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A STUDY OF THE DIAGENETIC ALTERATION OF BONE FROM THE CROWN MINES IN JOHANNESBURG, SOUTH AFRICA

Stacey Lee Lander

The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not to be attributed to the NRF.

Dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the degree of
Master of Science in Medicine

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Declaration

I, Stacey Lander, hereby declare that this dissertation is my own work and it is being submitted to the University of the Witwatersrand for the degree of Master of Science in Medicine. It has not been submitted before for any degree or examination at this or any other University.

Signed:

Date:

Abstract

Human skeletal remains of archaeological origin were rescued and exhumed from the Crown Mines site. Due to their poor preservation, estimates of age, sex, ancestry and stature could not be accurately assessed using the morphology of the bones. In such circumstances, histology has been shown to be a helpful technique used to acquire additional information. Assessing the histology of the Crown Mines' bone samples and their histological alterations associated with its poor bone preservation, also known as diagenesis, may lead to more accurate interpretations of the above estimates. The aim of this study was therefore to describe the histological integrity of the bones and assess the chemical interactions between the bones and the soil during diagenesis. Fifty femora were selected from the exhumed remains taken from the Crown Mines and cross-sections were manually ground. Using light and polarized microscopy a variation of microcracks, infiltrations, inclusions and staining were qualitatively identified mainly in the periosteal and endosteal zones, with the mesosteal zone being well preserved. Interestingly, no biodegradation was present. Chemically, there were a number of elements that were altered due to diagenesis and there was a transfer of element/s from the soil to the bone and vice versa. Histologically, the preservation of the bones was good, having sufficient microstructure for future assessment to improve the accuracies of age-at-death estimation and for descriptions of pathology and trauma. Future research would benefit from investigating the mesosteal zone for the estimation of age-at-death as the periosteal zone was often not well preserved in the Crown Mines' bone samples.

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Introduction

In 2010 a number of exposed human skeletal remains were discovered at an old Crown Mines dump in Johannesburg, South Africa. These exposed skeletons were rescued but upon further investigation more burial pits were identified. A full scale investigation with the assistance from Archaetnos, an independent archaeological company, and physical anthropologists from the University of the Witwatersrand was launched. Archaeology is the study of past societies using material remains such as pottery, stone tools, art and architecture to understand how these individuals may have lived (Dupras *et al*., 2006), while physical anthropology utilizes methods and techniques developed from skeletal biology and osteology to gain information pertaining to a specific skeleton (Adebisi, 2009). The role of the archaeologist and the anthropologists at the Crown Mines site was to acquire as much information about these buried individuals by assessing the site and the burial pits containing the skeletal material. Upon investigation, the site was found to be associated with the early mining history of Johannesburg due to the archaeological artefacts that were found and was dated to between the late 1890's and 1920's (Pelser and van Vollenhoven, 2011). Approximately 100 skeletons were exhumed with an estimated 550 still interred. Biological profiles were completed for the skeletal remains exhumed and while every attempt was made to describe their age-at-death, sex, stature and ancestry, very little information was collected due to the poor morphological preservation of the bones.

The preservation of bone can be analysed in one of two ways, morphologically and histologically. In circumstances where the morphological preservation of bone prevents conventional quantitative and qualitative skeletal analysis, other methods such as histology have been used with success to obtain more information (Hanson and Buikstra, 1987). Describing the preservation of bone refers to diagenesis and is defined as a process where the structural properties of bone are modified by a combination of the physical, chemical and biological environments which lead to an alteration in its preservation and/or eventual destruction (Wilson and Pollard, 2002). The four main parameters used when assessing diagenesis are the destruction of histological integrity, the change in porosities (water uptake potential), crystallinity (the infra-red splitting factor) and the loss of collagen (Hedges *et al.*, 1995; Nielsen-Marsh and Hedges, 2000; Hedges, 2002). These parameters have been shown to display clear patterns of diagenetic change in buried bone which are often site-dependent because each site has a specific environment with a number of factors that affect diagenetic change (Nielsen-Marsh and Hedges, 2000). These factors include bone composition, water

availability, the biological age of the individual, microorganisms, soil pH, temperature, the method of burial and oxygen availability to name a few (Gordon and Buikstra, 1981; Hackett, 1981; Von Endt and Ortner, 1984; Nicholson, 1996; Ubelaker, 1997; Byers, 2002; Hedges, 2002; Jans *et al*., 2002; Hollund *et al*., 2011).

Bone diagenesis has been investigated in a number of countries which have their own unique climate, environment and temperature. A few examples of such studies include the United States of America (Byers, 2002; Jans, 2008), Canada (Trueman and Martill, 2002), Africa - Nigeria (Trueman and Martill, 2002; Jans, 2008), the Middle East - Israel (Jans, 2008), Asia - Indonesia (Hackett, 1981), the United Kingdom – England (Trueman and Martill, 2002), Europe - Sweden, Italy, France and the Netherlands (Hackett, 1981; Nielsen-Marsh and Hedges, 2000; Jans *et al*., 2002; Trueman and Martill, 2002; Jans, 2008; Hollund *et al*., 2011) and Australia (Hackett, 1981). In South Africa, diagenesis has been investigated mainly with regards to the crystallinity of bone (Price *et al*., 1992; Sillen and Sealy, 1995; Sillen and Parkington, 1996). Very little research has been done on the histological integrity of bone which includes the study of biodegradation (tunnelling caused by bacteria and fungi), microcracks, birefringence (polarization), infiltrations and inclusions.

Bone and its histology is a topic of interest for paleoanthropologists, archaeologists and physical anthropologists as a large amount of information can be interpreted from skeletal remains. While better preserved bones can reveal links to human evolution for paleoanthropologists, provide insights into human history and culture for archaeologists, and provide specific information about an individual such as age, sex and ancestry for physical anthropologists; poorly preserved bone is known to inhibit accurate interpretations of skeletal trauma, pathology, dietary reconstructions and dating of skeletons by biochemical analyses (Piepenbrink, 1986; Hanson and Buikstra, 1987; Bell, 1990; Nawrocki, 1995). Understanding diagenetic alteration and how it affects the histological preservation of bone could highlight the possible usage of histology in obtaining more accurate descriptions of an individual's biological profile. Therefore, the aim of this study was to assess the diagenetic alterations, particularly the histological integrity, of the skeletal remains obtained from the Crown Mines site to establish if the histology of poorly preserved bone can be used in the future to improve the accuracies of age-at-death and sex estimation or describing pathology and trauma. In addition to this, an assessment of the chemical nature of the bone was done to obtain a better understanding of the potential effects of the surrounding soil in the burial environment during diagenesis.

Objectives

1. To assess the histological integrity of the skeletal remains obtained from the Crown Mines site by identifying and describing biodegradation, microcracks, birefringence, infiltrations and inclusions for each section of bone selected.
2. To determine the chemical composition of the bone and soil at the Crown Mines site to investigate whether a transfer of elements between the bone and the surrounding soil or vice versa had occurred during diagenesis.
3. To assess whether the diagenetic alterations of the Crown Mines' bones can be used more accurately in the future to assist with the estimation of age-at-death and various descriptions of pathology and trauma.

Literature Review

1. The Crown Mines Site

A number of exposed soil-buried skeletal remains located at an old Crown Mines dump on Crownwood Road in Johannesburg was discovered in 2010 and reported to the South African Police Service (SAPS). Upon investigation, the site was confirmed as an archaeological site and reported to the South African Heritage Resources Agency (SAHRA). In October 2010, Archaetnos, an independent archaeological company, was approached by SAHRA to launch an investigation into the origins of these remains and to rescue the uncovered remains. The initial survey revealed the remnants of a historical refuse dump associated with the mine which had been extensively disturbed over time, scattered for over 5400m² by water erosion and the work that had been done during the reclaiming of the old mine dump (Pelser and van Vollenhoven, 2011). Additionally, more burial pits were identified than those that were initially identified and rescued. These burial pits appeared to be more systematically organised and in April 2011 SAHRA granted Archaetnos permission to exhume the other burial pits on the site as well as the excavation of the historical refuse dump (Pelser and van Vollenhoven, 2011). The aims of the investigation were to determine the age of the dump and the age of the cemetery by recovering as much cultural material as possible from the site, and to provide information on the individuals buried at the site which included their possible origin, relationship to the mine and social status. The skeletal remains were analysed by anthropologists from the University of the Witwatersrand to compile biological profiles which consisted of the estimation of age-at-death, sex, stature and ancestry to provide additional information on the individuals buried at the site.

1.1. Archaeological Investigation

The Crown Mines site is located on a portion of the farm Langlaagte 224 IQ which is situated 4km south of Johannesburg (GPS coordinates: 26°13.522'S 28°0.357'E) on the property of a recycling demolition and construction waste company called Stones and Stones on Crownwood Road (**Fig. 1**). The farm Langlaagte was essentially linked with early gold mining in Johannesburg in 1886, with George Harrison discovering gold on the farm during July of that year that eventually led to the large scale mining along the Main Reef shortly thereafter (Pelser and van Vollenhoven, 2011). Seven mines were operating on the Langlaagte mine during that time, including Block B Langlaagte Estate, Consolidated Langlaagte, Langlaagte Royal, Langlaagte Deep, Paarl Central, Robinson Central Deep and South Langlaagte. On the

1st of July 1909 the Crown Mines Ltd was formed when the seven mining companies were joined by the Crown Reef Gold Mining Company. These included Paarl Central, Langlaagte Deep, Robinson Central Deep, South Langlaagte, New Vierfontein, South Rand Gold Mining Company and South Deeps Limited, of which the first four were located on Langlaagte (Pelser and van Vollenhoven, 2011). It was suggested by Pelser and van Vollenhoven (2011) that the cemetery found on the Langlaagte site was related to Robinson Central Deep based on the fragment of porcelain plate found on the site (**Fig. 2**).

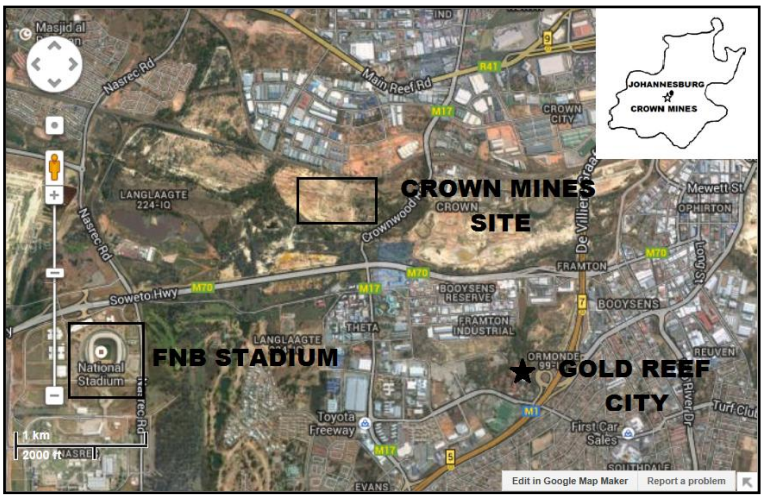


Figure 1: *A satellite map illustrating the location of the Crown Mines site situated south of Johannesburg in the Gauteng province (top right corner) (Google Maps, 2013).*



Figure 2: *Fragment of porcelain plate with Robinson Centr(al Deep) Johannesburg on it (Reproduced with permission obtained from A. Pelser).*

Most of the cultural material collected from the cemetery site was related to the early gold mining history of the area and that of the Witwatersrand which were all dated to between the late 19th and early 20th century (Pelser and van Vollenhoven, 2011). A few of the company names associated with some of the items found are mentioned in support of the time period given. Goldberg and Zeffert operated in Johannesburg between 1898 and 1910, Jackson and Gosling operated between 1866 and 1968, William Barnard and Sons operated approximately between 1860 and 1930, Chandler and Co. operated between 1887 and 1903 (Pelser and van Vollenhoven, 2011). The cultural material found at the site included ceramics, metals, glass and other miscellaneous items. A small brass tag with the words Crown Mines and the letters 1920 punched on to it (**Fig. 3**) was suggested by Pelser and van Vollenhoven (2011) to be an identity tag worn by the individuals who worked on the mine at the time. Chinese ceramics provided evidence for the presence of Chinese miners as it was known that they were imported to the Rand Gold Mines between 1904 and 1910 (Callinicos, 1981; Pelser and van Vollenhoven, 2011). Other household items were also recovered that provided insight into the society in which the individuals might have lived. These included fragments of plates, saucers, cups/mugs, serving dishes; bottles of rum, whiskey, beer, ginger beer, sodawater/mineral water and lemonade; Maclaren's cheese, fish paste, toothpaste, ointments (Ponds), perfume, porcelain dolls and porcelain figurines (Pelser and van Vollenhoven, 2011).



Figure 3: *Brass/copper tag with "CROWN MINES 1920" punched on to it (Reproduced with permission obtained from A. Pelser).*

Upon completion of the archaeological assessment of the site, the following was concluded. The Crown Mines site was suggested to date back to the late 19th/early 20th centuries - more specifically between the late 1890's and 1920's as deduced from the associated materials de-

scribed above (Pelser and van Vollenhoven, 2011). The site was suggested to be one of the largest cemeteries in Johannesburg in the early twentieth century (pers. comm. Anton Pelser) with the individuals believed to be migrant workers who had been working on the mines. It was estimated that a possible 650 bodies were buried in the cemetery of which approximately 100 skeletons were exhumed to date. Each of these skeletons were placed in separate coffins and stored at AVBOB, a specialist funeral insurance and burial service provider, in Johannesburg until a later stage when all the remains would be reburied.

1.2. Anthropological Assessment

When the biological profiles of the skeletons were compiled, it was found that the burials for each individual varied in nature and manner. The remains were buried in different positions with some skulls located north and others south, with some of their faces directing east and others west. Some individuals were buried in the supine position, lying on their backs, while others were buried in positions that resemble the foetal position (**Fig. 4**). The majority of the individuals were not buried in coffins but rather covered with canvas or blankets and only some graves included items such as jewellery which was buried with the individual.



Figure 4: *Archaeological image illustrating the burial position of the remains found in Grave 113 (Reproduced with permission obtained from A. Pelser).*

The preservation of all the skeletal remains was very poor which reduced the accuracy and reliability of interpretation drawn from the morphology of the skeletons during the compilation of the biological profiles. Poor preservation was characterised by skeletal elements that were severely flattened, for example the skull as illustrated in **Fig. 5**, and bones that were damaged, fragmented and appeared "cemented" together. Macroscopically, these elements had a thinned cortical surface that was much degraded, described as being brittle, flaky, rough

and uneven, with many of the skeletal elements and/or teeth having a blue discolouration to their external cortical surfaces (**Fig. 5** and **Fig. 6**). This poor preservation therefore resulted in quantitative analyses that were limited, with most of the measures taken *in situ*.

Overall, the anthropological assessment resulted in ninety-two written reports for the exhumed individuals. The other ±8 burial pits that were excavated were during the initial rescue operation where skeletal remains were protruding through the soil surface and needed to be removed resulting in no reported skeletal data. Sixty-five of these documented reports had biological profiles that had an estimated age, sex or stature. The other twenty-seven reports were photographically recorded due to time constraints which resulted in the remains being removed before the anthropological assessment was completed. These sixty-five biological profiles reported that the Crown Mines' individuals were all adult male ranging in stature from 1.52m - 1.90m with a mean value of 1.70m.



Figure 5: *An image illustrating a poorly preserved skull which was flattened from side to side with tooth roots characterised by a blue discolouration (Reproduced with permission obtained from D. Brits).*



Figure 6: *An image of a long bone from Grave 74 illustrating the blue discolouration of the bone cortex (Reproduced with permission obtained from D. Brits).*

1.3. Gold Mining (1890's -1920's)

As mentioned previously the Crown Mines site was believed to be associated with the early mining history of Johannesburg - particularly during the period 1890's – 1920's. To understand what life may have been like during that time, some background about how the mine workers were recruited, their living conditions and their health are discussed as described by Callinicos (1981). Due to the fact that black and Chinese unskilled labour workers were in the majority and most likely the individuals buried at the Crown Mines site, their history is discussed in more detail than the small group of white skilled workers.

1.3.1. Recruitment of Workers

The discovery of gold in the Transvaal in 1886 began what is known today as "The Gold Rush". As people from within South Africa and all over the world made their way to what was the richest gold mining area at the time, life began to change. The more gold that was found the more mining camps began to grow. One mining town in particular called Langlaagte, later known as Johannesburg, became the largest in the Witwatersrand area. In 1890 there were 14 000 labourers working on the gold mines and by 1899, only five years after deep-level mining started, nearly 100 000 labourers were employed on the Rand mines.

Labourers working in the mines were mainly black farmers needing money for taxes or supporting their families on the farm lands. At first there were enough black farmers willing to go to the Witwatersrand for a short time to earn some money, but as the mines got larger, the mine-owners needed more labourers. To increase this supply of cheap labour the government changed the laws on taxes which firstly stipulated that taxes had to be paid by money

and not cattle as was done previously, and that additional taxes such as hut tax, poll tax and labour tax had to be paid. This forced more black farmers to leave their lands to go and work on the mines, however, with long distance travel to the mines being dangerous and other companies such as the railways, municipalities, factories and diamond mines paying higher wages, the resultant increase in gold mine labourers was not substantial.

In 1901, the Chamber of Mines set up a recruiting organisation known as the Witwatersrand Native Labour Association (WNLA) who sent agents to villages all over South Africa, as well as Zambia, Tanzania, Malawi, Mozambique, Lesotho, Swaziland and Botswana. In return for working on the mines these agents offered to pay the taxes of the farmers to the government and gave them cash in advance. In 1902, after the Anglo-Boer war, the new government (The British) had a tighter control over the labour force on the mines, reducing their wages, resulting in unskilled workers looking elsewhere for jobs. This caused the mine-owners to look outside of Africa for additional unskilled labour by importing workers from China. In 1904, the first 10 000 Chinese labourers arrived in the Witwatersrand to work in the gold mines and more arrived throughout the next four years until in 1908 nearly 100 000 Chinese workers were working in the mines. In 1907 the new British government began sending the Chinese workers home because of the dislike concerning the reported slave conditions of the compounds and their low wages. At this time the mine-owners were happy about the progress made with the WNLA recruiting system and a sufficient amount of African workers had eased the labour shortage. By 1910 all the Chinese workers had left the mines.

Compared to the unskilled black workers, skilled work was reserved for whites only. They were imported from the Cornwall or Northumberland mines in England, mines of Scotland and Wales, and coal mines of Australia. After the 1907 skilled workers strike, many Afrikaners who had become very poor as a result of the war assisted in keeping the mines running by learning from experienced black and Chinese miners how to do the relevant jobs. As time went on, more and more Afrikaners were working in the mines and within 10 years there were just as many Afrikaners as English-speaking miners (Callinicos, 1981).

1.3.2. Living Conditions

As labour quantities increased over the years the living conditions at the mines became overcrowded, dirty and unhealthy. Although some mines provided better housing than others, most of them consisted of compounds made of wood and iron shacks containing 20 to 50 workers who slept on concrete bunks one above the other. In severe conditions where no space was available, workers had to sleep on the rotten/earth floors in the huts which turned

muddy in wet weather. Most compounds were badly built with no windows or lights, had no washing facilities, had cracks in the walls filled with rags to keep the wind and cold out, and the only source of heat came from a big tin of hot coal giving off highly dangerous smoke fumes.

Before 1903, living conditions at the compounds were atrocious for the black migrant workers. When the Chinese workers were brought into the country, the British government insisted that new and improved compounds be built. Although this consisted of better food, the Chinese workers had lower wages than the black workers. Eventually some of the richer mines built new compounds with bathing facilities, electric lights and higher ceilings for ventilation but many of the black workers were still housed in these older compounds. Although it was the compound who was supposed to provide enough food for the workers to stay alive, the food was either inedible due to it being rotten or it was in short supply. 5lbs (2.27kg) of mealiemeal and 2lbs (0.91kg) of meat were given to a worker per week which was not enough for a 10 hour day shift of hard manual labour, forcing the workers to spend almost half their wages on buying food. With such long work hours, workers did not have much spare time available and when they did they were either resting or in a hospital. Workers were not treated as men but rather as numbers with each one wearing a numbered bracelet around their arms. Many of the workers had little time to think of their families back home and spent their money on heavy drink and dagga to forget where they were. Placed under such unliveable conditions, violence was a common occurrence. Supervisors would often hit and kick the workers to get them to work harder which created anger and bitterness within the compounds. Fights would break out where people would get seriously wounded or end up dead.

White skilled workers were treated different to the large amounts of blacks and Chinese in the compounds. They were given much higher wages, they were free of the pass laws and other forms of labour control, and were allowed to live in houses in the towns (Callinicos, 1981).

1.3.3. Health

The poor food and medical care, and dangerous work underground caused the deaths of many miners every year. The growing spread of diseases such as pneumonia, tuberculosis and cerebrospinal meningitis was rampant due to increased numbers in the compounds. In 1903, 5022 black labourers died on the mines. 59% of them died from pneumonia and meningitis due to overcrowding, damp conditions, sudden changes in temperature and general weakness. 11.86% died from intestinal infections from bad food, 5.8% from scurvy due to lack of vege-

tables, 4.08% from accidents as no proper work clothes or protective helmets were given, 5.39% from Bacillosis (an infection caused by bacteria) and 5.39% from Tuberculosis. Pneumonia killed many workers from hot countries such as Zambia, the Congo and Tanzania who were not used to the cold Transvaal nights. In 1911, 67 out of 1000 mine workers died of pneumonia because of these circumstances and in 1913 these numbers were so shocking that the government prevented workers from these countries coming to the mines. In the Chinese compounds most of the workers had to be treated for syphilis obtained from prostitutes and of the 80 000 Chinese workers who were working on the Rand, 3000 men had died in accidents, suicide or from disease (Callinicos, 1981).

1.4. History of the Site Post-Burial

As described in the archaeological investigation (**p. 4**), the historical cemetery is situated beneath an old gold mine dump (**Fig. 7**) which has been extensively disturbed over the years. This includes the reclamation process by Crown Gold Recoveries (Pelser and van Vollenhoven, 2011). To understand the affects this mine dump may have had on the buried individuals, the process of gold mining and its reclamation process are briefly discussed.

