TITLE: Testing the Use of Portable X-Ray Fluorescence (pXRF) in the Identification of Pathological Conditions in Archaeological Bone

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ABSTRACT:

This study aims to investigate the potential of portable X-ray fluorescence spectrometry (pXRF) for identifying pathological conditions in archaeological human skeletal remains. Bone element distribution in relation to known disease categories is analyzed using pXRF from the femora of 99 individuals (34 adult; 63 non-adult) from the post-Medieval Coach Lane skeletal collection (Durham University). There were no significant differences in the elemental ratios of individuals with scurvy, rickets, and cribra orbitalia. Potential alterations in elemental content were observed in relation to syphilis (Mn/S, Mn/Cl, and Ba/Cl) and neoplastic disease (Ba/Sr, S/Sr, Mn/Fe, and Zn/Cl). It is likely that post-depositional diagenetic changes, potentially exacerbated by the industrial location of the burial site, altered the elemental content of the individuals sampled and thereby effectively obscured any pathological changes detectable by pXRF.

Keywords: elemental analysis, diagenesis, disease, palaeopathology, post-medieval

1. Introduction

The ability to accurately distinguish pathological conditions in human skeletal remains is essential for understanding the health, living conditions, and social landscape of individuals and populations in the past (Buikstra et al., 2017; Buzon, 2012). Currently, palaeopathological analysis is primarily dependent on macroscopic observations of skeletal lesions and their distribution patterns (Buikstra, 2019; Roberts and Manchester, 2005). Macroscopic analysis is supported with imaging techniques, such as radiographs and computed tomography (CT) scans, as well as by microscopic and chemical analyses (Buikstra, 2019). Chemical analyses contribute to pathological assessments through ancient DNA research, isotope studies and elemental analysis (Beaumont et al., 2015; Martin et al., 2013; Schuenemann et al., 2013).

The trace element composition of bone is dependent on multiple factors, including an individual's physiology, metabolism, diet, and the environment in which they lived (Darrah, 2009; Gonzalez-Rodriguez and Fowler, 2013). After death, the elemental composition of bone is further altered by post-depositional processes, known as diagenesis, which can affect the chemical structure of bone (Carvalho et al., 2004; Kendall et al., 2018). Although the exact roles and effects of many trace elements on the human skeletal system are poorly understood, some elements (e.g. iron, zinc and manganese) have been identified as necessary components for normal bone growth and function (Darrah, 2009; Maciejewska et al., 2014; Marquardt et al., 2012). Other elements (e.g. lead and aluminum) are known to be detrimental to human health, even at low concentrations (Aufderheide et al., 1988; Nayak, 2002).

The importance of trace elements to bone structure and function suggest that diseases that disrupt normal bone processes could alter the chemical composition of the skeleton, however, investigations into the relationships between disease and bone elemental content have so far been limited. These studies have focused almost exclusively on single-element research, with the majority of studies examining lead exposure (eg. Handler et al., 1986; Rebôcho et al., 2006; Swanston et al., 2018) and the medicinal use of mercury in medieval Europe (eg. Kepa et al., 2012; Pessanha et al., 2016; Rasmussen et al., 2008; Walser et al., 2019). Multi-element studies have investigated potential elemental changes associated with cribra orbitalia (CO), osteoarthritis (OA), and residual rickets (Fornaciari et al., 1981; Gleń-Haduch et al., 1997; Kerns et al., 2015; Nganvongpanit et al., 2016a). In an analysis of 24 skulls from Carthage, Fornaciari et al. (1981)

found lower iron levels in the skulls of individuals with CO than those without. In contrast, Gleń-Haduch et al. (1997) found no significant difference in iron concentration, though they did notice greater variability in iron levels among individuals with CO and significant distinctions in element ratios involving copper between CO and non-CO groups. It is important to note that the aetiology of CO is broad and not always associated with iron deficiency (Brickley, 2018; Walker et al., 2009). Raman analysis of several individuals from the *Mary Rose* shipwreck revealed significantly different elemental profiles between individuals identified as having residual rickets and those without observed pathological changes, though the specific elements responsible for the discrepancies were not determined (Kerns et al., 2015). Additionally, Nganvongpanit et al. (2016a) found increased levels of iron in the pelvic bones of dogs with OA when compared to individuals without OA.

This study seeks to further elucidate the relationship between bone chemistry and disease by investigating the use of portable X-ray fluorescence (pXRF) in the identification of specific pathological conditions in archaeological human skeletal remains. pXRF spectrometry is an inexpensive, mobile, and non-destructive analytical technique that allows for rapid multi-element analyses (Williams et al., 2020). It has been commonly utilized in the archaeological analysis of lithic, ceramic, and metallurgic artifacts, as well as in soil mapping and art conservation, largely due to its non-destructive and fast analysis abilities (Aimers et al., 2012; Cannell et al., 2018; Johnson, 2011; McGlinchey, 2012; Roxburgh et al., 2019). It has previously been successfully applied to the elemental analysis of human bone, particularly to demonstrate elemental distinctions between individuals in forensic instances of commingled remains (Finlayson et al., 2017; Gonzalez-Rodriguez and Fowler, 2013; Perrone et al., 2014).

In order to assess the utility of pXRF for pathological analysis, elemental ratios of bones were examined for patterns that could be linked to rickets, scurvy, CO, and pathological new bone formation. Rickets and scurvy result from nutritional deficiencies (of vitamins D and C, respectively), while CO is a non-specific condition defined by porous lesions in the orbital roof. CO has multiple possible aetiologies, including trauma, scurvy, rickets, and neoplastic disease, among other conditions or combinations of conditions (Brickley, 2018; Cole and Waldron, 2019). Elemental changes might be expected in response to these conditions as they involve alterations in osteoblastic (bone forming) and osteoclastic (bone destroying) processes, which could possibly result in deviations in the trace element composition of bones, in particular changes to calcium, phosphorus, iron, copper, and zinc values (Brickley and Ives, 2008; Endt and Ortner, 1982). This hypothesis is supported by *in vivo* chemical analyses of modern rickets patients, which found fluctuations in their fluid calcium, phosphorus, and zinc levels (Doğan et al., 2012). While bone element values tend to remain more stable than fluid, variation in bone can occur, as evidenced by the previously mentioned dry bone studies (Burton, 2008; Pate and Hutton, 1988).

Individual instances of neoplastic disease and "phossy jaw", as well as suspected instances of syphilis, tuberculosis, and smallpox, were observed in the studied group and their results were also examined to search for potential unique elemental signatures. Exploring variation in elemental distribution in relation to known disease categories may provide an additional tool for understanding health in the past, as well as further our understanding about possible alterations to bone element composition caused by pathological conditions.

2. Materials and methods

2.1. Skeletal material

The skeletal material analyzed was from the Coach Lane skeletal collection at Durham University. Coach Lane was the site of a post-Medieval Quaker cemetery located in North Shields in northeastern England, in use from AD 1711 until 1829 (Proctor et al., 2016). As a coastal community at the mouth of the Tyne, North Shields experienced expansion during the eighteenth century as sea trade and industrialization intensified throughout the region (Roberts et al., 2016). Contemporary descriptions of North Shields depict the area near the cemetery as narrow and crowded, with inadequate drainage and sewerage (Report of the Commissioners, 1845). Small houses lodged multiple families and had 'a deficiency of light and ventilation' and the entire North Shields area was troubled by poor air quality from industrial and domestic smoke and pollutants (Proctor et al., 2016; Report of the Commissioners, 1845:179). Continuous exposure to high levels of pollution would not only have affected the pulmonary health of people living in North Shields, but also, in combination with limited exposure to sunlight in the winter months, increased their risk of vitamin D deficiency (Macdonald et al., 2011; Newman et al., 2019; Pearce and Cheetham, 2010). Outbreaks of infectious disease were common in North Shields throughout the post-Medieval period, and a possible correlation between phases of intense cemetery use and smallpox/fever epidemics has been noted (Proctor et al., 2016). The individuals interred at Coach Lane were thus exposed to a number of diseases and health complications through their environment and living conditions that could have affected the trace element composition of their bones.

A total of 99 individuals were selected for pXRF analysis, of which 63 were non-adult and 34 were adult individuals (see *supplementary material*). The ages of the non-adult individuals ranged from perinates (36-38 weeks *in utero*) to older adolescents (17-19 years) (Gowland, unpublished). The adult individuals ranged from young adult (18-29 years) to older adult (45+ years), with the majority (56%) estimated to be of middle adult age (30-45 years). Males and females were almost evenly represented—17 male/probable male individuals and 16 female/probable female individuals were selected, as well as one of unknown biological sex (Gowland, unpublished).

