear testing (Comar et al. 1957). Emissions from the burning of oil and coal also can be a source of Sr inhalation and Sr can also be found in small amounts in drinking water as well as the soil (Agency for Toxic Substances and Disease Registry 2004).

#### Bioavailability of Se

Se is a minor essential trace element that is required for the proper functioning of a variety of Se based enzymes (Mariotti et al. 2012). These Se containing enzymes perform a variety of functions including: antioxidant functions, such as protecting spermatozoa against oxidative stress, regulating cell growth, synthesis of hormones, and glucose metabolism among others (Boitani and Puglisi 2008; Lu and Holmgren 2014; Schomburg 2012).

Se deficiency risks are increased in individuals who suffer from Crohn's disease and other gastrointestinal conditions. Individuals who are chronically ill and have been receiving intravenous nutrition may also be at increased risk for Se deficiency (Cooper et al. 2012). In general, Se deficiency is not common in the U.S. (Food and Nutrition Board 2000).

Food sources of Se include seafood and organ meat as well as muscle meat and grains (Table 8). Water is not a significant source of Se in the US (Peplow and Edmonds 2004). Se levels in water are highly dependent on the Se levels in the soil. Similarly, Se levels in plants depend heavily on the Se levels in the soil in which they are grown. Certain plants, however, are more susceptible to Se accumulation than others, such as Brazil nuts and garlic (Chang et al. 1995). Se supplements provide another source of Se (Food and Nutrition Board 2000), however, the bioavailability of organic Se found in food is much higher than that of inorganic Se found in supplements (Rayman et al. 2008). Se toxicity can also occur if levels exceed recommended values (Food and Nutrition Board 2000).

Table 8: Rich food sources of selenium, showing amount of selenium per serving, derived from USDA 2015

Source	Serving	Selenium (mg)	Percent Daily Value
Brazil Nuts (From Se Rich Soil)	1 ounce	0.543	777
Tuna	3 ounces	0.092	131
Clams	3 ounces	0.054	77
Pork	3 ounces	0.032	45
Sunflower Seeds	¼ cup	0.018	26
Whole-Wheat Bread	2 slices	0.016	22

## **CHAPTER THREE: RESEARCH MATERIALS AND METHODS**

The analysis of trace element content in human hair was carried out on 77 hair samples from three different sample groups. The first sample group consists of 32 archaeological samples from Kellis, Egypt; the second sample group consists of 25 cadaver samples from the UCF College of Medicine's Willard-Body Program; and the third sample group consists of 20 hair samples from living individuals in Florida. All 77 of the samples were prepared and analyzed by the author at the University of Central Florida. The female to male ratio in both the cadaver and living samples (this grouping of cadaver and living samples together will be referred to as the modern sample from here on out) are representative of the sex ratio in the U.S. as a whole and Florida in particular (Figure 3).

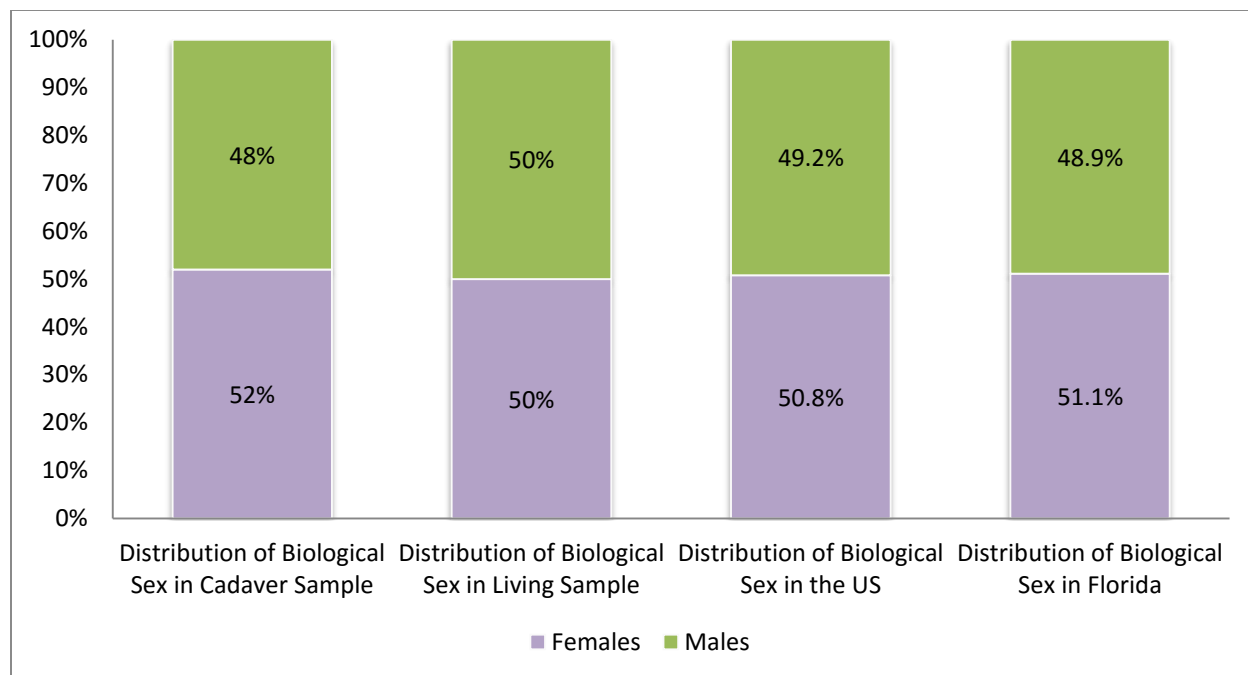


Figure 3: Comparison of the similar distributions of sex between the cadaver sample, living sample, Florida, and the United States

For the purposes of this study, individuals were classified by age as follows: 1) juveniles (<18 years; n = 7), 2) adults (18-50 years; n = 41), and 3) older adults (>50 years; n = 29). The 18 year mark was used to separate juveniles from adults based on the legal conventions in the U.S. which are of importance to forensic anthropology and a natural age gap within the sample. The 50 year mark was used to separate adults from older adults based on the decreased skeletal distinctions in individuals over the age of 50 and a natural age gap within the sample. However, it is important to note that these numbers were arbitrarily assigned by the author and do not necessarily accurately reflect social, chronological, or biological age. The purpose for defining these age groups is to detect any potential age related differences in trace elements within the total sample that would not be possible otherwise, due to limited sample size and

uneven age representation. Within the archaeological sample group seven were classified as juveniles, 20 were classified as adults, and five were classified as older adults. Within the cadaver sample group none were classified as juveniles, one was classified as an adult, and 24 were classified as older adults. Within the modern sample group none were classified as juveniles, 20 were classified as adults, and none were classified as older adults.

Age ratios of the modern sample group are not quite representative of the U.S. age distribution (Figure 4). IRB restrictions prevent the inclusion of living individuals under the age of 18, and a higher proportion of older individuals donate their bodies to willed body programs, which due to age restrictions do not allow individuals under 18 to donate their bodies. These factors directly impact the age ratios of the living sample group and the total sample group, particularly with regard to the number of juveniles included in this study.

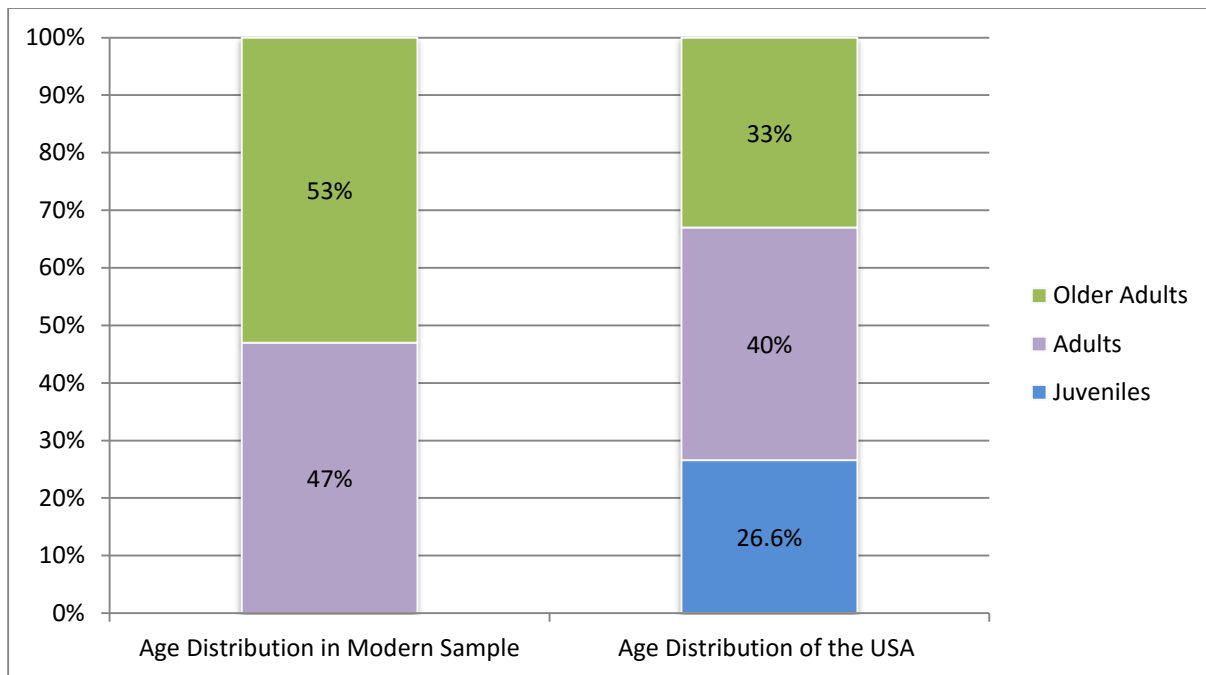


Figure 4: Comparison of the biological age distribution between the modern sample and the U.S. showing the differences in juveniles

All samples were coded with representative identifiers ('A' for archaeological sample, 'C' for cadaver sample, or 'L' for living sample) and numbered to distinguish one individual from another using existing program designations. For example, a sample identifier for an individual represented in the archaeological sample group would be A-57.

#### Archaeological Sample

The archaeological samples from the Kellis 2 cemetery, Dakhleh Oasis, Egypt were obtained for this study courtesy of the Dakhleh Oasis Project and Dr. Tosha Dupras. The Dakhleh Oasis is located 660km south west of Cairo and 330 km west of the Nile in the Western Desert of Egypt (Cook et al. 1998; Giddy 1987). The Oasis is



home to the ancient site of Kellis, known also as Ismant el-Kharab, which is located along an ancient trade route that ran through Dakhleh (Hope 2001). Much is known about Kellis as a result of the Dakhleh Oasis Project, which has been studying and excavating the ancient settlement since 1978 (Mills 1984). The archaeological sample consisted of hair from 32 individuals (seven juveniles, 20 adults, and five older adults). These samples were collected from the Kellis 2 cemetery by the Dakhleh Oasis Project Bioarchaeology Team. The author was blinded to the estimated sex of the individuals in the archaeological sample in order to test the reliability of using variance values from trace elements and stepwise binary logistic regression to predict biological sex. The adults were sexed by the Dakhleh Oasis Project Bioarchaeology Team using standard morphological assessment of the skeletal remains as described in Buikstra and Ubelaker (1994), while the sex of the juveniles was based on the preservation of genitalia soft tissue. Therefore, the archaeological sample consisted of 15 females, 16 males, and one unknown. The female to male ratio in the archaeological sample is representative of the sex ratio found in the Kellis 2 cemetery (Figure 5).

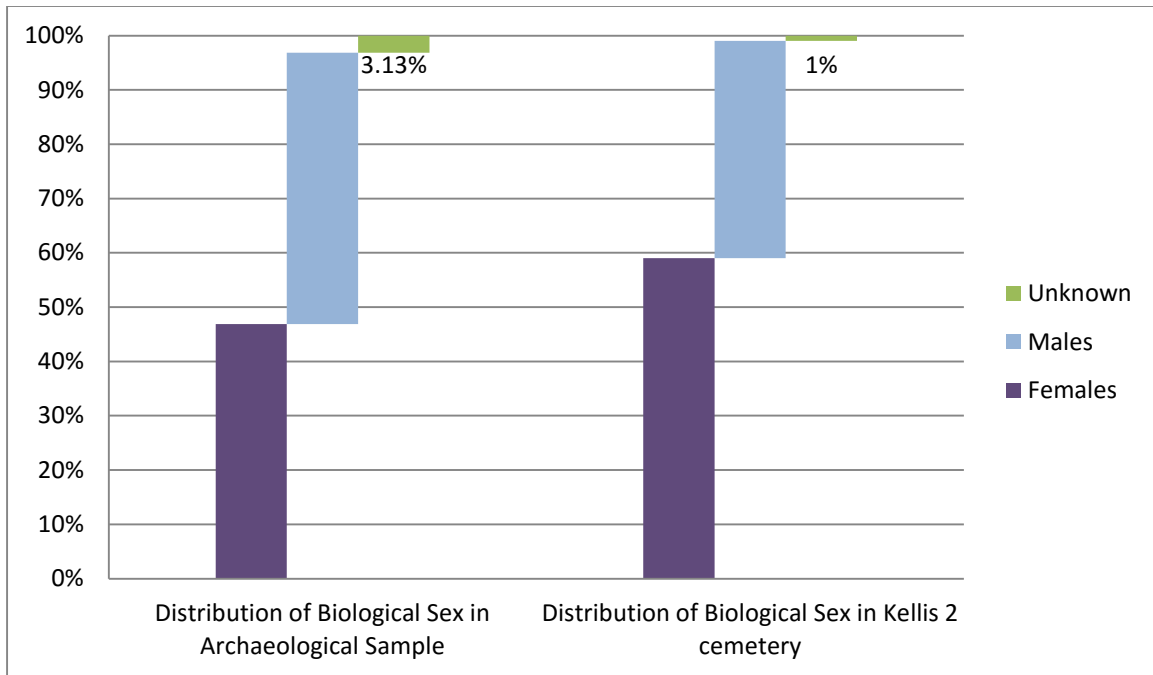


Figure 5: Comparison showing the similar biological sex distributions between the archaeological sample and Kellis 2 Cemetery

Ages of the individuals in the archeological sample were also unknown to the author prior to analysis of the hair samples and were estimated by the Dakhleh Oasis Project Bioarchaeology Team using standardized aging methods found in Buikstra and Ubelaker (1994). The average age of the archaeological sample was 28.26 years with the youngest individual being 40 gestational weeks old and the oldest individual being 61 years old. The archaeological hair samples were stored in individual plastic bags that were labeled with a sample identifier that corresponds to their burial project referenced number, such as A-131.

### Cadaver Sample

The modern cadaver samples from the UCF College of Medicine's Willard-Body Program were obtained for this study courtesy of the program and its director, Dr. Andrew Payer. The cadaver sample group consisted of 25 individuals (13 females, 12 males). These samples were collected under the supervision of the Director of UCF Anatomical Facilities, Jennifer Mark, and these samples were cut from the head of the individual upon arrival at the UCF College of Medicine's Anatomical Facility. Ages of the individuals in the cadaver sample were collected from medical or legal records obtained by the UCF College of Medicine's Willard-Body Program. The average age of the cadaver sample was 81.96 years with the youngest individual being 49 and the oldest individual being 101. The cadaver hair samples were stored in individual plastic bags that were labeled with a sample identifier, such as C-404.

### Living Sample

Samples from living individuals were obtained for this study from individuals over 18 from the state of Florida who were willing to participate. The living sample consisted of 20 individuals (10 females and 10 males). These samples were collected by the author with IRB approval (IRB00001138, see Appendix A for IRB approval letter). Ages of the individuals in the living sample were self-reported via survey (see Appendix B for pre-collection survey) at the same time as the hair sample. The average age of the living sample was 27.95 years with the youngest individual being 22 and the oldest individual being 45.

Other background information was also collected from the living individuals at the same time as sample collection. Individuals were asked to complete a pre-collection survey which included information such as age, exposure to specific environments, smoking habits, and hormonal contraceptive use. Following completion of the survey three to six strands of hair were collected either by running a comb through the individual's hair or, if preferred, by plucking. Unlike with stable isotope analysis and DNA testing, the growth phase, presence or absence of the root, and exposure to water will not affect the analysis of trace elements (McNevin et al. 2005; Williams et al. 2011). The hair sample was then placed in an individual plastic bag, which was labeled with a sample identifier that corresponded to the sample identifier on the top of the survey, such as L-10.

### Sample Preparation

All hair samples were prepared for analysis at the University of Central Florida using methods outlined by Williams (2008). The length of each sample was measured in centimeters and then placed into a two dram glass vial for cleaning. Shampoo, dust, bleach, conditioners, and sweat on hair can all skew the results of elemental analysis if not properly washed (Batista et al. 2009). Therefore, the cleaning process is important, otherwise it would be impossible to determine whether the detected trace elements were exogenous or endogenous in origin (Chojnacka et al. 2006). Samples were sonicated in distilled water for 30 minutes to remove surface contaminants and then soaked in a methanol:chloroform solution (2:1 v/v) for 24 hours. The samples were then

sonicated for 30 minutes in distilled water to rinse the cleaning solution and any remaining residues. The last two steps were repeated until the distilled water was clear and there were no visible residues present. The samples were then sonicated three more times with distilled water to ensure a complete rinse of solutions and residues. After the samples were clean they were left in the uncapped vials in an empty fume hood to air-dry for 48 hours.

### LIBS Analysis

After sample preparation was completed, each sample was analyzed using Laser Induced Breakdown Spectroscopy (LIBS). This method of analysis was chosen because of the ability to analyze multiple points along a single strand of hair. LIBS is an atomic emission spectroscopy that utilizes a pulsed laser as the source of excitation (Emara et al. 2013). A single strand of hair was fixed to the stage via double-sided tape, and a one-centimeter reference measure was placed alongside the strand. This measure was used to consistently and evenly space the sample points along one centimeter of hair, which corresponds with approximately one month of hair growth (Barth 1986; Harding and Rogers 1999; Hayashi et al. 1991; Valkovic 1977). This analysis was performed under a flow of argon (Ar) at a rate of 1.00 L/min. The diameter of the focal spot of the laser on the hair strand was 100  $\mu\text{m}$  with a laser output of 80% and seven pulse shots. For each individual, one hair strand was analyzed. Eleven evenly-spaced points were analyzed over the length of the one centimeter measure in a single strand of hair from each sample. The resulting spectra for each point were then assessed for the emission

lines at 10 specific wavelengths corresponding with nine specific trace elements as well as carbon (C). The nine trace elements were: Mg, Cu, Zn, Fe, Ca, Mn, Pb, Sr, and Se. The emission lines that were used in this analysis are as follows: the neutral C line at 247.8, the Mg II at 279.5, Cu I at 324.8, Zn I at 334.5, Fe I at 373.7, Ca II at 396.8, Mn I at 403.3, Pb I at 405.8, Sr I at 460.7, and Se I at 473.9. The relative concentrations of these different trace elements were determined by using C as a reference as it is the primary element in all hair. The concentrations of the remaining elements were then calculated as the ratio of the reference element to the element of interest, for example C/Mg. The ratio was calculated in this way because the resultant numbers were considerably easier to work with than if the concentrations had been calculated as the ratio of the element of interest to the reference element, for example Mg/C. However, this creates an inverse relationship where the ratio increases as the element decreases.

### Statistical Analysis

The statistical analyses for this project were performed using Excel and SPSS software. The average and variance of all 11 points for each trace element were calculated in Excel for all 77 individuals. Each statistical test was carried out once using averages (or mean) and then again using variances (or MV) in order to demonstrate and examine the differences between the average monthly concentrations and the monthly fluctuations. The mean and range were calculated for the total sample for each trace element using Excel. Descriptive statistics summarizing the variance data, including mean variance values (MV) and MV range, were also calculated. The mean

and MV for each trace element was also calculated separately for males and females in SPSS.

Box plots were created in order to assess outliers using SPSS. Outliers were only examined for the averages as variance is based on the same measurements. Individuals who were considered extreme outliers, designated by an asterisk (\*), were reexamined to exclude the possibility of an error in data recording (e.g. a typo). Individuals who were extreme outliers for more than one trace element (with the exclusion of Pb and Mn) had the measurements removed for those elements. Pb and Mn were not examined for outliers because exposure levels to both can be very variable throughout a sample group or population.

The associations between the trace element values and age group, sample group, context group, sex, and hormonal contraception use were evaluated in SPSS using a multivariate analysis of variance (MANOVA) with post-hoc analysis of variance (ANOVAs) or independent t-tests when necessary. The p-values were evaluated for significance at the 0.05 level.

The dependent variables included in the MANOVA were biological sex, age group, sample group, context group, and hormonal contraceptive use. For these analyses the biological sex of the individuals was coded with 0 indicating females and 1 indicating males. The age groups were coded as follows: 1 = juveniles, 2 = adults, and 3 = older adults. The sample groups were coded similarly with 1 indicating the archaeological sample, 2 indicating the cadaver sample, and 3 indicating the living sample. The context group coding was 1 for archaeological context and 2 for modern

context. Females who did not use hormonal contraceptives were coded with a 0, while females who did use hormonal contraceptives were coded with a 1. The independent variables were the trace element ratios mean and MV values.

Stepwise binary logistic regression was used to predict biological sex from the set of trace element ratio means and MVs that significantly contributed to the regression model using the known sexes of the living and cadaver samples. All of the outliers that were removed for the MANOVA analyses were returned to the data for the regression equation. A regression model was made for both averages and MVs to predict the natural log odds of being either male or female. These equations were then used to predict the sex of the unknown archaeological adult sample. The archaeological adult sex estimations were then compared to the previously estimated adult sexes.

Then another stepwise binary logistic regression was used to predict the biological sex from only the trace element ratio MVs using the now known sexes of the adult archaeological sample. Another regression model was made using only MVs to predict the natural log odds of being either male or female. This equation was then used to predict the sex of the unknown archaeological juvenile sample. These sex estimations were then compared to the known juvenile sexes.



## CHAPTER FOUR: RESULTS

The mean trace element ratio values for the total sample can be seen in Table 9 along with the minimum and maximum values. The mean C/Mg value for the entire sample was 1.24 with a range from 0.42 to 3.26. The mean value for C/Cu was 11.16 for the total sample with a range from -17.22 to 33.44. The total sample mean value for C/Zn was 41.79 with a range from -191.65 to 351.38. The mean value for C/Fe was 6.46 with a range from 0.85 to 25.73 for the total sample. The mean value for C/Ca for the total sample was 0.26 with a range from 0.08 to 0.84. The mean value for C/Mn was 20.82 for the total sample with a range of -489.97 to 735.57. The total sample mean value for C/Pb was 40.21 with a range of -790.31 to 884.79. The mean value for C/Sr was 13.16 with a range from 1.55 to 44.55 for the total sample. The mean value for C/Se for the total sample was 24.05 with a range of -14.80 to 119.90.