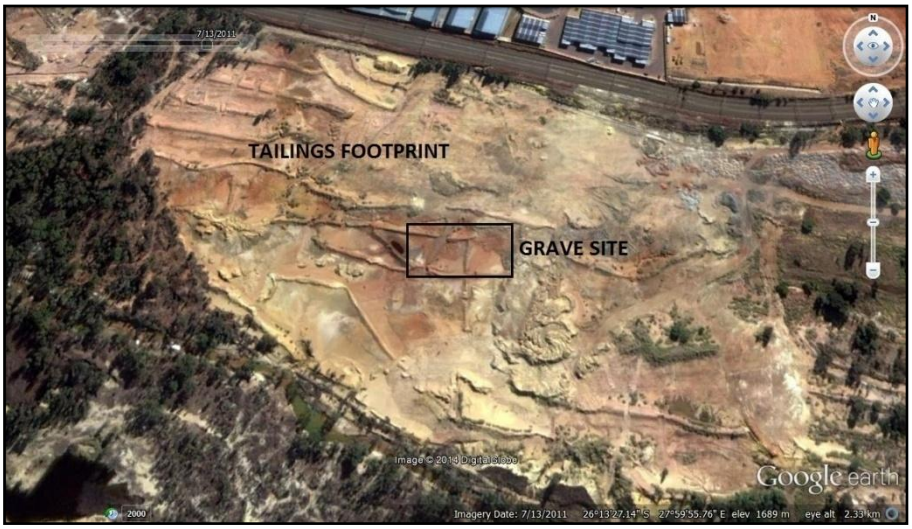


Figure 7: *Google earth image illustrating the position of the grave site in relation to the tailings footprint in 2011 (Google Earth, 2014).*

From the first discovery of gold in 1886, gold was extracted using a mercury amalgam method where ore mined underground was brought to the surface, milled to a fine sand and exposed to a mercury spread on copper plates; the tailings of which were transported to sand dumps (Naicker *et al*., 2003). Once deep-level mining was initiated, unoxidised ore contain-

ing pyrite (FeS_2) was encountered which interfered with the gold extraction. In 1915, this method was adjusted to the MacArthur-Forrest process where finer milling of the ore occurred which was treated with a cyanide containing solution to dissolve the gold, allowing the remaining tailings to be transported to nearby producing slime dumps (Naicker *et al*., 2003; Tutu *et al*., 2008). For almost a century, many of these tailing dumps have remained undisturbed, however, since 1983 many of them have undergone a reclamation process because the way in which gold was extracted in the past was not as efficient as it is today, resulting in gold still present in the tailings (Naicker *et al*., 2003; Strydom and King, 2009).

During the reclamation process, if the gold in the mine dump was coarser than slime, a front-end loader was used to transport the dump material to a nearby treatment plant by a conveyer. If the gold was finer than slime, the material was reprocessed by squirting the dump with a very high-pressure jet of water which eroded the dump material away into a sluice (an artificial channel for conducting water) (Strydom and King, 2009). The sluice allowed the material to be collected at a low point and pumped via a pipeline to the treatment plant. During this retreatment process, the underlying soil is limed (CaOH) to reduce the acidity of the footprint and immobilise metals (Tutu *et al*., 2008).

For environmental protection of the site, once the reclamation process is completed, dust pollution resulting from dry, windy conditions is prevented (Strydom and King, 2009) by constructing paddocks/tailing dams on the cleared site to minimize the run-off of rainwater and at the base of the dump to control erosion (Tutu *et al*., 2008). These paddocks can be seen in **Fig. 8** on a Google image taken in 2000.

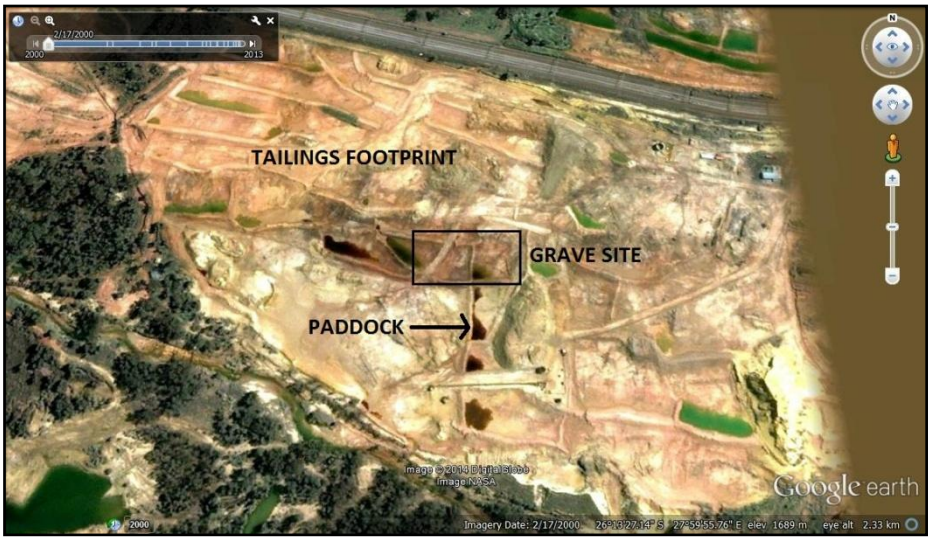


Figure 8: *Google earth image illustrating the position of the grave site in relation to the paddocks within the tailings footprint in 2000 (Google Earth, 2014).*

1.4.1. Implications on the Buried Remains

The old gold mine dump was situated directly on top of the historical cemetery which may have had many implications on the buried remains. Depending on where the skeletons were buried in the dump, they would have been physically and/or chemically changed. The weight of the tailings (consisting of waste rock) would have compressed the underlying soil, put pressure on the skeletons, causing some, if not all, the elements to be flattened (**Fig. 5, p. 8**), damaged, fragmented or even "cemented". Chemically, due to the gold extraction process, these tailings do affect the underlying soil and groundwater systems, known as acid mine drainage (Naicker *et al*., 2003; Akcil and Koldas, 2006; Tutu *et al*., 2008).

Acid mine drainage is produced when sulphide-bearing material (pyrite) is exposed to oxygen and water, causing the water to become acidified while percolating through the dumps and entering the groundwater system beneath the dumps (Naicker *et al*., 2003; Akcil and Koldas, 2006). In the study done by Naicker and colleagues (2003) the surface and groundwater drainage systems, as well as the soil, were assessed in a mining district south of Johannesburg. Their results indicated that the ground water within the mining district was heavily contaminated and acidified because of the oxidation of pyrite within the mine tailing dumps. This included a pH of 3.4 with high sulphate and heavy metal concentrations. Similarly this acidification of the water percolating through the mine dump, containing sulphates and heavy metals, may have chemically altered the skeletal remains at the Crown Mines, diagenetically affecting the bones.

As discussed previously, the reclamation process involved the removal of the tailings either mechanically or by high pressure water. This would have resulted in not only the tailings being removed but also the topsoil overlying the skeletons, causing the bones to become exposed, destroyed or washed out. As the paddocks were formed, the underlying skeletons may have been in close proximity or even lying within the paddock water which may have affected diagenesis. Paddocks can be contaminated due to the water table intersecting their surfaces which allows oxidation to occur resulting in the water containing elevated amounts of heavy metals (Tutu *et al*., 2009). In **Fig. 8** the contaminated water in the paddocks appear to be red-brown and green in colour possibly indicating some of these metal ions.

1.5. Environmental Conditions

Establishing the nature of the environment at the Crown Mines in the last 100 years is an important factor to take into consideration when the post-mortem alteration of the bones is

assessed. One of the factors that affect diagenesis, discussed at a later stage (**Ch. 3, subs. 3.2, p. 37**), are environmental factors such as temperature, moisture and soil. In this section the rainfall and temperature of the Crown Mines area are discussed, as well as the physical and chemical properties of the soil.

1.5.1. Rainfall and Temperature

Data pertaining to the average rainfall and temperature that occurred in the Crown Mines area were provided by the South African Weather Service. The closest weather station available that measured rainfall was the Turffontein station situated 4 km from the Crown Mines site and has been measuring rainfall since September 1909. **Fig. 9** displays the average rainfall per month in ±20 year intervals for the last ±100 years. The closest weather station available that measured temperature was the Johannesburg Botaniese Tuine (Botanical Gardens) station situated 6 km from the Crown Mines site. Unfortunately this station had only been measuring temperature since April 1985. **Fig. 10** and **Fig. 11** illustrate the average maximum and minimum temperatures respectively per month in ±10 year intervals for the last ±30 years.

The typical seasons expected in South Africa are summer from mid-October to mid-February, autumn from mid-February to April, winter from May to July and spring from August to mid-October (Brand South Africa). **Fig. 9**, **Fig. 10** and **Fig. 11** reveal that a typical Johannesburg summer in the Crown Mines is hot (high minimum temperatures; 11.37-15.08°C, and high maximum temperatures; 25.45-26.62°C) and wet (high rainfall; 69.06-134.45mm) whereas a typical winter in the Crown Mines is cold (low minimum temperatures; 2.64-6.20°C, and low maximum temperatures; 17.96-20.65°C) and dry (low rainfall; 7.09-21.42mm). The total average rainfall per year for the last ±100 years was 753mm.

From the above it is clear that periods of high rainfall at the Crown Mines site would have allowed water to percolate through the tailings, becoming contaminated by sulphates and heavy metals (Naicker *et al.*, 2003) as it filtered into the soil below where the skeletal remains were buried. The compressed soil between the level of the tailings and the buried individuals (caused by the tailing's weight) may have allowed the water to filter more slowly, allowing the contaminates to infiltrate into the skeletal remains. In additon to this, while controlling dust pollution created during the reclamation process, watering of the soil using water sprays from a high-pressure nozzle was often used (Cheng, 1973). This would have caused additional water to filter through the contaminated soil (discussed in the physical and chemical properties of soil section, **p. 17**), affecting the skeletal remains.

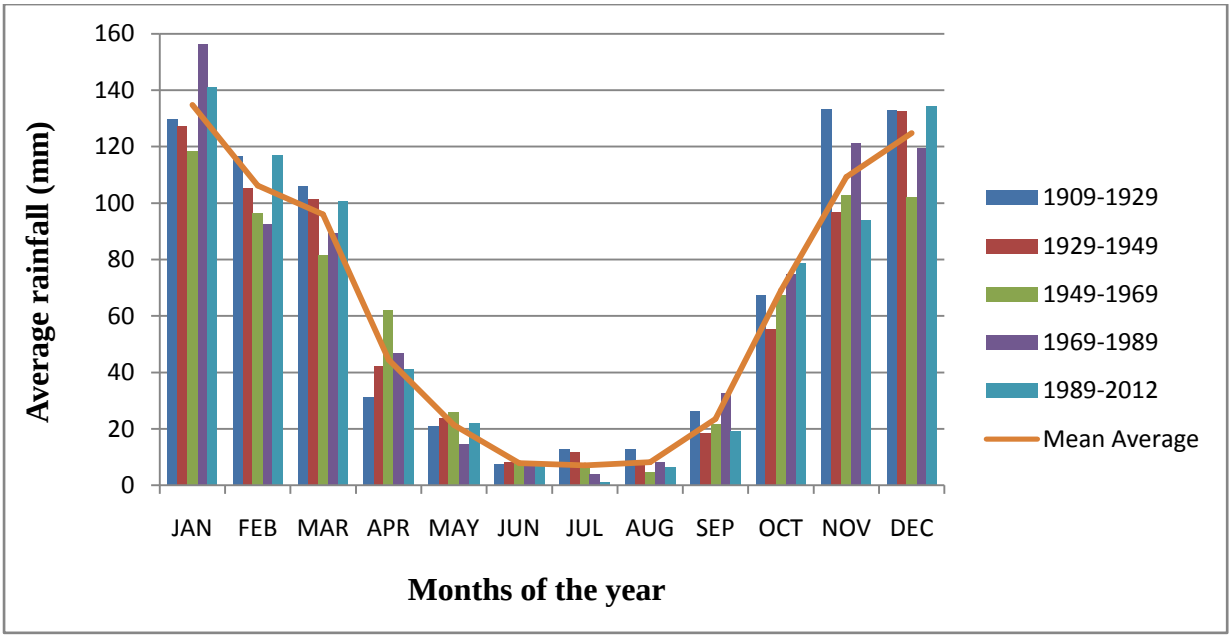


Figure 9: *Graph illustrating the average rainfall (mm) at the Crown Mines for the period 1909-2012.*

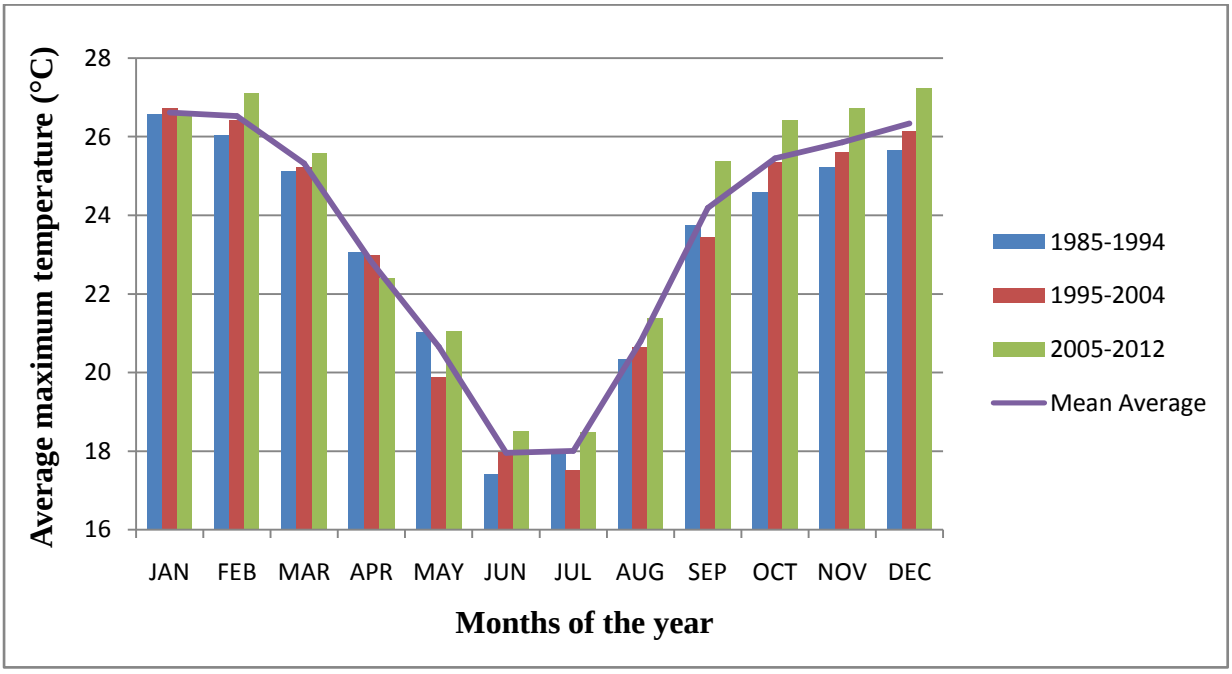


Figure 10: *Graph illustrating the average maximum temperature (°C) at the Crown Mines for the period 1985-2012.*

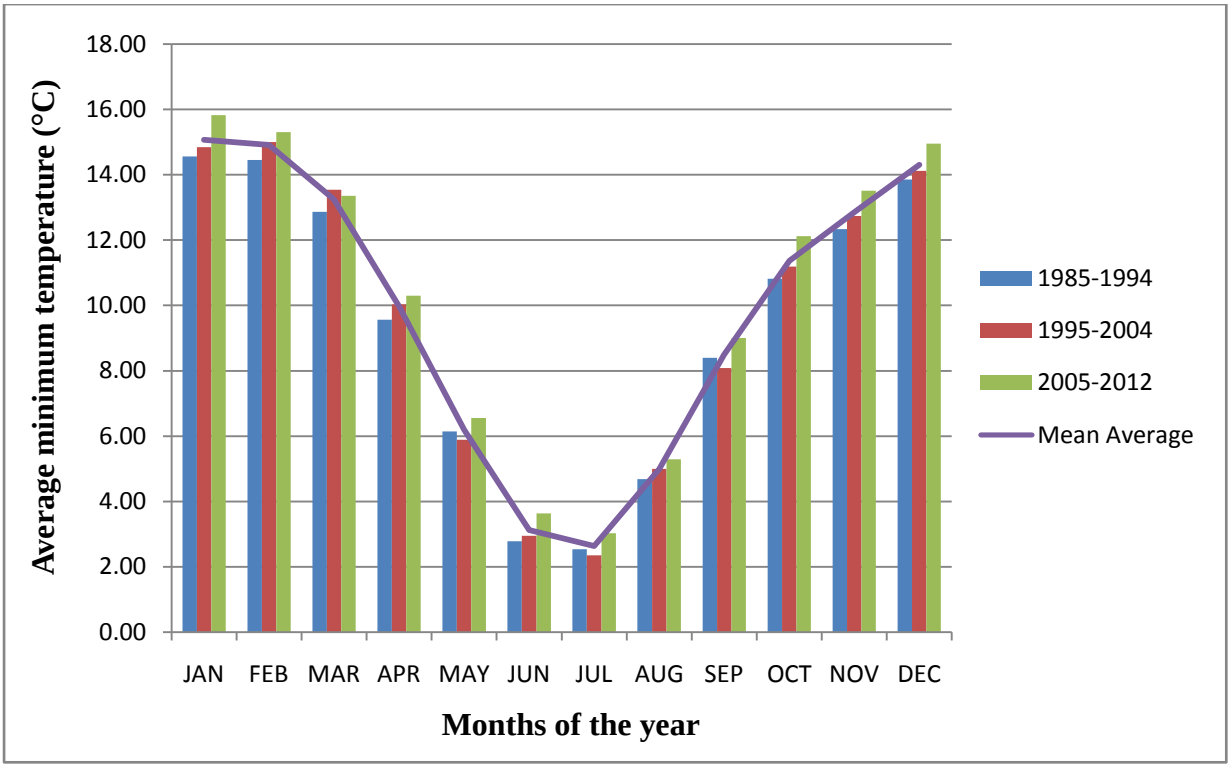


Figure 11: *Graph illustrating the average minimum temperature (°C) at the Crown Mines for the period 1985-2012.*

1.5.2. Physical and Chemical Properties of Soil

Soil mainly consists of a mineral, organic, water and air component. The mineral component has three parts - sand, silt and clay whereas the organic component is due to the decomposition of plants and animals. There are many variations concerning the types of soil that can be present, however, the three main ones are sandy soil, loam soil and clay soil. To identify the more complex soil types, further analysis needs to be done according to the soil's profile. This profile consists of a number of distinct layers, known as horizons, representing different degrees of decomposition of organic matter of which most soils will have three or more (Cranfield University, 2013). The soil layers include the O (humus), A (topsoil), E (eluviations layer), B (subsoil), C (regolith) and R (bedrock) horizons (Col, 2001).

The chemical nature of soil is dependent on the mineral composition, organic matter and environmental factors. An in-depth study on the soil done by Shacklette and Boerngen (1984) revealed major and trace elements. All the major elements that were identified included oxygen (O), silicon (Si), aluminium (Al), iron (Fe), carbon (C), potassium (K), calcium (Ca), sodium (Na), magnesium (Mg), titanium (Ti), nitrogen (N), sulphur (S), barium (Ba), manganese (Mn), phosphorus (P), and perhaps zirconium (Zr) and strontium (Sr) in decreasing

order of concentration. All the trace elements that were identified included chlorine (Cl), vanadium (V), zinc (Zn), chromium (Cr), boron (B), lithium (Li), copper (Cu), lead (Pb), nickel (Ni), cobalt (Co), arsenic (As), cesium (Cs), uranium (U), tin (Sn), molybdenum (Mo), beryllium (Be), silver (Ag), antimony (Sb), selenium (Se), cadmium (Cd), magnesium (Mg) in decreasing order of concentration. Major elements are those that exceed 100mg.kg^{-1} and trace elements are those that constitute less in concentration (Shacklette and Boerngen, 1984).

Analysing the soil at the old Crown Mines dump was an important factor to assess due to the suspected acid mine drainage that may have been caused by the tailings in the area. This acid mine drainage would not only have affected the rainwater percolating into underground systems and the skeletal remains, but also the underlying soil (Akcil and Koldas, 2006). This type of contamination could result in the soil having a low pH (acidic) (Naicker *et al*., 2003; Tutu *et al*., 2008) and higher concentrations of arsenic (As), cyanide (CN) and sulphate (SO_4^{2-}), uranium (U), aluminium (Al), copper (Cu), nickel (Ni), lead (Pb), cobalt (Co), zinc (Zn), iron (Fe), manganese (Mn), cadmium (Cd), chromium (Cr) and radium (Ra) present in the soil (Naicker *et al*., 2003; Akcil and Koldas, 2006; Tutu *et al*., 2008; Tutu *et al*., 2009).

2. Bone Biology

Bone is a hard, mineralised tissue that consists of two main components: the organic protein matrix (25%) and the inorganic mineral matrix (75%) (Kramer and Allan, 2005). The organic protein matrix is made up of mainly type I collagen (97%) and ground substance (3%) while the inorganic mineral matrix is made up of hydroxyapatite crystals. Bone is a living tissue that can repair and reshape itself in response to external stresses and is richly vascularised with calcium and phosphate content to help in the regulation of blood calcium levels (Kramer and Allan, 2005; White and Folkens, 2005). It is one of the strongest biological materials and is the main supporting tissue of the body that provides support and protection to important organs. In this chapter the basic morphology of bone and its histology is discussed in more detail.

2.1. The Morphology of Bone

The bones found in the human skeleton vary in shape and these include four main groups: long bones, short bones, flat bones and irregular bones (Kramer and Allan, 2005; White and Folkens, 2005). Long bones are tubular in shape with expanded ends and include the limb bones and many of the hand and foot bones (White and Folkens, 2005). Short bones are roughly oblong or quadrilateral in shape and are generally found where limited movement and compactness is required, for example the carpals of the hands and the tarsals of the feet. The bones of the cranial vault, shoulder (scapulae), pelvis and ribcage tend to be more flat and generally cover a greater area compared to their thickness. These bones mostly offer protection. Lastly, the irregular bones have peculiar shapes and include bones such as the maxilla, spinal column and bones of the viscerocranium (Kramer and Allan, 2005; White and Folkens, 2005).

At the microscopic level no matter what the shape of the bone, the same structural components are present: compact and spongy bone. The solid, dense bone found in the walls of the shafts and the external bone surfaces is called compact bone or cortical bone whereas spongy bone is porous, lightweight, has a honeycomb structure and is found under protuberances where tendons attach, in the vertebral bodies, in the epiphyses of long bones, in short bones and sandwiched within flat bones (White and Folkens, 2005). Due to the thin bony spicules (trabeculae) present in spongy bone the terms cancellous or trabecular bone are also often used.

The parts of long bones are often described according to their centres of ossification which occur as the skeleton matures with age (White and Folkens, 2005; Ross and Pawlina, 2006). Since the primary ossification centre develops in the shaft of long bones this region of bone is known as the diaphysis, and since the secondary ossification centres occur at each end of a long bone these regions are known as the epiphyses. The regions at the ends of the long bones where the epiphysis and the diaphysis join are known as the metaphysis (White and Folkens, 2005).

Microscopically, bones are also described according to specific regions such as the periosteum, mesosteum and endosteum. The periosteum is a connective tissue layer on the outer surface of bone which functions to nourish the bone. Similarly, the endosteum is generally one cell layer thick of connective tissue and is found lining the marrow (medullary) cavities of bone. The mesosteum on the other hand is the bone found between the periosteum and the endosteum and is composed of an outer section of compact bone and an inner section of spongy bone. In a bone cross-section of compact bone three ill-defined layers can be identified: the periosteal zone (outer surface), the mesosteal zone (central portion) and the endosteal zone (inner surface) (Hillier and Bell, 2007). It is important to note that the periosteum is missing in dry bones but is a thin tissue that coats all bone surfaces not covered by cartilage during life (White and Folkens, 2005).