Only one bone from each individual was selected for analysis, as multiple studies have demonstrated that inter-individual differences in trace element ratios are greater than those of bones from the same individual (Finlayson et al., 2017; Gonzalez-Rodriguez and Fowler, 2013; Perrone et al., 2014). Moreover, metabolic stress impacts individuals at a whole system level rather than affecting isolated regions (Eleazer and Jankauskas, 2016; Maciejewska et al., 2014; Raisz, 1999). Although tuberculosis and smallpox typically manifest skeletally as location-specific lesions (e.g. vertebrae and elbow joints, respectively) (Balaji, 2011; Roberts and Buikstra, 2014), both conditions can travel anywhere in the body and are possibly more widespread in the skeleton than macroscopic evidence suggests (Müller et al., 2014). Additionally, both conditions have systemic effects (for example, fever, malaise, weight loss, rashes), which could potentially affect the chemistry of the entire skeleton (Reynolds and Damon, 2017; Roberts and Buikstra, 2014). Right femora were preferentially selected for analysis, followed by left femora, then tibiae and humerii. Fibulae, radii and ulnae were used only when no other long bone was available. Femora were selected for analysis as they typically have the thickest cortical bone—making them more resistant to diagenetic changes and increasing their tendency to survive intact—and also have the largest surface area of the long bones, thereby improving the likelihood of getting a usable result from the pXRF (Croker et al., 2016; López-Costas et al., 2016; Perrone et al., 2014).

2.2. Assessment of pathological conditions

Rickets, scurvy, and CO were selected as the main conditions of interest because they have relatively high crude prevalence rates in the Coach Lane population and were considered likely to affect trace element ratios in bone. Pathological lesions were recorded in a previous study using standard methodologies (Gowland, unpublished). Rickets, scurvy, and CO were recorded as present or absent for the non-adults. Rickets was assumed to be active at the time of death unless otherwise noted. The presence or absence of residual rickets and CO was also recorded for the adult individuals (Gowland, unpublished). Table 1 presents a summary of the observed pathological conditions in both the non-adult and adult individuals (see *supplementary material* for more detail). Many individuals were identified as having multiple pathological conditions. Ten non-adult skeletons presented no evidence for either rickets or scurvy, and only two of the non-adult individuals had no identifiable pathological changes (Gowland, unpublished). Eleven adult skeletons without observable pathological conditions were included in the sample, though it should be noted that the lack of observable changes is not necessarily indicative of good health (Gowland, unpublished; Wood et al., 1992).

Periosteal new bone formation was present on five of the non-adult bones selected for analysis. Additionally, one of the non-adult individuals, COL069, likely suffered from phosphorus necrosis of the jaw, colloquially known as "phossy jaw," and had indicators suggestive of either smallpox or tuberculosis infections (Roberts et al., 2016). Two of the adult bones had areas of periosteal new bone formation. The presence of an osteoma, and, potentially, syphilis and diffuse idiopathic skeletal hyperostosis (DISH) were noted on three separate adult individuals.

Table 1. Number of individuals identified with each pathological condition. Note that many individuals were assessed with multiple pathological conditions. Scurvy was not assessed in the adult skeletal remains (Gowland, unpublished).

Age Group	**Scurvy**	**Rickets**	**Cribra Orbitalia**
Non-adult	34	31	20
Adult	N/A	22	5

2.3. Sample preparation

Gentle cleaning of the bones had previously occurred to remove dirt and enable macroscopic investigation. In order to avoid the destructive removal of woven bone and other pathological lesions, the skeletal remains in this study did not undergo any sanding or grinding.

2.4. pXRF analysis

Trace element analysis was performed using a Thermo Niton™ XL3t GOLDD+ pXRF with an Ag anode (6-50kV, 0-200 µA max X-ray tube) operating in Mining Cu/Zn mode (fundamental parameters). The instrument was warmed up and the system checked prior to all scanning sessions. An SiO_2 blank was analysed to test for contamination followed by NIST 2709a standard to test for drift and confirm factory calibration before all samples were analysed, and repeated every ten samples to confirm no new contamination. All samples were scanned with the pXRF held in a stand to maintain the stability of the machine and minimize the potential for error due to movement.

The pXRF window was held in contact with the anterior midshaft of each bone. Packing material, such as tissue paper, was placed under the bone to adjust its position and ensure full contact when necessary. Each sample was analysed with 30-second main, low and high filters, and a 60-second light filter, for a total of 150 seconds. The test was repeated on the same location three times and the results for each individual were averaged.

2.5. Statistical Analyses

Elements were plotted against each other on scatterplots using the mean values (in ppm) for each bone or lesion to visually examine the data for patterns. Where potential patterns in the data were noted, further statistical tests were computed using Past3 (v.3.24) statistical software, with the statistical significance set at $\alpha = 0.05$ (Hammer et al., 2001). Element values were converted to ratios, as ratios function to normalize the data, thereby limiting the effects of instrument fluctuation and chemical background noise (Craig et al., 2007; Gonzalez-Rodriguez and Fowler, 2013). A single case modified t-test was used to determine if there was a significant difference between a single value and the mean of the group (Hammer et al., 2001). In order to make comparisons between two groups, one of two tests were used. If data were normally distributed, a two-sample t-test was used to determine significant differences between the two groups. When this was not possible, a Mann Whitney U test was performed. The Shapiro-Wilk test was used to assess the normality of the data and decide which test was most appropriate. Where more than two groups were compared, ANOVA (analysis of variance) statistics were used to test for significantly different means between the groups.

3. Results

Internal comparison of elements detected in concentrations lower than 150 ppm, as well as elements with low atomic numbers—particularly magnesium—displayed higher error rates and were excluded from further analysis. The remaining fifteen elements were therefore identified as elements of interest. These elements were barium, strontium, lead, zinc, iron, manganese, titanium, calcium, potassium, aluminum, phosphorus, silicon, chlorine, sulphur, and copper. Elemental values that fell below the limits of detection of the machine were assigned a value of zero, following the methodology established by Perrone et al. (2014).

During pXRF analysis, it was determined that some of the infant bones were too small for accurate analysis because the entirety of the measurement window was not covered by the bone and could result in erroneous data. For this reason, individuals under the age of one year were removed from the study, as well as individuals COL095 and COL166. Although these two individuals were aged 1.0 to 1.5 years, the bones selected for analysis (humerus and fibula, respectively) were considerably smaller than the measurement window.

Individual COL205 was identified as having significantly higher levels of molybdenum relative to the other individuals (single case modified *t*-test: $t(74) = 6.80$, $p < 0.01$) and was the only individual with a nickel value above the limits of detection of the pXRF machine. As the femur tested from this individual was fragmented, it was determined that these results were likely due to soil contamination, as the fragmentation likely resulted in proportionally higher amounts of soil present in the area of analysis, relative to non-fragmented samples. Individual COL205 was therefore removed from the study and the total sample size reduced to 73 individuals (39 non-adult individuals and 34 adult individuals).

3.1. Rickets, scurvy, and cribra orbitalia

While there was variation in the element ratios between individuals, none of the variation is attributable to changes resulting from active, healed, or residual rickets. No patterns in the data could be correlated with scurvy or CO for any of the elemental ratios that were assessed. In the same way that no distinctions were observed in the elemental ratios comparing pathological and non-pathological states for rickets, scurvy and CO, no distinguishable differences were perceived between these conditions. No variations in the patterns or ranges of values were observed in the scatterplots for individuals identified with scurvy, rickets and CO to suggest deviations in the elemental composition between the groups (*Figure 1*).