Table 9: Mean, range, and standard deviation of the trace element ratio average values for all nine trace elements tested

	Mean	Std. Deviation	Minimum	Maximum	Range
C/Mg	1.237006	0.755401	0.416735	3.261386	2.844651
C/Cu	11.162821	6.744412	-17.219993	33.436138	50.656132
C/Zn	41.790233	70.346501	-191.645902	351.375389	543.021291
C/Fe	6.459853	5.880058	0.854509	25.729358	24.874849
C/Ca	0.260709	0.161656	0.084212	0.843210	0.759000
C/Mn	20.815145	147.278357	-489.968512	735.566612	1225.535124
C/Pb	40.211084	234.155371	-790.311254	884.787248	1675.098502
C/Sr	13.160334	10.983318	1.549211	44.553122	43.003911
C/Se	24.050415	16.956307	-14.795202	119.898407	134.693609

The outliers that fit the criteria of being extreme outliers in the average of more than one trace element are summarized as follows. Only individuals L-7, L-17, C-390, and C-396 fell into this category. Individual L-7 was an extreme outlier in C/Mg and C/Ca (Figures 6 and 9). Individual L-17 was an extreme outlier in C/Mg and C/Fe (Figures 6 and 10). Individual C-390 was an extreme outlier in C/Cu and C/Ca (Figure 7 and 9). Individual C-396 was an extreme outlier in C/Cu and C/Zn (Figures 7 and 8). Individuals are represented in the box plots by their individual number in SPSS. Individual L-7 is represented by the number 32, individual L-17 is represented by the number 42, individual C-390 is represented by the number 10, and individual C-396 is represented by the number 16. As you can see in Figure 8 there were more extreme outliers than what were excluded for the analyses. However, in this case the individual

represented by the number 9 was only an outlier for this one ratio, C/Zn. All of the outliers that were excluded were male, two were from the living sample and were adults, and two were from the cadaver sample and were older adults.

### Ave. Mg

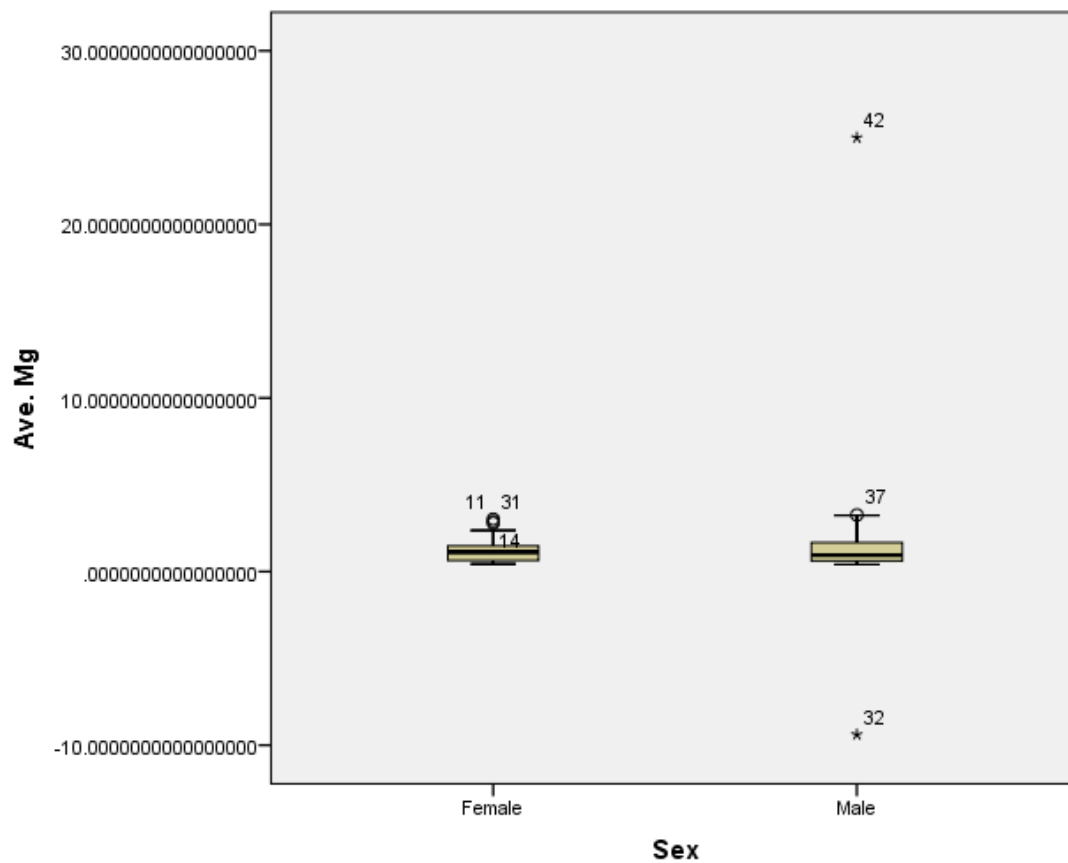


Figure 6: Box and whisker plot demonstrating the outliers for C/Mg based on sex. The extreme outliers are designated by an asterisk and were removed from analysis for this trace element. The number 42 designates individual L-17 and the number 32 designates individual L-7

## Ave. Cu

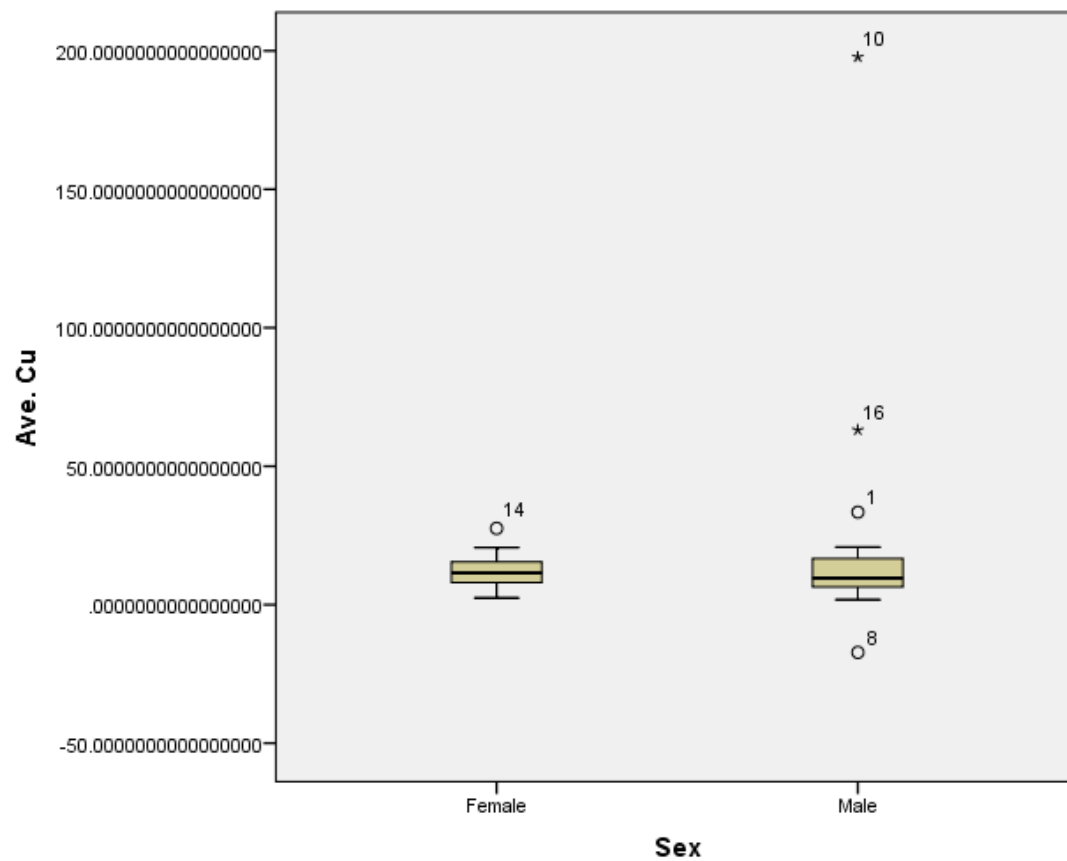


Figure 7: Box and whisker plot demonstrating the outliers for C/Cu based on sex. The extreme outliers are designated by an asterisk and were removed from analysis for this trace element. The number 16 designates individual C-396

## Ave. Zn

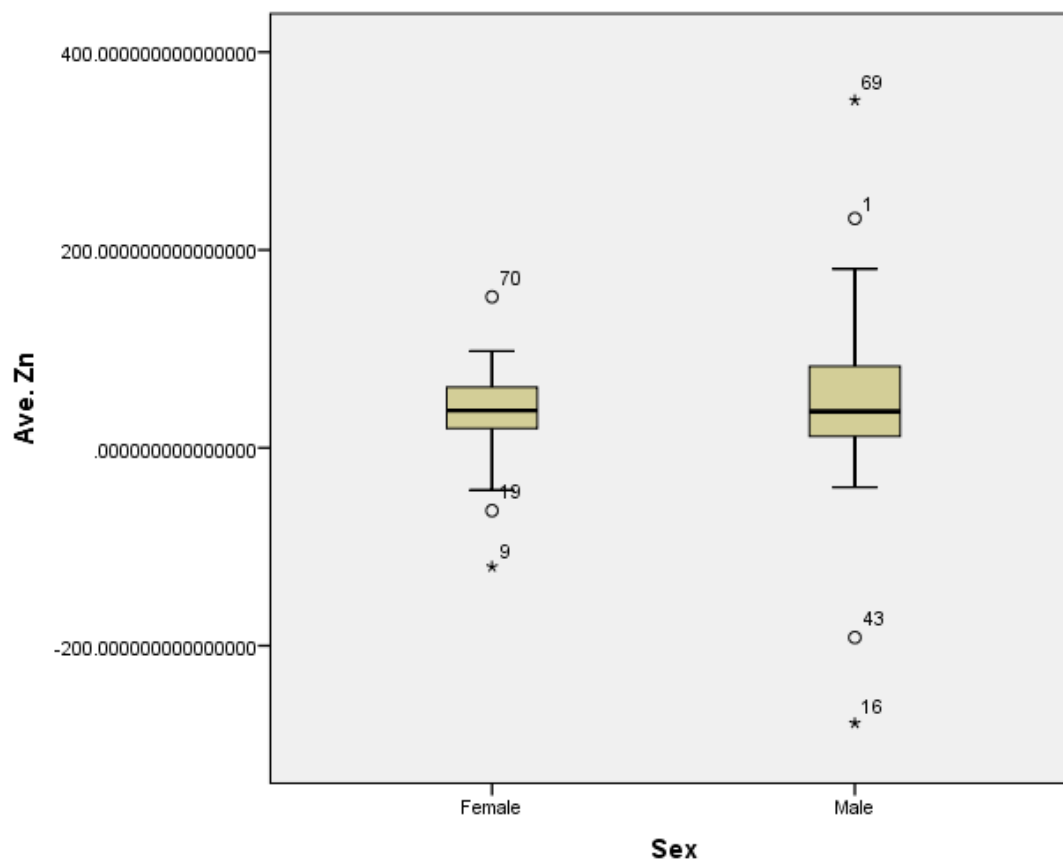


Figure 8: Box and whisker plot demonstrating the outliers for C/Zn based on sex. The extreme outliers are designated by an asterisk and were removed from analysis for this trace element. The number 16 designates individual C-396

## Ave. Ca

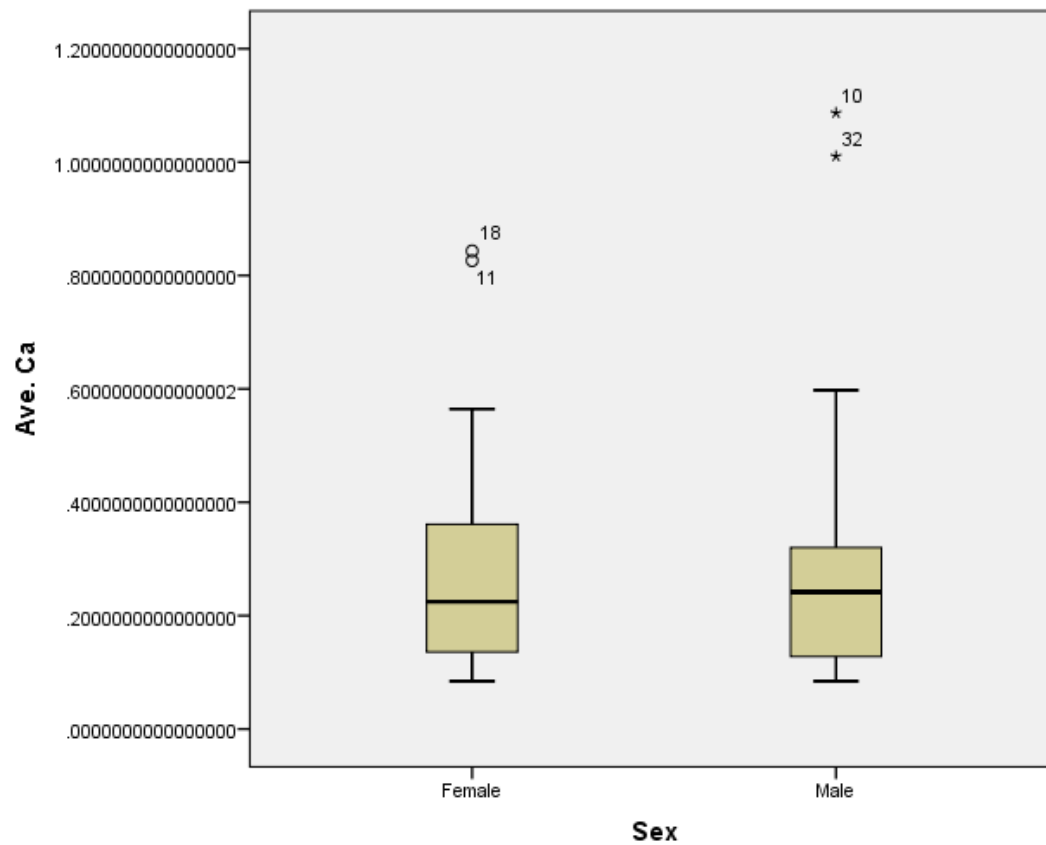


Figure 9: Box and whisker plot demonstrating the outliers for C/Ca based on sex. The extreme outliers are designated by an asterisk and were removed from analysis for this trace element. The number 10 designates individual C-390 and the number 32 designates individual L-7

## Ave. Fe

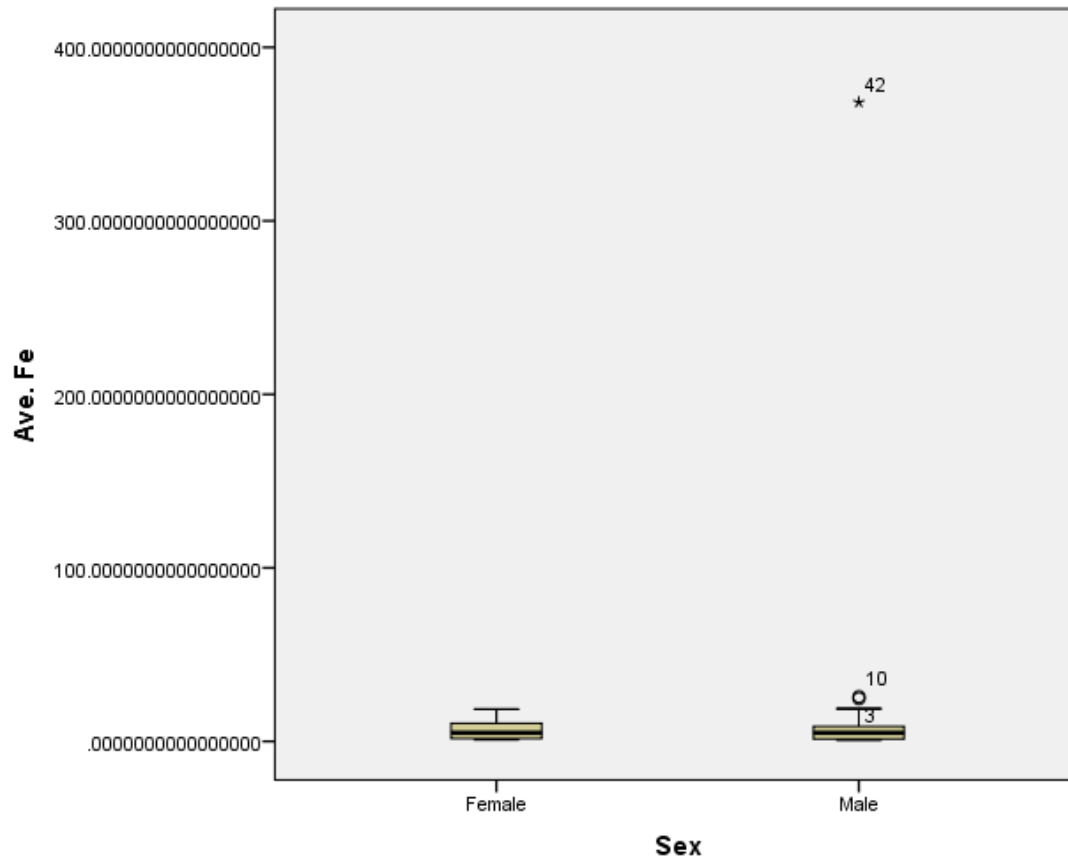


Figure 10: Box and whisker plot demonstrating the outliers for C/Fe based on sex. The extreme outliers are designated by an asterisk and were removed from analysis for this trace element. The number 42 designates individual L-17

In general, the mean trace element ratio values for C/Cu, C/Ca, and C/Se were higher in females, while the mean trace element ratio values for C/Mg, C/Zn, C/Fe, C/Mn, C/Pb, and C/Sr were higher in males (Figure 11). The independent t-test indicated that the trace element ratio means were not statistically significantly different for males and females in any of the trace elements analyzed at the 0.05 level of significance, although the average for C/Mn approached significance (p-value = 0.068).

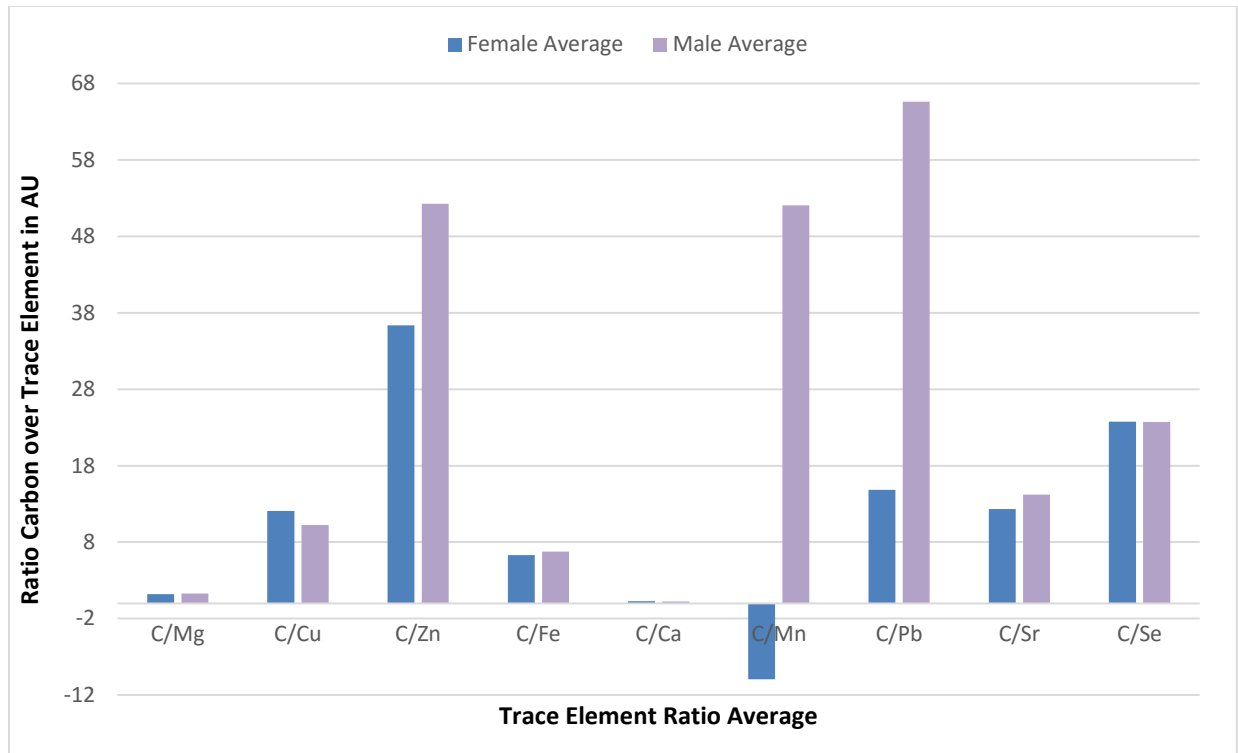


Figure 11: Comparison of the trace element averages between the males and females

In general, the mean trace element ratio values for C/Mn were higher in juveniles. The mean trace element ratio values for C/Se and C/Pb were higher in adults. The mean trace element ratio values for C/Mg, C/Cu, C/Ca, C/Sr, C/Zn, and C/Fe were higher in older adults (Figure 12). A MANOVA did not reveal a statistically significant multivariate main effect for age group at the 0.05 level (Table 10).