2.2. The Histology of Bone

To fully understand the microstructure of bone it is important to discuss what types of cells are present in bone tissue beforehand. The different cell types associated with bone tissue are: osteoprogenitor cells, osteoblasts, osteocytes and osteoclasts (Ross and Pawlina, 2006). Osteoprogenitor cells are derived from mesenchymal stem cells found in the bone marrow which have the ability to differentiate into many different cell types including osteoblasts. These cells are found on the external and internal surfaces of bones and their main function is osteogenesis (bone formation). During osteogenesis, osteoprogenitor cells respond to stimuli that transform them into bone-forming cells (osteoblasts) and are often concentrated just below the periosteum. Osteoblasts are responsible for producing large quantities of osteoid which is an uncalcified organic matrix rich in collagen and as the bone begins to calcify, hydroxyapatite crystals are deposited into the osteoid matrix (White and Folkens, 2005). When an osteoblast is completely surrounded by osteiod it is known as an osteocyte. These osteocytes are responsible for maintaining the bone matrix and calcium homeostasis as well as the repair or remodelling of bone. Lastly, osteoclasts which are derived from the fusion of mono-

nuclear haemopoietic progenitor cells are mainly responsible for bone resorption - the removal of bone tissue. As bone remodelling occurs, where the osteoclasts remove bone tissue and the osteoblasts build bone tissue, it gives bone the ability to maintain or change its shape and size during growth (White and Folkens, 2005).

When looking at the microstructure of bone there are two types of bone found in mammals: woven (immature) and lamellar (mature) bone (White and Folkens, 2005). Woven bone is the first bone that begins to develop in an embryo and is usually temporary because as the bone begins to grow the woven bone is replaced with lamellar bone. Woven bone is found in young human skeletons under the age of five and in adults mostly around bone fracture sites or in skeletal trauma or disease (Zernicke and Loitz-Ramage, 2003). Histologically, this type of bone is coarse and fibrous in appearance with collagen fibre bundles arranged in a nonoriented, random pattern (White and Folkens, 2005). Lamellar (mature) bone which is found in both compact and spongy bone has a more organized structure produced by the constant addition of lamellae (parallel collagen fibres) to the bone surfaces during appositional growth - the growth in diameter of bones around the diaphysis deep to the periosteum (White and Folkens, 2005). Although both compact bone and spongy bone are dense in nature they vary in structural organization with regards to their lamellae. Spongy bone is more porous, as was discussed previously, which allows for efficient nourishment of the bone due to the many marrow cavities present. This causes the lamellae to be organised/arranged in a parallel nature which differs in compact bone where concentric Haversian systems are found with no marrow cavities. There are no Haversian systems present in spongy bone (White and Folkens, 2005).

As bone matures there are two types of bone that develops: primary bone and Haversian bone. Primary bone is the newly laid layers of bone during appositional growth found in the periosteal and endosteal zones, histologically illustrated as circumferentially arranged lamellae. This bone contains primary Haversian systems which have large Haversian canals compared to their size, very few layers of concentric lamellae and are found during the transformation process from woven to lamellar bone (Hillier and Bell, 2007). Haversian or secondary bone found in the mesosteal zone is the new bone that is laid down in areas where previously existing bone had been resorbed or secondary reconstruction of the bone had occurred. This bone contains secondary Haversian systems which have small Haversian canals and many concentric lamellae which are surrounded by a cement line or reversal line (Kramer and Allan, 2005; Hillier and Bell, 2007). Majority of the Haversian systems seen in adult bone are secondary Haversian systems. The main differences between the primary and secon-

dary Haversian systems are that primary Haversian systems are smaller in size, they do not have a cement line, they form between the lamellae and have a smaller Haversian canal size compared to secondary Haversian systems. Secondary Haversian systems on the other hand are larger, they have a cement line, they intersect the circumferential lamellae and have a larger Haversian canal size compared to primary Haversian systems (**Fig. 12**) (Hillier and Bell, 2007). This cement line that is only present in secondary Haversian systems occur due to bone remodelling where bone resorption is ending and bone formation is beginning. As these Haversian systems are continuously being remodelled, pre-existing lamellae from "old" Haversian systems can be found between the Haversian systems and are known as interstitial lamellae (**Fig. 13**) (Kramer and Allan, 2005).

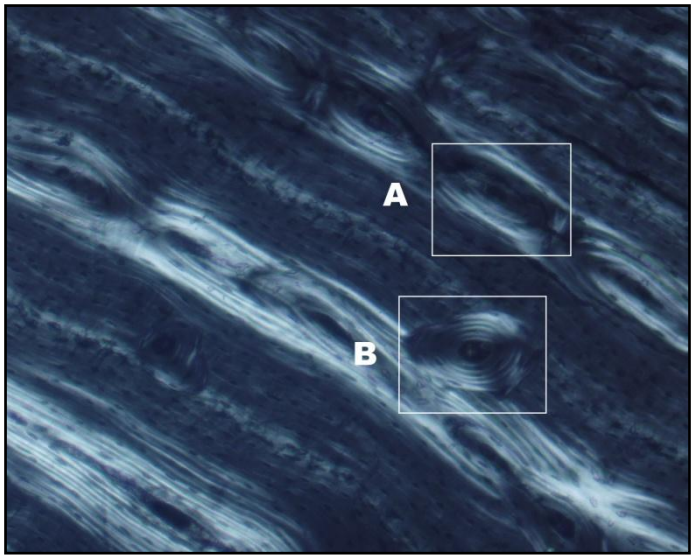


Figure 12: *A histological polarized image comparing A: primary and B: secondary Haversian systems in the lamellar bone of a donkey femur, 100X magnification (Taken from Brits, 2009).*

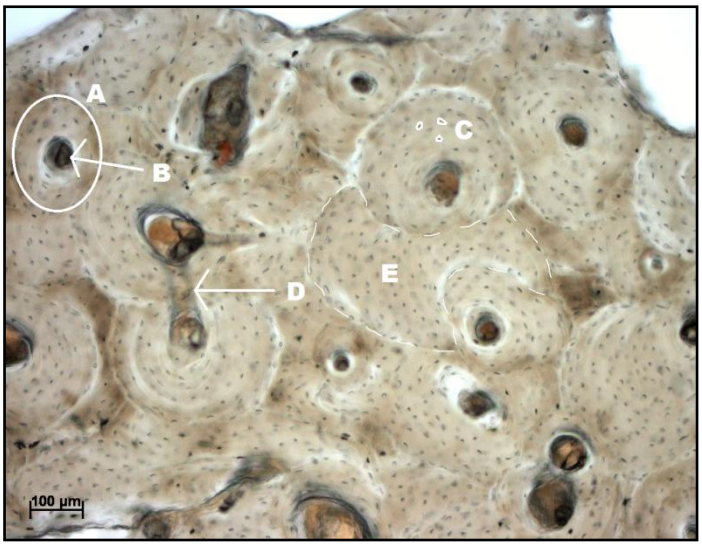


Figure 13: *A histological light microscope image displaying A: a Haversian system, B: Haversian canal, C: osteocytes in lacunae, D: Volkmann's canal and E: interstitial lamellae in the microstructure of Haversian bone, 100X magnification.*

Secondary Haversian systems in cross section consist of approximately 4-8 concentric rings surrounding a centrally placed Haversian canal which contains blood vessels, nerves and lymphatic vessels (White and Folkens, 2005). These Haversian systems are approximately 0.3mm in diameter but this is dependent on which bone is being assessed as the Haversian system size differs between the bones of the body (Evans and Bang, 1967). Secondary Haversian systems represent the structural units of compact bone and their long axes are parallel to the long bone of which they are part of. Other structures associated with Haversian systems are Volkmann's canals, osteocytes in lacunae and canaliculi (White and Folkens, 2005). Volkmann's canals are radially orientated vascular canals that lie at right angles to the Haversian canals through which blood vessels and nerves travel from the periosteal and endosteal surfaces to reach the Haversian canals connecting them to one another (Ross and Pawlina, 2006) (**Fig. 13**). Between the lamellae found surrounding the Haversian systems, osteocytes are found embedded in lacunae (**Fig. 13**). For the nutrients to be transported between these osteocytes, they are interconnected with one another and the Haversian canal by canaliculi which are long tunnel-like processes that extend into the lamellae (Kramer and Allan, 2005; White and Folkens, 2005). Overall, bone is a very complex organ with many aspects involved in its structure, function and development, and due to its complex nature there are many factors that can affect the histology of bone (Currey, 1964; Evans and Bang, 1966; Singh and

Gunberg, 1970; Burr *et al.*, 1990; Mulhern and Van Gerven, 1997; Pfeiffer, 1998; Currey, 2003; Pfeiffer *et al.*, 2006; Hillier and Bell, 2007).

2.2.1. Factors that Affect Bone Histology

A number of factors can affect the microscopic appearance of bone, for example age, the specific bone used (femur, humerus), the bone portion sampled (epiphysis, diaphysis), sex, pathology and diagenesis (discussed in **Ch. 3**) to name a few.

2.2.1.1. Age

Age is one of the most important factors that affect histological variation, as is particularly seen in the modelling and remodelling of bone. Hillier and Bell (2007) describe this histological variation in human bone from a foetus to an adult. During the 1st to the 9th month of foetal bone growth the cartilage model is gradually replaced by woven bone and eventually lamellar bone, consisting of many primary and a few secondary Haversian systems. This primary bone is present until two to three years after birth (Enlow, 1966). These secondary Haversian systems begin with wide Haversian canals with a small number of wide lamellae gradually developing into smaller but more numerous lamellae with narrower Haversian canals (Hillier and Bell, 2007). In sub-adults large amounts of lamellar bone are present and majority of the primary Haversian systems have been replaced by secondary Haversian systems. Some primary Haversian systems do remain but disappear completely by the age of 55 (Kerley, 1965). These younger individuals display complete and regular appearing Haversian systems lying next to one another with limited interstitial bone. As the Haversian systems begin the remodelling process Haversian system fragments, an overlapping of the Haversian systems seen in cross section due to osteoclasts burrowing channels through the Haversian bone, can be seen.

As development into adulthood occurs there is an increase in the number of secondary Haversian systems until the age of 95, the bone appears more disorganised in nature and there are more Haversian system fragments and interstitial lamellae present (Kerley, 1965; Hillier and Bell, 2007). As the individual gets older there is an increase in Haversian system density, the addition of lamellae discussed earlier, and continuous remodelling causes the Haversian system area to decrease with the Haversian canal taking up a greater percentage of the overall area of the Haversian system, allowing for the bone to become more porous with age (McCalden *et al*., 1993; Wang *et al*., 2003; Curtis and Nawrocki, 2010).

Histological differences related to age can also be found in the periosteal and endosteal surfaces, namely that a greater percentage of circumferential lamellae is present in children and decreases with age (Kerley, 1965). The periosteal surface, which has more circumferential lamellae compared to the endosteal lamellae due to appositional growth, becomes replaced by secondary Haversian systems. Due to a higher rate of remodelling at this surface these systems are smaller in size and more numerous as compared to the systems found nearest the endosteal surface. The endosteal circumferential lamellae are not replaced by Haversian systems but rather resorbed (Hillier and Bell, 2007).

2.2.1.2. Skeletal Elements

It is known that there are histological differences between the various skeletal elements found in humans. Pfeiffer (1998) and Pfeiffer *et al.* (2006) reported that rib Haversian systems are significantly smaller than the Haversian systems found in femora of the same skeleton. Evans and Bang (1967) showed that the femur has more Haversian systems/mm² than the tibia and the fibula has less Haversian systems/mm² than both the femur and the tibia. They also report that the tibia has more Haversian system fragments/mm² than the fibula while the femur has less Haversian system fragments/mm² than both the tibia and the fibula. Evans and Bang (1967) also concluded that the area of a Haversian system found in the fibula was greater than the area of a Haversian system found in both the tibia and the femur, with the femur having a greater Haversian system area than the tibia.

In addition to the histological differences between the specific skeletal elements, it is also known that a single bone is able to display random and systematic histological variation from one area of the cortex to another which influences the bone portion sampled. For example, an increase in the number of Haversian systems present or differences in bone remodelling. Establishing the reasons why such variation is found within a single bone is an ongoing process with suggestions including the biomechanical forces applied to the bone and/or the areas of muscle attachment (Currey, 2003). The areas of muscle attachment in particular show an increase in the number of Haversian systems present with an increase in tension and compression forces involved with the muscle movement and strain which can result in microcracks and fatigue. This allows for more remodelling of the bone to occur which leads to an increased number of Haversian systems (Currey, 2003; Chan *et al.*, 2007).

2.2.1.3. Sex

There are a number of significant differences found between the sexes when assessing the femur (Burr *et al.*, 1990; Mulhern and Van Gerven, 1997). A Medieval Nubian population displayed females with more fragmentary Haversian systems and an increased Haversian system area size as compared to the males that had more intact Haversian systems (Mulhern and Van Gerven, 1997). Females from an Archaic Native American population had larger Haversian systems and an increased Haversian system mean wall thickness (density) as compared to the males, who displayed greater Haversian system population density (Burr *et al*., 1990). It has been suggested that these significant differences between the sexes are contributed to increased activity patterns as males are involved in more physically strenuous tasks as compared to the females (Burr *et al.*, 1990; Mulhern and Van Gerven, 1997). It is important to note that not all the bones in the body have revealed sexual dimorphism, for example the ribs (Mulhern, 2000; Pfeiffer *et al.*, 2006).

2.2.1.4. Pathology

The pathological conditions that can affect cortical bone were briefly reviewed by Hillier and Bell (2007). These include, but weren't limited to, osteoporosis, Paget's disease, diabetes mellitus, osteomalacia, osteogenesis imperfecta, immobilization (paralysis and disuse atrophy), primary hyperparathyroidism, acromegaly, and mastocytosis. The histological effects these pathological conditions have on bone vary. Hyperparathyroidism for example results in an increase in the rate of bone remodelling which increases the number of Haversian systems and Haversian fragments present. Diabetes mellitus on the other hand results in a decrease in the rate of bone remodelling which decreases the number of Haversian systems and Haversian fragments present (Hillier and Bell, 2007).

Diseases such as Paget's disease and osteoporosis are the most common bone diseases occurring in modern times. Paget's disease is a disorder that involves the abnormal breakdown of bone tissue which is followed by the abnormal formation of bone tissue that is weaker and filled with new blood vessels, often resulting in a deformity (Lorenzo *et al*., 2011). Histologically, Paget's disease can be identified by excessive remodelling being present which results in numerous cement lines that produce a mosaic pattern (Collins, 1956), numerous Howship's lacunae and excessive amounts of osteiod (Stout and Teitelbaum, 1976). Furthermore, the bone in specific areas has an increased rate of resorption with the bone formation occurring

rapidly resulting in the bone being deposited in a very disorganised fashion, causing the bone to be of poor quality (Hillier and Bell, 2007).

Osteoporosis on the other hand is a condition where the existing bone is overly reabsorbed while new bone formation fails. This causes a loss in bone density (calcium and minerals) which leads to more fragile bone with a higher risk of fracturing (Alldredge *et al*., 2009). There are two types of osteoporosis, primary and secondary. Primary osteoporosis is most commonly found in the elderly and is more common in women than in men as it is mainly caused by hormonal imbalances, and secondary osteoporosis results from other disorders such as scurvy, diabetes mellitus, prolonged immobility or calcium loss (Hillier and Bell, 2007). Bone that is affected by osteoporosis appears histologically similar to normal bone, there is just less of it. There is an overall reduction in the amount of bone present where there is a slight loss of bone during each bone remodelling cycle which causes the bone to become thinner. This can particularly be seen in the trabecular bone (Cembrowicz and Allain, 2007; Hillier and Bell, 2007).

2.3. The Chemical Composition of Bone

As mentioned previously, bone consists of an organic component consisting of collagen and a mineral component comprising of calcium hydroxyapatite crystals [$Ca_{10}(PO_4)_6(OH)_2$]. The major chemical elements that form part of the organic component of bone are carbon (C), hydrogen (H), nitrogen (N), oxygen (O) and sulphur (S). The presence of these elements were mostly determined by genetics and partly by health status (Bocherens, 1997). The major chemical elements present in the mineral component of bone are calcium (Ca), phosphorus (P), oxygen (O), carbon (C), magnesium (Mg) and sodium (Na) (Grynpas *et al*., 1987; Bocherens, 1997). Other elements in the mineral component of bone are present in very small amounts (less than 1%), known as trace elements. These trace elements include iron (Fe), zinc (Zn), strontium (Sr), lead (Pb), copper (Cu), barium (Ba), manganese (Mn), aluminium (Al), arsenic (As), mercury (Hg) and iodine (I) in decreasing order of concentrations (Chamberlain 1994). Other trace elements that have also been mentioned in the literature are fluorine (F), molybdenum (Mo), silicon (Si) and chromium (Cr). All the above mentioned trace elements are predominantly derived from the diet (Grynpas *et al*., 1987; Sastri *et al*., 2001; Jugdaohsingh, 2007; Izci *et al*., 2013) and are controlled by the external environment (Bocherens, 1997). Some trace elements such as copper, manganese, zinc and iodine are essential for the normal functioning of the body and other trace elements such as lead, mercury and arsenic are non-essential or even toxic to the body (Chamberlain, 1994).

There are a number of factors that contribute to the variation of chemical elements present in bone. Some of which include the amount of the element present in the diet, the age and sex of the individual, whether any disease is present, and the extent of ingestion by inhalation and absorption through the skin (Chamberlain, 1994; Sheridan, 2001). Diagenetic alteration, discussed in the next section, can also change the amount of chemical elements present in bone. Once skeletonisation has occurred after an individual has been buried, these naturally occurring chemical elements can move between the bone and the surrounding soil or vice versa, with the elements mobility dependent on soil chemistry, ground water acidity and the length of time the bone has been buried (Chamberlain, 1994). Literature suggests that during this time of diagenesis, elements that are likely to contaminate the bone are iron, aluminium, potassium and manganese due to soil contamination whereas zinc, strontium and barium are relatively stable (Sheridan, 2001; Pollard and Heron, 2008).

3. Diagenesis

After death, the body undergoes two taphonomic processes, decomposition and diagenesis, to break the body down in order to incorporate the remains into the environment. These processes entail a number of chemical mechanisms which cause complex structures found in the human body made of proteins, carbohydrates, sugars, collagen and lipids to be broken down by protein degradation, carbohydrate degradation, lipid degradation, nucleic acid degradation and bone degradation (Vass, 2001). Decomposition is the general term given to the post-mortem breakdown of cells and organs through two major processes: autolysis (involving enzymes) and putrefaction (involving bacteria) whereas diagenesis is the physical and chemical changes that occur to teeth and bones after death, including the chemical and compositional effects of discovery, recovery, handling and storage (DiGangi and Moore, 2013).

Approximately 4 minutes after death, the human body begins to break down by initialising the first major process of decomposition - autolysis. This involves self-digestion where the cells of the body are deprived of oxygen causing the carbon dioxide in the blood to increase which in turn causes a decrease in the body's pH levels and an accumulation of waste which poisons or causes destruction of the cells (Vass, 2001). Cellular enzymes (lipase, amylase, etc.) or digestive fluids residing in the intestinal tract begin to dissolve the cells from the inside out causing them to rupture and release nutrient rich fluids. This release of nutrient rich fluid allows for the second major process of decomposition, putrefaction, to occur. Putrefaction involves the microorganisms (bacteria, fungi and protozoa) that normally reside in the body tissues to begin breaking down the soft tissues of the body under anaerobic conditions resulting in the formation of gases (e.g. methane, hydrogen sulphide, ammonia), liquids (e.g. propionic acid, lactic acid) and simple molecules (Vass, 2001; Fiedler and Graw, 2003; Dent *et al*., 2004). This results in an overall loss in the cadaver's mass where the liquefaction products, the most basic building blocks of protein, carbohydrates, etc., are eventually integrated within the water filtering through the surrounding soil and groundwater systems, leaving behind the skeletonised remains (Dent *et al*., 2004). Once skeletonisation has occurred, diagenesis is initiated where the proportions of organic (collagen) and inorganic (hydroxyapatite, calcium, magnesium) components of bone are altered and broken down until eventually nothing is left of the bone (Vass, 2001).

The term diagenesis has been extensively used in the field of geology, however it has filtered into the fields of anthropology, archaeology and palaeontology to describe the changes and alterations that take place in skeletal material when buried (Wilson and Pollard, 2002).

Many alterations to bone occur during the burial period including the uptake of cations and circulating organics, the exchange of ions, the breakdown and leaching of collagen, microbiological attack, alteration and/or leaching of the mineral matrix, and the infilling with mineral deposits (Hedges, 2002). These alterations are categorised into three main pathways of diagenesis based on the way in which the bone deteriorates: (1) chemical deterioration of the organic phase, (2) chemical deterioration of the mineral phase, and (3) biodegradation (Collins *et al*., 2002). A fourth pathway, biomolecular deterioration, is also suggested by Collins *et al.* (2002) however this is a relatively unexplored area that still requires much research.

Bone consists of three main components: a protein component mainly made up of collagen, a mineral component consisting of hydroxyapatite and ground substance consisting of organic compounds such as mucopolysaccharides and glycoproteins (Dent *et al*., 2004). These strong chemical bonds (protein-mineral) give bone its strength and hardness. For diagenesis to begin these protein-mineral bonds need to break down (dissolution) and this is done by the organic and inorganic pathways mentioned previously. The organic collagen phase of bone is altered by bacterial collagenases which cause hydrolysis of the protein-mineral bonds resulting in the breakdown of peptides into amino acids and the exposure of the collagen (Hackett, 1981; Von Endt and Ortner, 1984; Forbes, 2008). Once the collagen phase of bone has been affected, the inorganic mineral phase of bone is altered by the loss of the hydroxyapatitite where hydrogen ions found in the bone or surrounding soil replace the calcium (Ca) from the hydroxyapatite (Ca5(PO4)3(OH)) (White and Hannus, 1983). Not only is there removal of proteins and minerals from the bone but also substitution, infiltration and adsorption of ions (Dent *et al*., 2004). This changes the way in which the proteins bind to the minerals causing the bone structure to weaken and the disintegration process of the bone to begin by leaving the bone vulnerable to internal and external elements (Von Endt and Ortner, 1984; Dent *et al.*, 2004; Forbes, 2008). The biodegradation phase of diagenesis will be discussed at a later stage when the histological integrity of bone is reviewed.

When evaluating diagenesis, there are a few diagenetic parameters that are used to assess the extent to which the bone has degraded. The main parameters used are protein content/collagen loss, porosity, crystallinity and histological integrity (Nielsen-Marsh and Hedges, 2000; Hedges, 2002). It is known that the organic collagen phase of bone allows for the dissolution of minerals which exposes the collagen (Collins *et al*., 2002). Damage to this collagen, for example during biodegradation, leads to a change in organization and ultimately gelatinization and loss of collagen. This collagen loss is closely related and affects the poros-

ity and crystallinity of the bone (Hedges, 2002). Porosity relates to the changes in the physical structure of bone due to variations in either the organic or inorganic components (Nielsen-Marsh and Hedges, 2000). This involves the presence of micro and macro interconnecting pores which determines how the bone interacts with the groundwater in the surrounding soil and is suggested to affect the structural rearrangement of the remaining inorganic matrix (crystallinity) (Hedges *et al*., 1995). For example, microporosity decreases (smaller pores expand into larger pores) as collagen is lost which allows for a measure of the loss of the bone mineral to be taken from the macroporosity as compared to the microporosity. This in turn increases crystallinity (Hedges, 2002). Other chemical changes that affect crystallinity include the uptake of fluoride and carbonate, the crystallite size and strain, and the transformation of hydroxyapatite to brushite in acidic environments (Molleson, 1990). As the histological integrity of bone is the main component assessed in this study a complete review will be given in the following section.