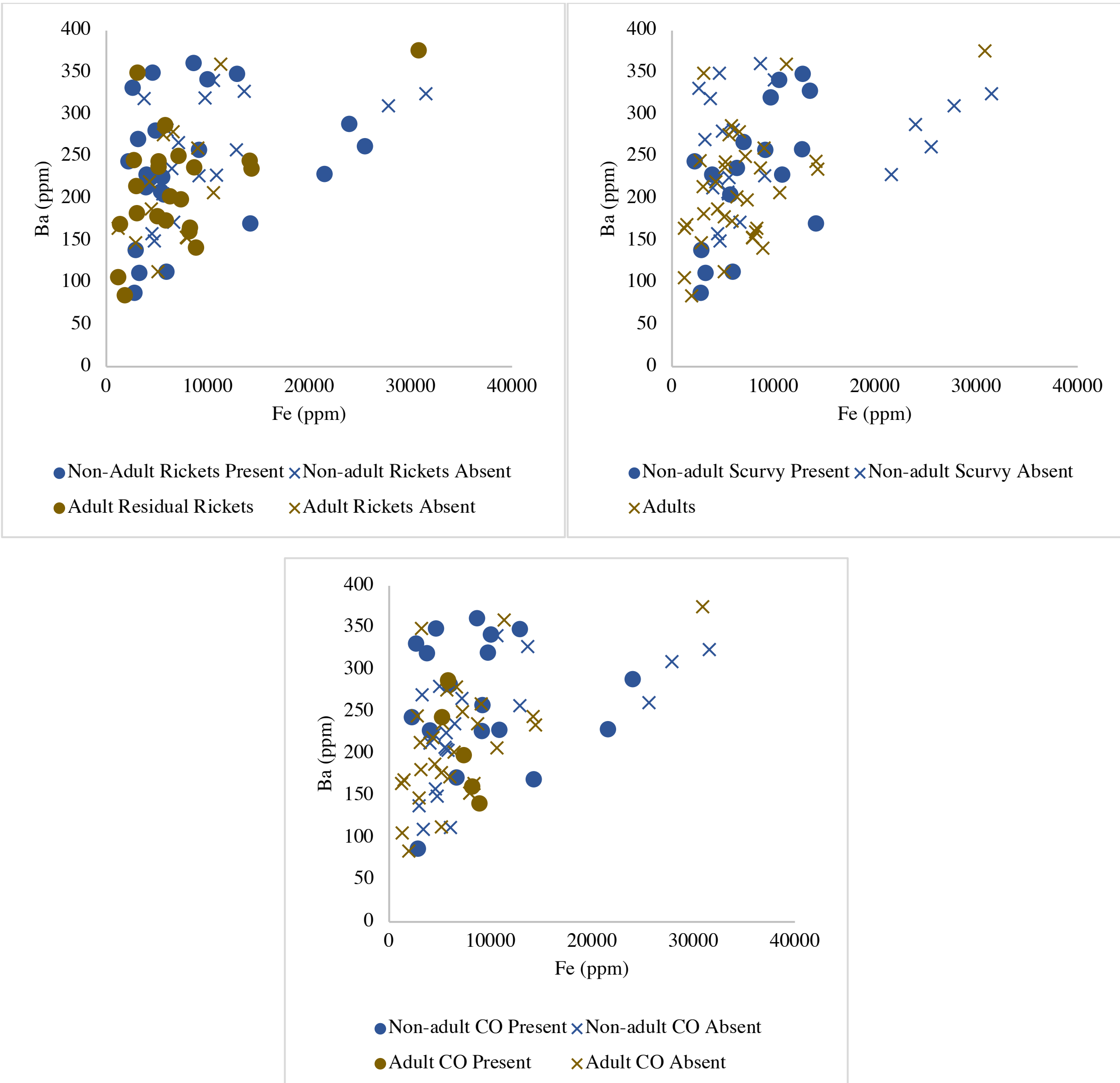

Figure 1*. Fe/Ba scatterplots for the rickets, scurvy, and CO disease categories demonstrating the similarity in the range and pattern of active conditions between the three groups.*

3.2. Periosteal new bone formation

Areas of periosteal new bone formation were observed on seven of the sampled femora. Femora with new bone formation had higher levels of silicon, potassium, titanium, and aluminum and lower levels of phosphorus and calcium, when compared with "normal bone"— bones without periosteal new bone formation. There was a strong linear correlation (Si/K: $r = 0.95$, $p < 0.05$) between these elements (*Figure 2*). Silicon and potassium formed ratios of new bone formation that significantly deviated from the remainder of the sample, except when paired with aluminum (Al/Si and Al/K), iron (Fe/Si and Fe/K), and each other (K/Si and Si/K) (see *supplementary material* for full statistical results). The aluminum ratios for periosteal new bone formation were also altered in most cases, with the exceptions being Ba/Al, Fe/Al, Pb/Al and Si/Al. Titanium ratios were significantly different between the two groups, excluding Ba/Ti, Pb/Ti, Fe/Ti and K/Ti. The proportions of phosphorus in the sampled bones showed significant variation between "normal" bone and bone with woven or transitional layers, relative to all elements of interest other than chlorine, manganese, lead, sulphur, and zinc. Calcium showed the same general pattern as phosphorus, with Cl/Ca, Mn/Ca, Pb/Ca, S/Ca, and Zn/Ca being the only tested ratios involving calcium to have no significant differences between the two groups.

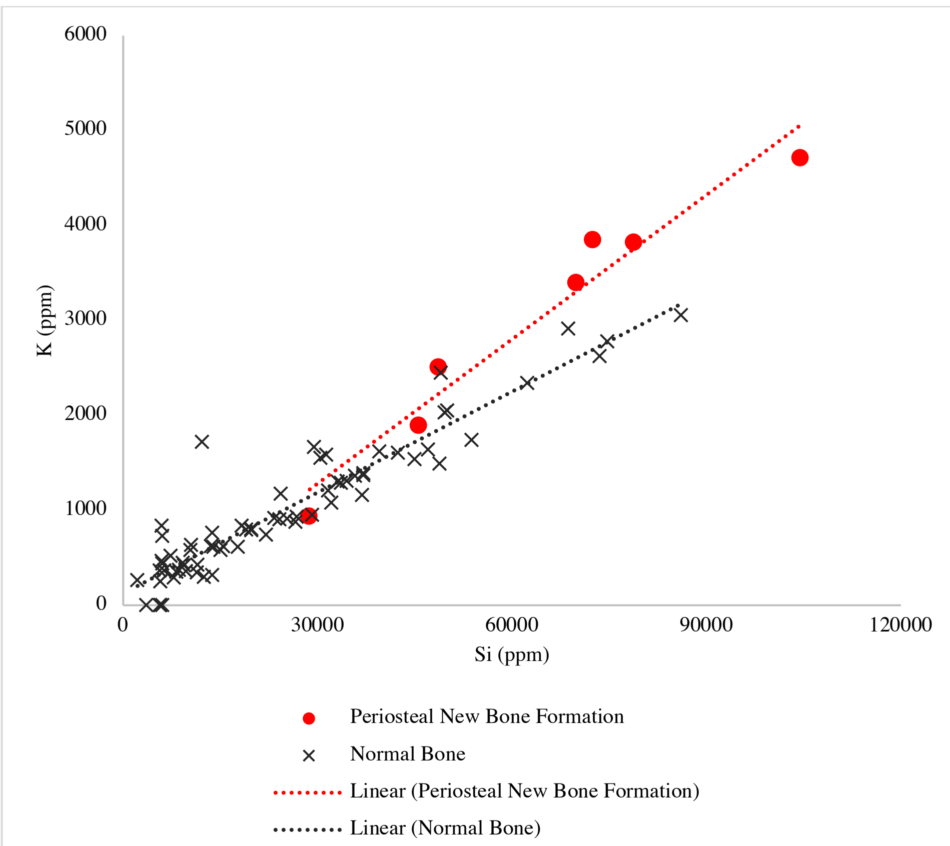

Figure 2. *Si/K scatterplot illustrating the linear relationship (r = 0.95, p < 0.05) between the two elements and the distinction between bones with periosteal new bone formation and those without.*

3.3. Individual instances of pathological conditions

3.3.1. COL008

COL008 was identified as having lesions consistent with syphilis. Analysis revealed that manganese and barium values for this individual were at the higher end of the sample range, whilst sulphur and chlorine values were lower, which altered the ratios involving those elements. The Mn/S, Mn/Cl, and Ba/Cl values for COL008 were significantly different from the sample mean (single case modified t-test—Mn/S: $t(79) = 2.35$, $p < 0.05$; Mn/Cl: $t(79) = 2.69$, $p < 0.01$; Ba/Cl: $t(79) = 3.46$, $p < 0.01$; *Figure 3*).