Table 10: MANOVA showing no significant relationship between any of the trace elements and age groups

<i>Effect</i>	<i>Test</i>	<i>Value</i>	<i>F</i>	<i>p-Value</i>	<i>Observed Power</i>
Age Group	Wilks' Lambda	0.766	0.984	0.483	0.667



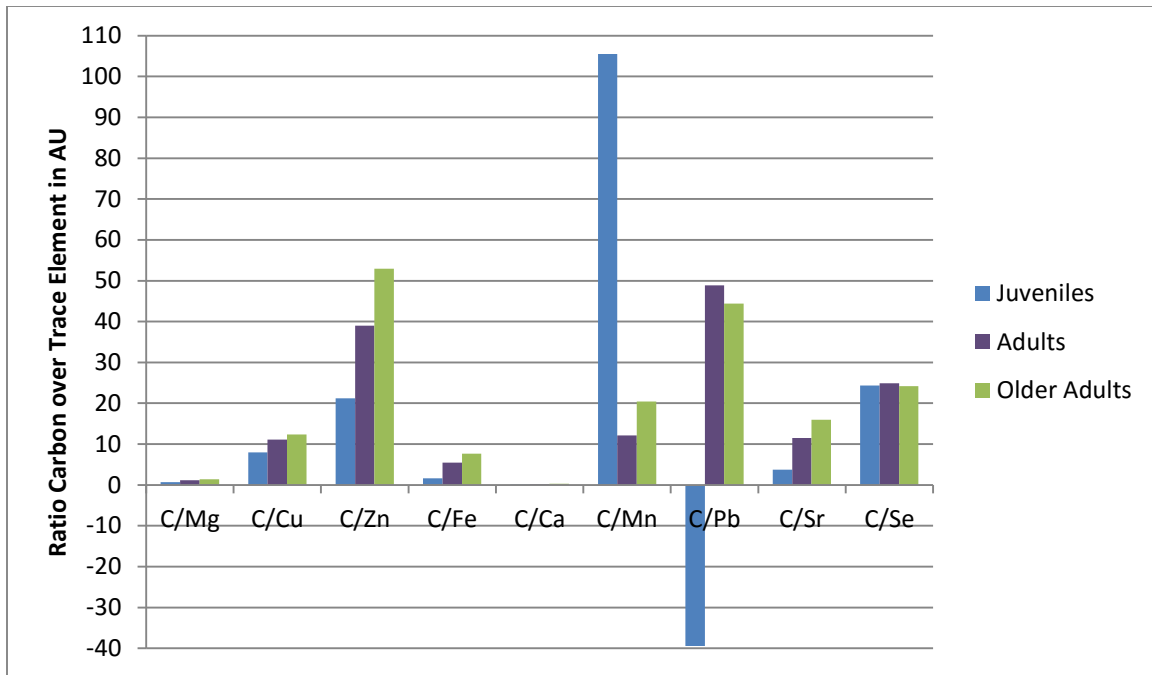


Figure 12: Comparison of trace element averages between age groups

In general, the mean trace element ratio values for C/Mn were higher in the archaeological sample. The mean trace element ratio values for C/Cu, C/Zn, C/Ca, C/Sr, and C/Se were higher in the cadaver sample. The mean trace element ratio values for C/Mg, C/Fe, and C/Pb were higher in the living sample (Figure 13). A MANOVA revealed a statistically significant multivariate main effect for sample group ( $p$ -value < 0.0001). The observed (or post-hoc) power of the MANOVA, given the sample size of 77, a two-tailed test, and alpha level of 0.05, was 1.00 (the highest it can be). A high observed power indicates that type II statistical decision errors (i.e. failing to reject a false null hypothesis) are minimized. Overall, these results confirm the hypothesis that sample group influences average trace element ratio values. Given the significant results of the MANOVA test, the univariate main effects were examined with post-hoc

ANOVA tests. Significant univariate main effects were obtained for C/Mg (p-value < 0.0001), C/Fe (p-value < 0.0001), C/Ca (p-value < 0.0001), and C/Sr (p-value < 0.0001) (Table 11). The observed power of the post-hoc ANOVA tests, given the sample size of 77, a two-tailed test, and alpha level of 0.05, was 1.00 for each significant variable.

Table 11: MANOVA output (top) showing statistically significant MANOVA main effect for sample group. Post-hoc ANOVA output (bottom) showing statistically significant univariate main effects for C/Mg, C/Fe, C/Ca, and C/Sr

<i>Effect</i>	<i>Test</i>	<i>Value</i>	<i>F</i>	<i>p-Value</i>	<i>Observed Power</i>
<i>Age Group</i>	Wilks' Lambda	0.300	5.689	0.000	1.000

	<i>FSTD (Dependent Variable)</i>	<i>F</i>	<i>p-Value</i>	<i>Observed Power</i>
<i>Age Group</i>	C/Mg	32.140	0.000	1.000
	C/Fe	40.239	0.000	1.000
	C/Ca	31.230	0.000	1.000
	C/Sr	49.035	0.000	1.000

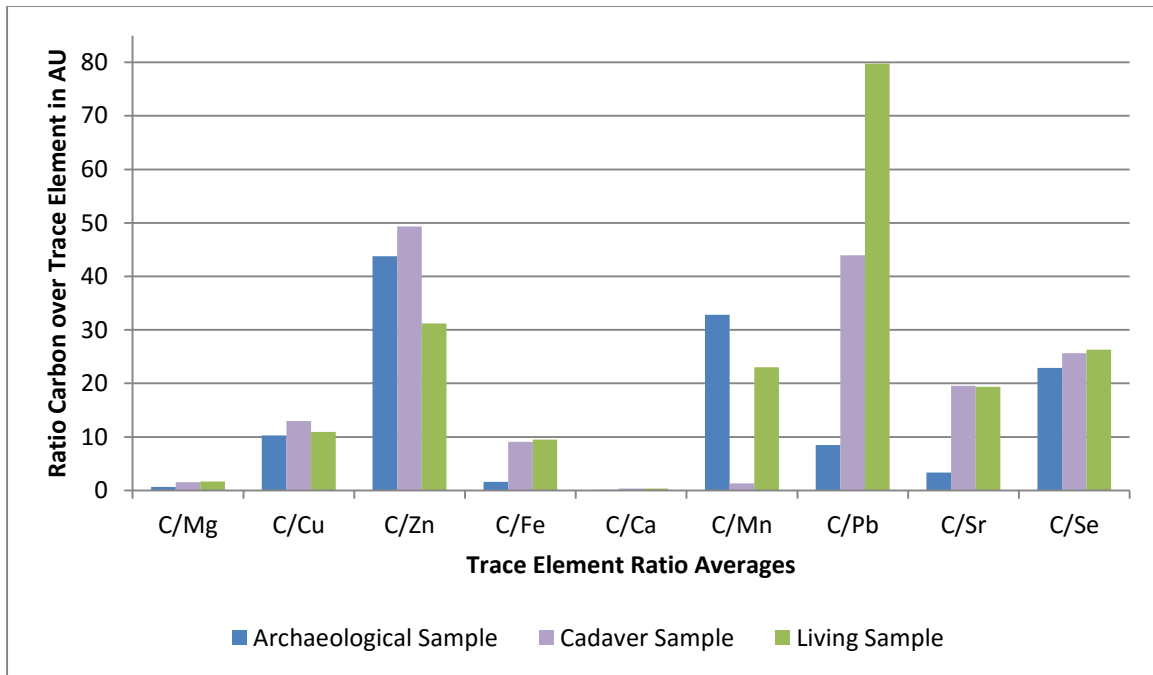


Figure 13: Comparison of the trace element averages between the three sample groups

In general, the mean trace element ratio values for C/Zn and C/Mn were higher in the archaeological context group. The mean trace element ratio values for C/Mg, C/Cu, C/Fe, C/Ca, C/Pb, C/Sr, and C/Se were higher in modern context group (Figure 14). The independent t-test indicated that the trace element ratio means were statistically significantly different for the archaeological context group and the modern context group for C/Mg (p-value < 0.0001), C/Fe (p-value < 0.0001), C/Ca (p-value < 0.0001), and C/Sr (p-value < 0.0001).

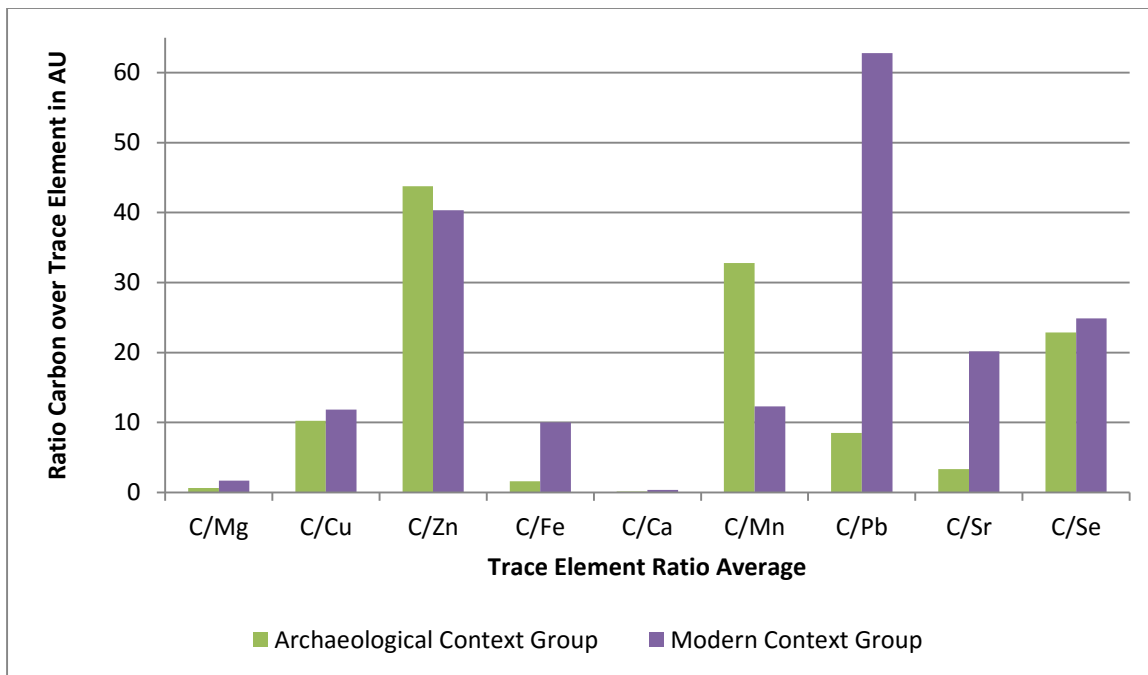


Figure 14: Comparison of trace element averages between context groups

The mean of the variance values (MV) for the trace element ratio values for the total sample can be seen in Table 12 along with the minimum and maximum variance values. The MV C/Mg value for the entire sample was 0.4232 with a range from 0.0014 to 10.2077. The MV value for C/Cu was 461.2331 for the total sample with a range from 0.0742 to 15065.2583. The total sample MV value for C/Zn was 56512.8670 with a range from 91.7480 to 1319290.2583. The MV value for C/Fe was 26.2256 with a range from 0.0330 to 444.5287 for the total sample. The MV value for C/Ca for the total sample was 0.0469 with a range from 0.0001 to 2.7386. The MV value for C/Mn was 250212.5053 for the total sample with a range of 6.1664 to 4774648.3750. The total sample MV value for C/Pb was 707406.8641 with a range of 3106.3997 to 16419323.6507. The MV value for C/Sr was 102.8656 with a range from 0.0767 to

2216.7966 for the total sample. The MV value for C/Se for the total sample was 2668.1930 with a range of 12.4745 to 103066.08496.

Table 12: Mean, range, and standard deviation of the trace element ratio variance values for all nine trace elements tested

	Mean	Std. Deviation	Minimum	Maximum	Range
C/Mg	0.423211	1.313430	0.001374	10.207689	10.206315
C/Cu	461.233075	2388.477321	0.074421	15065.258274	15065.18385
C/Zn	56512.867049	172567.072164	91.747956	1319290.8237	1319199.0757
C/Fe	26.225616	74.011234	0.032984	444.528742	444.495758
C/Ca	0.046852	0.315585	0.000141	2.737648	2.737507
C/Mn	250212.5052	751213.1672	6.166368	4774648.3749	4774642.2086
C/Pb	707406.8641	2202661.6204	3106.399731	16419323.650	16416217.250
C/Sr	102.865630	298.443731	0.076667	2216.796564	2216.7198970
C/Se	2688.193031	12510.333270	12.474452	103066.0849	103053.6105

In general, the MV for C/Mg, C/Cu, C/Ca and C/Pb were higher in females, while the MV for C/Zn, C/Sr, C/Se, C/Fe, and C/Mn were higher in males (Figures 15-17). The independent t-test indicated that the trace element ratio MV were not statistically significantly different at the 0.05 level of significance for males and females in any of the trace elements analyzed.

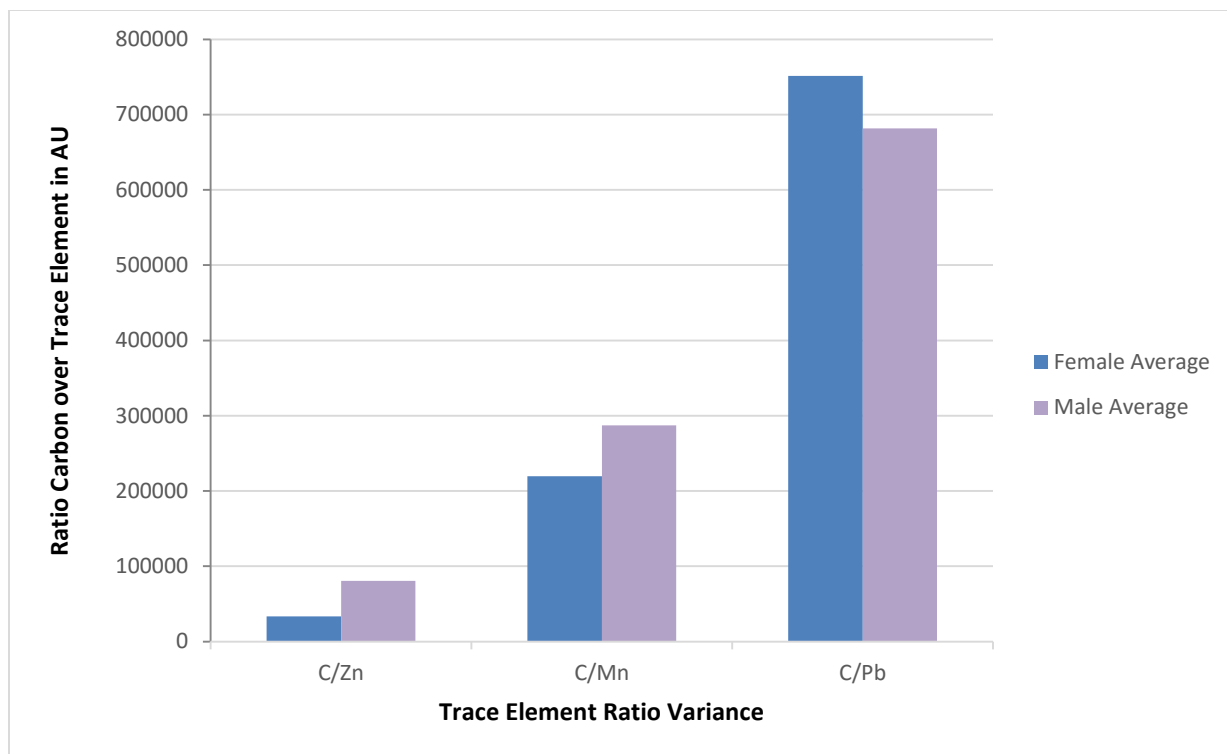


Figure 15: Comparison of trace element ratio variance for C/Zn, C/Mn, and C/Pb between males and females

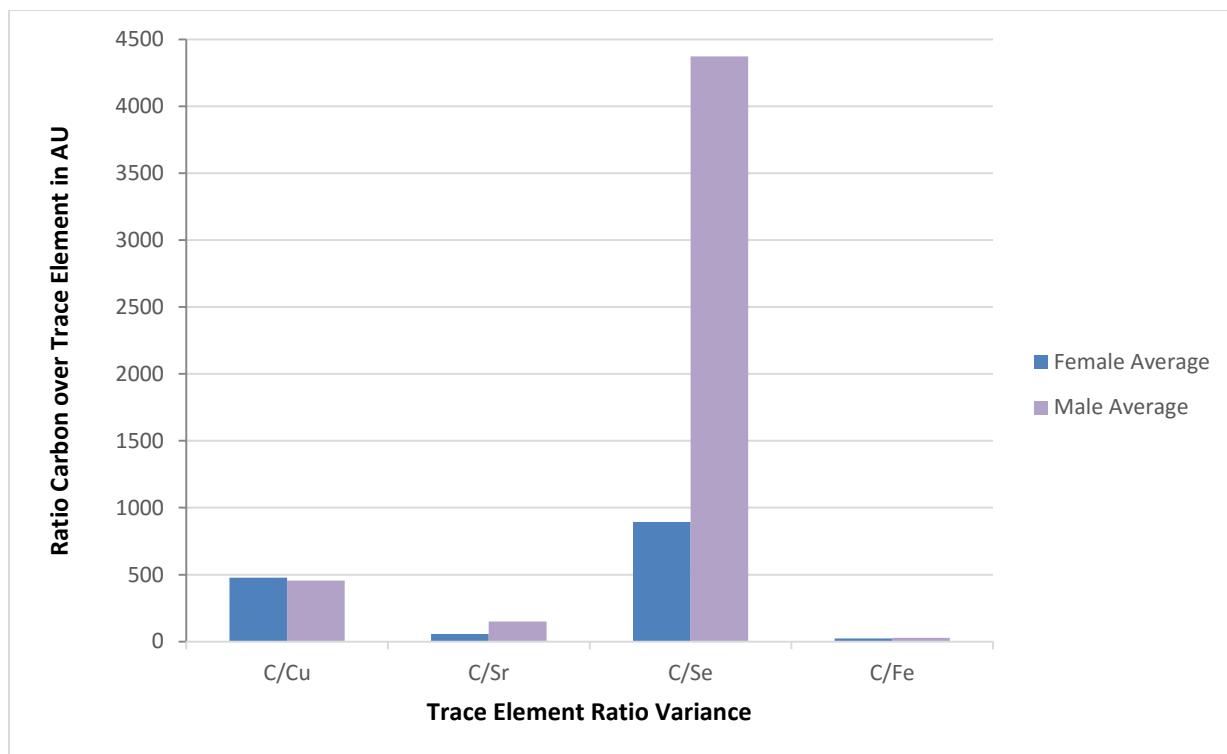


Figure 16: Comparison of trace element ration variance for C/Cu, C/Sr, C/Se, and C/Fe between males and females

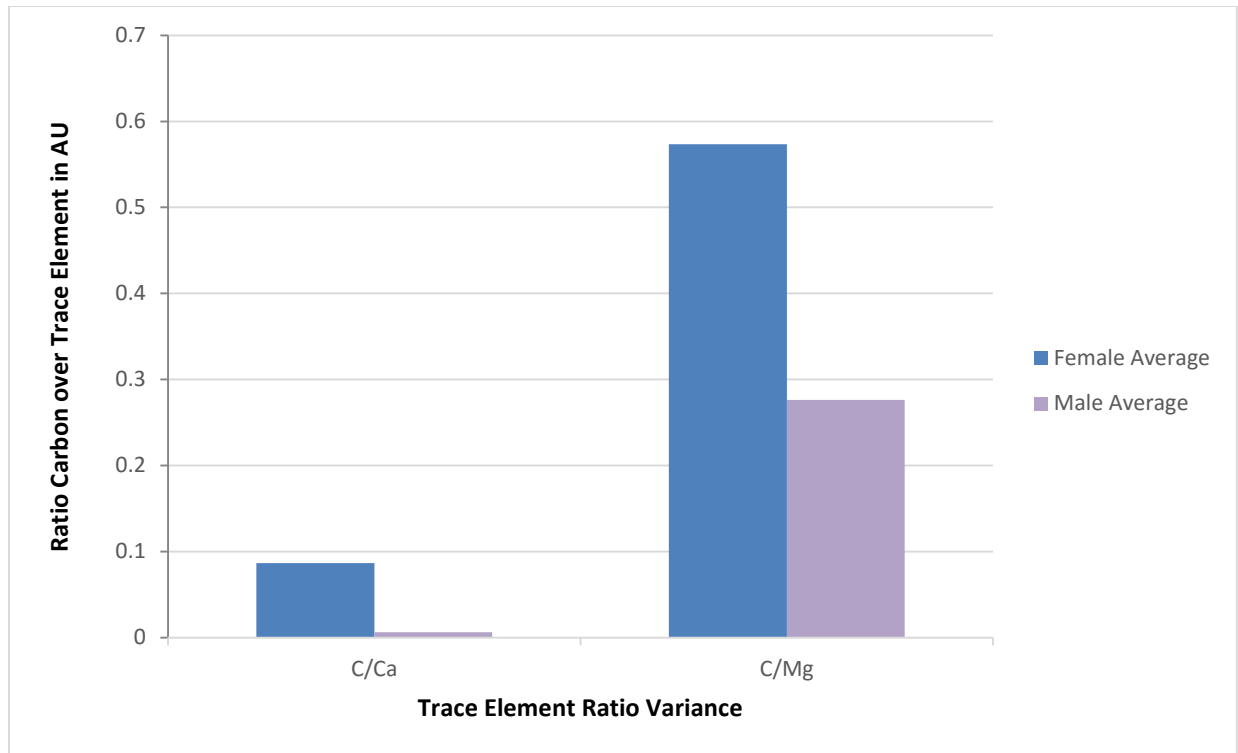


Figure 17: Comparison of trace element ratio variance for C/Ca and C/Mg between males and females

In general, the MV values for C/Mn and C/Cu were higher in juveniles. The MV values for C/Mg, C/Zn, and C/Pb were higher in adults. The MV ratio values for C/Fe, C/Ca, C/Sr, and C/Se were higher in older adults (Figures 18-22). A MANOVA did not reveal a statistically significant multivariate main effect for age group (Table 13).