3.1. Histological Integrity

The histological integrity of bone provides the overall pattern of diagenetic alteration at a microscopic level. This therefore gives a better description of the bone's preservation. When assessing histological integrity there are five main components that are examined. They are biodegradation, microcracks, birefringence, inclusions and infiltrations.

3.1.1. Biodegradation

Biodegradation, also known as bioerosion, is the chemical dissolution of bone by microorganisms which create what are known as microscopic focal destructions or tunnels (Hackett, 1981). Once the dissolution of mineral of the bone has exposed the collagen, microbial attack is the major cause of collagen loss (Collins *et al.*, 2002; Hedges, 2002). This gives extracellular microbial enzymes access to the collagen which allows microbial attack to occur similar to the organic and inorganic chemical deterioration pathways that were discussed previously (Collins *et al*., 2002). This leads to the formation of tunnels in specific regions of bone which appear early on in the diagenetic process but not immediately after death (Hedges, 2002). These specific regions include the inner and outer cortices of bone as these are the areas of easiest access for the microorganisms, as well as the areas closest to the Haversian canals through which the microorganisms extend from the cortices (Hackett, 1981). The extent of tunnel formation in these areas was dependent on how long the bone had been buried, envi-

ronmental conditions (temperature, humidity), the number of vascular canals, the orientation of the collagen fibre bundles, the osteocyte spaces and the cement lines (Hackett, 1981).

There are two main alterations of mineralization that are associated with biodegradation or the formation of tunnels. They are demineralization and mineral redeposition (remineralization) (Hackett, 1981). Demineralization was discussed previously with the exposure of collagen being the earliest sign of possible invasion by microorganisms. Mineral redeposition is the formation of a cuff, 3-6µm thick, most commonly found in the exposed surface areas of most of the tunnels from +5µm in thickness. It is important to note that cuffing is more frequent in smaller tunnels than larger ones and is not a deposit on the inner surface of the tunnel but rather the bone mineral passing into solution due to the annual tides of moisture, variation in moisture availability due to seasonal change, seeping into the open spaces of the tunnels during initial demineralization (Hackett, 1981). Once cuffing is completed, the annual tides of moisture allow for secondary demineralization to occur where the contents of the tunnels and the redeposited mineral from the refractile and hypermineralized rims are leached from the bone (Hackett, 1981). This is primarily done by the groundwater present in the surrounding soil which not only allows the organic material to be removed from the bone but it also allows for acids and other chemicals from the soil to enter the bone, changing its chemical and structural make-up (Nawrocki, 1995). Moreover, the moisture present within the bone provides the moisture needed for the microorganisms who rely on moist conditions to cause tunnelling in the bone (Hackett, 1981).

The microorganisms responsible for the formation of tunnels in the burial environment are mostly fungi and bacteria (Jans, 2008). The initiation of fungal tunnelling is very dependent on environmental conditions and can only begin when conditions are suitable. Bacterial tunnelling on the other hand is known to begin shortly after death due to bacteria already present within the body (intestines) which radiate through the vascular canals to assist in the putrefaction process. These microorganisms are categorised according to the specific pattern of tunnelling that they produce as they are usually no longer present within the tunnels upon excavation (Jans, 2008). The types of tunnelling that have been identified and described include: Wedl tunnels, linear longitudinal tunnels, budded tunnels and lamellate tunnels (Hackett, 1981; Trueman and Martill, 2002). Each of these types of tunnelling are caused by either bacterial or fungal invasion in the burial environment with one specific type of tunnel never passing or invading another's area (Hackett, 1981; Bell, 1990). Furthermore, neither bacteria nor fungal invasion are found to occur within the same bone and it is still unknown if this is due to competition between the organisms, a lack of nutrients due to alteration of the

opposing organism or environmental incompatibility (Jans *et al.*, 2004; Jans, 2008). **Table 1 (p. 37)** is a summary describing the different types of tunnelling with regards to the microorganisms thought to cause the tunnel, its size, appearance and organisation, particularly whether the distribution of the tunnelling appears to follow the microstructure of the bone, for example osteocytes and canaliculi, and whether the tunnel passes through the cement line of the Haversian system.

3.1.1.1. Fungal Tunnelling

These microscopic focal destructions were first described in 1864 by Wedl where tunnels measuring 8 µm in diameter were observed in fossil reptile bone and in modern human teeth that had been immersed in untreated well water for ten days. In later years Hackett (1981) described these fungal tunnels as centrifugal or Wedl tunnels passing away from the cortical surfaces and the Haversian canals with their direction of development never being smooth, straight or longitudinal (**Fig. 14**). These tunnels are branching tunnels of 5-10µm in diameter which appear to be unrestricted by the bone microanatomy allowing the tunnels to pass through cement lines (Jans, 2008). Wedl tunnelling was further described by Trueman and Martill (2002) as having two variations, Type 1 and Type 2 Wedl tunnels. Wedl type 1 tunnels are the more common of the two types of tunnels found concentrated towards the endosteum and periosteum of the bone, being unimpaired by the bone microstructure by easily crossing the Haversian system boundaries. These tunnels are branching, three-dimensional networks of 10-15µm in diameter with the edges of the tunnels appearing dense and mineralized. Wedl Type 2 tunnels are found exclusively associated with Haversian bone extending from the Haversian canals being unimpaired by cement lines with a diameter of 5µm. These tunnels differ in decreased size, increased branching frequency and more complex tunnel networks as compared to Wedl Type 1 (Trueman and Martill, 2002; Jans, 2008).

These variations in Wedl tunnelling are known to be of fungal origin and the process of identifying the specific species responsible for this type of tunnelling is still ongoing. The random distribution of this tunnelling is specific to fungal invasion and this is due to fungi having the ability to cross cement lines (Jans, 2008). Though it is unclear whether fungi dissolve the bone for nutritional value or for the use as a medium – substrate used for distribution purposes; fungal structures such as hyphae, fruit bodies and spores are regularly found associated with this tunnelling (Jans, 2008).

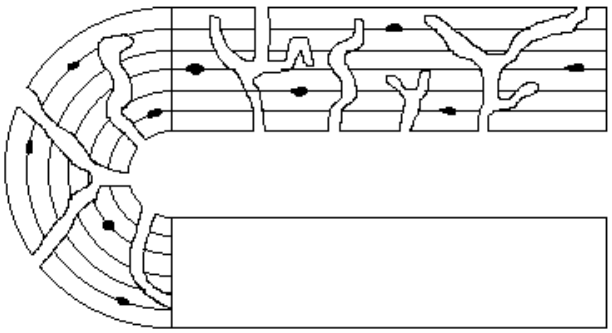


Figure 14: *An outline illustrating Wedl tunnels in transverse (semi-circular image) and longitudinal section (horizontal image) extending from a Haversian canal (Redrawn from Hackett (1981)).*

3.1.1.2. Bacterial Tunnelling

Hackett (1981) described three types of bacterial tunnelling that begin at the Haversian canal of the bone extending to 'fill' the Haversian system until it reaches the cement line. They are linear longitudinal, budded and lamellate tunnels. Linear longitudinal tunnels are mostly longitudinal in nature though horizontal ones can be found (**Fig.15**). The tunnel contents can appear empty once leaching out of redeposited mineral occurs, or filled; appearing streamed in longitudinal section (horizontal line pattern) and spotted in cross section with a pinkish tint under low magnification. The diameter of these tunnels has a wide range of sizes as they branch and dilate from the mid-zone of the cortex to the cortical surface, with diameters ranging from 5-10µm to 50µm or more the closer one gets to the external surface. The formation of these tunnels is hindered by the cement line and mineral redeposition is present from approximately 5µm in diameter, which is often lost at 20µm (Hackett, 1981).

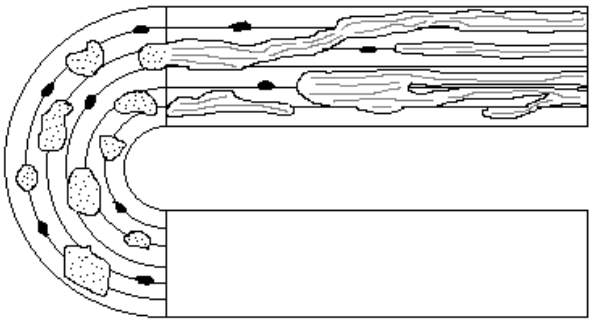


Figure 15: *An outline illustrating linear longitudinal tunnels in transverse (semi-circular image) and longitudinal section (horizontal image) (Redrawn from Hackett (1981)).*

Budded tunnels are frond-like tunnels (look like a large divided leaf) that are found along the Haversian canals where they extend to fill the Haversian system (**Fig. 16**). They measure 30µm or more in diameter with side shoots at acute angles budding off at irregular intervals of approximately 80-90µm (Hackett, 1981). These tunnels expand and appear palmate (having several lobes, 5-7, radiating from one point) containing contents that are streamed/spotted, demineralised and appear as a pinkish tint under low magnification. As the bacterial microorganism that penetrated the bone begins to grow, the denser the contents become. This allows for more mineral to be redeposited as cuffing around the tunnel and for more demineralization of the tunnel and the surrounding bone to occur (Hackett, 1981).

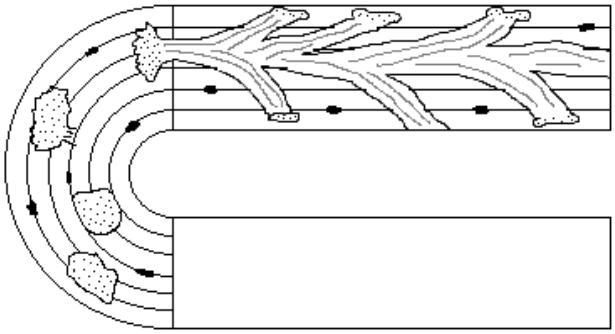


Figure 16: *An outline illustrating budded tunnels in transverse (semi-circular image) and longitudinal section (horizontal image) (Redrawn from Hackett (1981)).*

Lamellate tunnels are found most abundantly towards the cortical surface and may appear first near or away from the Haversian canal (Hackett, 1981). These tunnels are large, rounded and curved to conform to the circular pattern of the lamellae with a mono-lamellate or poly-lamellate organisation with size ranging from 10-20µm but can become as large as 250µm (**Fig. 17**). The appearance of this lamellation in the tunnels is thought to be from the solution and removal of the non-collagenous organic matrix which exposes but does not destroy the collagen fibres of the lamellae which over time is accompanied by the removal of some dissolved bone mineral. As expansion of the early tunnels cross the lamellate pattern in the Haversian system into the horizontal lamellae, the margins become very refractile and mineral redeposition increases until a narrow rim is left which prevents further intrusion. These initial refractile rims are uniform in thickness from 3-5µm. The contents of these tunnels contain fibrils that are thicker, darker and less regular than collagen fibre bundles with the outer and inner zones of bone having a mosaic pattern in transverse section. In longitudinal section, the lamellation of the tunnels is most often seen more clearly with the shapes of the tunnels appearing more round and squarish in nature (Hackett, 1981).

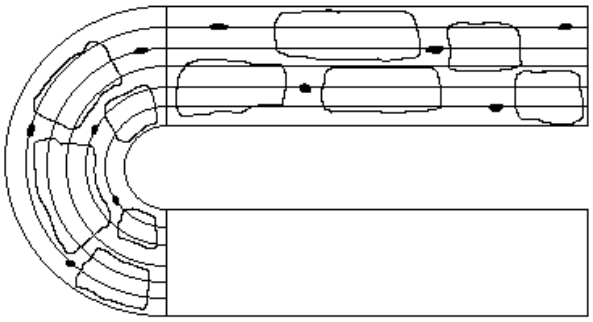


Figure 17: *An outline illustrating lamellate tunnels in transverse (semi-circular image) and longitudinal section (horizontal image) (Redrawn from Hackett (1981)).*

Table 1: *A summary of the types of tunnelling and their respective characteristics.*

Type of Tunnelling	Wedl (Hackett, 1981)	Wedl Type 1 (Trueman and Martill, 2002)	Wedl Type 2 (Trueman and Martill, 2002)	Linear Longitudinal (Hackett, 1981)	Budded (Hackett, 1981)	Lamellate (Hackett, 1981)
Size (µm)	5-10	10-15	5	5-10	30-60*	10-60*
Cement line	Yes	Yes	Yes	No	Yes	Yes
Branched	Yes	Yes	Yes	Yes	Side shoots from 80-90 µm	?
Mineral Redeposition (Cuffing)	No	No	No	Yes	Yes	Yes
Refractile Rim (Hypermineralized)	No	No	No	Yes	Little/No	Yes
Lamellation	No	No	No	No	No	Yes
Contents	Empty	Empty	Empty	Fibrils, Empty\Streamed	Streamed\Empty	Fibrils
Distribution	Random	Random	Random	Microanatomy	Microanatomy	Microanatomy
Microorganism	Fungal	Fungal	Fungal	Bacterial	Bacterial	Bacterial

*Larger tunnels up to 100-250 are occasionally found

3.1.1.3. Other

One other type of tunnelling that is described is not associated with buried remains but rather with remains that are associated with an aquatic environment. This type of tunnelling is described by Davis (1997) and Bell *et al*. (1996) and is produced by Cyanobacteria, also known as blue-green algae. These organisms are found most frequently in low energy, tropical shallow-marine environments and are influenced by environmental factors such as sunlight, current strength, temperature and salinity (saltiness) - most likely controlled by water depth (Davis, 1997). Bell *et al*. (1996) assessed these tunnels in teeth where they were found to be 5-7µm in diameter extending peripherally to a maximum depth of 300µm. No demineralization or remineralization was associated with the inner exposed surfaces of the tunnels. Davis (1997) on the other hand assessed these tunnels in bird bones where they were found in modern, archaeological and palaeological specimens. They were described as being similar to Wedl tunnels, found mostly parellel to the periosteal surface with a diameter of approximately 200-250µm. Due to the fact that Wedl tunnels are described as generally being perpendicular to the periosteal surface, the term Hackett tunnelling is used to describe these tunnels.

3.1.2. Microcracks

In diagenetically altered bone there are two types of cracks that can be present. Large cracks that are found radiating through large parts of the bone and microcracks that are on the scale of the Haversian systems (Jans *et al*., 2002). The larger cracks are generally associated with non-diagenetic factors such as physical stress due to changes in moisture or temperature in the environment (Grupe and Dreser-Werringloer, 1993) and processing or handling of the sample (Bell, 1990; Jans *et al.*, 2002). Microcracks on the other hand are directly linked to diagenetic alterations and are thought to be caused by the loss of the organic mineral component of the bone which causes shrinkage (Piepenbrink, 1989; Smith *et al.*, 2002), the disintegration of the microstructure due to the deposition of the calcium carbonates (Piepenbrink, 1986) and from the remineralisation processes (Piepenbrink, 1989; Grupe and Dreser-Werringloer, 1993). Research by Bell (1990) revealed that these cracks found in diagenetically altered bone follow both natural and non-natural fracture planes, providing insight into the orientation of the diagenetic process.

3.1.3. Birefringence

The term birefringence is used when looking at a section of bone under a polarised lens. Polarised light microscopy creates an image based on the sample's ability to refract light at multiple indices (Maggiano *et al*., 2009). This is done by normal light passing through a polarizing filter (polarizer), through the sample and then through another filter (analyzer) orientated 90° to the first. This polarized light allows for a specific pattern to become visible consisting of alternating bright and dark bands (Jans *et al.*, 2002; Maggiano *et al.*, 2009) (**Fig. 18**). These dark radial bands lie at right angles to each other forming a Maltese cross over the circular lamellae found within a Haversian system (Cook *et al*., 1962). In addition the adjacent corresponding lamellae display a series of fine alternating light and dark circumferential bands or striations which are commonly attributed to the arrangement of the collagen fibres.

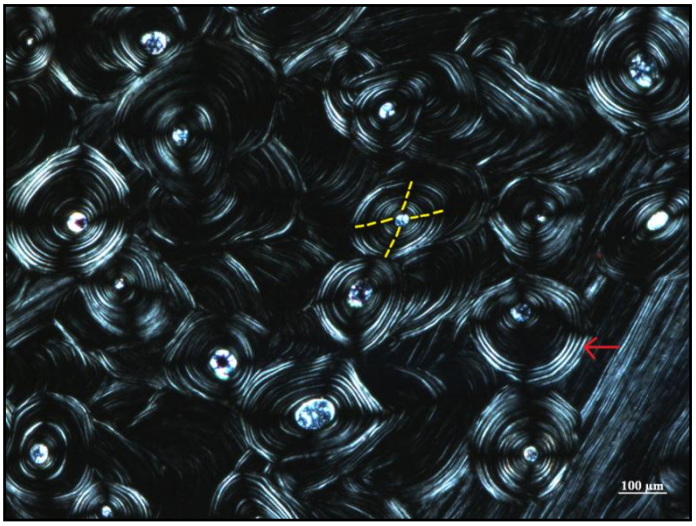


Figure 18: *A microscopic polarized image of Haversian bone displaying the alternating bright and dark bands of lamellae (red arrow) with the Maltese cross (yellow lines) formation of the Haversian system, 100X magnification.*

The overall variations in positive and negative birefringence in bone is due to the quantity and orientation of the collagen fibres, the presence of the bone mineral and the orientation of the section (Giraud-Guille, 1988). A positive birefringence is caused by the collagen fibres found in more of a transverse orientation (Bromage *et al*., 2003) or increased density compared with the adjacent lamellae (Marotti, 1993). A negative (reduction or absence of) birefringence is indicative of deterioration of collagen and/or the loss of orientation of hydroxyapatite crystals (Schoeninger *et al*., 1989).

3.1.4. Inclusions and Infiltrations

When analysing diagenetically altered bone, a section is often described as 'dirty' or stained. This is due to the presence of inclusions and infiltrations which are not normally found in bone. Inclusions are defined by Garland (1989) as the presence of externally derived material lying within the available bone spaces such as Haversian canals, osteocyte lacunae, medullary cavities, trabeculae and canaliculi. There are two types of inclusions found: biological and mineral (Garland, 1989). Biological inclusions are identified as externally derived material lying within the available bone spaces and mineral inclusions are identified with the use of staining under light microscopy or as birefringent material lining the inner surfaces of the bone spaces. Examples of inclusions include fungal cells, hyphae, rhizomorphs, bacteria, insect parts, calcite crystals, framboidal pyrite and sand to name a few (Garland, 1989; Jans *et al.*, 2002; Hollund *et al.*, 2011). Infiltrations are described by Garland (1989) as the presence of unrelated material within the bone substance itself which under low magnification is seen as a granular appearance to the section and under high magnification looks like the bone matrix has been replaced by non-osseous derived material. As collagen is lost from the bone, the hydroxyapatite which has a high affinity for amino acids, allows species of endogenous and exogenous origin to take up residence (Hedges, 2002). This is often visible as staining in the bone section (Jans *et al*., 2002). Major contributors that have been identified that can cause staining are microorganisms - particularly fungi, organic (humic) and inorganic (metals) constituents of soil. For example pink, red, black and violet-blue stains are related to fungal activity due to the presence of acid metabolites from the saprophytic microorganisms and the fact that fungi prefer acidic environments (Piepenbrink, 1986). Dark reddish brown stains are suggested to be associated with humic substances (Hedges, 2002; Jans *et al*., 2002), red-orange and brown stains are caused by iron oxides, and black and grey stains are a result of manganese oxides and oxyhydroxides (Reiche and Chalmin, 2008; Hollund *et al*., 2011).

Overall the histological integrity of bone is dependent upon a number of procedures which affect the bone in different ways. The effects of these processes can be variable as each process is dependent on the factors present within the burial environment thus affecting the degree of preservation of the bone.

3.2. Factors that affect Diagenesis

The factors that affect diagenesis are categorised as either intrinsic or extrinsic (Von Endt and Ortner, 1984). Intrinsic factors refer to those that take place within the bone as well as to the

chemical structure and reactivity of the tissue, while extrinsic factors are those which occur in the immediate environment of the bone after death (Von Endt and Ortner, 1984; Baxter, 2004). These intrinsic and extrinsic factors can either influence the process of diagenesis positively or negatively.

Factors that affect the diagenetic alterations of bone positively include high temperatures, a more acidic pH, an increase in water movement due to soil type and a smaller bone size to name a few. High temperatures, highly conductive soils such as sands and gravels which increase water movement and a more acidic pH soil increases the rate at which the dissolution of the inorganic matrix occurs (weakening of the protein-mineral bond) (Von Endt and Ortner, 1984; Hedges, 2002; Baxter, 2004; Dent *et al.*, 2004). This dissolution is further dependent on the size of the bone as it determines the degree of additional water movement within the bone. For example, the smaller the bone the easier water and minerals can penetrate into it enhancing degradation by causing an increase in the weakening of the protein-mineral bond, the destruction of more protein and an increase in the leaching of products during demineralization and mineral redeposition (Von Endt and Ortner, 1984).

Factors that affect the diagenetic alterations of bone negatively include a decrease in oxygen, the presence of heavy metals, the presence of staining, abundance or reduction in water availability and a decrease in temperature to name a few. The microorganisms involved in diagenesis are very meticulous and thus need favourable conditions to grow within the bone. A number of these factors listed above prevent this. For example, fungi and aerobic bacteria responsible for microbial attack need oxygen to survive (Forbes, 2008). A decrease in the amount of oxygen present would therefore prevent the microorganisms from tunnelling (Hollund *et al*., 2011). Other factors that specifically inhibit microbial attack include low temperatures, the presence of staining-especially by humic acids or irons, environments that are extremely wet or waterlogged, and well drained soils as the microorganisms need the exact amount of moisture to thrive (Hackett, 1981; Nielsen-Marsh and Hedges, 2000; Hedges, 2002; Hollund *et al.*, 2011).

Materials and Methods

For this project a permit for analysis (Ref: 9/2/228/0096) was obtained from the South African Heritage Resources Agency (SAHRA) for the period 03/09/2012 – 01/10/2013 which fell under the National Heritage Resources Act (Act 25 of 1999). This permit allowed for samples of bone to be taken from 50 individual coffins stored at AVBOB. The sampling process was random and depended on the identification or presence of the femora. The demographics of the individuals selected for this research, as described in their biological profiles were summarised in **Table 2**. Information regarding their estimated sex, age-at-death and stature is present as well as the appearance of their skeletal remains. Due to the poor preservation of the skeletal remains, interpretations of sex, age and stature could not be estimated for all the individuals. Interpretations of ancestry in particular were not possible for any of the individuals because the landmarks on the skull, commonly used for the estimation of ancestry were too poorly preserved and as such could not be used. Twenty of the fifty samples that were selected for this project are not described in **Table 2** due to two reasons. Firstly, there were no biological reports for these individuals as their exposed and protruding skeletal remains were exhumed and rescued before a full scale investigation was launched. Secondly, certain individuals were represented only by photographic reports of their remains as time constraints necessitated the removal of these remains before anthropological analyses could be completed. Most of the individuals in this study were considered males, older than 16 years of age, ranging in stature from 1.53m to 1.83m (mean value of 1.69m) with pieces of canvas, blanket and cow hide wrapping associated with their graves. The morphology of the individual's bones varied with majority of the bones being poorly preserved, having a blue discolouration to their external cortical surfaces, with a few individuals having bones that appeared cemented. Some individuals also had dark curly hair present on their skulls with one individual having shovel shaped incisors.