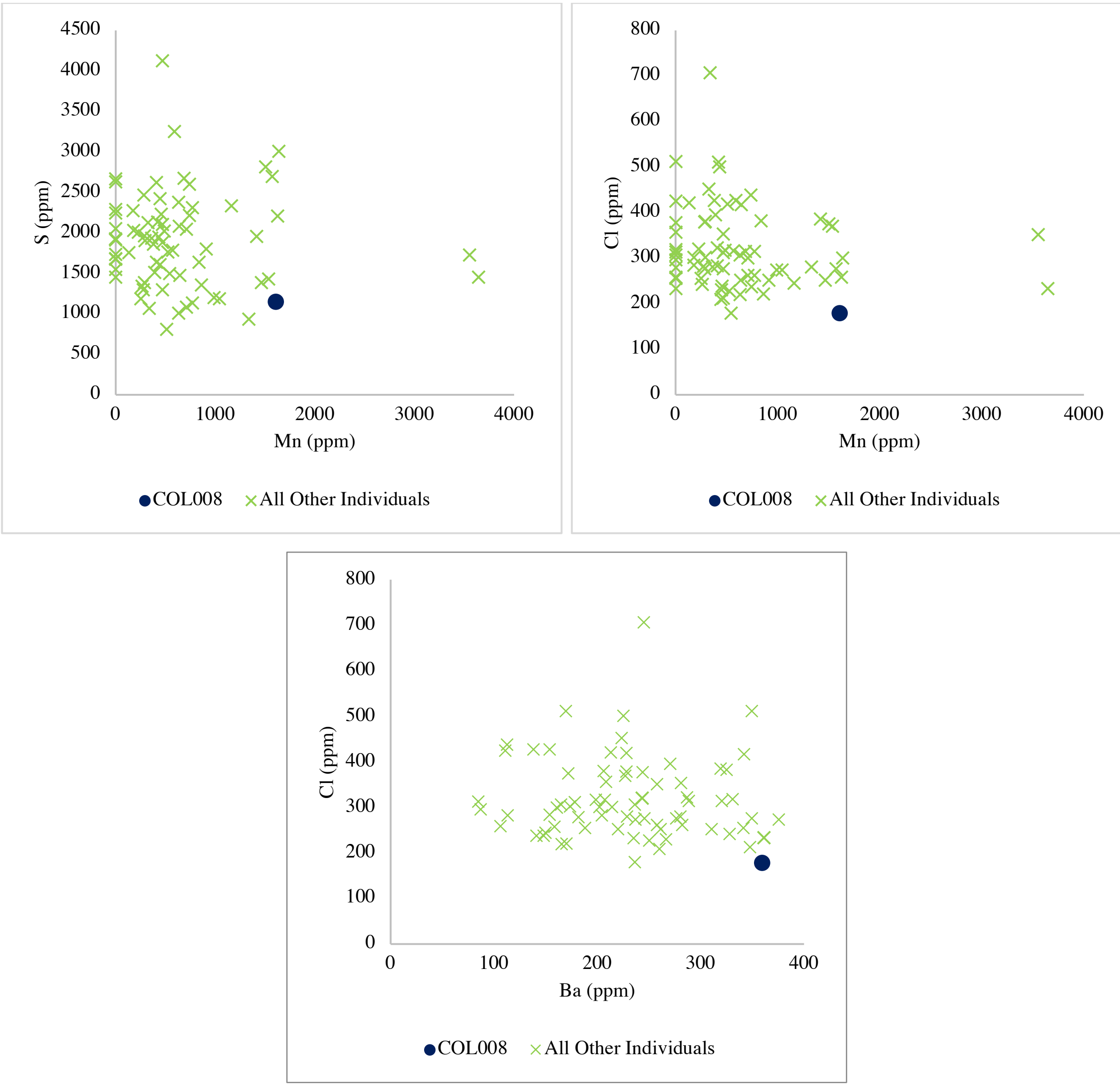

Figure 3. *Mn/S, Mn/Cl, and Ba/Cl scatterplots showing the significant difference in ratios between COL008 and the other samples.*

3.3.2. COL158

COL158 was diagnosed with diffuse idiopathic skeletal hyperostosis (DISH). The pXRF results showed higher levels of silicon, aluminum, and potassium relative to the rest of the sample population and lower comparative phosphorus levels (*Figure 4*). This resulted in a number of ratios that were significantly distinct from the sample mean, including the Al/S, K/P, Al/Fe and K/Ca ratios (see *supplementary material*).

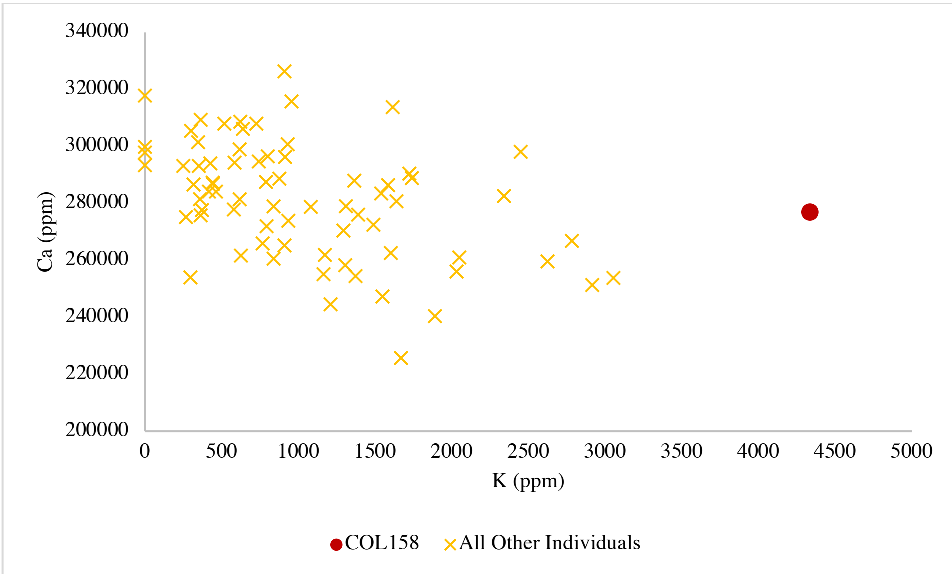

***Figure 4**. K/Ca scatterplot showing COL158's separation from the rest of the sample.*

3.3.3. COL069

COL069 was diagnosed with a number of potential conditions. This individual likely suffered from phosphorus poisoning and had skeletal lesions consistent with rickets, as well as either smallpox or tuberculosis infections. COL069 had lower levels of phosphorus, zinc, and calcium and higher levels of barium, relative to the other individuals in the sample population, however, none of the values were at the extremes of the range and the only ratio that deviated significantly from the mean was Ba/Ca (single case modified *t*-test: $t(79) = 2.08$, $p < 0.05$).

3.3.4. COL058

COL058 had a small osteoma on the right femur, which was tested in addition to the typical analysis location. The osteoma had higher manganese, zinc, barium, sulphur, and lead values than most of the sample, but not the most extreme values (*Figure 5*). The Ba/Sr, S/Sr, Mn/Fe, and Zn/Cl ratios were significantly altered as a result of these changes (see *supplementary material*).

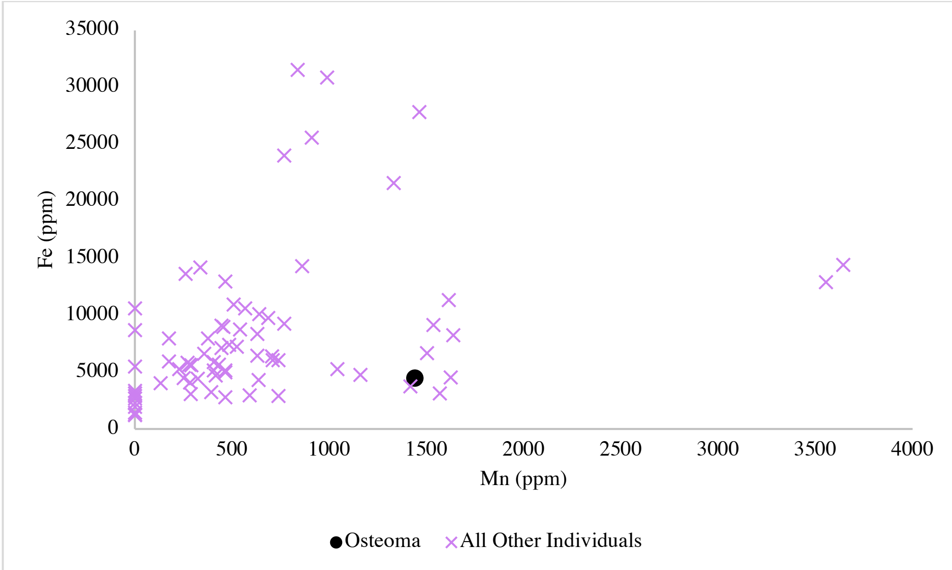

***Figure 5**. Mn/Fe scatterplot showing the osteoma compared with the rest of the sample.*

4. Discussion

4.1. Identification of Primary Conditions Through Elemental Ratios

The lack of distinguishable patterns relating to the primary disease categories analyzed suggests that pXRF cannot be utilized in the identification of rickets, scurvy, or cribra orbitalia in archaeological bone. There are a number of factors, working either individually or in combination, that could be impeding the detection of variation related to pathological conditions.

4.1.1. Impact of Diagenesis

Prior studies have identified diagenesis as a major limiting factor in the interpretation of chemical analyses of human remains (Sandford, 1993). Efforts have been made to identify unusual concentrations of specific elements, for example manganese or iron, or altered elemental ratios that could be used to evaluate diagenetic changes, frequently with contradictory results (Klepinger et al., 1986; Lambert et al., 1982; Zapata et al., 2006). The Ca/P ratio, however, is consistently utilized as an indicator of diagenesis (Burton, 2008; Kuzel et al., 2016; López-Costas et al., 2016). Fresh bone estimates for the ratio vary from 2.13 (Burton, 2008) to 2.18 (Skinner, 2005). In an archaeological context, the replacement of calcium and phosphate ions with alternative elements generally results in an increased Ca/P ratio, though the ratio is occasionally lowered instead

(Burton, 2008; López-Costas et al., 2016; Zapata et al., 2006). Zapata and colleagues (2006) noted considerable diagenetic alterations to the chemical profile of bones from two cemetery sites in Spain, with observed ratios between 2.30 and 2.50. The mean Ca/P value for the individuals in this study was 2.92 (± 0.20), which suggests diagenesis had a large impact on the elemental profile of the skeletal remains at the site.

The significant impact of diagenesis on the data is also indicated by high concentrations of elements generally associated with diagenetic change. Although some elements, like strontium and potassium, have been subject to much debate about their susceptibility to diagenesis—disagreements that are likely due in part to geographic variation in the availability of individual elements—other elements, including aluminum, silicon, and titanium, are almost universally considered to indicate diagenesis when present in elevated concentrations in human bone (Lambert et al., 1983; López-Costas et al., 2016; Zapata et al., 2006). While aluminum is normally present in bone and can have an inhibitory effect on bone metabolism (Ezzo, 1994), the average aluminum value of the Coach Lane sample (n=80, $\bar{x}$=16,857 ppm) is considerably higher than the typical *in vivo* bone value (<100 ppm), implying the influence of post-mortem contamination rather than metabolic factors. Titanium, which has no known function in human bone and is typically not present in any measurable amount, was also present in the Coach Lane sample (n=80, $\bar{x}$=248 ppm).