Table 13: MANOVA output showing no statistical significance between trace element variance and age group

<i>Effect</i>	<i>Test</i>	<i>Value</i>	<i>F</i>	<i>p-Value</i>	<i>Observed Power</i>
Age Group	Wilks' Lambda	0.703	1.326	0.83	0.830



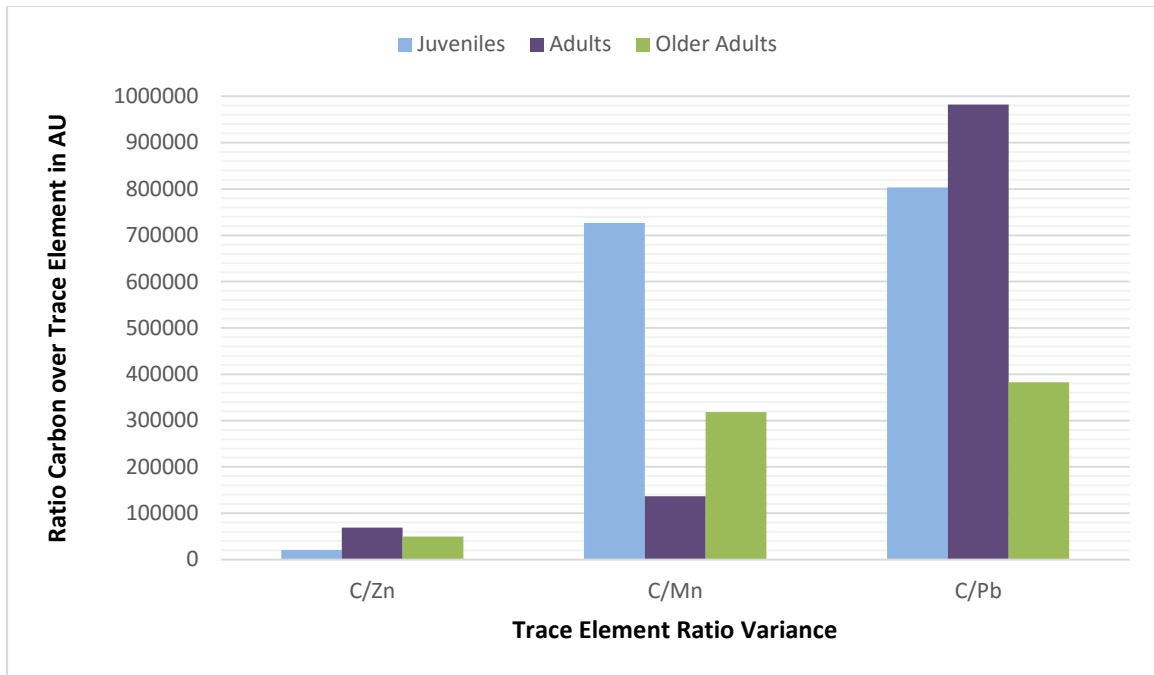


Figure 18: Comparison of trace element ratio variance for C/Zn, C/Mn, and C/Pb between age groups

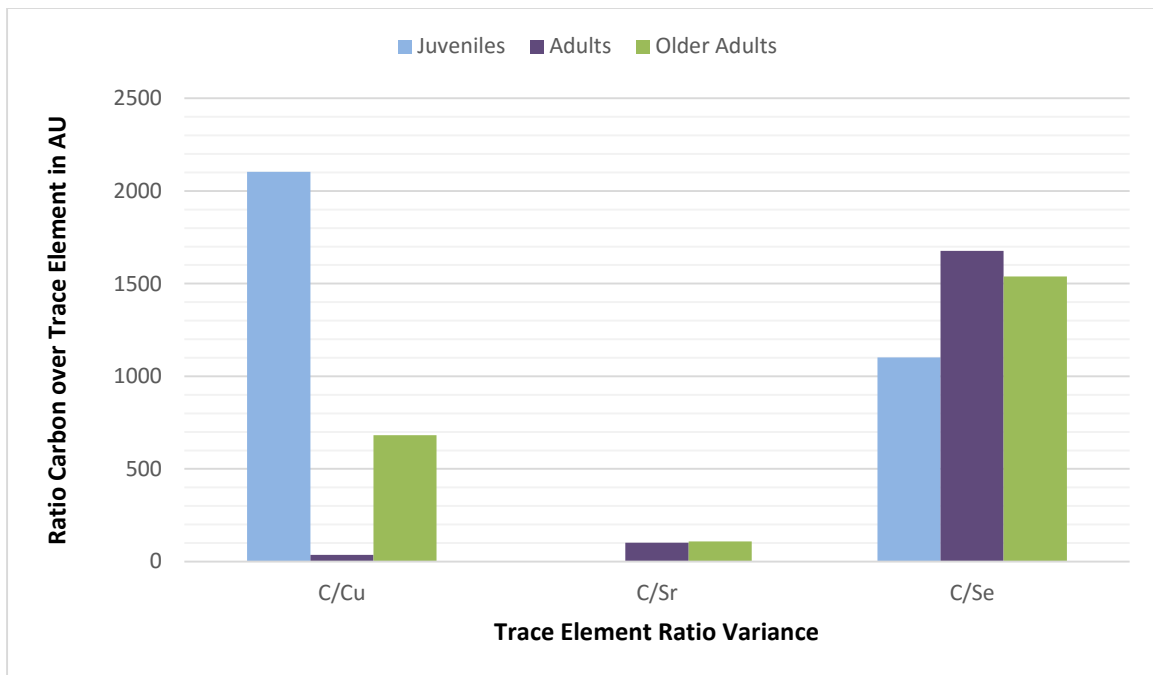


Figure 19: Comparison of trace element ratio variance for C/Cu, C/Sr, and C/Se between age groups

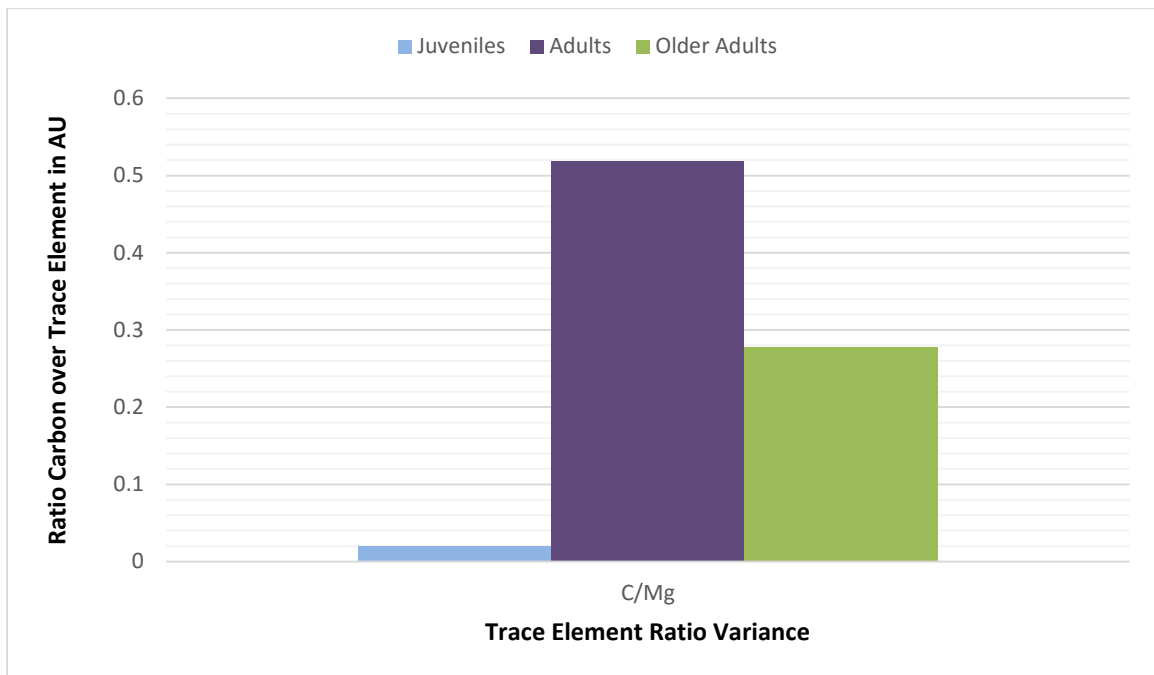


Figure 20: Comparison of trace element ratio variance for C/Mg between age groups

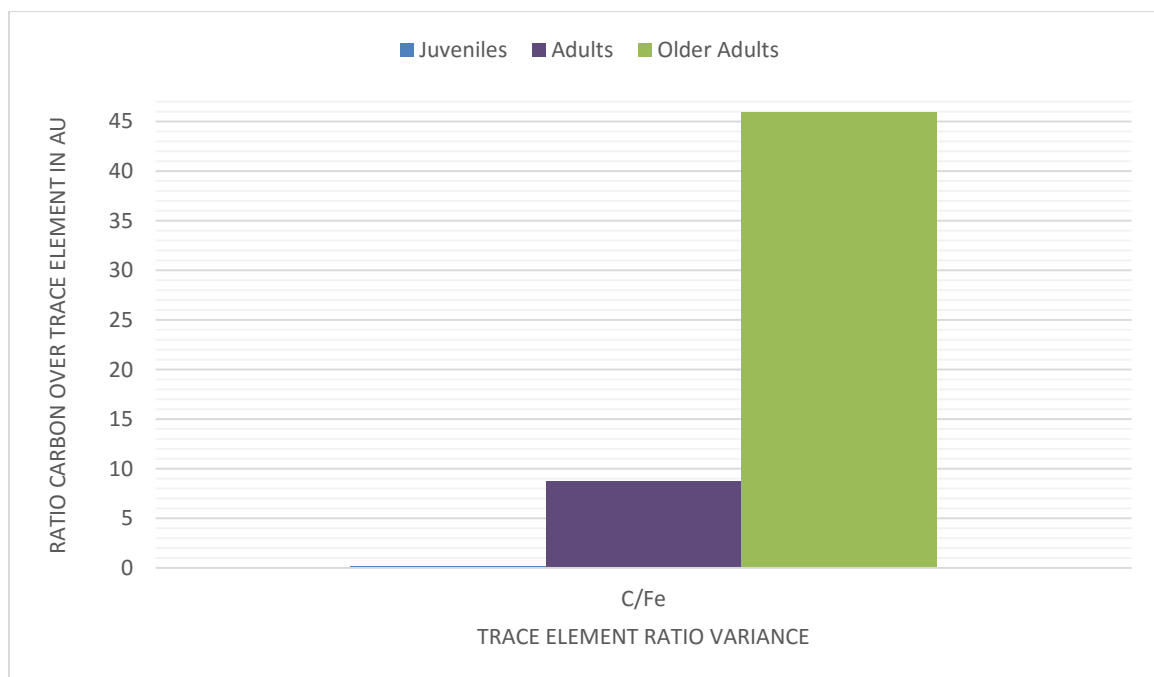


Figure 21: Comparison of trace element ratio variance for C/Fe between age groups

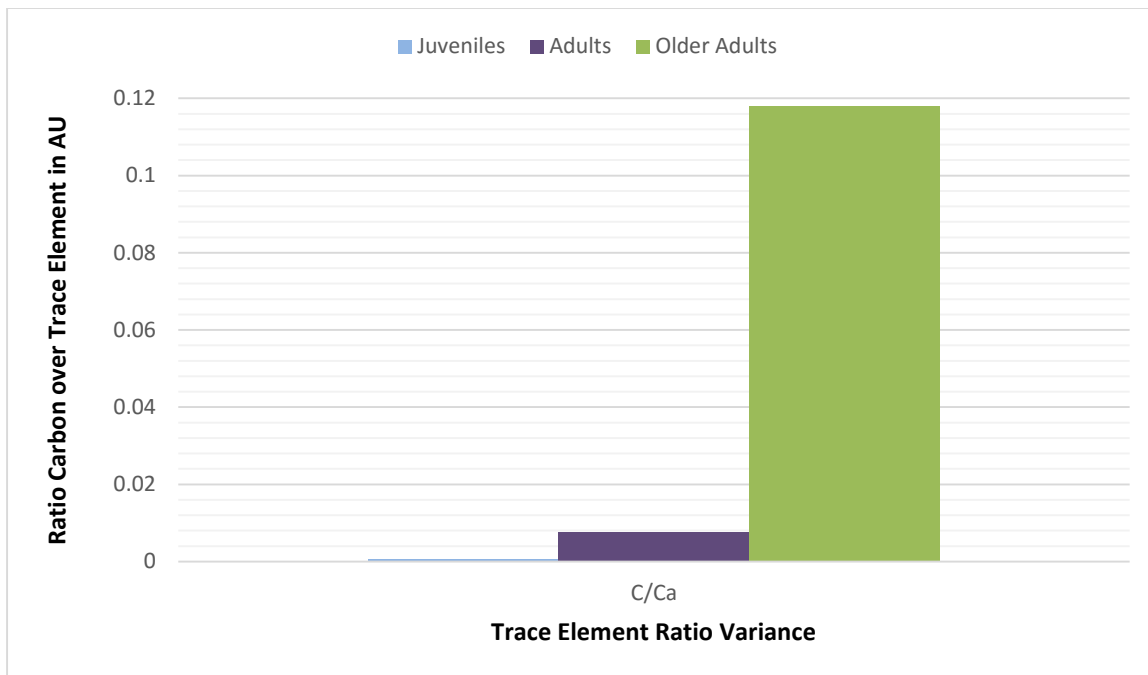


Figure 22: Comparison of trace element ratio variance for C/Ca between age groups

In general, the MV values for C/Zn were higher in the archaeological sample, while the MV values for C/Cu, C/Fe, C/Mn C/Ca, and were C/Se higher in the cadaver sample, while the MV values for C/Mg, C/Pb, C/Sr were higher in the living sample (Figure 17). A MANOVA revealed a statistically significant multivariate main effect for sample group (p-value = 0.018). The observed (or post-hoc) power of the MANOVA, given the sample size of 77, a two-tailed test, and alpha level of 0.05, was 0.962 (slightly below the maximum possible observed power of 1.00). A high observed power indicates that type II statistical decision errors (i.e. failing to reject a false null hypothesis) are minimized. Overall, these results support the hypothesis that sample group influences MV trace element ratio values. Given the significant overall MANOVA test, the univariate main effects were examined with post-hoc ANOVA tests. Significant

univariate main effects were obtained for C/Mg (p-value = 0.016), C/Fe (p-value = 0.010), and C/Sr (p-value = 0.042) (Table 23-28). The observed power of the post-hoc ANOVA tests, given a sample size of 77, a two-tailed test, and alpha level of 0.05, was 0.742 for C/Mg, 0.792 for C/Fe, and 0.611 for C/Sr.

Table 14: MANOVA output (top) showing statistically significant MANOVA main effect for sample group. Post-hoc ANOVA output (bottom) showing statistically significant univariate main effects for C/Mg, C/Fe, and C/S

<i>Effect</i>	<i>Test</i>	<i>Value</i>	<i>F</i>	<i>p-Value</i>	<i>Observed Power</i>
<i>Sample Group</i>	Wilks' Lambda	0.608	1.946	0.018	0.962

	<i>FSTD (Dependent Variable)</i>	<i>F</i>	<i>p-Value</i>	<i>Observed Power</i>
<i>Sample Group</i>	C/Mg	4.402	0.016	0.742
	C/Fe	4.940	0.010	0.792
	C/Sr	3.318	0.042	0.611

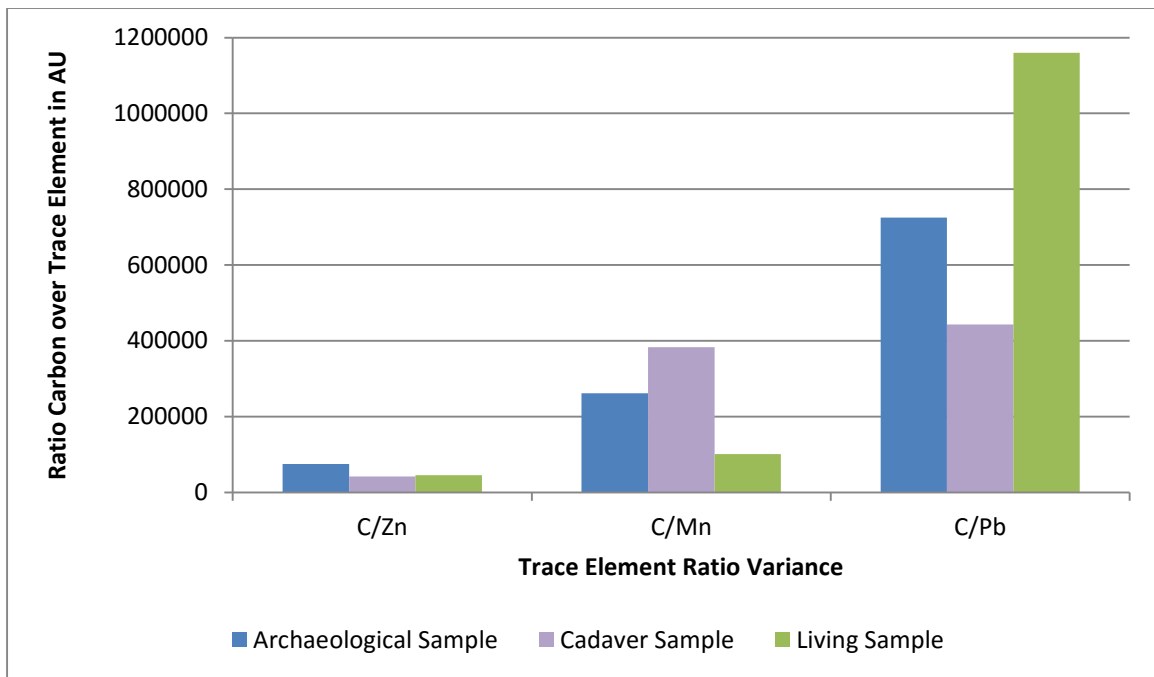


Figure 23: Comparison of trace element ratio variance for C/Zn, C/Mn, and C/Pb between sample group

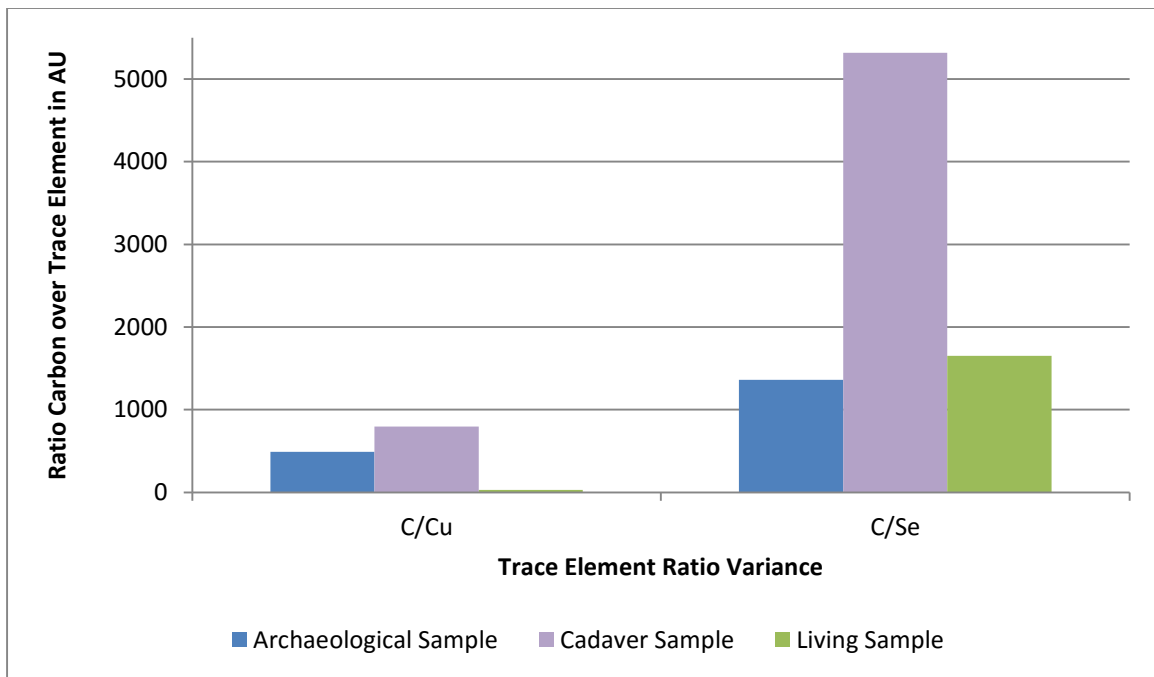


Figure 24: Comparison of trace element ratio variance for C/Cu and C/Se between sample group

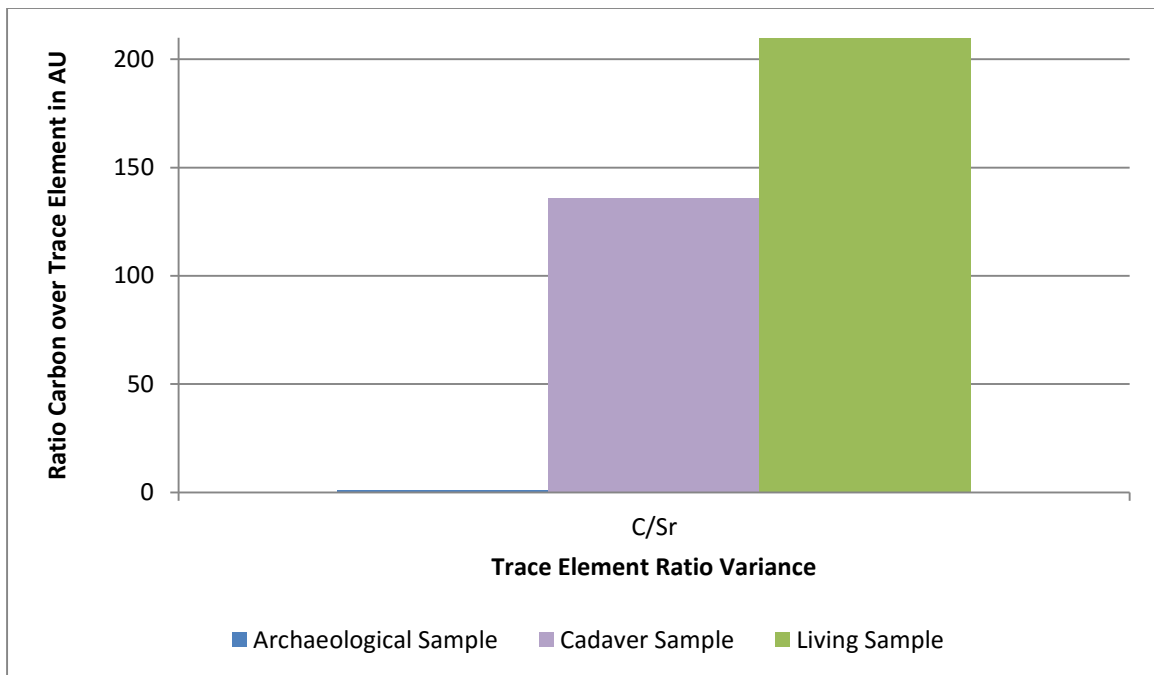


Figure 25: Comparison of trace element ratio variance for C/Sr between sample group