Table 2: *Sex, age, stature (m), preservation of skeletal remains and items associated with the graves of the selected sample.*

Burial no.	Sex	Age	Stature (m)	Preservation of Skeletal Remains	Items Associated with Grave
GR10	*	>16	1.57	Blue discolouration	Dark curly hair
GR18	Male	>25	1.72	Blue discolouration	Cow hide wrapping, canvas and dark curly hair
GR20	*	>16	1.65	Blue discolouration	Canvas and dark curly hair
GR21	*	>25	1.83		Canvas and dark curly hair
GR23	*	>17	1.77	Blue discolouration	Canvas and dark curly hair
GR24	*	>20	1.72	Blue discolouration	Canvas
GR25	Male	>17	1.80	Shovel shaped incisors	Dark curly hair
GR27	Male	>20	1.80	Blue discolouration	
GR31	*	>20	1.71	Blue discolouration	
GR32	Male	>25	1.77		Dark curly hair
GR34	*	>25	1.70	Blue discolouration	Canvas and dark curly hair
GR36	*	>17	1.61	Blue discolouration	Canvas and dark curly hair
GR37	*	>25	1.61	Blue discolouration	Canvas
GR38	*	>16	1.61	Blue discolouration	Canvas
GR49	*	*	*	Blue discolouration	
GR54	*	>25	*	Blue discolouration	Canvas
GR84	*	>25	1.65	Blue discolouration	Blanket
GR86	*	>12	*		Canvas
GR88	*	*	1.53	Blue discolouration	
GR89	*	>17	1.83	Blue discolouration	
GR96	*	>12	1.68	Blue discolouration	
GR100	*	*	1.80	Blue discolouration	Canvas
GR103	*	>17	1.61	Blue discolouration	
GR113	*	*	1.57		
GR116	*	>16	1.76		Dark curly hair
GR130	*	>18	1.78	Cemented	Blanket
GR136	Male	>17	1.68		
GR152	*	>16	*	Cemented	
GR153	*	>17	1.69		Dark curly hair
GR158	*	>16	1.61	Cemented	

***** Poor preservation prohibited estimations

1. Histological Integrity

1.1. Bone Sampling and Processing

Fifty coffins, where the femur could be identified, were selected and 5cm whole portions of bone were removed from the diaphysis using a hacksaw. A ground section for each sample collected was prepared according to stipulations by Maat *et al*. (2001) for archaeological bone. Cyanoacrylate glue (Bostik Blits Stik superglue) was placed on the anterior surface of the bone in the area from which a section was to be cut near the edge of the bone portion, as well as on the exposed perpendicular (cross section) surface that had been cut previously. After 24 hours of drying, a 4-5mm section of bone was cut transversely with a handsaw into the anterior surface of the bone portion that had been glued. The cut section was removed and glue was placed on the newly cut surface and left to dry overnight. The section was manually ground on both sides using P_{220} waterproof abrasive paper in a mixture of distilled water and Jik to smooth/polish the surfaces. Jik is household bleach cleaner that assists in degreasing the section, preventing an oily residue appearance under light microscopy. The ground section was washed thoroughly using a small paint brush in a glass Petri dish filled with a mixture of distilled water and Jik. This process was repeated twice with another Petri dish containing clean solution and left to dry overnight. The ground surface of the section that was the most evenly polished was glued to a clean slide with cyanoacrylate glue and a weight was placed on top of it for two hours to ensure that the section was adhesively attached to the slide. Paper was placed between the section and the weight to prevent them from sticking to one another. After two hours, the surface of the section that had the weight on top of it was ground while still attached to the slide until it was opaque. Two hours was ample time to ensure that the glue had set sufficiently to hold the bone section to the glass slide during wet grinding because if it was left longer the glue may have lost too much flexibility causing the section to peel off the glass slide during further grinding (Maat *et al*., 2001). The section attached to the slide was thoroughly washed twice, as described previously, with a mixture of distilled water and Jik, left to dry overnight and mounted with Entellan.

Macroscopic images were taken of the sections using a Nikon SMZ 1500 Stereomicroscope at 5X magnification to compare and illustrate the degree of cortical loss between the sections as each section varied in cortical thickness due to degradation. These images were also taken to illustrate the presence of infiltrations permeating from the periosteal and endosteal cortical surfaces. Microscopic images were taken using the Zeiss Axiolab at 50X and

100X magnifications to give a visual representation of the histological integrity assessed in the descriptive analysis section.

1.2. Descriptive Analysis

The five main components associated with the histological integrity of bone were qualitatively and/or quantitatively assessed by describing biodegradation (tunnelling), birefringence, inclusions, infiltrations and microcracks, as well as the general histological destructions to summarize the degree of diagenetic change. Using light microscopy the four types of tunnelling, i.e. linear longitudinal, budded, lamellate and Wedl tunnels, were qualitatively assessed by looking at their shape, size and their distribution, abundance and direction of their formation. Shape was assessed by visually comparing the structural relationships of the tunnels described by Hackett (1981) with those found in this sample (**Fig. 19**), i.e. long and elongated or round. Size was assessed using Image J version 1.44p (Rasband, 1997-2008) by measuring the thickness of the tunnel in three random areas to obtain a mean value in Microsoft Excel which was subsequently compared to the sizes summarized in **Table 1** (**Ch. 3, p. 37**) for each tunnel. The distribution, abundance and direction of the formation of the tunnels were visually assessed by determining the amount of tunnels present per Haversian system, whether the tunnels crossed the cement line of the Haversian system, if the tunnels appeared to follow a random pattern of organisation or the microanatomy itself, for example the path of canaliculi joining osteocyte lacunae, and in which direction they associated themselves to the Haversian system, i.e. did they originate from the Haversian canal and radiate outwards or were they associated with the concentric lamellae surrounding the Haversian canal (**Table 1, p. 37** and **Fig. 19**). Using polarized microscopy, the presence of cuffing (mineral redeposition) was also qualitatively assessed for each of the tunnels by visually evaluating the exposed surface areas found within each tunnel. If cuffing was present, an intensely bright white 3-6µm thick birefringent border was present in the exposed surface areas of tunnels that were wider than 5µm thick. If no cuffing was present these exposed surface areas were completely black.

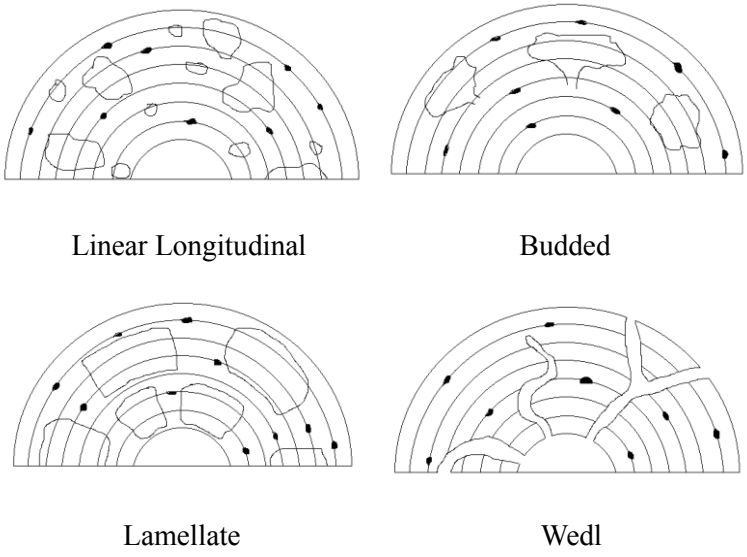


Figure 19: *Diagrams illustrating the shape of the four types of tunnels as seen in cross section (Redrawn from Hackett, 1981).*

The periosteal (outer surface), mesosteal (central portion) and endosteal zones (inner surface) of each slide was evaluated using polarised microscopy at 100X magnification to assess the intensity of birefringence in these regions. The entire area of the section was also evaluated at 50X magnification to get an overall assessment. Each area of interest was categorised as 0 (no birefringence), 0.5 (reduced birefringence) or 1 (perfect birefringence) (Hollund *et al*., 2011). Perfect birefringence was viewed as alternating dark and bright bands of concentric and interstitial lamellae with a Maltese cross in each Haversian system (**Fig. 18, p. 39**). Perfect birefringence was considered absent if the alternating bands and Maltese cross were not evident. The overall birefringence of each slide and its respective zones was inserted into a Microsoft Excel spreadsheet into tables and the overall mode (value occurring most frequently) for each area of the section and the section as a whole was determined.

Inclusions are the presence of externally derived material lying within available bone spaces such as Haversian canals and osteocyte lacunae. Infiltrations are the presence of unrelated material within the bone substance itself which under low magnification is seen as a granular appearance to the section and under high magnification looks like the bone matrix has been replaced by non-osseous derived material (Garland, 1989). Stained bone is also classified as an infiltration and was included in this analysis but from here forth it will be referred to as staining and not an infiltration to prevent confusion. The entire area of each section was qualitatively assessed at 35X, 100X and 400X magnification for inclusions, infil-

trations and staining. Infiltrations were described by location, colour, shape and appearance. Location revealed the pattern of origin of the infiltrations (i.e. originated from the Haversian canal or periosteal surface) and colours such as pink, red, black, violet-blue, red-brown or red-orange were identified to compare with the literature. The shape and appearance of the infiltrations (i.e. round, thin and long, clustered, spotted) were visually assessed and described to distinguish the infiltrations from one another and to illustrate any differences there might be in the organisation of the infiltration by describing what it looked like under high magnification. Staining and inclusions were described as mentioned above by location (i.e. originated from the Haversian canal or periosteal surface) and colour (i.e. pink, red, black, violet-blue, red-brown or red-orange). All infiltration and staining data were inserted into a Microsoft Excel spreadsheet in tables illustrating in which zones (periosteal, mesosteal or endosteal) they were found. An overall percentage for each zone was calculated by dividing the number of samples that had infiltrations and staining present in the periosteal, mesosteal and endosteal zones by 10 (overall sample size of rescued individuals) and 40 (the overall sample size of the buried individuals) respectively. The reason for this sample separation is discussed in the results section. Lastly, the general appearance of the microcracks was qualitatively assessed by identifying in which areas of bone (i.e. interstitial lamellae, Haversian systems) they were present. The microcracks were not quantified because it was difficult to differentiate between the microcracks resulting from sample preparation, the grinding of the bone, and those resulting from postmortem alteration.

To evaluate the overall general histological destruction, infiltrations and cracks were qualitatively assessed in the periosteal, mesosteal and endosteal zones at 100X magnification using the General Histological Index (GHI) developed by Hollund *et al*.(2011) with slight modifications (**Table 3**). The entire area of the section was also assessed at 50X magnification to illustrate the overall general histological destruction. Modifications made to the GHI included inclusion values (≥ instead of >) of approximate percentages of intact bone to allow for a clearer representation, especially in areas such as 50% where infiltrations were found to accommodate 49-51% of the bone section and a clearer interval of interpretation was needed. Other modifications occurred in the descriptions of index 0, 1 and 2 where the descriptions were reworded slightly for a clearer understanding, making it applicable to the specific characteristics (i.e. infiltrations) being assessed. This index gave an overall assessment of the altered versus unaltered microstructure of the entire section and each specific area of interest. For consistency, this analysis was repeated twice by the same observer. All index values were

inserted into a Microsoft Excel spread sheet in tables and the overall mode of each area of the section and the section as a whole was determined.

Table 3: *The General Histological Index values assigned to summarize the degree of diagenetic change (Modified from Hollund et al. (2011)).*

Index	Approximate % of intact bone	Description
0	≤5	No original features identifiable, other than some Haversian canals
1	≤15	Some lamellar structure preserved between destroyed areas, or some lamellar structure preserved by pattern of infiltration
2	<50	Smaller areas of well preserved bone present between destroyed areas
3	≥50	Large areas of well preserved bone present between destroyed areas
4	≥85	Bone is fairly well preserved with minor amounts of destroyed areas
5	≥95	Very well preserved, similar to modern bone

2. Soil Analysis

Analysing the soil in which the skeletal remains were buried is an important factor to assess. Depending on the type of soil present, for example clay soil versus sandy soil, more or less moisture may come into contact with the bone affecting the process of degradation. Depending on the soil's pH this could affect what type of organisms, for example fungi, inhabiting the surrounding area of the bone as they thrive in a specific pH, therefore, affecting how the bone degrades. With this in mind soil samples were collected from the grave wall (roughly from the middle of the excavated grave pit) and floor (from underneath the exhumed skeleton) of two burial pits (GR89 and GR100) at the Crown Mines site. Originally the skeletons would have been buried between 1.2m and 1.8m but due to the mine reclamation process and soil erosion, the depth of the skeletons varied between just below the surface to not more than between 0.8m and 1m below the present surface level (pers. comm. Anton Pelser).

The soil from these two burial pits was tested for soil type and pH. Soil from the four soil samples collected was tested to determine the soil type by using a volume ratio of 2/3 water

to 1/3 soil which was vigorously mixed for a few minutes and left to stand for 24 hours. Layers of sand, silt and clay from the suspended soil settled in this particular order from the bottom of the bottle which was used to determine the soil type by estimating the percentage of each layer present. Additional soil was taken from the four soil samples mentioned previously and were tested to determine pH using two solutions, neutralised tap water and distilled water to confirm the result. 1.5g of calcium chloride ($CaCl_2$) was dissolved in 1 litre of each solution. Soil and solution were then mixed in a 100ml bottle for an hour in a ratio of 1:5 respectively. The addition of salt to the solutions provided calcium ions (Ca^{2+}) which replaced the hydrogen ions (H^+) on the soil particles making the concentration in the solution closer to that found in the field (Anon, 1997).

Not only were the soil samples assessed by type and pH but also chemically to determine which chemical elements were present to compare with the chemical elements found in the bone sections described below. A small amount of soil from one sample of each grave, GR89 and GR100, varying in particle size was mounted onto aluminium stubs with double sided carbon tape. Each stub was sprayed with nitrogen to remove any loose soil present before being coated with carbon using the EMITECH K950X carbon coater to create an electron conductive surface. As the particles of soil were not flat in nature, four coats of carbon were sprayed onto the soil from one angle and four coats from another angle to cover the particles effectively. A broad spectrum analysis was done on 5 random soil particles from each sample using a JOEL JSM-5800 LV scanning electron microscope fitted with a Thermo Scientific electron dispersive spectrometer (SEM-EDS) at an excitation voltage of 20 keV, dead time of 40% and cycle time of 100 live seconds. Using Microsoft Excel, the weight percentages obtained for each of the chemical elements from the five particles taken from each soil sample were averaged. An overall mean weight percentage was subsequently determined from both soil samples and their results were displayed in bar graphs.

3. Chemical Analysis

In this study a chemical analysis of the Crown Mines bone samples was completed because it is known that the breakdown of bone involves changing the bone's chemical make-up which was of interest to this study. Additionally, determining whether the macroscopic blue discolouration present on the periosteal surface was chemically associated due to acid mine drainage was also of importance. Six of the fifty portions of bone used for the analysis of this project were selected for chemical analysis. Four portions of bone were randomly selected and two portions of bone that corresponded to their grave soil analysis samples were included

to compare the data and determine if there had been a transfer of elements from the soil to the bone. A 3-4mm section was cut transversely into the anterior surface of the bone with a hand-saw, adjacent to the area where the previous section was removed. Each section was ground according to stipulations by Maat *et al*. (2001) for non-archaeological bone. This grinding technique was similar to the grinding technique described previously for archaeological bone, however, no cyanoacrylate glue was used because very thin sections were not needed, therefore, reducing the possibility of the section falling apart during the grinding process. In addition to this, Jik was not added to the distilled water to prevent contamination of the section by sodium hypochlorite. Once grinding was complete, the section was washed twice in two Petri dishes, as described previously, in distilled water and left to dry for 3 days.

Each section was mounted onto aluminium stubs with double sided carbon tape and coated twice with carbon using the EMITECH K950X carbon coater to create an electron conductive surface. Since the sections were thick the flat horizontal surface of the section was grounded to the stub by placing conducting carbon paint (DAG) on two vertical surface areas of no interest. A broad spectrum analysis was done on the periosteal, mesosteal and endosteal zones of each section using a JOEL JSM-5800 LV scanning electron microscope fitted with a Thermo Scientific electron dispersive spectrometer (SEM-EDS) at an excitation voltage of 20 keV, dead time of 40% and cycle time of 100 live seconds. Using Microsoft Excel, the weight percentages of each of the chemical elements obtained for the periosteal, mesosteal and endosteal zones of all six bone samples were averaged and an overall mean weight percentage was determined for the periosteal, mesosteal and endosteal zones. Similar to this, the overall mean weight percentages for the two grave soil samples, corresponding to their respective bone samples, were determined. Each chemical element and their corresponding mean percentage was displayed in bar graphs for visual illustration.

Results

Macroscopically, all the cross sections of bone were diagenetically altered to some degree, with some sections being more degraded than others. The extent of degradation of each section was characterised by the loss of bone microstructure and the amount of discolouration (infiltrations) present. One section, GR136, was completely degraded with almost no intact bone identifiable (**Fig. 20**) while another section, GR79, had large degraded spaces present in the mesosteal and endosteal zones (**Fig. 21**). Overall, the cortical thickness of the anterior aspect of each bone varied depending on the extent of degradation present, with majority of the sections displaying a reduction in cortical thickness. Majority of the sections displayed an infiltrated periosteal surface while a number of sections displayed an infiltrated periosteal and/or endosteal surface as illustrated by the dark discoloured areas (**Fig. 22**). It is important to note that during bone sampling and processing many of the sample's periosteal surface was lost due to it being severely degraded, becoming detached during sectioning with the hacksaw. Histologically, this was illustrated by the loss of circumferential lamellae present along the periosteal surface in majority of the sections. This therefore resulted in bone cross sections displaying a discontinuous, less infiltrated periosteal surface than was originally present *in situ*.



Figure 20: *Macroscopic cross section of completely degraded bone from GR136, 5X magnification.*



Figure 21: *Macroscopic cross section of GR79 illustrating the large open spaces in the mesosteal zone, 5X magnification.*

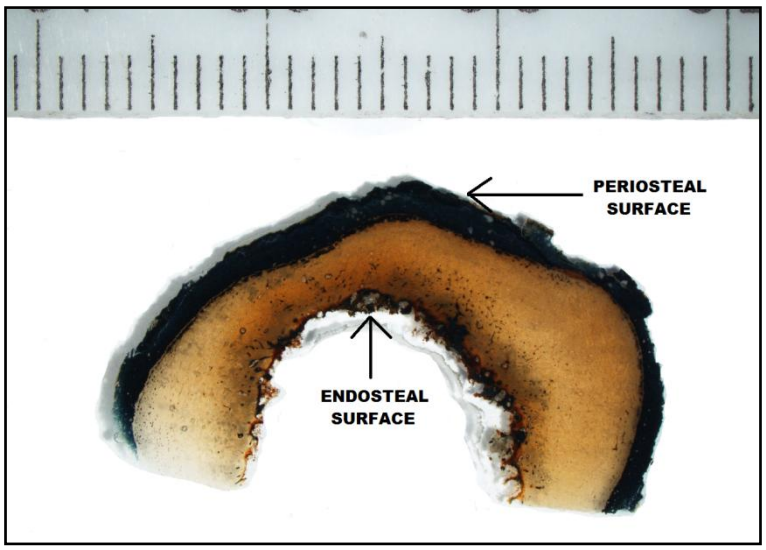


Figure 22: *Macroscopic cross section of the infiltrated periosteal and endosteal surfaces of GR31, 5X magnification.*

1. Histological Integrity

Histologically, all the sections from the Crown Mines displayed a variety of diagenetic alterations such as different types of infiltrations, staining, inclusions, microcracks and overall destruction. Each section varied with regards to the diagenetic alterations present, with some sections displaying more combined alteration than others. For example, some sections displayed only infiltrations whereas others displayed infiltrations, staining and inclusions. Interestingly, no biodegradation (tunnels) was found throughout the entire sample. One sec-

tion, GR79, displayed rather large Haversian canals and holes (resorption spaces) in son to the other sections as illustrated in **Fig. 23**.

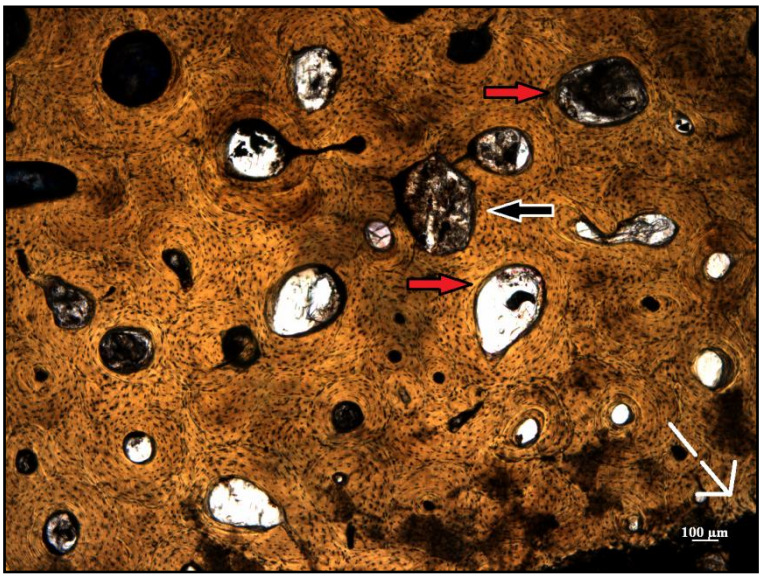


Figure 23: *Histological light microscope image illustrating the large Haversian canals surrounded by concentric lamellae (red arrows) and a resorption space surrounded by no concentric lamellae (black arrow) in the mesosteal zone of GR79, 50X magnification. Dotted white arrow indicating the direction of the periosteal surface.*

Table 4 and **Table 5** show the results obtained for the general histological index and birefringence analyses for the periosteal, mesosteal and endosteal zones, as well as the entire area of the sections for rescued and buried samples respectively. Also included are the samples that displayed infiltrations and staining, and in which region(s) it was found. An overall mode was given for the general histological index and birefringence results, and an overall percentage was given for the infiltrations and staining results. The specimens that had been rescued prior to formal excavations were separated from the other official excavation specimens to illustrate a difference that was noted in the mesosteal zone. The rescued specimens that were protruding through the soil surface had a less intact mesosteal zone with a GHI mode value of 2, as compared to the fully interred specimens with a GHI mode value of 5. This was mainly due to the superficially buried remains having large amounts of infiltrations (50% of the sections) in their mesosteal zone as compared to the fewer infiltrations that were present (20% of the sections) in the deeper buried remains.

In general, the Crown Mines' sections revealed very diagenetically altered periosteal and endosteal zones. The periosteal zone had a GHI mode value of 2 (16-49% intact bone) where 83% of the sections had infiltrations present and 64% of the sections were stained (**Table 6**).