The elevated concentrations of these elements could potentially be a result of the industrial location of the burial ground. From the early 1800s through to the 1980s, the North Shields area was used extensively for coal mining and heavy industry (North Tyneside Council, 2014). In the 19th century, multiple ironworks foundries and a ropery were located within several blocks of the site (English Heritage et al., 2004). Modern land management strategies have acknowledged the extreme likelihood of soil contamination along the Tyne (North Tyneside Council, 2014). It therefore seems very likely that environmental pollution has influenced the bone elemental ratios of the Coach Lane individuals through diagenesis.

It has been suggested that non-adult bones are more vulnerable to diagenetic changes than adult bones because of their relatively thin cortical bone and lower bone mineral density (Edward and Benfer, 1993; Zapata et al., 2006). Given the high proportion of non-adult bones in the current sample, this was considered as a potential factor impacting the element ratios. There was no statistically significant difference, however, between the Ca/P ratios of the adult and non-adult individuals (Mann-Whitney U: U = 615, p = 0.60) in the Coach Lane sample. Assuming the Ca/P ratio to be a reliable indicator of the degree of diagenesis, the absence of a distinction in the ratio between the two age groups suggests that, in this instance, the diagenetic impact was similar for both non-adult and adult individuals.

4.1.2. Impact of Normal Variation and the Bodily Response to Stress

Differences in the trace element profiles between studied conditions could be concealed by the presence of comorbidities among individuals. There were a limited number of individuals identified with a single condition (rickets: n = 7; scurvy: n = 5; CO: n = 4). However, almost half of the non-adult individuals analyzed (46%, n = 39) had evidence for more than one of the specified pathological conditions, and, of those, 15% were identified with all three conditions. If the same elements are affected, it is possible that the co-occurrence of multiple metabolic diseases in the same individual could result in the amplification of elemental changes. However, if the disease processes affect different elements or ratios, or have opposing effects on the same elements, there is the potential for deviations to be obscured or cancelled out.

Alternatively, there could be little variation in the chemical responses of scurvy, rickets, and CO, despite their differing aetiologies. Bone has a limited range of responses to stress and disease—in general bone can either be formed or destroyed, with macroscopic distinctions occurring predominantly in the pattern of the changes (Brickley and Ives, 2008; Buikstra, 2019). It is possible that this is also true of the chemical reactions that occur in bone. However, this seems unlikely to be the case when comparing rickets with scurvy or CO, as the osteoclastic and osteoblastic activity levels associated with the bone changes of each condition are very different (Brickley and Ives, 2008). Trace element levels would, therefore, be expected to have corresponding differences linked to variations in their function. Regardless, the narrow scope of macroscopic osteological reactions to disease could also be reflective of a comparably limited elemental response.

It is also probable that the sample contains individuals who experienced pathological conditions in life that did not result in observable macroscopic changes to bone, which could further contribute to indistinctions in the data. In some instances, death may occur before there is a skeletal response (Wood et al., 1992). Furthermore, many diseases, such as tuberculosis, primarily affect soft tissues and have limited or no involvement of the skeleton—some studies have indicated that tuberculosis generally affects the skeleton in only 3-5% of cases (Davies et al., 1984; Roberts and Buikstra, 2003). Although macroscopic indications of particular conditions may not be present, there is still the possibility that the elemental composition of the bones was altered. For example, there could be individuals in the sample who experienced vitamin C or D deficiencies in life and had corresponding changes to the elemental composition of their bones, but lacked identifiable macroscopic skeletal changes.

Normal variation, in the form of age and sex differences, may also prevent the observation of pathological distinctions in the pXRF data. Sex- and age-related hormone fluctuations are known to influence bone turnover rates (eg. Walsh et al., 2010), which presumably have a resulting impact on the elemental composition of bone. Variation has been noted in phosphorus, sulphur, calcium, and zinc values between the biological sexes (Nganvongpanit et al., 2016b). Clinical observations of strontium and zinc levels in growing rats observed age-related increases in the element levels corresponding to periods of intensive growth (Maciejewska et al., 2014). Age-related variations in strontium and zinc levels have also been reported in prehistoric North American populations (Lambert et al., 1979). There is a slight difference in the Sr/Zn ratio between adults and non-adults in the Coach Lane population, primarily related to a lower mean and decreased variation among the adult individuals, but it is not statistically significant (two-sample t-test: $t = 1.88$, $p = 0.06$) (*Figure 6*). This demonstrates that age-related differences could be occuring in the population, but the extent to which this variation is obscuring other relationships is unknown.

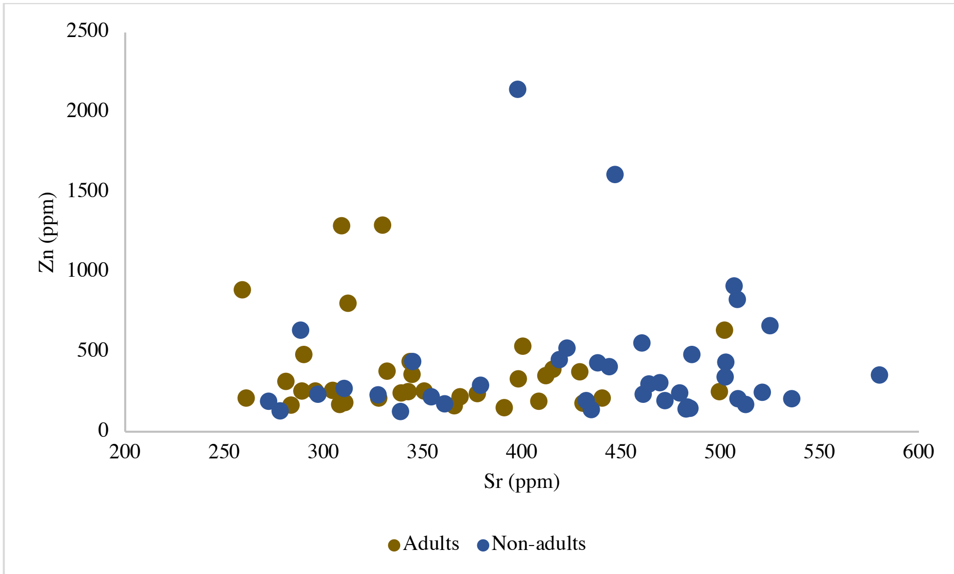

***Figure 6**. Sr/Zn scatterplot comparing adult and non-adult individuals from Coach Lane.*

4.1.3. Impact of Dietary and Environmental Influences

Distinctions in bone elemental content resulting from pathological conditions could be masked by dietary and environmental element signals. Barium and strontium values, often expressed relative to calcium in archaeological bone, have been used to make dietary inferences about past people—particularly in regard to trophic level effects and the determination of marine versus terrestrial subsistence (Burton et al., 2003; Burton and Wright, 1995; Price et al., 1985). The *in vivo* uptake of environmental barium and strontium through the consumption of food and drink results in the replacement of some calcium in the bone apatite by those elements, which causes alterations to the Sr/Ca, Ba/Ca and, Ba/Sr ratios (Burton and Wright, 1995; Perrone et al., 2014). As concentrations of these elements are highly correlated with geographic regions, changes to these element ratios have also been used to make interpretations about mobility (Burton et al., 2003). The impact of the environment on Sr/Ca, Ba/Ca, and Ba/Sr ratios continues after death, with replacement continuing in post-mortem contexts (Ezzo, 1994). As previously noted, strontium also displays a degree of variation related to age, making the interpretation of strontium levels in archaeological bone very difficult. The example of strontium highlights the complex series of interactions that occur in bone mineral in life and after death, which could be concealing differences related to pathology.

4.2. Periosteal New Bone Formation

Fifty-four ratios with significant differences between areas of pathological new bone formation and "normal" bone were observed, all involving silicon, titanium, aluminum, potassium, calcium, and phosphorus. The linear relationship (*Figure 2)* of these elements, which are strongly associated with diagenetic change, suggests a replacement of *in vivo* phosphorus with geologically abundant elements (Ezzo, 1994; Zapata et al., 2006). The Ca/P ratio of new bone formation is significantly higher than the already high ratio of the remainder of the Coach Lane individuals (two-sample t-test: $t = 2.96$, $p < 0.01$). As previously mentioned, titanium and aluminum occur in very low quantities in fresh bone and any considerable presence in archaeological bone should be questioned (Darrah, 2009; Ezzo, 1994). Although silicon and potassium occur in fresh bone in larger quantities than aluminum and titanium, they are still highly susceptible to diagenetic transfer due their substantial presence in soils and high geological mobility (Ezzo, 1994). The elements involved in the altered ratios suggests that they are the result of post-mortem changes, not *in vivo* biological processes. Additionally, the increased porosity of new bone growth makes diagenetic alterations to those areas more probable than to compact cortical bone (López-Costas et al., 2016). Since it can be concluded that abnormal concentrations of these elements in the Coach Lane sample are likely the result of diagenesis, any significant distinctions in ratios involving aluminum, silicon, titanium, potassium, calcium or phosphorus should be treated with caution.