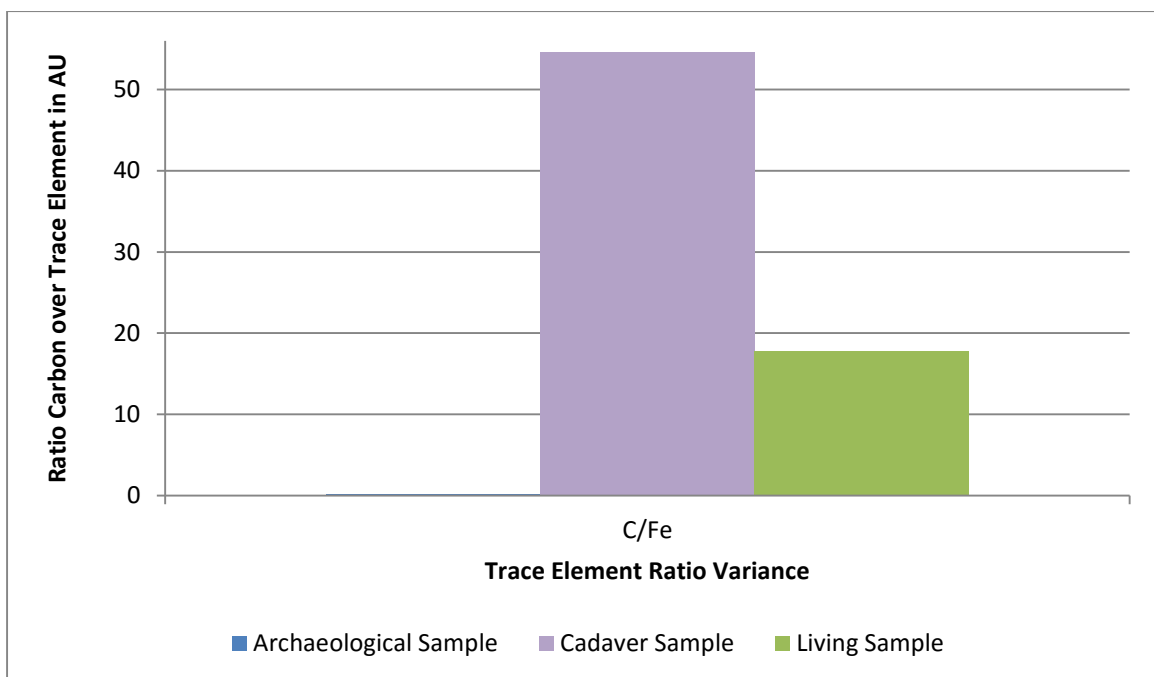


Figure 26: Comparison of trace element ratio variance for C/Fe between sample group



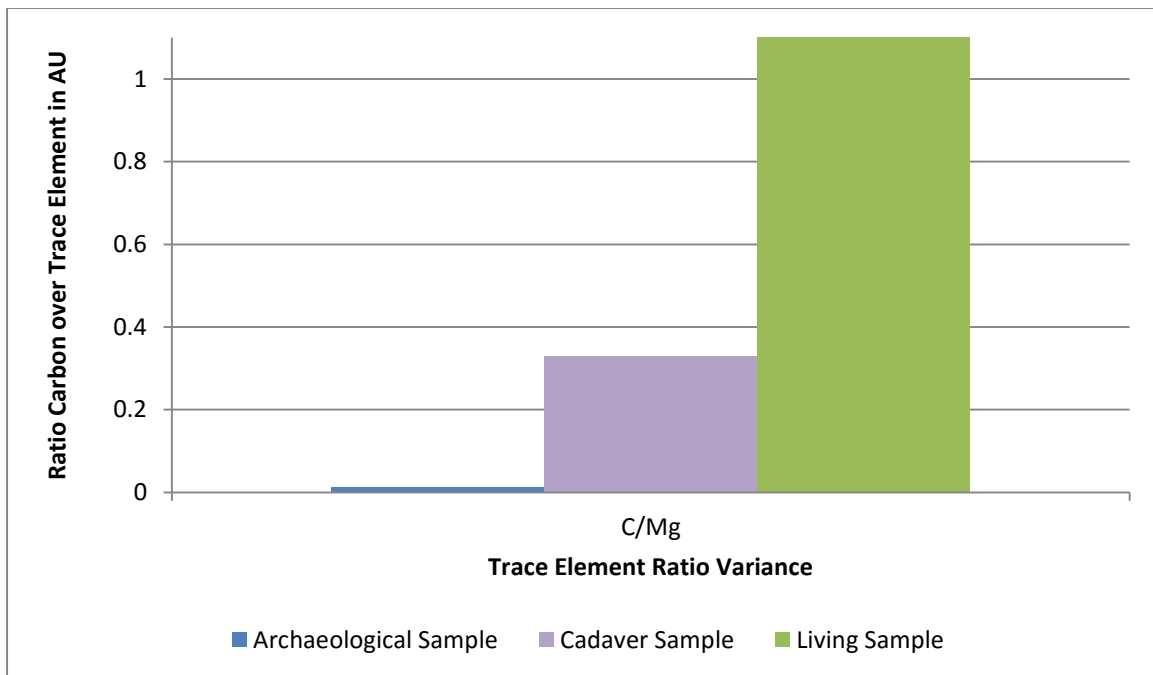


Figure 27: Comparison of trace element ratio variance for C/Mg between sample group

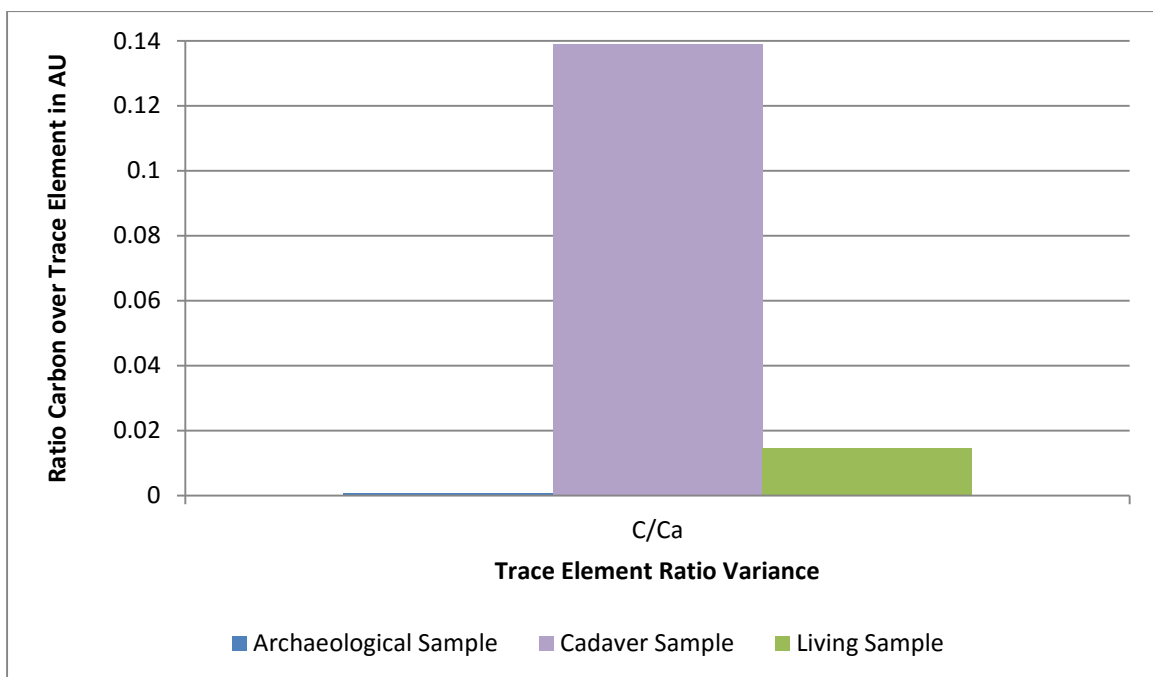


Figure 28: Comparison of trace element ratio variance for C/Ca between sample group

In general, the MV trace element ratio values for C/Mn, C/Cu, C/Zn and C/Pb were higher in the archaeological context group, while the MV trace element ratio values for C/Mg, C/Fe, C/Ca, C/Sr, and C/Se were higher in modern context group (Figure 29-34). The independent t-test indicated that the trace element ratio MVs were statistically significantly different for the archaeological context group and the modern context group for C/Mg (p-value = 0.008), C/Fe (p-value = 0.003), and C/Sr (p-value = 0.003).

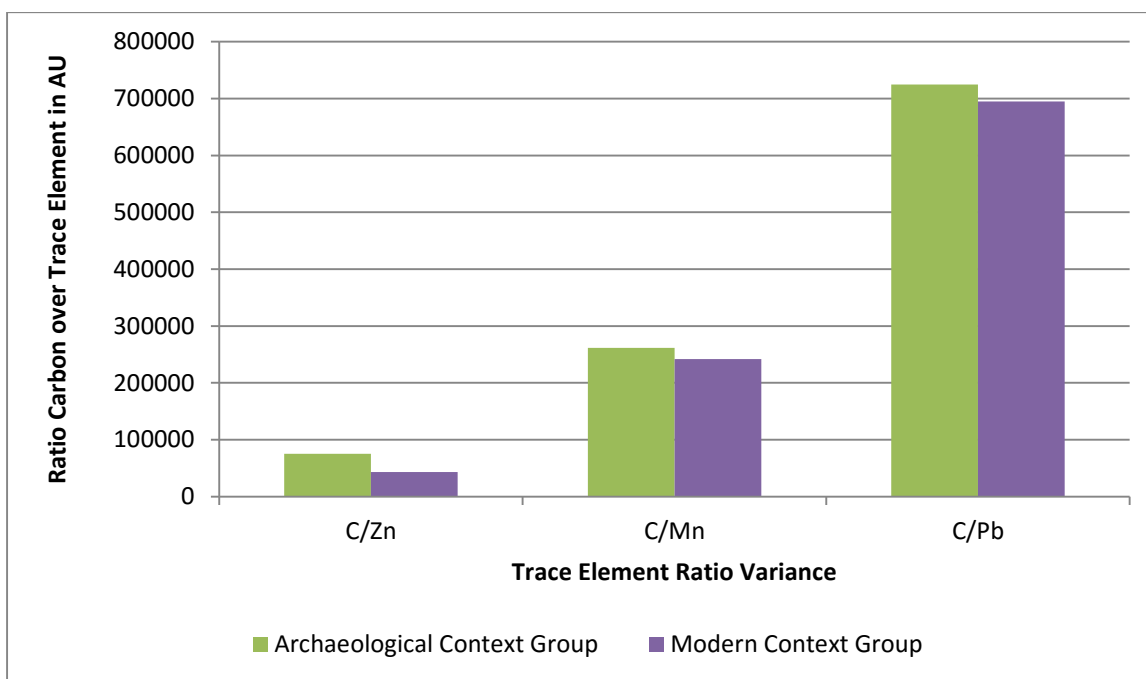


Figure 29: Comparison of trace element ratio variance for C/Zn, C/Mn, and C/Pb between context groups

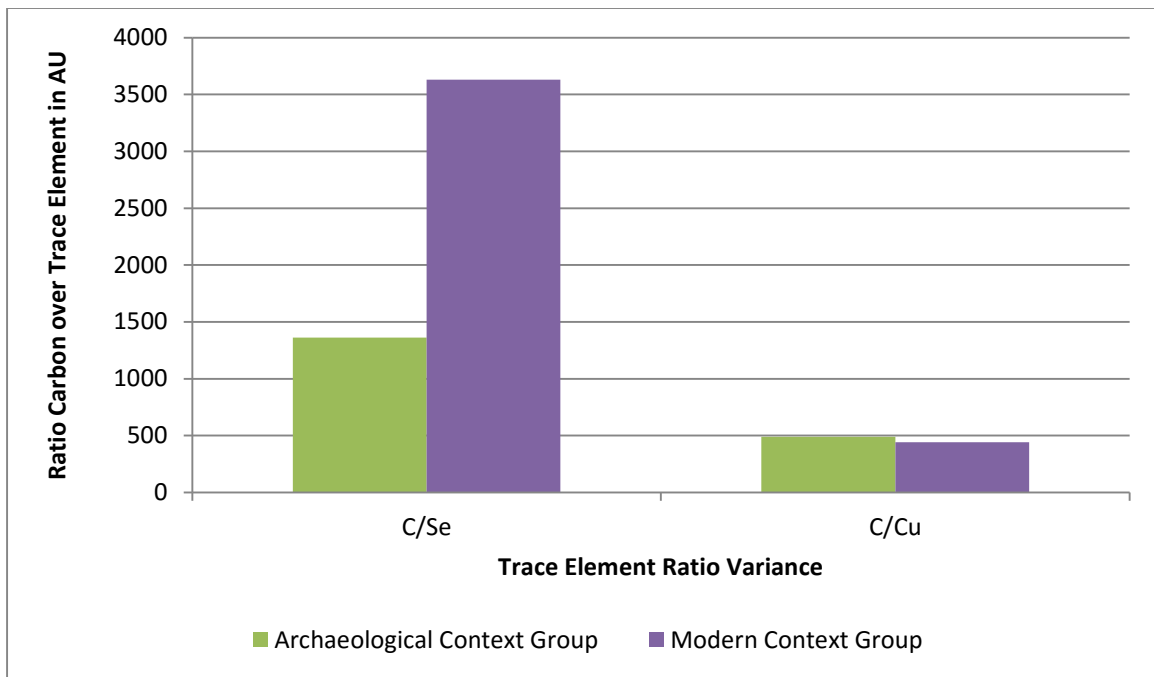


Figure 30: Comparison of trace element ratio variance for C/Se and C/Cu between context groups

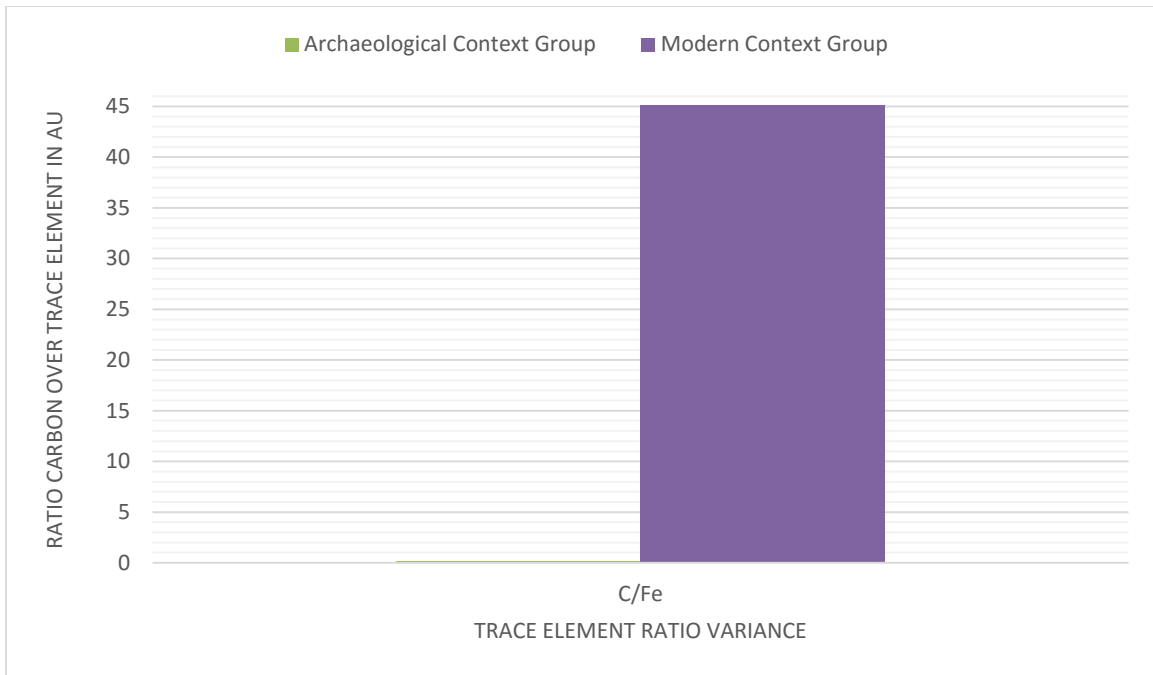


Figure 31: Comparison of trace element ratio variance for C/Fe between context groups

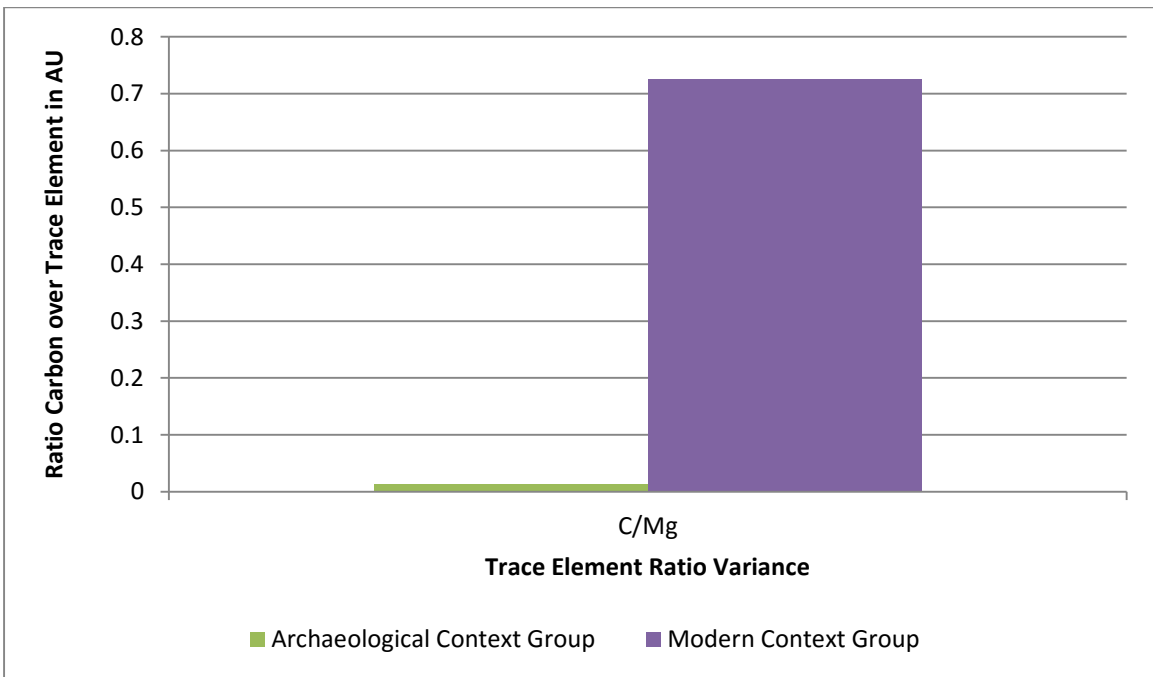


Figure 32: Comparison of trace element ratio variance for C/Mg between context groups

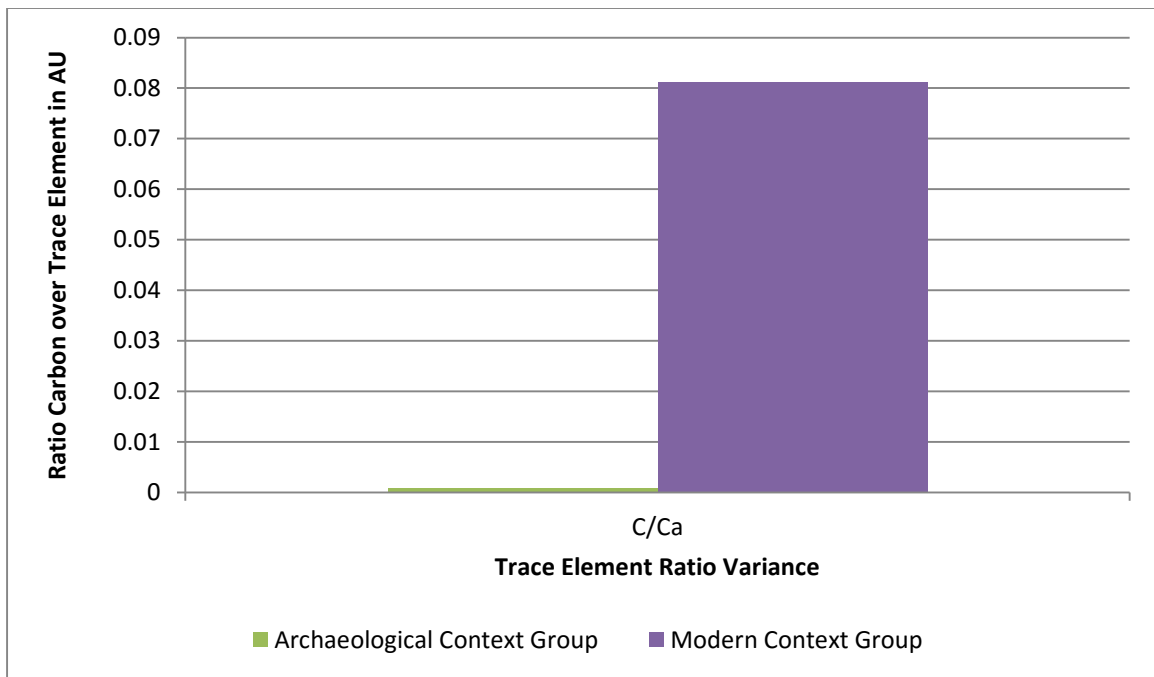


Figure 33: Comparison of trace element ratio variance for C/Ca between context groups

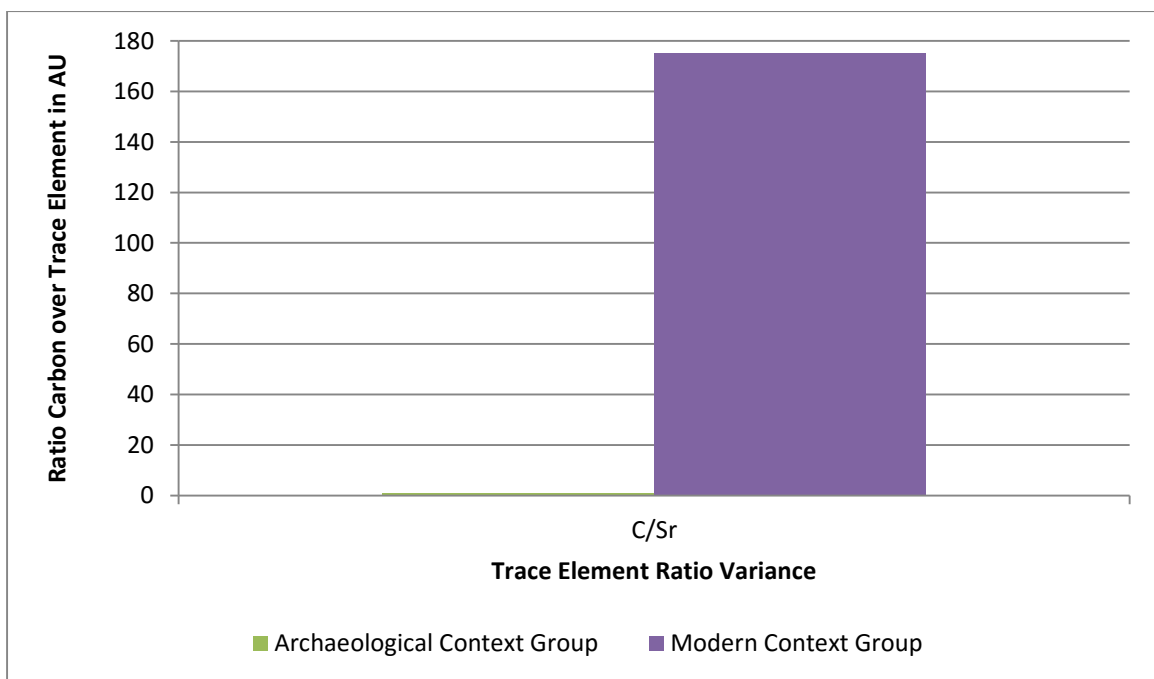


Figure 34: Comparison of trace element ratio variance for C/Ca between context groups

In general, there was no pattern found between hormonal birth control use and any of the trace element ratio means or MV and the independent t-test indicated that the trace element ratio means and MVs were not statistically significantly different for users and non-users of hormonal birth control in any of the trace elements analyzed at the 0.05 level of significance.