The endosteal zone had a GHI mode value of 3 (50-84% intact bone) where 52% of the sections had infiltrations present and 89% of the sections were stained. The mesosteal zones on the other hand displayed more intact bone with minor diagenetic alteration. They had an overall GHI mode value of 5, 95-100% intact bone, where 44% of the sections were stained and 35% of the sections had infiltrations. It is important to note that neither the infiltrations nor the staining affected the histological organisation of the bones and therefore resulted in an overall birefringence of 1 for the periosteal, mesosteal, endosteal and the entire area of the sections (**Table 6**). **Fig. 24** illustrates this perfect birefringence in the mesosteal zone of one of the samples, GR84. From the entire sample only four sections displayed reduced birefringence: GR130, GR134, GR136 and GR153 (**Table 5**). Section GR136 was discussed previously where the whole section had no original features identifiable other than some Haversian canals (GHI value of 0) which resulted in a reduced birefringence (BF value of 0.5) (**Fig. 25**). It was unclear whether infiltrations or staining were present in this section due to severe diagenesis. GR153 was the second most severely degraded section in the sample where overall the section had less than 50% of intact bone present with only small areas of well preserved bone (GHI value of 2) (**Fig. 26**). The histological organisation of the endosteal zone of GR153, GR130 and GR134 was very similar to the overall histological organisation of GR136, resulting in a reduced birefringence (BF value of 0.5). This suggested that the process of degradation was further along in these endosteal zones, with GR153 having 15% or less intact bone present (GHI value of 1), and GR130 and GR134 having 5% or less intact bone present (GHI value of 0) as compared to the other zones of each section which displayed a perfect birefringence (BF value of 1). On the whole, GR153 and GR130 had areas of severely degraded bone with reduced birefringence that took up more than 50% of the entire section which resulted in an overall section birefringence of 0.5. GR134 on the other hand had areas of severely degraded bone with reduced birefringence that took up less than 50% of the entire section and thus resulted in an overall birefringence of 1.

Table 4: *Results of the General Histological Index (GHI), Birefringence (BF), Infiltrations (I) and Staining (S) analysis for the rescued Crown Mines samples displaying mode values for the General Histological Index and Birefringence, and an overall percentage for the Infiltrations and Staining.*

Burial no.	GHI-P	GHI-M	GHI-E	GHI	BF-P	BF-M	BF-E	BF	I-P	I-M	I-E	S-P	S-M	S-E
BL4	2	2	2	2	1	1	1	1	√	√	√	√		√
BL12	2	2	3	3	1	1	1	1	√	√	√		√	√
BL13	2	3	3	3	1	1	1	1	√	√	√	√		√
BL24	1	2	2	2	1	1	1	1	√	√	√			
BL27	3	5	3	4	1	1	1	1	√		√	√	√	√
BL29	1	2	1	2	1	1	1	1		√				
G3	3	4	3	3	1	1	1	1	√		√		√	√
G5	4	4	3	4	1	1	1	1					√	√
G8	2	5	3	3	1	1	1	1	√			√		√
G9	2	5	4	3	1	1	1	1	√			√	√	√
Mode	2	2	3	3	1	1	1	1	-	-	-	-	-	-
Percentage	-	-	-	-	-	-	-	-	80%	50%	60%	50%	50%	80%

Abbreviations: GHI-P, General Histological Index-Periosteal; GHI-M, General Histological Index-Mesosteal; GHI-E, General Histological Index-Endosteal; GHI, Overall General Histological Index; BF-P, Birefringence-Periosteal; BF-M, Birefringence-Mesosteal; BF-E, Birefringence-Endosteal; BF, Overall Birefringence; I-P, Infiltrations-Periosteal; I-M, Infiltrations-Mesosteal; I-E, Infiltrations-Endosteal; S-P, Staining-Periosteal; S-M, Staining-Mesosteal; S-E, Staining-Endosteal.

Table 5: *Results of the General Histological Index (GHI), Birefringence (BF), Infiltrations (I) and Staining (S) analysis for the buried Crown Mines samples displaying mode values for the General Histological Index and Birefringence, and an overall percentage for the Infiltrations and Staining.*

Burial no.	GHI-P	GHI-M	GHI-E	GHI	BF-P	BF-M	BF-E	BF	I-P	I-M	I-E	S-P	S-M	S-E
GR6	2	4	3	3	1	1	1	1	√	√		√	√	√
GR10	3	5	4	4	1	1	1	1				√	√	√
GR18	3	5	2	3	1	1	1	1	√		√	√		√
GR20	2	5	4	4	1	1	1	1	√			√	√	√
GR21	3	5	3	3	1	1	1	1				√		√
GR23	3	3	1	2	1	1	1	1	√		√	√		√
GR24	1	3	2	2	1	1	1	1	√		√	√	√	√
GR25	2	5	3	3	1	1	1	1	√				√	√
GR27	1	3	1	2	1	1	1	1	√	√	√			√
GR31	2	5	3	3	1	1	1	1	√		√	√		√
GR32	3	5	3	4	1	1	1	1	√			√	√	√
GR34	3	4	3	3	1	1	1	1	√			√	√	√
GR36	2	5	3	3	1	1	1	1	√			√		√
GR37	3	5	3	4	1	1	1	1	√			√	√	√
GR38	1	2	1	2	1	1	1	1	√	√	√			√
GR49	1	3	3	2	1	1	1	1	√	√	√	√	√	√
GR54	3	4	3	3	1	1	1	1				√		√
GR71	2	5	3	3	1	1	1	1	√		√	√		√
GR78	2	5	3	3	1	1	1	1	√			√	√	√
GR79	3	4	3	4	1	1	1	1	√		√	√		√
GR80	4	5	3	4	1	1	1	1				√	√	√
GR84	2	5	2	3	1	1	1	1	√		√			
GR85	1	4	3	3	1	1	1	1				√	√	√
GR86	3	3	3	3	1	1	1	1	√		√	√		√

Table 5: *(continued)*

Burial no.	GHI-P	GHI-M	GHI-E	GHI	BF-P	BF-M	BF-E	BF	I-P	I-M	I-E	S-P	S-M	S-E
GR87	1	3	3	3	1	1	1	1	√			√		√
GR88	2	5	3	3	1	1	1	1	√			√	√	√
GR89	2	5	2	3	1	1	1	1	√		√	√		√
GR96	3	5	3	3	1	1	1	1	√				√	√
GR100	2	5	3	3	1	1	1	1	√					√
GR103	3	4	2	3	1	1	1	1	√		√	√		√
GR113	2	4	3	3	1	1	1	1	√		√	√		√
GR116	3	5	3	3	1	1	1	1	√		√	√	√	√
GR119	2	3	3	3	1	1	1	1	√	√		√		√
GR123	3	4	3	3	1	1	1	1	√			√		√
GR130	2	2	0	2	1	1	0.5	0.5	√		√	√		√
GR134	1	3	0	2	1	1	0.5	1	√	√	√	√		√
GR136	0	0	0	0	0.5	0.5	0.5	0.5	?	?	?	?	?	?
GR152	3	5	3	3	1	1	1	1	√			√		√
GR153	2	2	1	2	1	1	0.5	0.5	√	√				√
GR158	2	3	3	3	1	1	1	1	√	√				√
Mode	2	5	3	3	1	1	1	1	-	-	-	-	-	-
Percentage	-	-	-	-	-	-	-	-	85%	20%	43%	78%	38%	95%

Abbreviations: GHI-P, General Histological Index-Periosteal; GHI-M, General Histological Index-Mesosteal; GHI-E, General Histological Index-Endosteal; GHI, Overall General Histological Index; BF-P, Birefringence-Periosteal; BF-M, Birefringence-Mesosteal; BF-E, Birefringence-Endosteal; BF, Overall Birefringence; I-P, Infiltrations-Periosteal; I-M, Infiltrations-Mesosteal; I-E, Infiltrations-Endosteal; S-P, Staining-Periosteal; S-M, Staining-Mesosteal; S-E, Staining-Endosteal.

Table 6: *Summary of the General Histological Index (GHI), Birefringence (BF), Infiltrations (I) and Staining (S) for the entire Crown Mines sample displaying mode values for the General Histological Index and Birefringence, and an overall percentage for the Infiltrations and Staining.*

Analyses	Rescued Samples	Buried Samples	Mode	Mean
GHI-P	2	2	2	-
GHI-M	2	5	5	-
GHI-E	3	3	3	-
GHI	3	3	3	-
BF-P	1	1	1	-
BF-M	1	1	1	-
BF-E	1	1	1	-
BF	1	1	1	-
I-P	80%	85%	-	83%
I-M	50%	20%	-	35%
I-E	60%	43%	-	52%
S-P	50%	78%	-	64%
S-M	50%	38%	-	44%
S-E	80%	95%	-	89%

Abbreviations: GHI-P, General Histological Index-Periosteal; GHI-M, General Histological Index-Mesosteal; GHI-E, General Histological Index-Endosteal; GHI, Overall General Histological Index; BF-P, Birefringence-Periosteal; BF-M, Birefringence-Mesosteal; BF-E, Birefringence-Endosteal; BF, Overall Birefringence; I-P, Infiltrations-Periosteal; I-M, Infiltrations-Mesosteal; I-E, Infiltrations-Endosteal; S-P, Staining-Periosteal; S-M, Staining-Mesosteal; S-E, Staining-Endosteal.

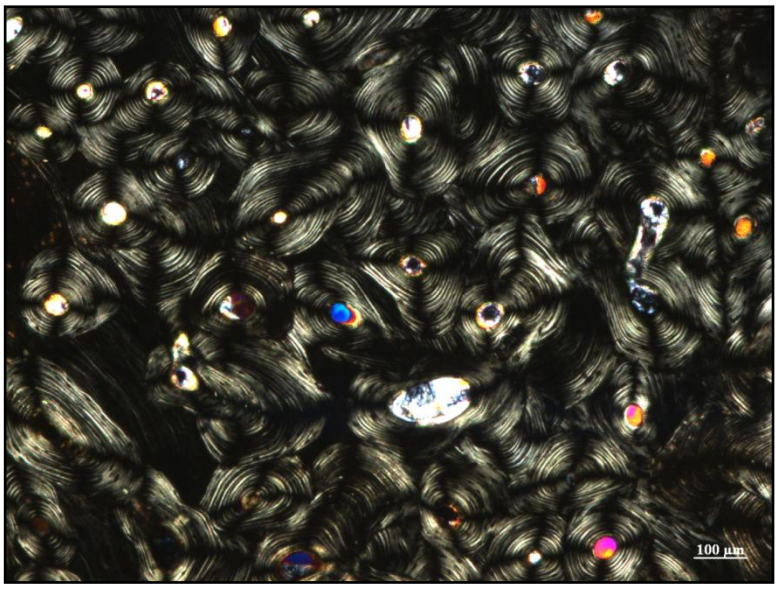


Figure 24: *Polarized image displaying perfect birefringence of the mesosteal zone of GR84 which can be seen by the characteristic bright and dark bands of lamellae and a Maltese cross in the Haversian systems, 100X magnification.*

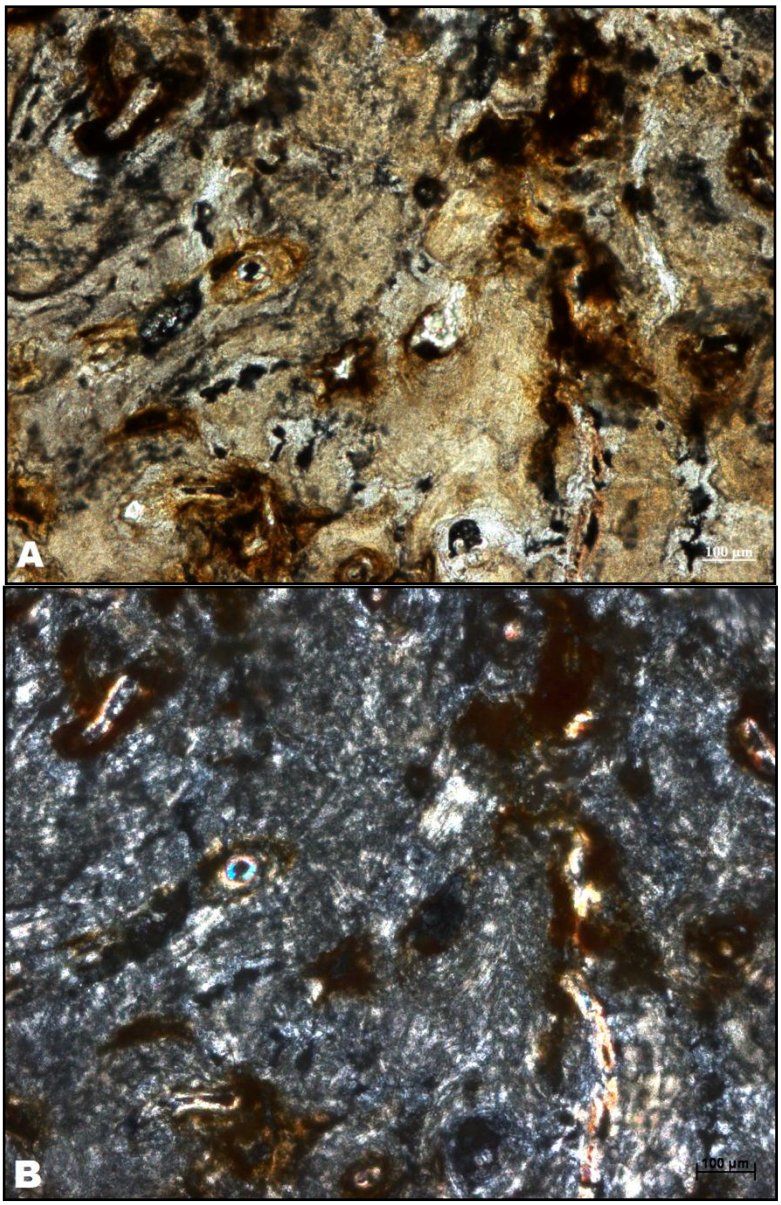


Figure 25: *A: Histological light microscope image. B: Polarized image of GR136 illustrating severe diagenetic alteration in the mesosteal zone which is evident from the severe loss of microstructure, 100X magnification.*

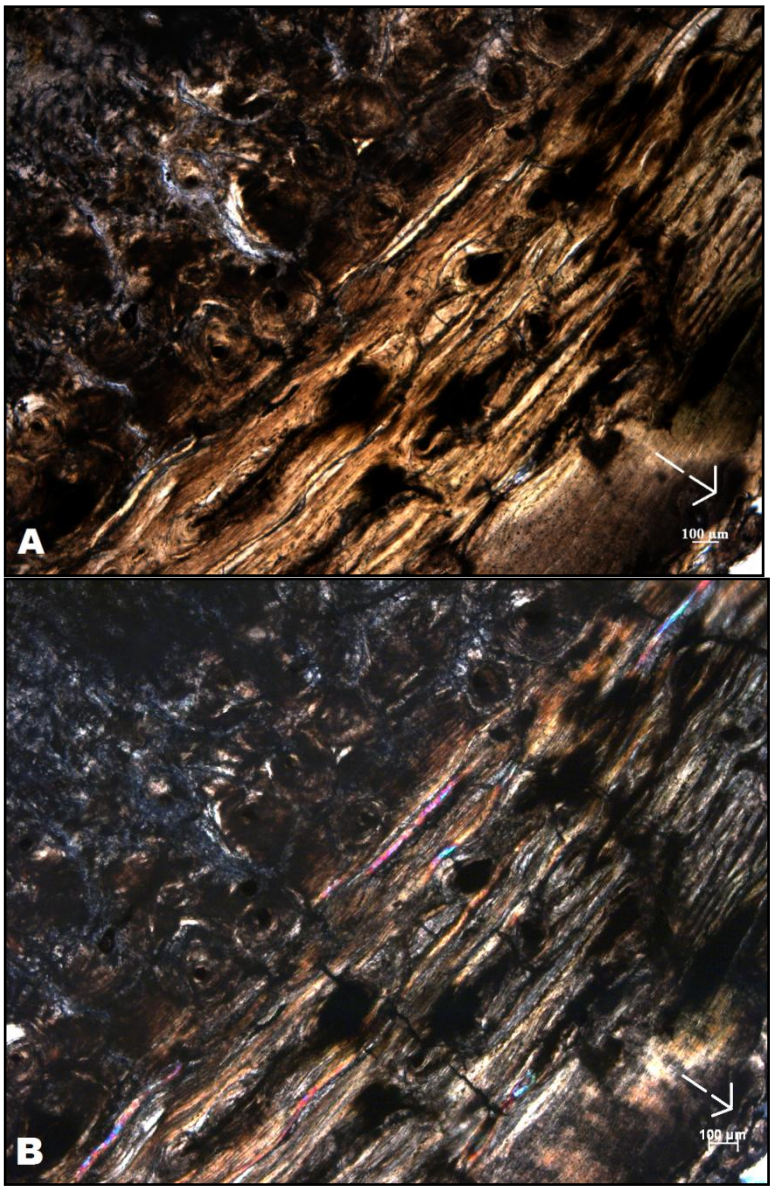


Figure 26: *A: Histological light microscope image. B: Polarized image of GR153 with severe diagenetic alteration of the mesosteal zone characterised by the lack of identifiable histological features, 50X magnification. White dotted arrow indicating the direction of the periosteal surface.*

As previously mentioned, a variety of diagenetic alterations were found in the periosteal, mesosteal and endosteal zones of the Crown Mines' sections. These included infiltrations, staining and inclusions. Infiltrations were found mainly in the periosteal and endosteal zones, with majority in the periosteal zone (**Table 6**). Two main types of infiltrations were identified. The first type of infiltration was spherical in shape, had variable sizes, with a granular appearance at high magnification, displaying green, blue, brown, orange and red colours (**Fig. 27**). The second type of infiltration was long and thin in shape with variable thicknesses, appearing

solid at high magnification with a dark brown or black colour (**Fig. 28**). Both types of infiltrations appeared to radiate/originate from the Haversian canals and/or the osteocyte lacunae in the mesosteal zone which allowed the entire Haversian system to eventually be filled and groupings of infiltrations to be found in the concentric lamellae. Generally the samples displayed majority type one (spherical) infiltrations, especially in the periosteal zone. In this region the entire histological organisation of the bone became filled with infiltrations, resulting in the macroscopically blue discoloured area (**Ch. 1, Fig. 6, p. 9**) and the microscopically infiltrated area (**Fig. 29**). The microscopic infiltrations in the periosteal zone appeared to follow the microstructure of the bone tissue, illustrated as parallel rows of infiltrations along the circumferential lamellae in **Fig. 29**. These infiltrations were not birefringent and did not appear to change the microstructure of the bone although in many areas the infiltrations did obscure visualisation of the bone microstructure, making it almost impossible to determine birefringence.

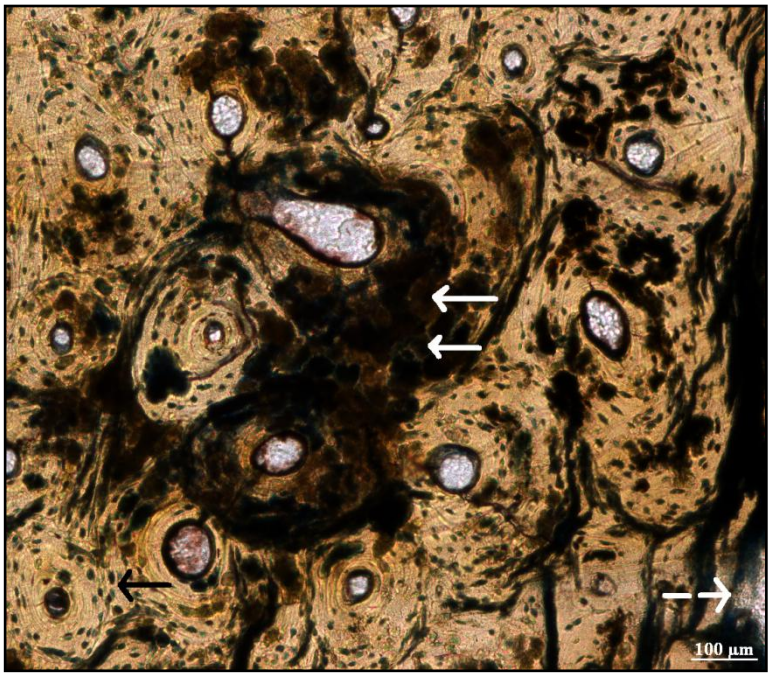


Figure 27: *Histological light microscope image of GR119 displaying the blue-green and brown spherical infiltrations (two of the many infiltrations are indicated by white arrows) in the mesosteal zone grouping in and around Haversian systems with some occurring in smaller groups within neighbouring Haversian systems, 100X magnification. Black arrow illustrating the coloured inclusions present in the osteocyte lacunae and the white dotted arrow indicates the direction of the periosteal surface.*

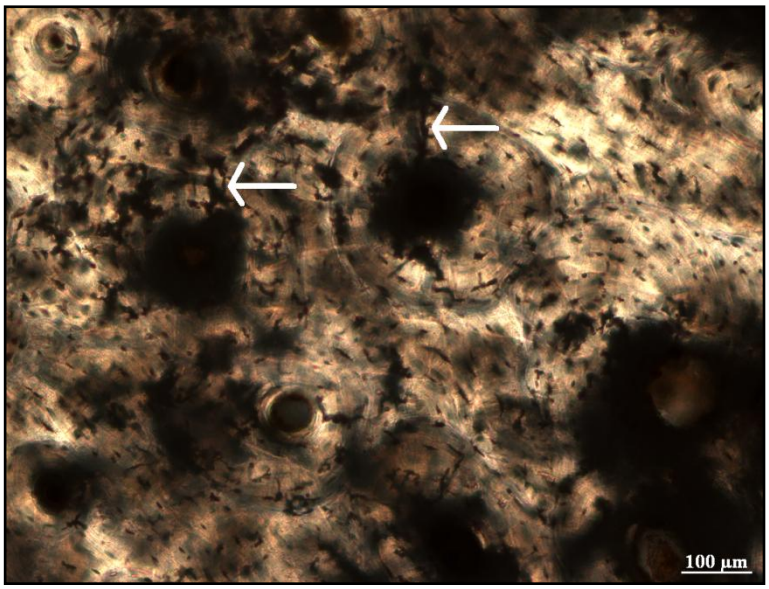


Figure 28: *Histological light microscope image of BL13 focusing on the long, thin dark brown/black infiltrations (two of the many infiltrations are indicated by white arrows) in the mesosteal zone radiating from some Haversian canals and osteocyte lacunae, 100X magnification.*

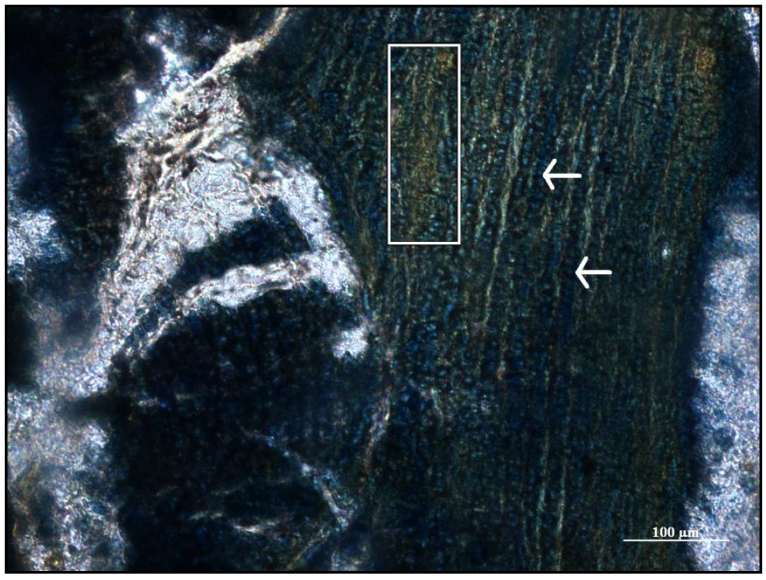


Figure 29: *Histological light microscope image of the periosteal zone of GR158 displaying many blue-green type one infiltrations (two parallel rows of infiltrations are indicated by white arrows) in the lamellae with orange-brown stained bone (white box) beneath the infiltrations, 400X magnification.*

In a few of the sections (GR23, GR24, GR27, GR38, GR49, GR85, GR87 and GR134), a pattern was noted with regards to these periosteal and/or endosteal infiltrations revealing a

progression of diagenesis. **Fig. 30** illustrates that as the blue infiltrations moved further into the intact bone, the remaining bone on the periphery became less intact (GHI value of 1), with reduced birefringence (**Table 5**), almost amorphous in structure, shown by a brown endosteal and periosteal border. Moreover, this movement of infiltrations from the periosteal and endosteal zones often resulted in the infiltrations overlapping in the mesosteal zone or allowed very little intact bone in the mesosteal zone which resulted in a lower mesosteal GHI value, such as GR38 which had a GHI value of 2 (**Table 5**). It was suspected that more sections had this brown, less intact bone border but as mentioned previously, because of the severe state of degradation in this region, many of the samples lost this part of the periosteal zone during bone sampling and processing.