4.3. Other Conditions

The ratios that significantly deviated from the rest of the group for COL158 and COL069 all involved elements determined to be altered through diagenesis. It is probable, therefore, that the variation in these ratios is also the result of diagenetic changes. Since the femur of COL069 was extensively covered with diffuse new bone formation, this is not surprising. Interpretations of the elemental ratios of COL069 were also likely to be hindered by problems of co-morbidity, as this non-adult individual was diagnosed with multiple conditions. As a result, no information about alterations in the elemental composition of bones with DISH, phossy jaw, tuberculosis or smallpox could be determined. Analysis of specific skeletal elements with macroscopic pathological changes could potentially have offered more clarity. However, the destructive nature of the lesions associated with these pathologies increases the likelihood of diagenetic alterations to bone elemental composition.

The osteoma on the right femur of COL058 had altered Ba/Sr, S/Sr, Mn/Fe, and Zn/Cl ratios. Given the high degree of diagenetic alteration to other elements, post-mortem modification of these ratios cannot be discounted. High levels of barium relative to the rest of the sample could be indicative of diagenesis, as it is incorporated from the environment and has no known metabolic function (Burton et al., 2003). Understanding the potential implications of increased strontium values is complicated, as it is influenced by a multitude of factors. While dietary differences could be one possible cause, the more likely explanations are either post-depositional transfer from the soil or elevated levels connected to bone formation processes or a combination of multiple factors. Elevated levels of iron, manganese, and zinc could likewise be the result of either diagenetic or bone formation processes, given their typical levels in soil and known involvement in bone growth (Bentley et al., 1976; Ezzo, 1994; Maciejewska et al., 2014).

COL008 was identified as having potential indicators of syphilis and had altered Mn/S, Mn/Cl, and Ba/Cl levels, caused by increased levels of manganese and barium and lower concentrations of sulphur and chlorine. The decreased chlorine and sulphur levels of COL008 could be the result of elemental leaching into the soil (Hancock et al., 1989). There is, however, a relationship between sulphur and syphilis. Sulphur baths and sulphur ointment were used historically for the treatment of skin conditions, including syphilis (Lane, 1875; Osterberg et al., 1929). Although the effects of this treatment on bone chemistry are unknown, an early study suggested that absorption of sulphur did occur, as indicated by an increase in blood and urine sulphur levels (Osterberg et al., 1929). As with the osteoma, the elevated manganese levels could reflect either diagenetic or bone forming processes, while barium levels are likely to be the result of diagenesis. The presence of extensive new bone formation on the tested bone implies that changes to the elemental ratios of COL008 could be influenced by diagenesis. The differences in which specific elements are most affected by diagenetic changes between individuals could be a result of discrepancies in soil composition around the burial area, variation in element composition of an individual's bones in life, or a combination of both factors.

5. Conclusions

While pXRF analysis allowed for the exploration of variation in the trace element concentrations of skeletal remains from the Coach Lane burial site, the deviations could not be linked to the primary pathological conditions under study. The inability to distinguish between disease categories was likely caused by a combination of factors, most notably post-depositional diagenetic changes. Even where a pattern could be observed, as in the case of periosteal new bone formation, the alterations were probably a product of diagenetic transformations after death rather than biological processes occurring in life. There are a large number of interconnected factors that contribute to elemental profiles of bone, including age- and sex-related variation, environmental and dietary influences, and post-mortem fluctuations. The multitude of possible influences limit the ability to make definitive conclusions about the sources of elemental variation.

Although this study did not provide promising results regarding the use of pXRF technology in the study of pathological conditions like scurvy, rickets and cribra orbitalia, there are some avenues for future research, including the further examination of conditions like syphilis, DISH or neoplastic disease. Potential differences were noted in this investigation but, as there was only one individual with each condition and the effects of diagenesis appeared pronounced, no definitive links could be made. Specific attention to iron, manganese, zinc, and, in the case of syphilis, sulphur, could be beneficial as these elements had possible alterations to their values. Future research could also expand the sample to include individuals under one year of age; the use of a pure metal plate behind small bones could allow for the testing of an increased demographic (Byrnes and Bush, 2016). The replication of the study at another site less affected by diagenetic processes could possibly find patterns between disease conditions that were obscured by the environmental conditions at Coach Lane.

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References

Aimers, J., Farthing, D., Shugar, A.N., 2012. Handheld XRF analysis of Maya ceramics: a pilot study presenting issues related to quantification and calibration, in: Shugar, A.N., Mass, J.L. (Eds.), Handheld XRF for Art and Archaeology, Studies in Archaeological Sciences. Leuven University Press, Leuven, pp. 423–448.

Aufderheide, A.C., Wittmers, L.E., Rapp, G., Wallgren, J., 1988. Anthropological applications of skeletal lead analysis. Am. Anthropol. 90, 931–936. https://doi.org/10.1525/aa.1988.90.4.02a00110

Balaji, D., 2011. Osteomyelitis Variolosa: A Case Report. J. Orthop. Surg. Hong Kong 19, 120–122. https://doi.org/10.1177/230949901101900128

Beaumont, J., Montgomery, J., Buckberry, J., Jay, M., 2015. Infant mortality and isotopic complexity: New approaches to stress, maternal health, and weaning. Am. J. Phys. Anthropol. 157, 441–457. https://doi.org/10.1002/ajpa.22736

Bentley, G., Dodson, E., Dodson, G., Hodgkin, D., Mercola, D., 1976. Structure of insulin in 4-zinc insulin. Nature 261, 166–168. https://doi.org/10.1038/261166a0

Brickley, M., Ives, R., 2008. The Bioarchaeology of Metabolic Disease. Academic Press, Oxford.

Brickley, M.B., 2018. Cribra orbitalia and porotic hyperostosis: A biological approach to diagnosis. Am. J. Phys. Anthropol. 167, 896–902. https://doi.org/10.1002/ajpa.23701

Buikstra, J.E., 2019. Ortner’s Identification of Pathological Conditions in Human Skeletal Remains, 3rd ed. Academic Press, London.

Buikstra, J.E., Cook, D.C., Bolhofner, K.L., 2017. Introduction: scientific rigor in paleopathology. Int. J. Paleopathol. 19, 80–87. https://doi.org/10.1016/j.ijpp.2017.08.005

Burton, J., 2008. Bone chemistry and trace element analysis, in: Katzenberg, M.A., Saunders, S.R. (Eds.), Biological Anthropology of the Human Skeleton. John Wiley & Sons, New Jersey, pp. 443–460.

Burton, J.H., Price, T.D., Cahue, L., Wright, L.E., 2003. The use of barium and strontium abundances in human skeletal tissues to determine their geographic origins. Int. J. Osteoarchaeol. 13, 88–95. https://doi.org/10.1002/oa.661

Burton, J.H., Wright, L.E., 1995. Nonlinearity in the relationship between bone Sr/Ca and diet: Paleodietary implications. Am. J. Phys. Anthropol. 96, 273–282. https://doi.org/10.1002/ajpa.1330960305

Buzon, M., 2012. The bioarchaeological approach to paleopathology, in: Grauer, A.L. (Ed.), A Companion to Paleopathology. John Wiley & Sons, Oxford, pp. 58–75.

Byrnes, J.F., Bush, P.J., 2016. Practical Considerations in Trace Element Analysis of Bone by Portable X-ray Fluorescence. J. Forensic Sci. 61, 1041–1045. https://doi.org/10.1111/1556-4029.13103

Cannell, R.J.S., Gustavsen, L., Kristiansen, M., Nau, E., 2018. Delineating an Unmarked Graveyard by High-Resolution GPR and pXRF Prospection: The Medieval Church Site of Furulund in Norway. J. Comput. Appl. Archaeol. 1, 1–18. https://doi.org/10.5334/jcaa.9

Carvalho, M.L., Marques, A.F., Lima, M.T., Reus, U., 2004. Trace elements distribution and post-mortem intake in human bones from Middle Age by total reflection X-ray fluorescence. Spectrochim. Acta Part B At. Spectrosc., 10th Symposium on Total Reflection X-Ray

Fluorescence Analysis and 39th Discussion Meeting on Chemical Analysis 59, 1251–1257. https://doi.org/10.1016/j.sab.2004.01.019

Cole, G., Waldron, T., 2019. Cribra orbitalia: Dissecting an ill-defined phenomenon. Int. J. Osteoarchaeol. 29, 613–621. https://doi.org/10.1002/oa.2757

Craig, N., Speakman, R.J., Popelka-Filcoff, R.S., Glascock, M.D., Robertson, J.D., Shackley, M.S., Aldenderfer, M.S., 2007. Comparison of XRF and PXRF for analysis of archaeological obsidian from southern Perú. J. Archaeol. Sci. 34, 2012–2024. https://doi.org/10.1016/j.jas.2007.01.015

Croker, S.L., Reed, W., Donlon, D., 2016. Comparative cortical bone thickness between the long bones of humans and five common non-human mammal taxa. Forensic Sci. Int. 260, 104.e1-104.e17. https://doi.org/10.1016/j.forsciint.2015.12.022

Darrah, T., 2009. Inorganic trace element composition of modern human bones: Relation to bone pathology and geographical provenance (PhD). University of Rochester, New York.