#### Summary of C/Mg Results and Data Trends

C/Mg results indicated significant differences in mean values between context groups ( $p\text{-value} < 0.0001$ ), and significant univariate main effects for sample group mean values were obtained for C/Mg ( $p\text{-value} < 0.0001$ ). C/Mg results indicated significant differences in MV values between context groups ( $p\text{-value} = 0.008$ ), and significant univariate main effects sample group MV values for C/Mg ( $p\text{-value} = 0.016$ ). C/Mg results indicated no significant differences for mean values between the biological sexes or age groups, and no significant differences in MV between the biological sexes or age groups. A general trend of C/Mg mean values and MV being the lowest in juveniles was observed. A potentially related pattern of C/Mg mean values and MV being highest in the living sample and lowest in the archaeological sample was observed. Another pattern of C/Mg mean values and MV being higher in the modern context group than the archaeological context group was observed. Generally, C/Mg mean values were higher in males while C/Mg variance was higher in females.

### Summary of C/Cu Results and Data Trends

C/Cu results indicated no significant differences in either mean values or variance between the biological sexes, age groups, sample groups, or context groups. A general trend of C/Cu mean values and variance being highest in the cadaver group was observed. Another pattern of C/Cu mean values and variance being higher in females than males was observed. Generally, C/Cu mean values were higher in the modern context group while C/Cu variance was higher in the archaeological context group.

### Summary of C/Zn Results and Data Trends

C/Zn results indicated no significant differences in either mean values or variance between the biological sexes, age groups, sample groups, or context groups. Generally, both C/Zn mean values and variance was higher in males. A general trend of C/Zn mean values and variance being higher in the archaeological context group than the modern context group was observed. Another trend of C/Zn mean values and variance being lowest in the juvenile sample was observed.

### Summary of C/Fe Results and Data Trends

C/Fe results indicated significant differences in mean values between context groups ( $p\text{-value} < 0.0001$ ) and significant univariate main effects for sample group mean values were obtained for C/Fe ( $p\text{-value} < 0.0001$ ). C/Fe results indicated significant differences in MV values between context groups ( $p\text{-value} = 0.003$ ) and significant univariate main effects sample group MV values for C/Fe ( $p\text{-value} = 0.010$ ). C/Fe results

indicated no significant differences in either mean values or variance between the biological sexes or age groups. A general trend of C/Fe mean values and variance increasing with age was observed. Another pattern of C/Fe mean values and variance being lowest in the archaeological sample was observed. A related pattern of C/Fe mean values and variance being higher in the modern context group than the archaeological context group was also observed. Generally, both C/Fe mean values and variance was higher in males.

#### Summary of C/Ca Results and Data Trends

C/Ca results indicated significant differences in mean values between context groups ( $p\text{-value} < 0.0001$ ) and significant univariate main effects for sample group mean values were obtained for C/Ca ( $p\text{-value} < 0.0001$ ). C/Ca results indicated no significant differences for mean values between the biological sexes or age groups and no significant differences in variance between the biological sexes, age groups, sample groups, or context groups. A general trend of C/Ca mean values and variance increasing with age was observed. A related pattern of C/Ca mean values and variance being highest in the cadaver sample and lowest in the archaeological sample was also observed. Another pattern of C/Ca mean values and variance being higher in the modern context group than the archaeological context group was observed. Generally, both C/Ca mean values and variance was higher in females than males.



### Summary of C/Mn Results and Data Trends

C/Mn results indicated no significant differences in either mean values or variance between the biological sexes, age groups, sample groups, or context groups. However, C/Mn mean values approached significance between the biological sexes (p-value = 0.068). A general trend of C/Mn mean values and variance being highest in juveniles and lowest in adults was observed. A related pattern of C/Mn mean values being highest in the archaeological sample and lowest in the living sample was also observed. However, C/Mn variance was higher in the cadaver sample than the archaeological sample. Another pattern of C/Mn mean values and variance being higher in the archaeological context group than the modern context group was observed. Generally, both C/Mn mean values and variance was higher in males.

### Summary of C/Pb Results and Data Trends

C/Pb results indicated no significant differences in either mean values or variance between the biological sexes, age groups, sample groups, or context groups. A general trend of C/Pb mean values and variance peaking in adulthood was observed. A related pattern of C/Pb mean values and variance being highest in the living sample was also observed. Generally, C/Pb mean values were higher in males while C/Pb variance was higher in females. C/Pb mean values were higher in the modern context group while variance was higher in the archaeological context group.

### Summary of C/Sr Results and Data Trends

C/Sr results indicated significant differences in mean values between context groups ( $p\text{-value} < 0.0001$ ) and significant univariate main effects for sample group mean values were obtained for C/Sr ( $p\text{-value} < 0.0001$ ). C/Sr results indicated significant differences in MV values between context groups ( $p\text{-value} = 0.003$ ) and significant univariate main effects sample group MV values for C/Sr ( $p\text{-value} = 0.042$ ). C/Sr results indicated no significant differences in either mean values or variance between the biological sexes or age groups. A general trend of C/Sr mean values and variance increasing with age was observed. Another pattern of C/Sr mean values and variance being higher in the modern context group than the archaeological context group was observed. A related trend of C/Sr being lowest in the archaeological sample was observed. Generally, both C/Sr mean values and variance was higher in males.

### Summary of C/Se Results and Data Trends

C/Se results indicated no significant differences in either mean values or variance between the biological sexes, age groups, sample groups, or context groups. A general trend of C/Se mean values and variance peaking in adults was observed. A potentially related pattern of C/Se mean values and variance being lowest in the archaeological sample was also observed. Another pattern of C/Se mean values and variance being higher in the modern context group than the archaeological context group was observed. Generally, C/Se mean values were higher in females while C/Se variance was higher in males.

### Regression Results

A logistic regression analysis was conducted to predict biological sex using mean values of trace element ratios from human head hair as predictors. A test of the full model against a constant only model was not statistically significant (chi square = 3.759,  $p = 0.053$  with  $df = 1$ ). Prediction success overall was 50% (100% for male and 0% for female). The Wald criterion demonstrated that none of the trace element ratios made a significant contribution to prediction at a 0.05 level of significance.

A similar logistic regression analysis was then conducted using MVs of trace element ratios rather than mean values. A test of the full model against a constant only model was statistically significant (chi square = 19.458,  $p = 0.007$  with  $df = 7$ ). Prediction success overall was 84.4% (91.3% for females and 77.3% for males). The Wald criterion demonstrated that none of the trace element ratios made a significant contribution to prediction at a 0.05 level of significance. The logistic regression equation produced from this analysis is as follows in Equation 1:

$$\begin{aligned} \text{Log Odds} = & 0.221702 + 0.003006 \times (\text{C/Mg}) + 0.000277 \times (\text{C/Cu}) + \\ & 0.000005 \times (\text{C/Zn}) + 0.003265 \times (\text{C/Fe}) + (-85.296683) \times (\text{C/Ca}) + \\ & (-0.000002) \times (\text{C/Mn}) + 0.005877 \times (\text{C/Sr}) \end{aligned} \quad (1)$$

The numerator was then determined using Equation 2:

$$\text{Numerator} = e^{(\text{Log Odds})} \quad (2)$$

The denominator was then determined using Equation 3:

$$\text{Denominator} = (1 + e^{(\text{Log Odds})}) \quad (3)$$

The probability of the individual being a male was determined using Equation 4:

$$\text{Probability(Male)} = \text{Numerator} \div \text{Denominator} \quad (4)$$

The probability of the individual being a female was determined using Equation 5:

$$\text{Probability(Female)} = 1 - \text{Probability(Male)} \quad (5)$$

However, the sex estimation can be determined without the probability. A negative Log Odds indicates a sex estimation of male and a positive Log Odds indicates a sex estimation of female. However, it may be important for researchers to know the actual probability, especially in situations where estimated sex is barely greater than 50% for one sex and barely below 50% for the opposite (e.g. 51% for one sex and 49% for the other sex), or, essentially not much different from a fair coin flip.

The sex probabilities, predictions, and actual biological sexes for the cadaver sample group can be seen in Table 15. Twenty of the 25 individuals in the cadaver sample group, or 80%, were correctly predicted for known biological sex. Of the five incorrectly predicted biological sexes in the cadaver sample group, four were known males who were incorrectly predicted to be females. In general, the percentage of correctly predicted biological sex is higher than that of incorrectly predicted biological sex for the cadaver sample group.

Table 15: Sex predictions with probabilities for the cadaver sample group, with corresponding known biological sex. Correct predictions are highlighted in purple

Indicator	Probability of being Male	Probability of being Female	Sex Estimation	Known Sex
C-380	75%	25%	Male	Male
C-381	43%	57%	Female	Male
C-382	51%	49%	Male	Male
C-383	47%	53%	Female	Female
C-384	51%	49%	Male	Male
C-385	28%	72%	Female	Female
C-386	42%	58%	Female	Male
C-388	92%	8%	Male	Male
C-389	0%	100%	Female	Female
C-390	100%	0%	Male	Male
C-391	2%	98%	Female	Female
C-392	63%	37%	Male	Male
C-393	73%	27%	Male	Male
C-394	64%	36%	Male	Female
C-395	32%	68%	Female	Female
C-396	100%	0%	Male	Male
C-397	48%	52%	Female	Male
C-398	0%	100%	Female	Female
C-399	27%	73%	Female	Female
C-400	38%	62%	Female	Female
C-401	10%	90%	Female	Male
C-402	13%	87%	Female	Female
C-403	32%	68%	Female	Female
C-404	9%	91%	Female	Female
C-407	41%	59%	Female	Female

The sex probabilities, predictions, and known biological sexes for the living sample group can be seen in Table 16. Seventeen of the 20 individuals in the living

sample group, or 85%, were correctly predicted for known biological sex. Of the three incorrectly predicted biological sexes in the living sample group, two were known males who were incorrectly predicted to be females. In general, the percentage of correctly predicted biological sex is higher than that of incorrectly predicted biological sex for the living sample group.

Table 16: Sex predictions with probabilities for the living sample group, with corresponding known biological sex. Correct predictions are highlighted in purple

Indicator	Probability of being Male	Probability of being Female	Sex Estimation	Known Sex
L-1	52%	48%	Male	Male
L-2	29%	71%	Female	Female
L-3	46%	54%	Female	Female
L-4	49%	51%	Female	Female
L-5	52%	48%	Male	Male
L-6	1%	99%	Female	Female
L-7	100%	0%	Male	Male
L-8	18%	82%	Female	Female
L-9	79%	21%	Male	Female
L-10	32%	68%	Female	Male
L-11	16%	84%	Female	Female
L-12	53%	47%	Male	Male
L-13	100%	0%	Male	Male
L-14	44%	56%	Female	Female
L-15	49%	51%	Female	Female
L-16	44%	56%	Female	Female
L-17	100%	0%	Male	Male
L-18	98%	2%	Male	Male
L-19	53%	47%	Male	Male
L-20	39%	61%	Female	Male

The sex probabilities, predictions, and estimated and/or known biological sexes for the archaeological sample group can be seen in Table 17. Twenty-two of the 31 individuals of known sex in the archaeological sample group, or 71%, were correctly predicted for known biological sex. Of the nine incorrectly predicted biological sexes in

the archaeological sample group, four were known males who were incorrectly predicted to be females. In general, the percentage of correctly predicted biological sex is higher than that of incorrectly predicted biological sex for the archaeological sample group. Individual A-512/1 was given a probability of 50% for both sexes but was estimated to be a male due to a positive Log Odds.

Table 17: Sex predictions with probabilities for the archaeological sample group, with corresponding estimated or known biological sex. Sex is known for juveniles only (designated by an asterisk (\*)), all other sexes are estimated. Correct predictions are highlighted in purple

Indicator	Probability of being Male	Probability of being Female	Sex Estimation	Estimated or Known Sex
A-19	60%	40%	Male	Female
A-21	23%	77%	Female	Female
A-26	1%	99%	Female	Female
A-57*	0%	100%	Female	Male
A-60	44%	56%	Female	Male
A-81	80%	20%	Male	Male
A-089	70%	30%	Male	Male
A-93	43%	57%	Female	Male
A-107	65%	35%	Male	Male
A-131	52%	48%	Male	Female
A-143	54%	46%	Male	Male
A-177	60%	40%	Male	Female
A-222	17%	83%	Female	Male
A-269	70%	30%	Male	Female
A-278*	42%	58%	Female	Female
A-282	44%	56%	Female	Female
A-291	22%	78%	Female	Female
A-318A	10%	90%	Female	Female
A-336*	91%	9%	Male	Male
A-402	100%	0%	Male	Male
A-434	53%	47%	Male	Female
A-452	79%	21%	Male	Male
A-457	51%	49%	Male	Male
A-461	96%	4%	Male	Male
A-463	46%	54%	Female	Female
A-511	41%	59%	Female	Female
A-512/1	50%	50%	Male	Male
A-512/2	78%	22%	Male	Male
A-519*	100%	0%	Male	Unknown
A-520*	17%	83%	Female	Female
A-555*	66%	34%	Male	Male
A-593A*	0%	100%	Female	Female



## CHAPTER FIVE: DISCUSSION

Due to differences in bioavailability, as well as metabolic and hormonal differences, variability was expected between all of these groups. The use of this variation in trace elements as a predictor of biological sex is discussed, and multiple techniques are compared.

### Trace Element Differences between Females and Males

Generally, C/Mg mean values were higher in males, while C/Mg variance was higher in females, this may be related to the prevalence of osteoporosis in females. Furthermore, adult males require almost 100% more Mg than females per day, likely as a result of the role of Mg in cellular energy production and increased muscle mass in males (Food and Nutrition Board 1997). Inversely, C/Se mean values were generally higher in females while C/Se variance was generally higher in males. However, none of these differences were significant.

A pattern of C/Cu mean values and variance being higher in females than males was observed. Cu levels have been shown to be linked to estrogen levels (Baig et al. 2003; Kilinc et al. 2010). Furthermore, a relationship between parity and increased Cu levels has been noted, particularly among women who birthed multiple children (El Ati-Hellal et al. 2016). This is likely due to the rise in Cu levels that occurs during pregnancy, possibly as a result of increased estrogen levels (Baig et al. 2003; Izquierdo Alvarez et al. 2007; Kilinc et al. 2010). Women who have had multiple births are much more likely to have increased Cu levels as a result of the increased estrogen (El Ati-

Hellal et al. 2016). Interestingly, menopause was found to correlate with a significant increase in Cu levels (Fischer et al. 1990). This is supported by the trend of C/Cu mean values and variance being highest in the cadaver sample group. This may mean that biological sex plays a greater role in Cu levels and absorption than age does, something that is supported by a lack of a trend between the age groups concerning C/Cu mean values and variance. Another possible factor is the use of oral and hormonal contraceptives by females. These contraceptives contain estrogen which has been shown to raise Cu levels (Kowalska et al. 2015).

A general trend of C/Zn mean values and variance being higher in males was observed. Statistically significant differences in Zn levels have been found in previous studies and were contributed to dietary and metabolic differences between the sexes (Stipišić et al. 2014). Food sources of Zn include red meats and seafood, such as oysters, and as a result vegetarians are at increased risk for Zn deficiency (Food and Nutrition Board 2001). It has been found that in Western cultures, females tend to eat less meat and are more likely to be vegetarian than males, however, this is not necessarily true of other modern non-Western cultures or archaeological populations (O'Doherty Jensen and Holm 1999). Furthermore, due to the physical demands of carrying and feeding a child, pregnant and lactating women often have lowered Zn levels (Fung et al. 1997). The general trend of C/Ca mean value and variance being higher in females may also be a contributing factor since high Ca levels may inhibit the absorption of Zn (Wood and Zheng 1997). Another potential factor could be the increased potential of Zn intake via inhalation for males. Zn inhalation can occur as a

result of smelting or welding (Food and Nutrition Board 2001; King and Cousins 2006). Typically, more men would be exposed to these situations or environments, however, it is important to underscore that this is actually a gendered difference rather than a difference in biological sex.

A general pattern of C/Fe mean values and variance being higher in males than in females was observed. Typically, men do have higher stores of Fe than women do (Finley et al. 1994). Menstruating women are at increased risk of Fe deficiency due to repetitive blood loss. Similarly, pregnant women are more susceptible to Fe deficiency (Brody 1999). In Western-cultures where both the living sample group and the cadaver sample group are from women have been found more likely to be vegetarians.

Vegetarians very often have low levels of Fe and suffer from some level of Fe deficiency (Food and Nutrition Board 2001; O'Doherty Jensen and Holm 1999). However, the archaeological group does not necessarily adhere to this same trend, and therefore a vegetarian diet cannot be solely responsible for this trend. High levels of Ca have been shown to decrease the absorption of Fe (Food and Nutrition Board 2001). The general trend of C/Ca mean value and variance being higher in females than males may support this. This idea is also supported by comparing the pattern of C/Ca absorption and C/Fe absorption in the hair. There is a noticeable pattern of C/Ca values corresponding to C/Fe values within many of the same individuals (Figure 35). However, as can be seen in Figure 36, although the absorption patterns are the same, the absorption levels are quite different. This supports the idea that Fe levels are directly related and, to some extent, controlled by Ca level.

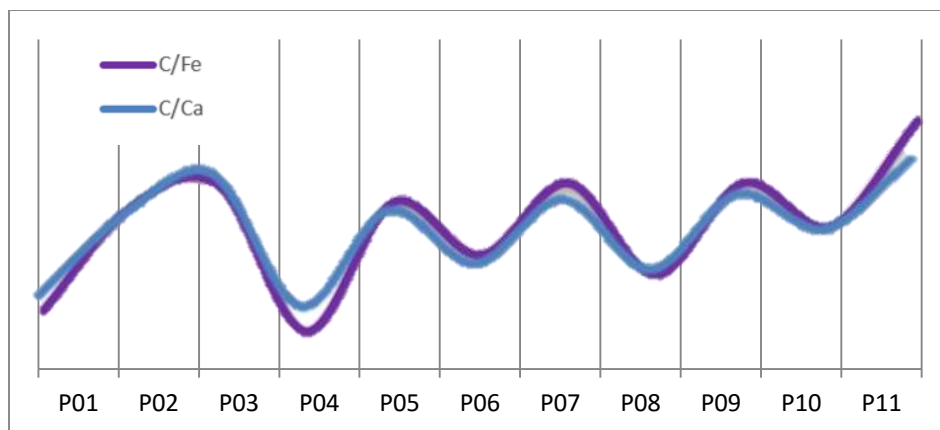


Figure 35: Overlap of C/Fe and C/Ca data in individual A-089 (61 year old male) over 11 (i.e., P01 – P11) points along 1 cm of hair shaft, demonstrating similarity in absorption patterns of the two trace elements in the same individual

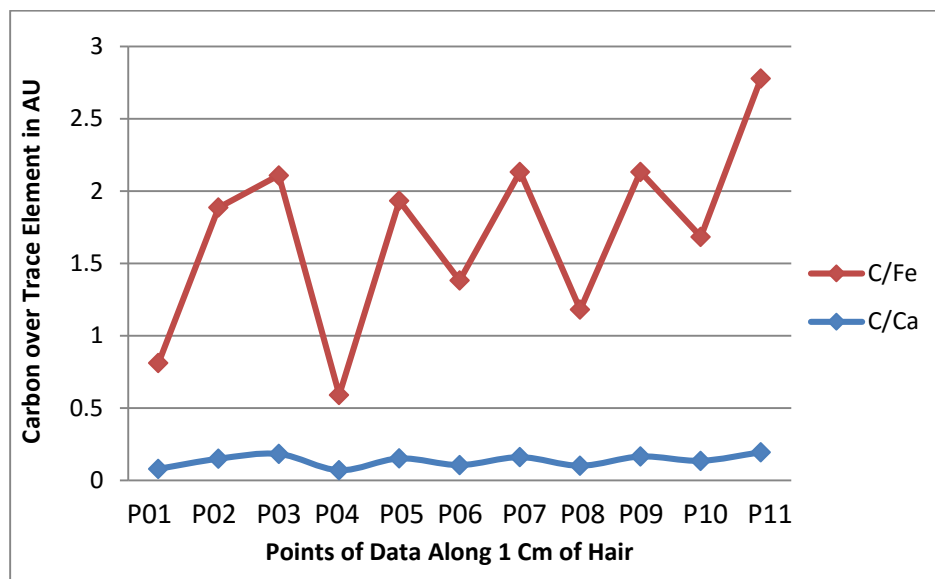


Figure 36: C/Ca and C/Fe data for individual A-089 (61 year old male) over 11 points (i.e., P01 – P11) along 1 cm hair shaft, demonstrating the difference in the levels between the two trace elements when shown on the same scale, despite having very similar absorption patterns

A general trend of C/Ca mean values and variance being higher in females than in males was observed. It is likely that the main cause for this is the increased consumption of dairy and intake of Ca supplements as a direct result of concern for

osteoporosis. Osteoporosis is a big medical concern for women and many women take preventative measures, which include increasing Ca intake naturally and pharmaceutically. In fact, women are much more likely to use Ca supplements than men (Bailey et al. 2010). Another factor, is that the body goes through homeostatic actions to maintain the Ca level within the blood which will keep Ca levels steady between the two sexes for as long as possible by demineralizing the bone to maintain blood Ca levels (Weaver 2012).

A general trend of C/Mn mean values and variance being higher in males than in females was observed. Daily recommended intakes for most trace elements including Mn are typically higher for men, with the exception of pregnant or lactating women and occasionally menstruating women, due to lower absorption rates and larger body size (Finely et al. 1994). Females need less Mn than males because they are better at absorbing Mn than males and due to lower Fe stores than males (Finley et al. 1994). This is supported by the relationship between Fe and Mn, low levels of Fe can result in heightened levels of Mn (Aschner and Dorman 2006; Keen and Zidenberg-Cherr 1996). However, the half-life of Mn within males is much longer than it is within females, meaning Mn levels for males depleted more slowly than they do for females. Finley et al. (1994) found that while females absorbed more Mn and excreted less Mn, levels of Mn were overall higher in men due to increased intake and increased half-life. Also postmenopausal women with osteoporosis were found to have decreased Mn, however, whether this was caused by the individuals' sex, the osteoporosis, or the individuals'

ages could not be determined (Freeland-Graves and Llanes 1994; Reginster et al. 1988).