Figure 30: *Macroscopic image of GR134 illustrating the progression of infiltrations and thus diagenesis from the periosteal and endosteal zones, 5X magnification.*

Stained bone was found mainly in the endosteal zone and displayed no change in the microstructure of the bone itself, only its colour. Majority of the stained bone displayed perfect birefringence (GH value of 1) with the exception of areas of severely degraded bone, mentioned previously. Four colours of staining were noted throughout the samples: green, brown, orange and red. The orange stain was found most abundantly (**Fig. 31**) and the green stain was only found in one section, GR153, which was discussed previously (**Fig. 26**). All stained bone appeared to originate from open surfaces such as the periosteal and endosteal surface (orange and red stain), as well as some Haversian canals and inclusions in osteocyte lacunae, staining the Haversian systems within the mesosteal zone (brown stain) (**Fig. 32**). Upon further assess-

ment, it was noted that because the periosteal zones of many of the sections were completely infiltrated, it obscured visualisation of the bone and therefore the stained regions which were only partly visible but not clear for assessment. In **Fig. 29** brown-orange stained bone was seen between areas of complete infiltration in the periosteal zone. This demonstrated that more than 64% (**Table 6**) of the periosteal zones were stained than was previously assessed. Nevertheless, some sections did reveal that the degree of colour change found within the respective regions was related to one another. For example, the bone displayed a dark red stain towards the periosteal surface becoming more orange and eventually brown in the Haversian systems of the mesosteal zone, becoming lighter from its point of origin.

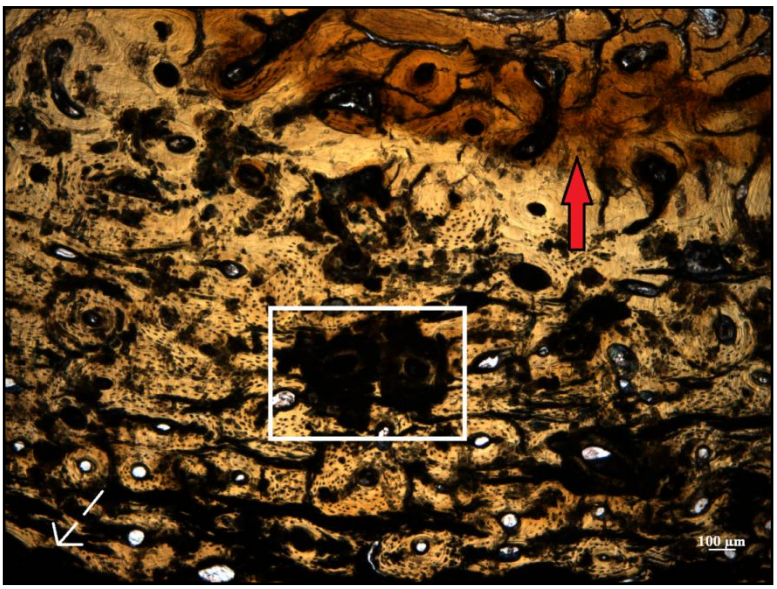


Figure 31: *Histological light microscope image of GR119 displaying groupings of spherical infiltrations (white box) in the mesosteal zone and the orange staining (red arrow) towards the endosteal surface, 50X magnification. White dotted arrow indicating the direction of the periosteal surface.*

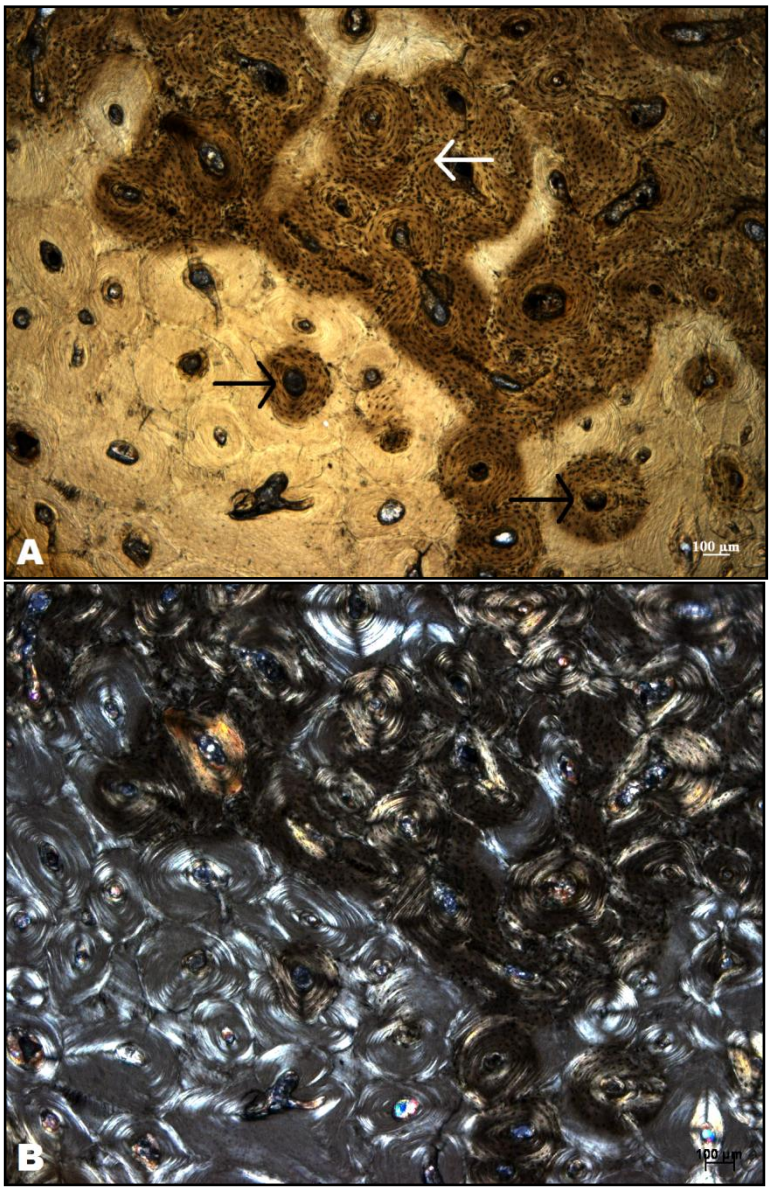


Figure 32: *A: Histological light microscope image. B: Polarized image of GR20 illustrating brown stained bone in the mesosteal zone spreading outwards from the Haversian canals (black arrows) and inclusions in osteocyte lacunae (white arrow), 50X magnification.*

With regards to the assessment of inclusions, there was externally derived material present in all the sections, particularly in the medullary cavity/spongy bone, Volkmann's canals, Haversian canals, osteocyte lacunae and canaliculi. The distribution of the inclusions was very selective, present in some areas of the bone and not others, while a particular pattern of distribution was noted. Stained bone for example in **Fig. 32** had many Haversian systems that were completely filled with brown-orange stain, yet it appeared that the osteocyte lacunae which contained inclusions were primarily found in this stained region only. This was found to be consistent in another section, BL12, where small groupings of round, black inclusions were

present in the medullary cavity/spongy bone closely associated with a thin orange stained rim of bone (**Fig. 33**). In comparison, **Fig. 34** displayed inclusions present in the canaliculi which appeared to spread from the originally filled osteocyte lacunae to their connecting canaliculi, osteocyte lacunae, canaliculi and so forth, whereas **Fig. 35** displayed inclusions in the osteocyte lacunae only, with none found in the canaliculi. These patterns in which the inclusions are distributed appear very random in orientation but for some sections suggest an association with staining. Colours such as red, orange, blue and green observed for the inclusions found in the Haversian canals and osteocyte lacunae were the same as the colours portrayed in the infiltrations within the same section (**Fig. 27**), however, in general, most of the section's inclusions were black (**Fig. 32**, **Fig. 34** and **Fig. 35**).

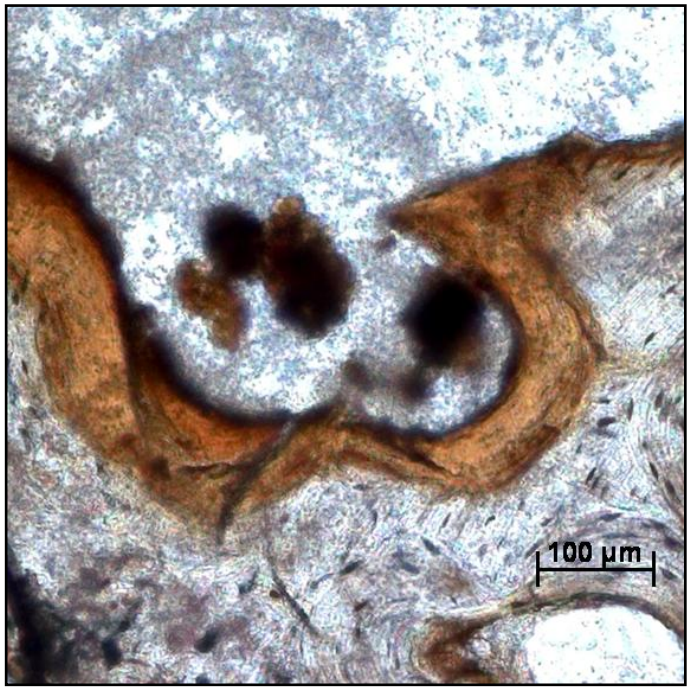


Figure 33: *Microscopic light microscope image of BL12 indicating the round, black inclusions and thin orange stained rim of bone in the medullary cavity/spongy bone, 100X magnification.*

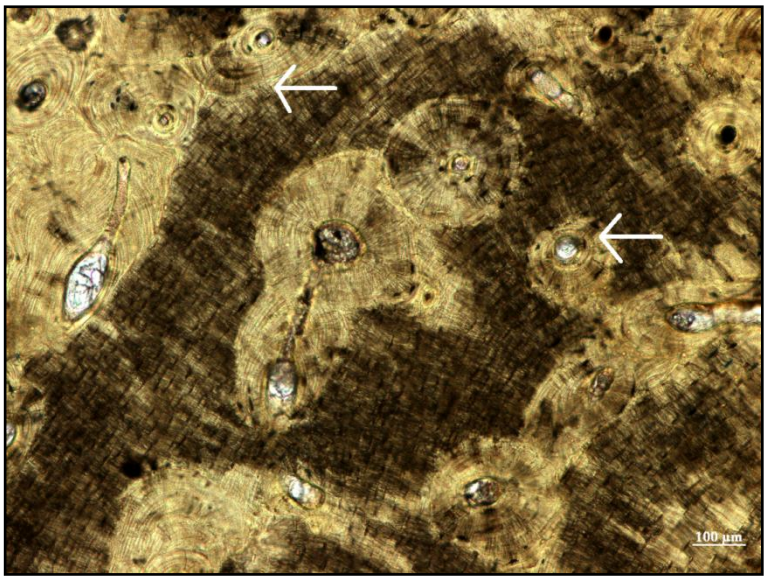


Figure 34: *Histological light microscope image of GR32 illustrating the spread of inclusions from selective osteocyte lacunae, via canaliculi (white arrows) to other osteocyte lacunae in the mesosteal zone, 100X magnification.*

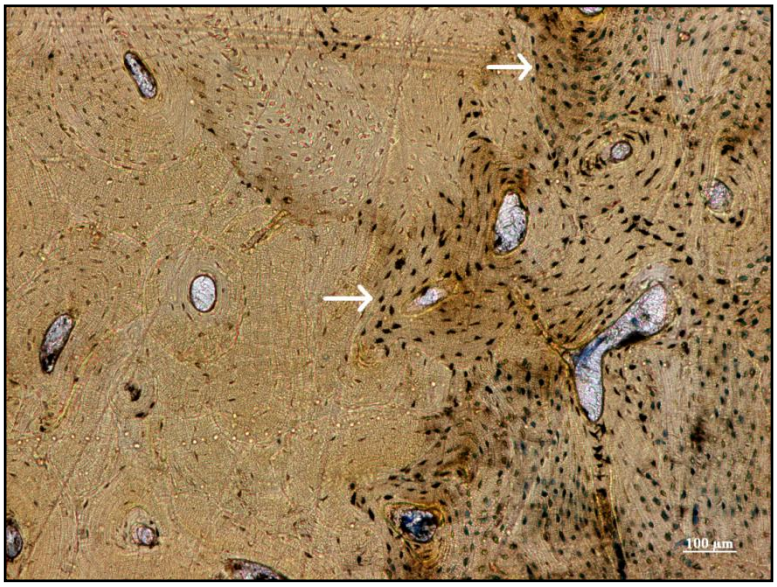


Figure 35: *Histological light microscope image of G8 displaying black inclusions (white arrows) in selective osteocyte lacunae in the mesosteal zone, 100X magnification.*

When assessing the diagenetic alterations of bone, microcracks are known to contribute to this process but cracks are not. Nonetheless, a point is made to mention cracks in this study as they are important in describing the overall general histological destruction of the samples. It was mentioned previously that the periosteal and endosteal zones were the most diagenetically altered, with GHI mode values of 2 and 3 respectively (**Table 6, p. 58**). It is important to note

that this alteration was mainly due to the presence of infiltrations and cracks which affected the overall percentage of intact bone. The cracks present in many of the sections were thought to be associated with bone sampling and processing where due to excessive grinding on sandpaper and not enough support from the glue, the most exposed surfaces, namely the periosteal surface, had large cracks extending into the bone, sometimes completely separating the bone into sections. This is important because some of the GHI values given to the periosteal and endosteal zones may have resulted in a different value if another preparation technique was used which resulted in less crack formation and more intact bone being present.

Importantly, artefact scratches appearing as cracks were also found on the sections which resulted in the inability to assess the extent of microcracking. Microcracks appeared to be located in the concentric lamellae, interstitial lamellae and radiating from the Haversian canals (**Fig. 36**). When histologically analysing the sections, however, it was not known if these microcracks radiating from the Haversian canals were due to diagenesis or due to sample preparation. Therefore the cracking index developed by Jans (2005) was not completed on any of the samples as misinterpretations could have led to inaccurate results.

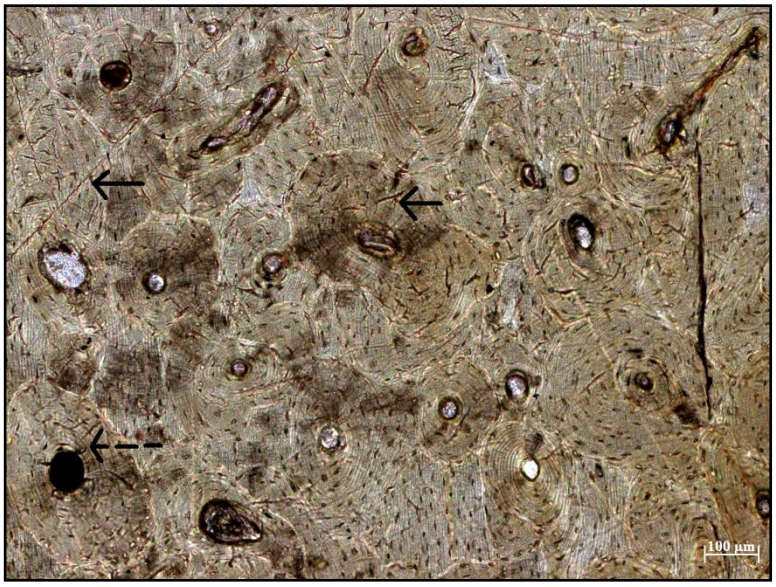


Figure 36: *Histological light microscope image of GR87 illustrating artefact cracking (black arrow) and potential microcracks associated with sample preparation radiating from a Haversian canal (black dotted arrow) in the mesosteal zone, 100X magnification.*

2. Soil Analysis

The soil collected from the wall and floor of the two burial pits at the Crown Mines site was assessed for soil type and pH. All the samples were of the sandy soil type due to the larger estimated percentage of sandy layer present. Sandy soil is characterised as having less than 18% clay and more than 68% sand in 100cm of solum (subsoil layer) (Bruand *et al*., 2007). It is weak in structure, has poor water retention properties, high permeability, high sensitivity to compaction, a small reservoir of bases (buffer capacity) due to low clay and organic contents, and a particle grain size of between 2 and 0.0625mm (Waters, 1992; Bruand *et al*., 2007). The pH of all the samples resulted in a value of 3.5 which is acidic.

3. Chemical Analysis

A broad spectrum analysis was completed on the bone and soil samples obtained from the Crown Mines site. In general fourteen elements were found in the bone samples which included: oxygen (O_2), fluorine (F), magnesium (Mg), phosphorus (P), sulphur (S), calcium (Ca), potassium (K), iron (Fe), molybdenum (Mo), manganese (Mn), sodium (Na), chromium (Cr), aluminium (Al) and silicon (Si) (**Fig. 37**). The major contributing elements, which had the greatest mean weight percentages, were oxygen (38.66%), calcium (33.30%), phosphorus (18.21%) and iron (10.04%). The trace contributing elements included sulphur (1.50%), molybdenum (0.32%), sodium (0.29%), manganese (0.10%), aluminium (0.08%), silicon (0.06%), fluorine (0.05%), potassium (0.04%) and chromium (0.02%). Of these trace elements molybdenum and sodium were the major contributors. The trace elements were represented in a separate graph to compare the contributing weight percentages of the elements (**Fig. 38**).

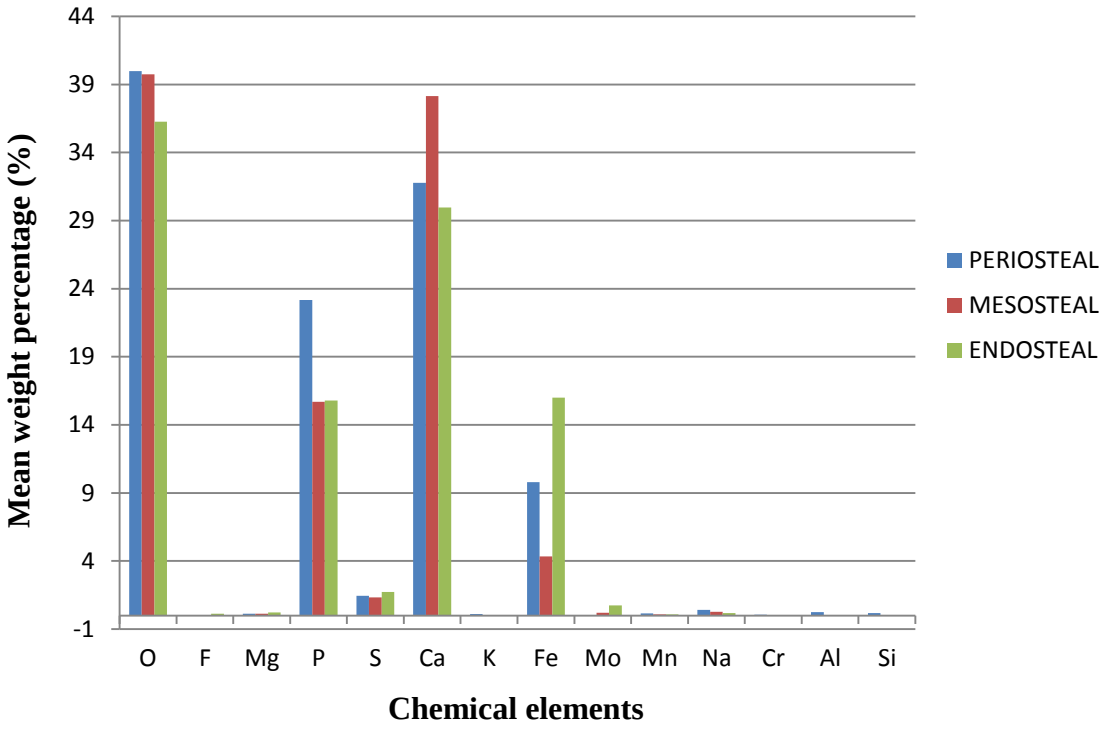


Figure 37: *Bar graph displaying the chemical composition of the periosteal, mesosteal and endosteal zones of the bone samples acquired from the Crown Mines.*

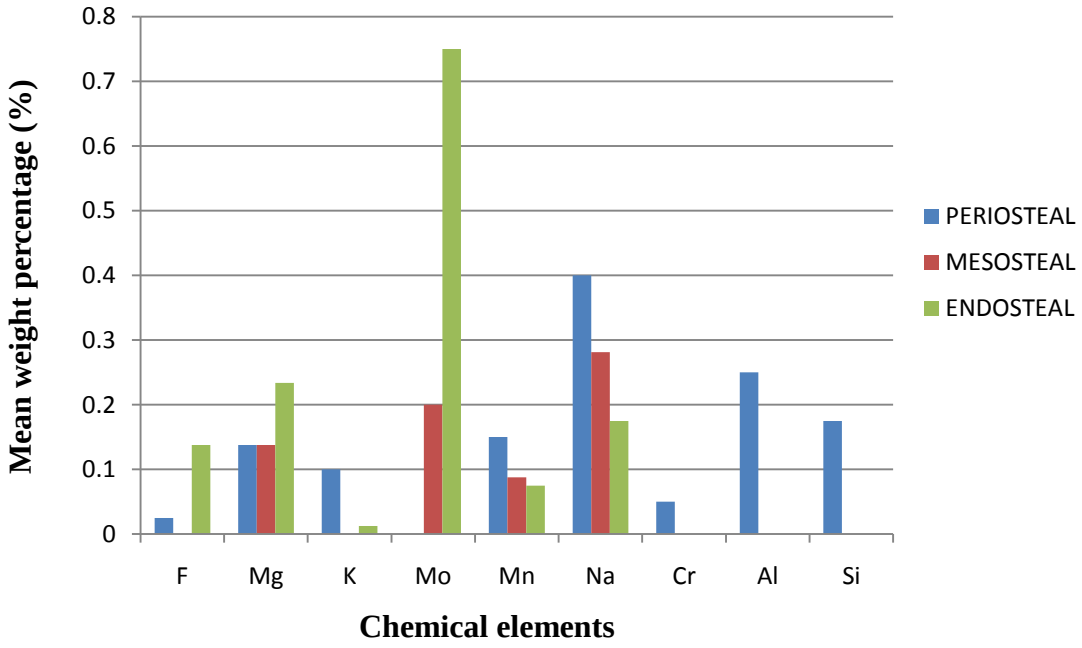


Figure 38: *Bar graph displaying the trace elements contributing to the chemical composition of the Crown Mines bone samples.*

When assessing the chemistry in each respective region (periosteal, mesosteal and endosteal zones) the major chemical elements of the periosteal zone were oxygen (39.98%), calcium (31.78%), phosphorus (23.16%) and iron (9.79%), and the trace chemical elements were sulphur (1.45%), sodium (0.40%), aluminium (0.25%), silicon (0.18%), manganese (0.15%), magnesium (0.14), potassium (0.10%), chromium (0.05%) and fluorine (0.03%) in descending percentages (**Fig. 37** and **Fig. 38**). No molybdenum was found in the periosteal zone. The major chemical elements of the mesosteal zone were oxygen (39.74%), calcium (38.16%), phosphorus (15.69%) and iron (4.34%), and the trace chemical elements were sulphur (1.34%), sodium (0.28%), molybdenum (0.20%), magnesium (0.14%) and manganese (0.09%) in descending percentages. No fluorine, potassium, chromium, aluminium and silicon were present in the mesosteal zone. Lastly, the major chemical elements of the endosteal zone were oxygen (36.27%), calcium (29.96%), iron (16.00%), phosphorus (15.79%), and the trace chemical elements were sulphur (1.73%), molybdenum (0.75%), magnesium (0.23%), sodium (0.18%), fluorine (0.14%), manganese (0.08%) and potassium (0.01%) in descending percentages. No chromium, aluminium and silicon were present in the endosteal zone.