Davies, P., Humphries, M., Byfield, S., Nunn, A., Darbyshire, J., Citron, K., Fox, W., 1984. Bone and joint tuberculosis. A survey of notifications in England and Wales. J. Bone Joint Surg. Br. 66-B, 326–330. https://doi.org/10.1302/0301-620X.66B3.6427232

Doğan, M., Cesur, Y., Zehra, D.Ş., Kaba, S., Bulan, K., Cemek, M., 2012. Oxidant/antioxidant system markers and trace element levels in children with nutritional rickets. J. Pediatr. Endocrinol. Metab. 25, 1129–1139. https://doi.org/10.1515/jpem-2012-0153

Edward, J.B., Benfer, R.A., 1993. The effects of diagenesis on the Paloma skeletal material, in: Sandford, M.K. (Ed.), Investigations of Ancient Human Tissue: Chemical Analyses in Anthropology, Food and Nutrition in History and Anthropology. Gordon and Breach Science Publishers, Amsterdam, pp. 183–268.

Eleazer, C.D., Jankauskas, R., 2016. Mechanical and metabolic interactions in cortical bone development. Am. J. Phys. Anthropol. 160, 317–333. https://doi.org/10.1002/ajpa.22967

Endt, D.W.V., Ortner, D.J., 1982. Amino acid analysis of bone from a possible case of prehistoric iron deficiency anemia from the American Southwest. Am. J. Phys. Anthropol. 59, 377–385. https://doi.org/10.1002/ajpa.1330590407

English Heritage, North Tyneside Council, City of Newcastle, Northumberland County Council, 2004. North Shields: An archaeological assessment and strategy.

Ezzo, J.A., 1994. Putting the “Chemistry” Back into Archaeological Bone Chemistry Analysis: Modeling Potential Paleodietary Indicators. J. Anthropol. Archaeol. 13, 1–34. https://doi.org/10.1006/jaar.1994.1002

Finlayson, J.E., Bartelink, E.J., Perrone, A., Dalton, K., 2017. Multimethod Resolution of a Small-Scale Case of Commingling. J. Forensic Sci. 62, 493–497. https://doi.org/10.1111/1556-4029.13265

Fornaciari, G., Mallegni, F., Bertini, D., Nuti, V., 1981. Cribra orbitalia, and elemental bone iron, in the Punics of Carthage. Ossa 8, 63–77.

Gleń-Haduch, E., Szostek, K., Głąb, H., 1997. Cribra orbitalia and trace element content in human teeth from neolithic and early bronze age graves in southern Poland. Am. J. Phys. Anthropol. 103, 201–207. https://doi.org/10.1002/(SICI)1096-8644(199706)103:2<201::AID-AJPA5>3.0.CO;2-W

Gonzalez-Rodriguez, J., Fowler, G., 2013. A study on the discrimination of human skeletons using X-ray fluorescence and chemometric tools in chemical anthropology. Forensic Sci. Int. 231, 407.e1-407.e6. https://doi.org/10.1016/j.forsciint.2013.04.035

Gowland, R.L., unpublished. Coach Lane Skeletal Records.

Hammer, Ø., Harper, D., Ryan, P., 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeotologica Electronica.

Hancock, R.G.V., Grynpas, M.D., Pritzker, K.P.H., 1989. The abuse of bone analyses for archaeological dietary studies. Archaeometry 31, 169–179. https://doi.org/10.1111/j.1475-4754.1989.tb01012.x

Handler, J.S., Aufderheide, A.C., Corruccini, R.S., Brandon, E.M., Wittmers, L.E., 1986. Lead Contact and Poisoning in Barbados Slaves: Historical, Chemical, and Biological Evidence. Soc. Sci. Hist. 10, 399–425. https://doi.org/10.1017/S014555320001556X

Johnson, P.R., 2011. Elemental Analysis of Fine-Grained Basalt Sources from the Samoan Island of Tutuila: Applications of Energy Dispersive X-Ray Fluorescence (EDXRF) and Instrumental Neutron Activation Analysis (INAA) Toward an Intra-Island Provenance Study, in: Shackley, M.S. (Ed.), X-Ray Fluorescence Spectrometry (XRF) in Geoarchaeology. Springer New York, New York, NY, pp. 143–160. https://doi.org/10.1007/978-1-4419-6886-9_7

Kendall, C., Eriksen, A.M.H., Kontopoulos, I., Collins, M.J., Turner-Walker, G., 2018. Diagenesis of archaeological bone and tooth. Palaeogeogr. Palaeoclimatol. Palaeoecol. 491, 21–37. https://doi.org/10.1016/j.palaeo.2017.11.041

Kepa, M., Kozłowski, T., Szostek, K., Drozd, A., Walas, S., Mrowiec, H., Stepańczak, B., Głab, H., Grupa, M., 2012. Analysis of mercury levels in historical bone material from syphilitic subjects--pilot studies (short report). Anthropol. Anz. Ber. Uber Biol.-Anthropol. Lit. 69, 367–377. https://doi.org/10.1127/0003-5548/2012/0163

Kerns, J.G., Buckley, K., Parker, A.W., Birch, H.L., Matousek, P., Hildred, A., Goodship, A.E., 2015. The use of laser spectroscopy to investigate bone disease in King Henry VIII's sailors. J. Archaeol. Sci. 53, 516–520. https://doi.org/10.1016/j.jas.2014.11.013

Klepinger, L.L., Kuhn, J.K., Williams, W.S., 1986. An elemental analysis of archaeological bone from Sicily as a test of predictability of diagenetic change. Am. J. Phys. Anthropol. 70, 325–331. https://doi.org/10.1002/ajpa.1330700307

Kuzel, A.R., Christensen, A.M., Marvin, S.M., 2016. Calcium and Phosphorus Detection Using Benchtop Versus Handheld X-ray Fluorescence Spectrometers. J. Forensic Sci. 61, S190–S192. https://doi.org/10.1111/1556-4029.12951

Lambert, J.B., Simpson, S.V., Buikstra, J.E., Hanson, D., 1983. Electron microprobe analysis of elemental distribution in excavated human femurs. Am. J. Phys. Anthropol. 62, 409–423. https://doi.org/10.1002/ajpa.1330620406

Lambert, J.B., Szpunar, C.B., Buikstra, J.E., 1979. Chemical Analysis of Excavated Human Bone from Middle and Late Woodland Sites. Archaeometry 21, 115–129. https://doi.org/10.1111/j.1475-4754.1979.tb00248.x

Lambert, J.B., Vlasak, S.M., Thometz, A.C., Buikstra, J.E., 1982. A comparative study of the chemical analysis of ribs and femurs in woodland populations. Am. J. Phys. Anthropol. 59, 289–294. https://doi.org/10.1002/ajpa.1330590308

Lane, J.R., 1875. The Treatment of Syphilis by Moist Mercurial Fumigation. Br. Med. J. 1, 137.

López-Costas, O., Lantes-Suárez, Ó., Martínez Cortizas, A., 2016. Chemical compositional changes in archaeological human bones due to diagenesis: Type of bone vs soil environment. J. Archaeol. Sci. 67, 43–51. https://doi.org/10.1016/j.jas.2016.02.001

Macdonald, H.M., Mavroeidi, A., Fraser, W.D., Darling, A.L., Black, A.J., Aucott, L., O'Neill, F., Hart, K., Berry, J.L., Lanham-New, S.A., Reid, D.M., 2011. Sunlight and dietary contributions to the seasonal vitamin D status of cohorts of healthy postmenopausal women living at northerly latitudes: a major cause for concern? Osteoporos. Int. 22, 2461–2472. https://doi.org/10.1007/s00198-010-1467-z

Maciejewska, K., Drzazga, Z., Kaszuba, M., 2014. Role of trace elements (Zn, Sr, Fe) in bone development: Energy dispersive X-ray fluorescence study of rat bone and tooth tissue. BioFactors 40, 425–435. https://doi.org/10.1002/biof.1163

Marquardt, M.L., Done, S.L., Sandrock, M., Berdon, W.E., Feldman, K.W., 2012. Copper deficiency presenting as metabolic bone disease in extremely low birth weight, short-gut infants. Pediatrics 130, e695–e698. https://doi.org/10.1542/peds.2011-1295

Martin, R.R., Naftel, S., Macfie, S., Jones, K., Nelson, A., 2013. Pb distribution in bones from the Franklin expedition: synchrotron X-ray fluorescence and laser ablation/mass spectroscopy. Appl. Phys. A 111, 23–29. https://doi.org/10.1007/s00339-013-7579-5

McGlinchey, C., 2012. Handheld XRF for the examination of paintings: proper use and limitations, in: Shugar, A.N., Mass, J.L. (Eds.), Handheld XRF for Art and Archaeology, Studies in Archaeological Sciences. Leuven University Press, Leuven, pp. 131–158.