A general trend of C/Pb mean values being higher in males was observed. This is unsurprising as men are more commonly associated with industrial activities and jobs such as those of smelting and mining, which may cause higher levels of Pb (Rieuwerts et al. 2000). Furthermore, men are more likely to work in construction and automotive industries, which may expose them to higher levels of Pb. Men are also more likely to smoke cigarettes, which may increase their Pb intake (Sekulic et al. 2014). However, it is important to remember that this difference is therefore gender-based, rather than based on biological sex.

A general pattern of C/Sr mean values and variance being higher in males than in females was observed. Studies have shown that there are significant differences in Sr levels between males and females, which were attributed to dietary and metabolic differences (Stipišić et al. 2014). While trace elements differ from isotopic values, it is important to note that many archaeological studies utilize Sr isotopic values for analyzing migration in past populations. However, it is clear that biological sex differences between these individuals may skew these results if not carefully considered.

#### Trace Element Differences between Age Groups

A general trend of C/Mg mean values and variance being the lowest in juveniles was observed. This may be related to the trend of C/Ca mean values and variance

being lowest in juveniles, because decreased Ca levels have been found to correspond with decreased Mg levels (Rude and Shils 2006; Spencer et al. 1994). Another potentially related trend is that of C/Mg mean values and variance being highest in the living sample and lowest in the archaeological sample. These trends are likely related because all of the juveniles fall within the archaeological sample. This is true also of the C/Mg trend of mean values and variance being higher in the modern context sample than the archaeological sample. It is likely that this trend of juveniles being the lowest in both C/Mg mean value and variance can be attributed to their archaeological status rather than their juvenile status since their numbers are not extremely different from the other archaeological individuals. However, in general, infants and children need considerably less Mg than do adults (Food and Nutrition Board 1997).

There were no trends observed in C/Cu mean values and variance between the age groups. However, there was a pattern of C/Cu mean values and variance being highest in the cadaver sample group, which is comprised mostly of older adults. This is likely because sex is a larger contributing factor in Cu levels than age is. This is supported by the observation that female levels were higher across all age groups. It may also be a result of the archaeological individuals, who generally have lower C/Cu mean values, being included within the older adult group but not within the cadaver group. Furthermore, age has not been found to be associated with any significant changes in the requirement for Cu (Wood et al. 1995). However, menopause has been found to correlate with increased levels of Cu, which may account for the higher C/Cu mean values and variance in the cadaver sample group (Fischer et al. 1990).

A general pattern of C/Zn mean values and variance being lowest in the juvenile sample was observed. This trend is not surprising since children and adolescents are at significantly increased risk for Zn deficiency. In fact, Zn deficiency is the cause of almost 5% of all juvenile deaths (Fischer Walker et al. 2009). Of the six juveniles, four are under the age of three (Table 18). In ancient Kellis children were breast fed almost exclusively for the first six months of life, after which they were gradually weaned until approximately three years of age (Dupras et al. 2001). Generally, important weaning foods include honey, cow or goat milk, bread, and even wine (Dupras 1999). Therefore, there is evidence to suggest these children would have consumed considerably less meat, a large source of Zn, than adults which may contribute to the lower C/Zn mean values and variance. However, these juveniles would have also been consuming milk and wheat which are sources of Ca and Fe both of which inhibit Zn absorption (Dupras 1999; Food and Nutrition Board 2001; Wood and Zheng 1997). Although it is important to note that all of the juveniles come from the archaeological sample and context group and so the pattern we see concerning Zn may be related to their archaeological status rather than their juvenile status. For example, a source of Zn in the modern world is the inhalation Zn oxide fumes created during welding and smelting, which are seen with greater frequency in modern populations (King and Cousins 2006). Although, Cu and Pb ores in the Eastern Desert of Egypt contain Zn which could have potentially been smelted in ancient Kellis (Nicholson and Shaw 2000) However, it is more likely to do with their juvenile status since a pattern of C/Zn mean values and variance being higher within the archaeological context group than the modern context group was observed.



A general pattern of C/Fe mean values and variance increasing with age was observed. This pattern is in line with medical literature that states older adults are more likely to have high Fe stores, which is due to intracellular Fe buildup that occurs slowly over time (Fleming et al. 2001). This pattern means that the juveniles tend to have the lowest C/Fe mean values and variance, which is supported by the fact that infants and adolescents are extremely susceptible to Fe deficiency. This is due to the importance of Fe during periods of rapid growth (Brody 1999). Furthermore, postmenopausal women, who make up the majority of the women in this age group, do not suffer from the same lack of Fe that is caused by menstruation and pregnancy (Brody 1999).

A trend of C/Ca mean values and variance increasing with age was observed. While this may seem counterintuitive, it is likely due to the Ca feedback loop. This occurs when Ca levels in the blood drop so low that the body begins demineralization of the bones to maintain the Ca blood levels (Weaver 2012). Therefore, while bone Ca levels are decreasing, blood Ca levels, which nourish the hair, remain the same (Robbins 2012). On top of this, many older individuals, over 67% of females and over 51% of males, are taking Ca supplements, which will increase their Ca levels above the balanced amount (Bailey 2010).

A general trend of C/Mn mean values and variance being highest in juveniles and lowest in adults was observed. However, a related trend of C/Mn mean values and variance being highest in the archaeological sample and lowest in the living sample was also observed. Because the entire juvenile sample is comprised of individuals from the archaeological sample it was important to distinguish the cause of these trends. The

cause of these trends was determined to be a result of high levels in juveniles in particular, rather than high levels throughout the entire archaeological sample. The juvenile sample was found to have one individual, A-57, who had much higher levels of C/Mn than the other juveniles and was causing the higher averages and variance (Table 18). These increased C/Mn concentrations may be due to the estimated age of the juvenile, 40 gestational weeks, which is at or around the time of birth. Infants at this age are extremely susceptible to high levels of Mn even to the point of Mn toxicity due to high absorption rates and low excretion rates (Food and Nutrition Board 2001; Ljung and Vanter 2007). Furthermore, the main source of Mn available to infants is breastmilk. The Mn found in breastmilk is more bioavailable than the Mn found in cow's milk (8.2% vs. 2.4%) (Davidsson et al.1989). This falls in line with weaning and breastfeeding patterns in the Dakhleh Oasis (Dupras 1999; Dupras and Tocheri 2007). A57 was likely being fed exclusively through breastfeeding because he was less than 6 months of age which when combined with increased Mn absorption of infants likely explains his elevated concentrations and the elevated Mn rates for the juvenile age group.

Table 18: Age, Sex, and average C/Mn levels for the seven juveniles from the archaeological sample

Identifier	Average C/Mn	Sex	Age
A-57	735.57	M	40 gestational weeks
A-278	1.57	F	3 years
A-336	-33.89	M	6 years
A-519	2.26	?	2.5 years
A-520	-16.08	F	7.5 years
A-555	18.87	M	5 years
A-593A	30.06	F	1.5 years

The trend of the adult age group having lower C/Mn mean values and variance than the older adult group is less obvious but is likely due to a number of factors, a few possible factors will be discussed. Mn is a common ingredient in many antacids and laxatives (Hendler and Rorvick 2001). It has been documented that laxative overuse, even to the point of abuse, is common within older populations. A similar pattern has been seen, to a lesser extent, with overuse of antacids in older populations (Roering et al. 2010). Similarly, it has been shown that older individuals take mineral and other dietary supplementation, including the use of Mn supplements, at much higher rates than younger age groups (Gordon and Schaffer 2005). This could account for the increased C/Mn levels as well as the increased variance in the older age group as well as the increased variance in the cadaver sample.

A general trend of C/Pb mean values and variance peaking in adulthood was observed. This is likely a result of increased intracellular buildup of Pb overtime. This is also likely influenced by the fact that the juvenile sample is completely made up of individuals from the archaeological sample, which would have had less exposure to Pb.

A general trend of C/Sr mean values and variance increasing with age was observed. This trend is likely due to the related metabolic pathways that Ca and Sr share as this same trend was observed for Ca.

A general trend of C/Se mean values and variance peaking in adults was observed. This is consistent with other studies which have found that Se values tend to increase with age (Skalnaya et al. 2015; Soroko et al. 2014). However, studies have

also shown that as age continues to increase Se levels will decline to lower levels once again (Savarino et al. 2001).

### Trace Element Differences between Sample Groups

A general pattern of C/Mg mean values and variance being highest in the living sample and lowest in the archaeological sample was observed. Due to the strongly related pattern of C/Mg mean values and variance being higher in the modern context group than the archaeological context group, the reasons for these trends will be discussed in the following section concerning context groups. However, there is also the trend of C/Mg mean values and variances being higher in the living sample than the cadaver sample. In general, older individuals, who make up the bulk of the cadaver sample, are especially susceptible to Mg deficiency because Mg absorption capabilities within the intestines decrease with age (Moshfegh et al. 2009; Sebastian et al. 2007). Furthermore, older individuals tend to have lowered dietary consumption of Mg (Food and Nutrition Board 1997; Moshfegh et al. 2009). It is also possible that this trend is caused by increased Zn levels within the cadaver sample which can interfere with Mg absorption (Rude and Shils 2006; Spencer et al. 1994). This may be supported by the general trend of C/Zn mean values being highest within the cadaver sample and lowest within the living sample. Also women suffering from osteoporosis have been found to have lower Mg levels than women who do not. Since older women tend to suffer from osteoporosis at higher rates, this may be a contributing factor.

A pattern of C/Cu mean values and variance being highest in cadaver samples was observed. This is similar to other studies that have reported increased levels of Cu in older individuals as opposed to younger individuals (Fischer et al. 1990; Sánchez et al. 2010; Sauberlich 1999). This is also likely a result of sustained exposure to inorganic Cu, such as that from exposure to industrial operations and use of Cu pipes to transfer water supply (Brewer 2009; Herman et al. 2013). Organic Cu from food is processed by the liver whereas inorganic Cu typically bypasses the liver and enters the bloodstream (Brewer 2009). This is a pattern that has been reported in other studies (Fischer et al. 1990; Sánchez et al. 2010; Sauberlich 1999) and is likely a result of continuous exposure to Cu from inorganic sources as well as increase in Cu levels following multiple childbirths and menopause (Fischer et al. 1990). The likely reason that the C/Cu mean values and variance were highest in the cadaver sample but not highest in the older adult sample is because the cadaver group, unlike the older adult group, contained exclusively modern individuals, who typically have higher C/Cu mean values and variance than those within the archaeological context group.

A general trend of C/Fe mean values and variance being lowest in the archaeological sample was observed. Part of this trend may be due to the juveniles in this research all falling into the archaeological sample because juveniles tend to have lower Fe levels and are more susceptible to Fe deficiency (Brody 1999). The lower number of older adults could also be playing a small part in this trend. One of the best sources of Fe is seafood, which is unlikely to have been a large portion of the diet in ancient Kellis (Dupras 1999).

A general pattern of C/Ca mean values and variance being highest in the cadaver sample and lowest in the archaeological sample was observed. This is likely due to the high number of older individuals in the cadaver sample group. Over half of all individuals in the US over the age of 50 take Ca supplements, and that number, as well as the amount of Ca in the supplements, increases along with age (Bailey et al. 2010). Not only does the archaeological group not have as many older individuals but they did not have access to Ca supplements.

A pattern of C/Mn mean values being highest in the archaeological sample and lowest in the living sample was observed. While the elevated C/Mn levels observed in the archaeological sample are most likely a result of the elevated C/Mn levels within the juvenile sub-sample, as was previously discussed, there are other potential explanations for this pattern. Water is often an important source of trace elements; this is particularly true of undeveloped countries and ancient civilizations that did not have water regulations in place. The water source of the Dakhleh Oasis is a sandstone aquifer which is accessed by drilling deep wells (Dupras 1999). The source of this water is of importance because of the mineral content within the ground that then becomes bioavailable to the inhabitants of the Oasis. Figure 37 shows the differences in trace element availability in drinking water for the Dakhleh Oasis, Egypt and the U.S. (Azoulay et al. 2001; Clarke 1979; Morr et al. 2006; Soltan 1997; United States Environmental Protection Agency 1986 U.S. Geological Survey 1989). Geological surveys of water sources from both the Dakhleh Oasis and the U.S. demonstrate that

Mn levels are higher in the Oasis which could be contributing to the higher C/Mn mean values in the archaeological sample (Clarke 1979; U.S. Geological Survey 1989).

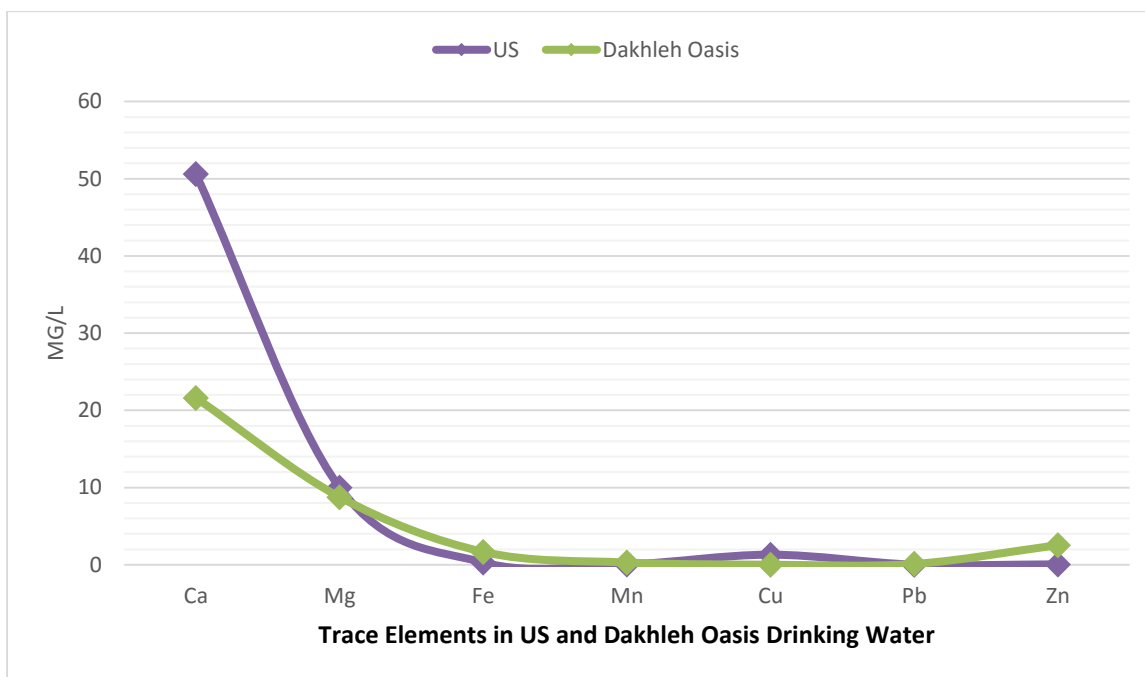


Figure 37: Mineral levels in typical U.S. drinking water in comparison with Dakhleh drinking water, derived from Azoulay et al. 2001, Clarke 1979, Morr et al. 2006, Soltan 1997, United States Environmental Protection Agency 1986, and U.S. Geological Survey 1989

Studies into the diet of the population of ancient Kellis have been augmented by the discovery of the Kellis Agricultural Account Book (AD 350), which has been used to create a list of available food sources for the Oasis (Table 19) (Bagnall 1997; Dupras 1999). In fact, many of the best food sources of Mn were available to the people of ancient Kellis, including nuts, whole grains, and legumes (USDA 2015; Dupras 1999). The average American takes in approximately 1.8-2.3 mg of Mn per day, which is under the recommended daily intake, likely due to increased reliance on processed foods. In fact, one serving of many of the food products present at Dakhleh would provide more

Mn than the average American receives all day (Table 20). However, C/Mn variance was higher in the cadaver sample than the archaeological sample, which could be explained by the more restricted diet of the ancient people of Kellis and the propensity of older individuals to supplement their mineral intakes.

Table 19: Potential food sources available to the individuals of the Dakhleh Oasis, Egypt. Derived from Bagnall 1997 and Dupras 1999.

Animal Protein	Field Crops	Fruits	Garden Plants	Nuts	Other
Cow	Wheat	Dates	Garlic	Almonds	Honey
Pig	Barley	Doum Palm Nuts	Onions	Walnuts	Ami
Goat	Millet	Carob	Turnips	Pistachios	Safflower
Camel	Sesame	Jujube	Celery	Hazelnuts	Coriander
Donkey		Fig	Legumes	Pine Nuts	Cinnamon
Gazelle		Olives	Artichokes		Cumin
Hartebeest		Pomegranates	Cucumber		Dill
Oryx		Pears	Gourds		Fennel
Chicken		Peaches			Thyme
Pigeon		Apricots			Marjoram
Duck		Cherry			Rosemary
Geese		Apples			Mint
Ostrich		Citron			Mustard
Eggs					Anise
Fish					Laurel
Hare					Pepper
					Caper



Table 20: Level of manganese per serving for different food sources

Food Source	Manganese (mg)	Serving
Almonds	0.53	¼ cup
Walnuts	3.99	1 cup
Pistachios	1.476	1 cup
Hazelnuts	5.6	2 ounces
Fava Beans	2.739	1 cup
Wheat Flour	4.88	1 cup
Millet	3.264	1 cup

A general pattern of C/Pb mean values and variance being highest in the living sample was observed. This pattern is likely related to the pattern seen concerning C/Pb between the age groups. Likely this is due to increased exposure to Pb due to a modern environment that has many different possible sources of Pb including paint and water from Pb containing pipes (United States Environmental Protection Agency 1986).

A general pattern of C/Sr mean values and variance being lowest in the archaeological sample was observed. This pattern is also seen in C/Ca and this is supported by the fact that C/Sr and C/Ca share metabolic pathways (Comar et al. 1957). This is likely related to the pattern of C/Sr mean values and variance being higher in the modern context group than the archaeological context group that was observed. Furthermore, atmospheric Sr from nuclear testing is something that is a major source of Sr for both the cadaver and living sample groups (Agency for Toxic Substances and Disease Registry 2004).

A general trend of C/Se mean values and variance being lowest in the archaeological sample was observed. This is most likely associated with the lack of seafood in the ancient Kellis diet. Seafood is one of the best sources of Se, and while

the people of ancient Kellis had some access to dried fish it is likely that modern consumption is significantly higher (Dupras 1999). Furthermore, Se supplements were available to both the living and cadaver samples, which may contribute slightly to this trend (Food and Nutrition Board 2000).

### Trace Element Differences between Context Groups

A pattern of C/Mg mean values and variance being higher in the modern context group than the archaeological context was observed. There are multiple potential contributing factors, one of which is the Mg levels in drinking water. The water in the Dakhleh Oasis has lower Mg levels than those found in typical U.S. drinking water (Clarke 1979; Soltan 1997; U.S. Geological Survey 1989). It is also possible that this trend is caused by increased Zn levels within the archaeological context group, which can interfere with Mg absorption (Rude and Shils 2006; Spencer et al. 1994). This may be supported by the general trend of C/Zn mean values and variance being higher within the archaeological context group than the modern context group. Similarly, it is possible that this trend is caused by decreased Ca levels within the archaeological context group, because decreased Ca levels have been found to correspond with decreased Mg levels (Rude and Shils 2006; Spencer et al. 1994). This may be supported by the general trend of C/Ca mean values and variance being lower within the archaeological context group than the modern context group. Furthermore, many modern over the counter supplements and medicines, such as laxatives and antacids, contain Mg (Hendler and Rorvick 2001). Food source of Mg are not likely to be a

contributing factor in the difference between modern and archaeological context groups because the food sources that are good sources of Mg, such as nuts, animal meats, and whole grains were available to individuals in ancient Kellis (Dupras 1999).

A pattern of C/Cu mean values and variance being higher in the modern context group than the archaeological context group was observed. There are multiple possible explanations. Food such as oysters, seeds, liver, whole grains, nuts, and other organ meats are the main source of Cu in the body (Fraga, 2005; Sadhra et al. 2007). It is likely that modern populations consume considerably higher amounts of oysters than the ancient populations of the Dakhleh Oasis, due to its inland location and lack of modern food transport abilities. However, the people of Kellis also had access to many varieties of nuts, seeds, grains, and animal meats (Table 19) (Dupras 1999). Therefore, it is unlikely that the difference between the modern context group and the archaeological context group is solely related to diet. Another source of Cu may be water coming from Cu piping that is commonly used in modern buildings (Brewer 2009). Another source of Cu is industrial operations such as steel production plants, municipal incinerators, and smelters (Herman et al. 2013; Newhook et al. 2003; Pope et al. 2007). These are likely the main source of C/Cu differences between the modern context group and the archaeological context group. Another possible factor is the use of oral and hormonal contraceptives in modern context groups. These contraceptives contain estrogen, which has been shown to raise Cu levels (Kowalska et al. 2015).

A general trend of C/Zn mean values and variance being higher in the archaeological context group than the modern context group was observed. This may

be the result of the related pattern of C/Ca mean values and variance being higher within the modern context group than the archaeological context group that was observed. High Ca values have been found to inhibit the absorption of Zn (Wood and Zheng 1997). Therefore, the elevated Ca values in the modern context group may be impairing the ability of this group to absorb Zn. Similarly, high levels of Fe can impair the absorption of Zn (Sandström 2001). C/Fe mean values and variance was found to be higher in the modern context group, which may contribute to the lower C/Zn mean values and variance within the modern context group. Furthermore, many of the best sources of Zn were available to the people of ancient Kellis, including red meats such as beef, nuts, and legumes (Table 21) (USDA 2015; Dupras 1999). The average American takes in approximately 9-13 mg of Zn per day, which is slightly less than the recommended daily intake. Furthermore, the Zn content of water from the Dakhleh Oasis is considerably higher than that of the average American drinking water (Figure 37) (Clarke 1979; Patterson et al. 2013; Soltan 1997; U.S. Geological Survey 1989).