Müller, R., Roberts, C.A., Brown, T.A., 2014. Biomolecular Identification of Ancient Mycobacterium tuberculosis Complex DNA in Human Remains From Britain and Continental Europe. Am. J. Phys. Anthropol. 153, 178–189. https://doi.org/10.1002/ajpa.22417

Nayak, P., 2002. Aluminum: Impacts and Disease. Environ. Res. 89, 101–115. https://doi.org/10.1006/enrs.2002.4352

Newman, S.L., Gowland, R.L., Caffell, A.C., 2019. North and south: A comprehensive analysis of non-adult growth and health in the industrial revolution (AD 18th–19th C), England. Am. J. Phys. Anthropol. 169, 104–121. https://doi.org/10.1002/ajpa.23817

Nganvongpanit, K., Buddhachat, K., Brown, J.L., 2016a. Comparison of Bone Tissue Elements Between Normal and Osteoarthritic Pelvic Bones in Dogs. Biol. Trace Elem. Res. 171, 344–353. https://doi.org/10.1007/s12011-015-0556-4

Nganvongpanit, K., Buddhachat, K., Brown, J.L., Klinhom, S., Pitakarnnop, T., Mahakkanukrauh, P., 2016b. Preliminary Study to Test the Feasibility of Sex Identification of Human (Homo sapiens) Bones Based on Differences in Elemental Profiles Determined by Handheld X-ray Fluorescence. Biol. Trace Elem. Res. 173, 21–29. https://doi.org/10.1007/s12011-016-0625-3

North Tyneside Council, 2014. Contaminated Land Strategy.

Osterberg, A.E., Rubenstein, M.W., Goeckerman, W.H., 1929. The absorption of sulphur compounds during treatment by sulphur baths. Arch. Dermatol. Syphilol. 20, 158–166. https://doi.org/10.1001/archderm.1929.01440020010002

Pate, F.D., Hutton, J.T., 1988. The use of soil chemistry data to address post-mortem diagenesis in bone mineral. J. Archaeol. Sci. 15, 729–739. https://doi.org/10.1016/0305-4403(88)90062-3

Pearce, S.H., Cheetham, T.D., 2010. Diagnosis and management of vitamin D deficiency. BMJ 340, b5664. https://doi.org/10.1136/bmj.b5664

Perrone, A., Finlayson, J.E., Bartelink, E.J., Dalton, K.D., 2014. Chapter 7 - Application of Portable X-ray Fluorescence (XRF) for Sorting Commingled Human Remains, in: Adams, B.J., Byrd, J.E. (Eds.), Commingled Human Remains. Academic Press, San Diego, pp. 145–165. https://doi.org/10.1016/B978-0-12-405889-7.00007-1

Pessanha, S., Carvalho, M., Carvalho, M.L., Dias, A., 2016. Quantitative analysis of human remains from 18th–19th centuries using X-ray fluorescence techniques: The mysterious high content of mercury in hair. J. Trace Elem. Med. Biol. 33, 26–30. https://doi.org/10.1016/j.jtemb.2015.08.004

Price, T.D., Schoeninger, M.J., Armelagos, G.J., 1985. Bone chemistry and past behavior: an overview. J. Hum. Evol. 14, 419–447. https://doi.org/10.1016/S0047-2484(85)80022-1

Proctor, J., Gaimster, M., Young Langthorne, J., 2016. A Quaker burial ground in North Shields: Excavations at Coach Lane, Tyne and Wear. Pre-Construct Archaeology Limited, London.

Raisz, L.G., 1999. Physiology and Pathophysiology of Bone Remodeling. Clin. Chem. 45, 1353–1358.

Rasmussen, K.L., Boldsen, J.L., Kristensen, H.K., Skytte, L., Hansen, K.L., Mølholm, L., Grootes, P.M., Nadeau, M.-J., Flöche Eriksen, K.M., 2008. Mercury levels in Danish Medieval human bones. J. Archaeol. Sci. 35, 2295–2306. https://doi.org/10.1016/j.jas.2008.03.003

Rebôcho, J., Carvalho, M.L., Marques, A.F., Ferreira, F.R., Chettle, D.R., 2006. Lead post-mortem intake in human bones of ancient populations by 109Cd-based X-ray fluorescence and EDXRF. Talanta, Colloquium Spectroscopicum Internationale XXXIV 70, 957–961. https://doi.org/10.1016/j.talanta.2006.05.062

Report of the Commissioners, 1845. Second Report of The Commissioners for inquiring into the state of large towns and populous districts, Vol. II. Clowes and Sons, London.

Reynolds, M.G., Damon, I.K., 2017. Smallpox, in: Quah, S.R. (Ed.), International Encyclopedia of Public Health (Second Edition). Academic Press, Oxford, pp. 524–533. https://doi.org/10.1016/B978-0-12-803678-5.00411-2

Roberts, C.A., Buikstra, J.E., 2014. The History of Tuberculosis from Earliest Times to the Development of Drugs, in: Davies, P.D.O., Gordon, S.B., Davies, G. (Eds.), Clinical Tuberculosis. CRC Press, Boca Raton, USA, pp. 3–18.

Roberts, C.A., Buikstra, J.E., 2003. The bioarchaeology of tuberculosis: a global perspective on a re-emerging disease. University Press of Florida, Gainesville.

Roberts, C.A., Caffell, A., Filipek-Ogden, K.L., Gowland, R., Jakob, T., 2016. 'Til Poison Phosphorous Brought them Death': A potentially occupationally-related disease in a post-medieval skeleton from north-east England. Int. J. Paleopathol. 13, 39–48. https://doi.org/10.1016/j.ijpp.2015.12.001

Roberts, C.A., Manchester, K., 2005. Archaeology of disease., Third. ed. Sutton Publishing, Stroud.

Roxburgh, M.A., Heeren, S., Huisman, D.J., Os, B.J.H.V., 2019. Non-Destructive Survey of Early Roman Copper-Alloy Brooches using Portable X-ray Fluorescence Spectrometry. Archaeometry 61, 55–69. https://doi.org/10.1111/arcm.12414

Sandford, M.K., 1993. The biogenic-diagenetic continuum, in: Sandford, M.K. (Ed.), Investigations of Ancient Human Tissue: Chemical Analyses in Anthropology, Food and Nutrition in History and Anthropology. Gordon and Breach Science Publishers, Amsterdam, pp. 3–57.

Schuenemann, V.J., Singh, P., Mendum, T.A., Krause-Kyora, B., Jäger, G., Bos, K.I., Herbig, A., Economou, C., Benjak, A., Busso, P., Nebel, A., Boldsen, J.L., Kjellström, A., Wu, H., Stewart, G.R., Taylor, G.M., Bauer, P., Lee, O.Y.-C., Wu, H.H.T., Minnikin, D.E., Besra, G.S., Tucker, K., Roffey, S., Sow, S.O., Cole, S.T., Nieselt, K., Krause, J., 2013. Genome-wide comparison of medieval and modern Mycobacterium leprae. Science 341, 179–183. https://doi.org/10.1126/science.1238286

Skinner, H., 2005. Mineralogy of bone, in: Selinus, O., Alloway, B., Centeno, J., Finkelman, R., Fuge, R., Lindh, U., Smedley, P. (Eds.), Essentials of Medical Geology: Impacts of the Natural Environment on Public Health. Elsevier Academic Press, Amsterdam, pp. 667–693.

Swanston, T., Varney, T.L., Kozachuk, M., Choudhury, S., Bewer, B., Coulthard, I., Keenleyside, A., Nelson, A., Martin, R.R., Stenton, D.R., Cooper, D.M.L., 2018. Franklin expedition lead exposure: New insights from high resolution confocal x-ray fluorescence imaging of skeletal microstructure. PLOS ONE 13, e0202983. https://doi.org/10.1371/journal.pone.0202983

Walker, P.L., Bathurst, R.R., Richman, R., Gjerdrum, T., Andrushko, V.A., 2009. The causes of porotic hyperostosis and cribra orbitalia: A reappraisal of the iron-deficiency-anemia hypothesis. Am. J. Phys. Anthropol. 139, 109–125. https://doi.org/10.1002/ajpa.21031

Walser, J.W., Kristjánsdóttir, S., Gowland, R., Desnica, N., 2019. Volcanoes, medicine, and monasticism: Investigating mercury exposure in medieval Iceland. Int. J. Osteoarchaeol. 29, 48–61. https://doi.org/10.1002/oa.2712

Walsh, J.S., Henry, Y.M., Fatayerji, D., Eastell, R., 2010. Hormonal determinants of bone turnover before and after attainment of peak bone mass. Clin. Endocrinol. (Oxf.) 72, 320–327. https://doi.org/10.1111/j.1365-2265.2009.03606.x

Williams, R., Taylor, G., Orr, C., 2020. pXRF method development for elemental analysis of archaeological soil. Archaeometry n/a. https://doi.org/10.1111/arcm.12583

Wood, J.W., Milner, G.R., Harpending, H.C., Weiss, K.M., Cohen, M.N., Eisenberg, L.E., Hutchinson, D.L., Jankauskas, R., Cesnys, G., Katzenberg, M.A., Lukacs, J.R., McGrath, J.W., Roth, E.A., Ubelaker, D.H., Wilkinson, R.G., 1992. The Osteological Paradox: Problems of Inferring Prehistoric Health from Skeletal Samples [and Comments and Reply]. Curr. Anthropol. 33, 343–370. https://doi.org/10.1086/204084

Zapata, J., Pérez-Sirvent, C., Martínez-Sánchez, M.J., Tovar, P., 2006. Diagenesis, not biogenesis: Two late Roman skeletal examples. Sci. Total Environ. 369, 357–368. https://doi.org/10.1016/j.scitotenv.2006.05.021