Table 21: Level of Zinc per serving in different food sources

Food Source	Zinc (mg)	Serving
Beef	6.98	3 ounces
Almonds	3.37	1 cup
Pigeon	1.87	3 ounces
Hazelnuts	3.31	1 cup
Fava Beans	4.71	1 cup
Pork	2.63	3 ounces
Wheat Flour	3.79	1 cup

A general pattern of C/Fe mean values and variance being higher in the modern context group than the archaeological context group was observed. Part of this trend

may be due to the higher level of older adults in the modern context group, who are more likely to have high levels of Fe stores (Fleming et al. 2001). Another part of this trend may be due to the lack of juveniles within the modern context group, who are at increased risk for Fe deficiency (Brody 1999). Constant and easy access to fish and other seafood, regardless of geographic location, is likely to be a contributor to this trend, as seafood is a good source of heme Fe (Lynch 1997). Similarly, the grain in modern U.S. populations has been fortified with Fe and may be contributing to this pattern (Food and Nutrition Board 2001). There are also Fe supplements available to the modern context group that were not available to the archaeological context group (Hendler and Rorvick 2008).

A general trend of C/Ca mean values and variance being higher in the modern context group than the archaeological context group was observed. This is likely due to the prevalence of Ca supplements, particularly within the older adults. Another potential factor is the lower C/Mg mean values and variance within the archaeological context group. This could be contributing to their lower C/Ca mean values and variance since Ca deficiency often results from low levels of Mg (Weaver 2012). Although dairy products, which are a good source of Ca, were available in ancient Kellis, it is possible that adults did not consume as much dairy as infants and children due to lactose intolerance. Although nearly all children are born with the ability to digest lactose, this ability will often dissipate as an individual ages (Scheindlin 2007). Lactose tolerance has been linked with the practice of pastoralism, in which individuals from geographic regions where pastoralism was common are more likely to possess lactose tolerance.

Typically, this includes individuals from Europe, while individuals from Africa and Asia, as well as Native Americans are often lactose intolerant (Scheindlin 2007). This idea is supported by a study that assessed the level of lactose intolerance in present day Egypt. The study compared lactose intolerance levels between populations in the Eastern Egyptian Desert and the Western Egyptian Desert, where the Dakhleh Oasis is located. The study found that not only do individuals from the Western Desert have a higher rate of lactose intolerance at 51%, but the highest rate of lactose intolerance came from the sample population from present day Dakhleh at 64.4% (Hussein and Ezzilarab 1994). High levels were recorded in spite of the fact that the average household from the Dakhleh Oasis population had approximately 2.1 milking animals per household (Hussein and Ezzilarab 1994). This study supports the idea that although ancient Kellis had access to milking animals, and although milk was important in feeding and weaning infants, it is very possible that many adults did not consume high levels of dairy, which could in turn affect their Ca levels.

A pattern of C/Mn mean values and variance being higher in the archaeological context group than the modern context group was observed. This is likely related to the previously discussed patterns of C/Mn mean values and variance being highest in juveniles and C/Mn mean values being higher in the archaeological sample. While it is likely the juvenile results that are driving this difference, it is possible that this Mn levels are higher within the archaeological context as a whole. As was discussed previously Mn rich foods were present in Kellis and modern individuals are known for not meeting the daily recommended intake for Mn (Dupras 1999; USDA 2015). Therefore, these

results are a part of a pattern of increased juvenile and archaeological Mn levels which is supported by the literature.

A general trend of C/Pb mean values and variance being higher in the modern context group than the archaeological context group was observed. The trend is likely related to the pattern seen between the age groups and the sample groups concerning Pb. Increased exposure over time from multiple sources is likely the cause. Although Pb levels are slightly higher in the water found at Dakhleh than typical U.S. drinking water, the use of Pb based piping may cause Americans to also be exposed through drinking water (Figure 37) (Brewer 2009; Herman et al. 2013). Furthermore, other kinds of exposure including paint based, cigarette, mining, smelting, and importantly vehicle emission are certainly much higher amongst modern populations (Sekulic et al. 2014).

A general pattern of C/Sr mean values and variance being higher in the modern context group than the archaeological context group was observed. The pattern is also seen in C/Ca and this is supported by the fact that C/Sr and C/Ca share metabolic pathways. This is likely related to the pattern of C/Sr mean values and variance being lowest in the archaeological sample group that was observed. This difference is likely due to the increased atmospheric Sr levels caused by fallout from nuclear testing and potentially from increased levels of coal and oil burning (Agency for Toxic Substances and Disease Registry 2004; Comar et al. 1957).

A general trend of C/Se mean values and variance being higher in the modern context group than the archaeological context group was observed. This trend is likely related to the general pattern of C/Se mean values and variance being lowest in the

archaeological sample. This is likely due to a lack of Se rich foods, such as fish and seafood, available to the people of ancient Kellis (Dupras 1999). Also, individuals within the modern context group are likely getting the correct amount of daily Se, as Se deficiency is fairly rare within the U.S. (Food and Nutrition Board 2000). Furthermore, Se supplements may be playing a small role in the trend that was observed.

### Sex Estimation

The stepwise binary logistic regression analysis to predict biological sex from human hair was conducted multiple ways with varying degrees of success. The first technique used averages and was modeled after many of the current studies that use trace elemental analysis of hair (Huang and Beauchemin 2014). However, the results of this were little better than a guess at 50% prediction success. This technique does not work because of the cyclical nature of human hormones and metabolism, which regulate our trace element requirements and absorption. Figure 38 demonstrates the problems with using averages in trace elemental analysis of biological sex. The figure shows the average C/Mg values and the C/Mg variance of two individuals; individual M-12, who is a male, and individual C-390, who is a female. If these two individuals had been categorized as either male or female based on the average of C/Mg, they would have been classified as being the same sex because their average C/Mg values are very similar. However, if these two individuals had been categorized based on their variances, which are extremely different, they would have been classified as being of opposite sexes, which is in fact the case here.



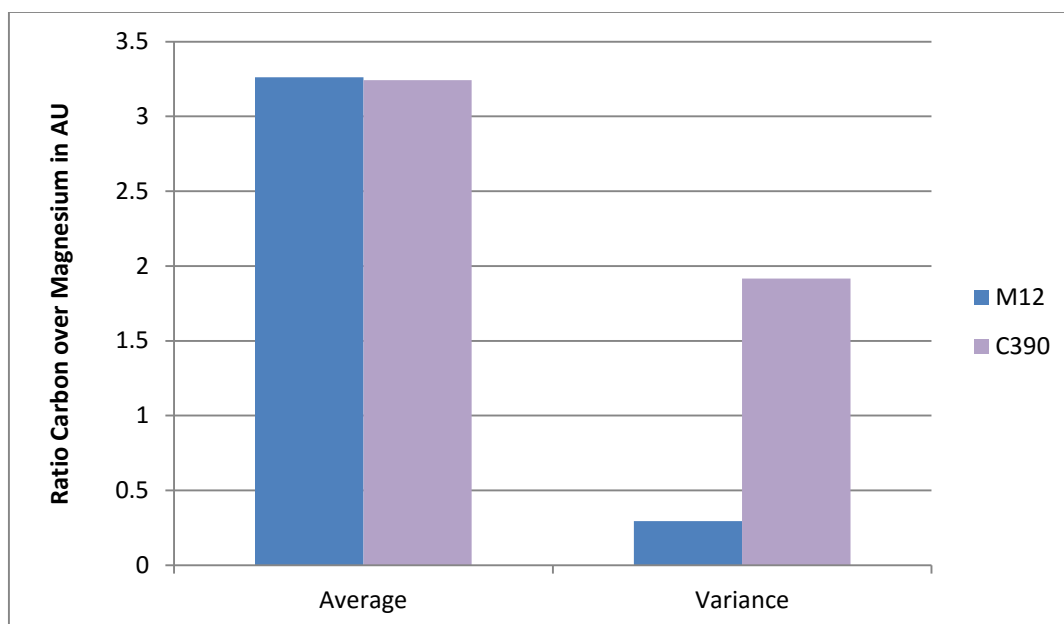


Figure 38: Chart showing the similar C/Mg averages but extremely different C/Mg variances between individuals of different sexes, demonstrating the problems with estimating sex based only on trace element averages

The second stepwise binary logistic regression analysis to predict biological sex from human hair used mean variances values (MVs) from individual hair strands in order to capture the different cycles that occur within the human body, and was much more successful with an approximately 85% prediction success rate. One of the 77 hair samples, A-512/2, was actually beard hair, and it was correctly predicted for sex. This logistic regression is as follows in Equation 1:

$$\begin{aligned} \text{Log Odds} = & 0.221702 + 0.003006 \times (\text{C/Mg}) + 0.000277 \times (\text{C/Cu}) + \\ & 0.000005 \times (\text{C/Zn}) + 0.003265 \times (\text{C/Fe}) + (-85.296683) \times (\text{C/Ca}) + \\ & (-0.000002) \times (\text{C/Mn}) + 0.005877 \times (\text{C/Sr}) \end{aligned}$$

The resultant log odds can then be classified as male if they are negative and female if they are positive. This equation, however, did not work as well when applied to the archaeological population. Due to the reduced predictive accuracy, a new archaeologically specific equation with a much higher success rate was created and is shown in Equation 6:

$$\begin{aligned} \text{Log Odds} = & -1.233498 + 78.198965 \times (\text{C/Mg}) + 0.002579 \times (\text{C/Cu}) + \\ & 0.000002 \times (\text{C/Zn}) + -2.729181 \times (\text{C/Fe}) + (1333.127887) \times (\text{C/Ca}) + (-0.000002) \times (\text{C/Mn}) + \\ & -0.852564 \times (\text{C/Pb}) + 0.006203 \times (\text{C/Sr}) + -0.0000002343 \times (\text{C/Se}) \end{aligned} \quad (6)$$

This demonstrates the need for population specific equations using this technique, particularly between archaeological populations. It is likely that the population differences between modern populations would be less drastic; however, if possible a population specific equation would be ideal. However, it is apparent that equations derived from modern populations are not adequate for use with archaeological populations.

This new archaeological equation was then applied to the juvenile archaeological sub-population with great success. Out of the seven juveniles one was of unknown sex and five out of the other six were accurately predicted for biological sex using the archaeological population specific variance method. Excluding the individual of unknown sex this technique produced an 83% success rate when predicting the biological sex of juveniles. There are many other studies that have attempted to provide a reliable method for estimating the biological sex of juveniles with varying success (Table 22). Many of these methods, however, fail in replication, have unbalanced

samples with regard to biological sex, and small age ranges. Furthermore, in many cases the reliability of the method is dependent on the experience of the researcher. For example, Loth and Henneberg (2001) had accuracy varying from 74%-89% depending on the individual performing the analysis.

Table 22: Table summarizing multiple methods for estimating juvenile sex within biological anthropology and their associated % accuracies. Asterisk (\*) denotes a validation study of a previously published method.

Study	% Accuracy	Sample Size (Female/Male)	Age Range	Method
Woltering 2016	83%	6 (3/3)	0-7.5 years	Trace element analysis of hair
Molleson et al.1998	78%	20 (6/14)	1-14 years	Morphology of orbit, mandibular angle, and mentum
Mittler and Sheridan 1992*	74.1%	58 (24/34)	0-18 years	Morphology of auricular surface
Schutzkowski 1993	70-90%	61 (24/37)	0-5 years	Morphology of chin, dental arcade, and greater sciatic notch
Vlak et al. 2008*	61%	56 (23/33)	0-15 years	Morphometric analysis of greater sciatic notch
Loth and Henneberg 2001	81%	19 (12/7)	0.5-3.5 years	Morphology of mandibular body and sympheseal base
Gonzalez 2012	78-89%	83 (36/47)	5-16 years	Craniofacial measurements

As can be seen in Table 22, this study has a very small sample size in comparison to the other studies. For this reason, it is necessary that this method be replicated with a larger sample before this method can be confidently utilized within anthropology. Another issue with all of these studies, including this one, is that the methods require the presence of a particular skeletal element or biological tissue. However, this method is much less subjective or reliant on researcher experience than many of the other proposed methods.

It should be noted, however, that the one individual in this study who was incorrectly estimated to be female when they were actually male needs to be considered separately. The individual was predicted with 100% probability to be female, however, the individual was approximately 40 gestational weeks old, which is at or around the time of birth. This likely indicates that the trace element data that was being analyzed was that of the mother. As previously discussed multiple trace elements are passed from mother to fetus and it is likely that this is evidence of that process. This must be taken into consideration when evaluating the success of this technique.

This demonstrates the viability of this technique with relation to juveniles. The logistic equation that was created from adult data was able to accurately predict the sex of the majority of the juvenile sample. This shows promise for future application of this technique particularly with modern samples where creating a large amount of adult data is possible. Furthermore, the possibility of creating a juvenile specific equation for modern populations is extremely encouraging for future research and for enhancing this method.

This technique has importance within both forensic and bioarchaeological contexts as a result of the promising potential for estimating juvenile biological sex. This is particularly true of bioarchaeology where DNA analysis has been utilized for sex determination of juveniles with variable results (Cunha et al. 2000; De La Cruz et al. 2008; Faerman et al. 1998). However, DNA analysis for determining biological sex in a forensic context is much more reliable. While this technique can typically be completed more quickly and cost effectively than DNA analyses, DNA analysis is still relatively quick and is constantly decreasing in price (Nelson 2010). Furthermore, DNA analysis may provide a definitive answer for biological sex as opposed to an estimate or probability. Nevertheless, DNA in forensic anthropology has many potential issues including the presence of enzymatic inhibitors within DNA extracts, degradation of nucleic acids, contamination risks, faint amplification of Y band, or small amount of DNA present (Keyser-Tracqui and Ludes 2005; Quincey et al. 2013). These issues can be exacerbated by small, incomplete, fragmentary, degraded, and/or altered skeletal remains. For these reasons, morphological assessment is the preferred method for adult biological sex estimation of forensic anthropologists, however, this is not possible for juveniles. This demonstrates the importance of this potential technique concerning juvenile biological sex in forensic anthropology.

When considering the usefulness of this technique for either adults or juveniles within a forensic context it is important to remember that in forensic anthropology the methods used for creating the biological profile are always case driven. Therefore, the forensic value of this technique will vary greatly depending on the case under

consideration. Therefore, forensic anthropology is only strengthened by access to a variety of techniques for estimating any aspect of the biological profile, including sex.

However, in order for this technique to be a definitive study for use in forensic anthropology it will need to be replicated through a validation study which will require a larger sample size of both adults and modern juveniles. Furthermore, the location of the LIBS analysis along the hair shaft will have to be explored further and the replication of the process on different locations along the hair shaft for each individual will be required to confirm the biological sex estimates. Nevertheless, this technique for trace element analysis of hair has been shown to be a better method than other techniques because it relies on variances rather than averages, which can be problematic considering the cyclical nature of human metabolism and hormones. In addition, the use of LIBS, as opposed to LA-ICP-MS, is preferable for the ability to take multiple samples along the shaft of the hair. This allows for the use of variances and also for tracking of trace element cycles for future research.

## CHAPTER SIX: CONCLUSIONS

The trace element analysis of hair conducted on 77 individuals from three different sample groups demonstrates the viability of this technique for biological sex estimation. Generally, the modern sample group had the best predictive success rate, followed by cadaver sample group, and then the archaeological sample group. However, the juvenile age group had the highest predictive success rate of any of the categories. While no one trace element was predicative of biological sex, by analyzing a combination of trace elements we can get a fairly accurate sex estimation. Additionally, many of these trace elements interact with one another. There were many differences between the sample groups that are based on diet, metabolic differences, water source, contamination levels, etc. This is another reason for the use of multiple trace elements. This minimizes the effects of these variables on the sex predictions.

The outliers that were present in this sample were eliminated when comparing averages and variance between the different populations and groupings. However, they were included in the regression equation because they represent natural variation that may occur in either a forensic or bioarchaeological context. All four of the outliers C-390, C-396, M-7, and M-17 were correctly classified using this technique. This demonstrates that although these individuals may be considered outliers, they are not so drastically different from the rest of the sample that they were incorrectly predicted for biological sex. However, had only one point been taken, instead of 11, these individuals could very well have been too different to be accurately predicted for

biological sex. This is another reason why taking multiple points for evaluation of the cycles is the better of the two techniques.

### Limitations

While hair is a relatively stable biological tissue, it is important to consider that many things can still affect an individual's ability to absorb certain trace elements, such as disease, diet, and geographic location. Furthermore, these three sample groups overlapped pretty consistently with one age group or context group. For example, the older adult age group consisted almost exclusively of individuals from the cadaver sample group. This could limit the ability to make completely accurate comparisons between groups. Also the small sample size of juvenile individuals limits the applicability of those findings.

Furthermore, limitations exist that could affect the implementation of this technique. For example, this technique may have to be repeated for every specific archaeological population to achieve the best possible results. Also, this technique is reliant on the availability of human head hair to analyze. In cases where no head hair is present or preserved this method cannot be used. Furthermore, although hair is very resilient to degradation and destruction, diagenesis can occur and can reduce the reliability of this method. Although trace elemental analysis is cheaper than DNA analysis, it does require sophisticated equipment that may not be readily available.



### Future Directions

More research into the trace elements contents of juvenile hair is necessary to confirm the preliminary results produced here. Research including a larger sample size of individuals, with a wider variety of juvenile ages, and including living individuals will need to be done to further confirm this technique as reliable. Furthermore, research into the trace element content of fetal and newborn hair is necessary to determine whether all fetuses and newborns are classified as females under this technique and also the age at which their own sex begins to affect the trace element content seen in hair. Additionally, it is possible that similar research could answer important questions about the age of menarche in ancient populations.

Future research into the trace element content in the hair of other ancient populations will be required to create population specific equations for use in multiple bioarchaeological contexts. Furthermore, research into other trace elements in hair for potential sex based differences may add to the current research and create an even more reliable equation for future use. Research into other types of hair, such as arm hair, beard hair, and pubic hair could build off and add to this research. Finally, research into hormonal cycles and their effect on trace element content in hair would be helpful in further understanding and refining this technique.

There is also potential for use of trace elements in the hair as a tool for health investigations. Trace elements in hair have been used successfully in the past to track pollution and exposure that may cause certain health issues for affected individuals (Kales 2005). The existing literature sheds light on the how certain values of trace

elements are expected to change when an individual has a particular disease or condition. Therefore, this particular method may have future potential for use in tracking diseases or conditions as well as assisting in diagnoses. The ability to track changes in trace elements along the length of the hair shaft using this technique would be particularly helpful in this research. However, there is a need for more research into the many different and complex relationships between trace elements, diseases, diet, population variation, and other variables before this could be beneficial.

### Implications

This study has shown that trace elemental analysis of human head hair is a viable technique for estimating biological sex in both forensic and bioarchaeological contexts. This is significant due to the current underuse of hair as an avenue of research and evidence, particularly in forensic anthropology considering the prevalence of hair recovered in forensic contexts. The high percent of successful sex predictions within the living sample demonstrates the viability of this technique in forensic cases when other lines of evidence are not possible, are uncertain, or even when a quick tentative answer is required while other analyses are conducted. The high percent of successful sex predictions within the archaeological sample demonstrates the viability of this technique in bioarchaeology when other analyses are not possible or too expensive.

This study also revealed a promising technique for estimating biological sex of juveniles. This is significant because there are currently no reliable methods for

accomplishing this. Furthermore, this study uncovered interesting questions concerning the transmission of maternal metabolic signatures to the hair of male newborns which will require further research.

This research has provided a new technique for sex estimation within forensic anthropology and bioarchaeology which can be used regardless of follicle presence or even heavy biodegradation. These findings have suggested a new reliable technique for the biological sex estimation of juveniles as well, which could be of incredible usefulness throughout the sub-disciplines of biological anthropology.

## **APPENDIX A: IRB APPROVAL LETTER**



University of Central Florida Institutional Review Board  
Office of Research & Commercialization  
12201 Research Parkway, Suite 501  
Orlando, Florida 32826-3246  
Telephone: 407-823-2901 or 407-882-2276  
[www.research.ucf.edu/compliance/irb.html](http://www.research.ucf.edu/compliance/irb.html)

### Approval of Human Research

From: UCF Institutional Review Board #1  
FWA00000351, IRB00001138

To: Abigail M. Woltering

Date: February 11, 2016

Dear Researcher:

On 02/11/2016, the IRB approved the following human participant research until 02/10/2017 inclusive:

Type of Review: UCF Initial Review Submission Form  
Project Title: Biological Sex Determination from Trace Element Analysis in Human Hair  
Investigator: Abigail M Woltering  
IRB Number: SBE-16-12042  
Funding Agency:  
Grant Title:  
Research ID: N/A

The scientific merit of the research was considered during the IRB review. The Continuing Review Application must be submitted 30 days prior to the expiration date for studies that were previously expedited, and 60 days prior to the expiration date for research that was previously reviewed at a convened meeting. Do not make changes to the study (i.e., protocol, methodology, consent form, personnel, site, etc.) before obtaining IRB approval. A Modification Form cannot be used to extend the approval period of a study. All forms may be completed and submitted online at <https://iris.research.ucf.edu>.

If continuing review approval is not granted before the expiration date of 02/10/2017, approval of this research expires on that date. When you have completed your research, please submit a Study Closure request in iRIS so that IRB records will be accurate.

Use of the approved, stamped consent document(s) is required. The new form supersedes all previous versions, which are now invalid for further use. Only approved investigators (or other approved key study personnel) may solicit consent for research participation. Participants or their representatives must receive a copy of the consent form(s).

All data, including signed consent forms if applicable, must be retained and secured per protocol for a minimum of five years (six if HIPAA applies) past the completion of this research. Any links to the identification of participants should be maintained and secured per protocol. Additional requirements may be imposed by your funding agency, your department, or other entities. Access to data is limited to authorized individuals listed as key study personnel.

In the conduct of this research, you are responsible to follow the requirements of the [Investigator Manual](#).

On behalf of Sophia Dziegielewski, Ph.D., L.C.S.W., UCF IRB Chair, this letter is signed by:

Signature applied by Joanne Muratori on 02/11/2016 04:43:06 PM EST

IRB Manager

## **APPENDIX B: PRE-COLLECTION SURVEY**

**Pre-Collection Survey**

**Specimen #:** \_\_\_\_\_

Biological Sex:                      Male                      or                      Female

Age: \_\_\_\_\_

Is the sample you are providing today your own natural grown hair:

Yes                                      No

Have you recently been exposed to a laboratory environment for an extended period of time?

Yes                                      No

Have you recently been exposed to a heavy industrial environment for an extended period of time? (i.e. factories, automotive shops, etc.)

Yes                                      No

Have you recently been exposed to heavy-strength cleaning products?

Yes                                      No

Are you a smoker?

Yes                                      No

Do you use any leave-in conditioners or hair products (i.e. hairspray, gel, etc.)?

Yes                                      No

Have you recently used or are you currently using any hormonal contraceptives (i.e. oral birth control, intrauterine devices, birth control shot, etc.)?

Yes                                      No

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