Overall, the periosteal and endosteal zones contained majority of the chemical elements, with the periosteal zone having the most elements present. The periosteal zone contained thirteen of the chemical elements identified, with chromium, aluminium and silicon being only found in this region. Interestingly, molybdenum was only found in the mesosteal and endosteal zones, with the largest amount being present in the endosteal (**Fig. 38**). In comparison, the mesosteal zone in addition to missing chromium, aluminium and silicon as in the endosteal zone, it did not contain fluorine or potassium.

When assessing the chemical composition of the soil, nine elements were identified which included: oxygen (O_2), aluminium (Al), silicon (Si), phosphorus (P), sulphur (S), potassium (K), titanium (Ti), iron (Fe) and molybdenum (Mo) (**Fig. 39**). The major contributing elements which had the greatest mean weight percentages were oxygen (46.30%), silicon (31.85%), iron (10.23%) and aluminium (8.82%), and the trace contributing elements included molybdenum (2.05%), sulphur (1.11%), potassium (0.67%), titanium (0.60%) and phosphorus (0.10%) in descending percentages (**Fig. 39** and **Fig. 40**). When visually comparing the soil elements to those found in the two soil corresponding bone sample elements, there were both similarities and differences present. The soil corresponding bone samples had a very similar chemical composition as compared to the other bones portrayed in **Fig. 37** and **Fig. 38**. The chemical elements oxygen, aluminium, silicon, phosphorus, sulphur, potassium, iron and molybdenum

were present in both the bone and the soil. Titanium was only present in the soil and chromium, sodium, manganese, calcium, magnesium and fluorine were only present in the bone.

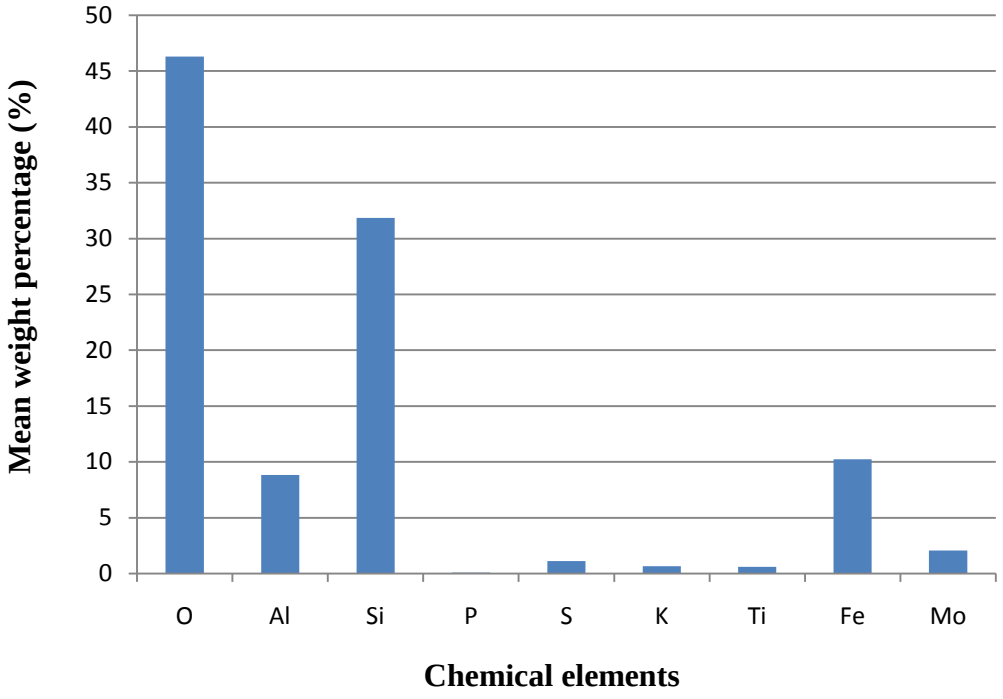


Figure 39: *Bar graph illustrating the chemical composition of the soil from the Crown Mines.*

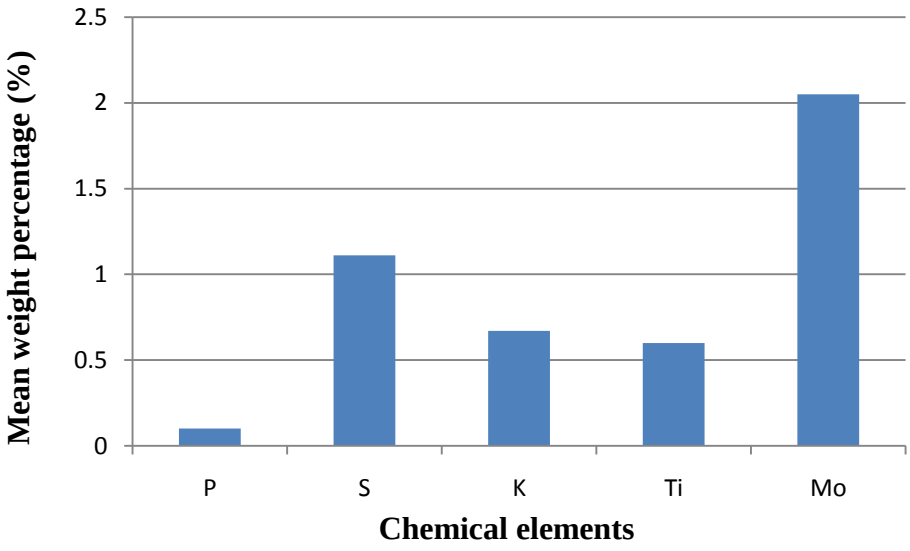


Figure 40: *Bar graph illustrating the trace elements contributing to the chemical composition of the soil from the Crown Mines.*

Discussion

The aim of this study was to describe the overall histological integrity of the skeletal remains from the Crown Mines site by giving a general description of microcracks, infiltrations, inclusions, staining, birefringence and biodegradation associated with the bones. Furthermore, a chemical analysis was done to determine if these diagenetic alterations had affected the bone chemically and whether there had been a transfer of elements between the bone and the surrounding soil or vice versa. By understanding these diagenetic alterations, a suggestion as to whether the bones could be used in the future for more accurate descriptions of the individual's characteristics, such age-at-death, can be established.

In the discussion chapter, a few of the limitations noted while completing the project are discussed and the overall histological integrity is reviewed with regards to microcracks, inclusions, infiltrations, staining, birefringence and biodegradation. Other sections that are also examined are the soil analysis and chemical analysis for the bones and soil samples obtained from the Crown Mines site.

1. Bone Sampling and Processing

Sampling bone and processing the sections is an important first step in analysing and interpreting data. The researcher must understand how sampling and processing using a specific technique can affect the results interpreted during analysis. As a result of using archaeological bone, a few limitations were noted during bone sampling and processing in this study. This includes the modification of the periosteal zone of many of the samples and the manual grinding technique used which caused artefact scratches and cracks radiating from the periosteal and endosteal surfaces.

The initial archaeological and anthropological investigations found that the cortical surfaces of the skeletal remains were badly degraded. During bone sampling, many of these cortical surfaces became detached due to the hacksaw's vibrations which resulted in the loss of the periosteal surface and as such, no circumferential lamellae are present in most of the sections. This is similar to findings by Bell (1990) who noted that archaeological bone displayed a destructive removal of the circumferential lamellae, however, it was not indicated whether this was from diagenesis or sample processing. Hillier and Bell (2007) noted that in large mammals there was a removal of bone tissue from the periosteal zone which may have been due to post-mortem damage such as fire, burial and gut digestion. Since there is no evidence at the Crown Mines site of fire damage, the periosteal loss in this study is most likely due to diagenesis

which was further agitated by the processing of the samples with a hacksaw. It is therefore important to note that the periosteal zone described in this study was more likely the superficial mesosteal zone than the periosteal zone that was present *in situ*.

The manual grinding technique described by Maat *et al*. (2001) for archaeological bone was used in the current study to process the archaeological bone samples. Although this technique had some limitations, it was used because it was cheap, fast, did not require any specialised equipment and resulted in quality samples that were adequate for histological analysis. During the processing of sections for this study, the degraded sections (specifically those where majority of the sample was filled with infiltrations) could not withstand the grinding process, especially during the final stage when the sections became reasonably thin. During grinding, the small rim of glue surrounding the outer borders of the sample (resulting from excess glue being present during attachment of the bone sample to the slide) would lift/peel off the slide causing the bone section to break. In a study done by Beauchesne and Saunders (2006) the authors tested Maat *et al*.'s (2001) grinding technique on samples that varied in preservation. The authors found that in the very fragile bone the cyanoacrylate glue failed to keep the sample intact causing it to consistently fall apart and disintegrate in the final stages of grinding which was consistent with what was observed in the current study. To prevent breakage, the thickness of the sections were increased slightly which resulted in some sections displaying an overlap of infiltrations due to an inability to have all the areas of the section in focus at a time. This was seen in **Fig. 28** (**p. 63**) where only a few of the many long and thin infiltrations were identifiable and in focus. Nonetheless, this thickness increase did not appear to affect birefringence or the general histological appearance and the interpretations thereof.

In addition to the above, the manual grinding of the sections also resulted in cracks originating from the periosteal and endosteal surfaces, and artefact "scratches" (**Fig. 36, p. 69**). It is suggested that large cracks are caused by the grinding motion on the sandpaper and the inability of the cyanoacrylate glue (Bostik Blits Stik superglue) used to attach the bone section to the slide, from keeping the section intact while grinding. As mentioned previously, most of the periosteal zones were lost during bone sampling thus resulting in a discontinuous surface that may have created weak spots for cracks to appear. Maat *et al*. (2001) indicated that sandpaper with a finer grit size (i.e. P1200) was not necessary for fragile specimens which corresponded with Beauchesne and Saunders (2006) whom indicated that most of their archaeological bone samples with a normal cortical thickness and better preservation held up well in the grinding process. However, based on observations in the current study it is suggested that fragile sec-

tions which do not have continuous periosteal surfaces are ground using finer sandpaper as this may decrease the number of surface cracks present.

Artefact scratches similar to those in the current study were observed by Keough (2007), Papageorgopoulou *et al*. (2010) and de Boer *et al*. (2012). In Keough (2007) artefact cracks distorted the images slightly and obscured the concentric lamellae of the Haversian systems used for histological age estimation. Papageorgopoulou *et al*. (2010) observed the artefact scratches as artificial cracks caused by the histological preparation of sections and de Boer *et al*. (2012) described them as coarse superficial scratches observed at low magnification. de Boer *et al*. (2012) suggested that the superficial scratches were due to the short time period of polishing the section and advised that the grinding of the bone section be lengthened and a finer grit sandpaper of P1200A be used to reduce/remove the presence of the artefact scratches for a clearer identification of the microcracks associated with diagenesis.

2. Diagenetic Alteration

Morphologically, the cortical surfaces of the Crown Mines' skeletons were very poorly preserved and were described as being brittle, flaky, rough and uneven. Compared to the literature, poorly preserved bone displays extreme cracking, breakage of the skeletal elements, cortical surface weathering and surface erosion (Hanson and Buikstra, 1987; Byers, 2002) which is similar to the cortical surfaces observed in this study. A study done by Hanson and Buikstra (1987) further revealed that histologically the preservation was independent of the whole bone preservation, by revealing that the thin sections displaying histological alteration were just as likely to have come from a morphologically well preserved bone as from a bone that was less well preserved. Similarly, although the morphology of the Crown Mines skeletal remains appeared badly degraded, the histology/microstructure was better preserved.

Of the 50 samples investigated in this study, only 4 samples (8%) (**Table 5**, **p. 57**) were so severely diagenetically altered that no qualitative information could be observed. The remaining 92% of the samples had periosteal and endosteal zones that showed signs of diagenetic alteration, with the periosteal zone being the most affected zone while the mesosteal zone was very well preserved (**Table 6, p. 58**). Similar results to those identified in this study were found by Bell (1990) and Hollund *et al*. (2011). Bell (1990) found extensive diagenetic alterations present in the subperiosteal (the superficial mesosteal zone) and endosteal regions with the mid-cortical regions (mesosteal zone) remaining well preserved. The subperiosteal region was further found to be more extensively altered than the endosteal region, consistent with the results from this study, which is suggestive of a more protective location within the medulla

(Bell, 1990). Similarly in Hollund *et al*. (2011), staining, cracking and overall destruction was found mainly in the outer cortical zone (periosteal zone) and lower cortex (endosteal zone), subcortical/trabecular bone with the middle cortex (mesosteal zone) appearing well preserved. This diagenetic alteration in the periosteal and endosteal zones of the samples in this study reveals a progression of diagenesis from the periosteal and endosteal surfaces into the bone's cortex (**Fig. 30, p. 64**) consistent with what was observed by Bell (1990) and Hollund *et al*. (2011). Considering that the periosteal surface would have had first contact to any contaminants filtering through the soil, this may also explain why this zone was more degraded than the other zones. Furthermore due to some of the remains being flattened due to the weight of the tailings, this may have created weak spots in the bones, exposing the endosteal zone to the soil contaminants.

The well preserved mesosteal zone identified in this study's samples may lead to future research being done on the mesosteal zone to determine whether or not better interpretations of age-at-death, sex, pathology and trauma can be obtained. Although the mesosteal zone was well preserved in this study, age-at-death may be difficult to interpret because 83% of the samples had infiltrations present in the periosteal zone (**Table 6, p. 58**) which obscured the bone microstructure in this area. To a certain extent, a general age can be estimated by assessing the mesosteal and endosteal zones, for example young versus old individuals, by assessing secondary Haversian systems and Haversian system fragments (Kerley, 1965; Hillier and Bell, 2007). However, considering that the outer 1/3 of the cortex is the most important region to assess when estimating the age-at-death for the individuals buried at the Crown Mines (Kerley, 1965; Keough *et al*., 2009), further research is needed. Similarly for sex estimation, the Haversian system area size and number of fragmentary Haversian systems in the periosteum are used, when comparing males and females (Burr *et al*., 1990; Mulhern and Van Gerven, 1997). Interestingly, a possible pathology was identified in the samples as it appeared as though one individual, GR79, had osteoporosis, which is discussed at the end of the diagenetic alteration section. Other pathological conditions such as Hyperparathyroidism result in an increased number of Haversian systems and Haversian fragments present whereas Diabetes mellitus results in a decrease in the number of Haversian systems and Haversian fragments present (Hillier and Bell, 2007). Since the bone microstructure was preserved well enough for the assessment of pathology in majority of the samples in the mesosteal zone, this may lead to better interpretations in the future. Hillier and Bell (2007) also showed that traumatic events resulting in immediate fracture or long-term immobility will result in new immature bone forming and changes in the normal remodelling parameters respectively. This results in differences in size

and number of the Haversian systems which could clearly be seen in the mesosteal zone of the bone samples in this study which may lead to better interpretations of trauma in the future.

In the periosteal, mesosteal and endosteal zones of the Crown Mines samples, the diagenetic alterations varied with regards to microcracks, inclusions, infiltrations and staining (**Table 6, p. 58**). Each of the alterations differed with respect to quantity and distribution throughout the zones in each sample. For example, it was noted that the rescued samples displayed more diagenetic alteration, specifically with regards to infiltrations, in the mesosteal zone than in the buried samples (**Table 4** and **Table 5**). This may be due to the rescued remains being closer to the surface than the buried remains, resulting in a variation of depth most likely due to the reclamation process and formation of paddocks causing soil erosion. This caused the skeleton to be more exposed to the environment, resulting in poorer preservation and fewer skeletal elements found. It is not known if the missing elements had completely degraded due to diagenesis or if they were removed from the soil as the bones became exposed during the reclamation process. The latter scenario is most likely because the reclamation process involves the use of a front-end loader or high-pressure jet of water (Strydom and King, 2009) which may have removed skeletal remains or the overlying soil in the grave, exposing the bones to the surface. Additionally, these rescued remains display poorer preservation possibly because there was more oxygen available less than 1 metre from the surface (discussed at a later stage in **subs. 2.6**, **p. 87**), were more exposed to contaminated water during the watering of the soil to control dust pollution (Cheng, 1973) or situated within or just below the overlying contaminated paddock water, promoting the presence of infiltrations.

This variability illustrated in the samples with regards to diagenetic alteration show the specific interactions that occur between the bone and its external environment. This close association allows for physical and molecular changes to occur during diagenesis which produce different diagenetic alterations depending on the conditions the archaeological bone was subjected to. These similarities and differences observed between the samples are consistent with what is known about diagenetic alteration varying considerably within the same bone, within individual bones from the same grave and skeletons within the same archaeological site (Garland, 1989).

In this section the variations of each of the main factors, namely microcracks, inclusions, infiltrations, staining, birefringence and biodegradation, associated with the histological integrity of the bone samples, are discussed.

2.1. Microcracks

Although the manual grinding technique of bone is successfully used for archaeological bone preparation (Maat *et al*., 2001; Beauchesne and Saunders, 2006; de Boer *et al*., 2012), analysing microcracks for diagenetic alteration proved to be problematic in this study. Due to the artefact scratches present on the bone sections, described in the bone sampling and processing section (**p. 71**), it was not possible to distinguish between the microcracks associated with diagenesis and those related to sample processing. Although the presence of diagenetic microcracks was not quantified in this study, the qualitative results revealed that microcracks in general were located in the interstitial lamellae, concentric lamellae and radiating from the Haversian canals in the mesosteal zone (**Fig. 36, p. 69**).

Similar microcracks to those identified in this study were observed by Papageorgopoulou *et al*. (2010) and Pfretzschner and Tütken (2011). Papageorgopoulou *et al*. (2010) investigated pre-existing microdamage sustained *in vivo* from microdamage caused by sample processing in archaeological bone. Microcracks were found mostly in the interstitial lamellae which is consistent with the results from this study. Unfortunately it is unknown whether these microcracks were naturally occurring in the bone ante-mortem or caused by diagenesis post-mortem. Microcracks known as short central radial cracks radiating from the Haversian canals were identified and described in diagenetically altered fossil bones by Pfretzschner and Tütken (2011). These short central radial cracks were similar to the microcracks found in **Fig. 36** for this study and resulted from the shrinkage of bone collagen due to desiccation/very dry conditions (Pfretzschner and Tütken, 2011). These very dry conditions may have occurred during the processing of the sample or at the Crown Mines site itself. de Boer *et al*. (2012) described a meshwork of microcracks observed at high magnification suggestively caused by drying strain within the section, affirming the suggestion made by Pfretzschner and Tütken (2011). The Crown Mines site was shown to have sandy soil that does not hold much moisture and there were long periods of time before the reclamation process where very little water was present (**Sect. 1.5.1, p. 15**). This may have caused very dry environmental conditions resulting in the shrinkage of bone collagen and the creation of central radial cracks radiating from the Haversian canals.

2.2. Inclusions

Foreign matter that has entered the bone from the external environment during diagenesis is commonly found in the open spaces of bone such as Haversian canals and osteocyte lacunae.

This foreign matter is known as an inclusion and for the purpose of this study inclusions were qualitatively described with reference to their location and their colour. Inclusions in this study were located in the medullary cavity/spongy bone, Volkmann's canals, Haversian canals, osteocyte lacunae and canaliculi (**Fig. 32**, **Fig. 33, Fig. 34** and **Fig. 35, p. 66-68**), and their colours were mostly black with some red, orange, blue and green. Hollund *et al*. (2011) noted similar results of location and colour where black inclusions were present in osteocyte lacunae, canaliculi and spongy bone consistent with the results in this study. Hollund *et al*. (2011) identified the inclusions in the osteocyte lacunae and canaliculi as manganese (Mn) and iron sulphide (FeS_2), and the inclusions present in the spongy bone as framboidal pyrite, which are crystals of iron sulphide (FeS_2) in the form of small aggregates of finely divided grains, shaped as raspberries (Hollund *et al*., 2011). The most common mineral in gold mine tailings is pyrite (FeS_2), which makes up 3-5% of the tailings (Tutu *et al*., 2008), and the occurrence of acid mine drainage, which may have contaminated the bones from the Crown Mines, has a high concentration of manganese present (Akcil and Koldas, 2006). Large amounts of iron and sulphur and trace amounts of manganese were found in the bone samples (**Fig. 37** and **Fig. 38, p. 71**) and large amounts of iron and trace amounts of sulphur were found in the soil samples (**Fig. 39** and **Fig. 40, p. 73**). Very little comparisons could be made to the red, orange, blue and green coloured inclusions but it is suspected that they may be associated with the infiltrations present in the same section. This is discussed in more detail in the infiltrations section below.

While investigating the inclusions in this study, a pattern was noted in **Fig. 34, p. 68** which illustrates the spread of inclusions from one osteocyte through the canaliculi to another neighbouring osteocyte. This may suggest a diffusion gradient which allowed the inclusions to move through their natural structural pathways, such as the tunnel network joining Haversian canals to canaliculi and eventually osteocyte lacunae, to become distributed through the bone section.

2.3. Infiltrations

Infiltrations differ from inclusions in that they are not present in the available bone spaces but rather constitute the bone substance itself, i.e. foreign matter from the external environment that appears to be incorporated into the bone matrix. In the literature, the infiltrations described for archaeological bone include staining of the bone observed (Jans *et al*., 2002; Hollund *et al*., 2011). In this study, staining was described separately to provide additional clarity of the qualitative infiltrations identified in the bone. Two types of infiltrations were identified: type one, spherical infiltrations and type two, long and thin infiltrations (**Fig. 27** and **Fig. 28, p. 62-63**).

On the whole, infiltrations were found in the periosteal, mesosteal and endosteal zones, with the periosteal zone containing majority of the infiltrations (**Table 6, p. 58**). The type one infiltrations were the most common infiltrations present in the periosteal zones (**Fig. 29, p. 63**), with the type two infiltrations found less abundantly throughout all the samples.

The infiltrations in the current study were qualitatively described by location, colour, shape and appearance. When attempting to qualitatively compare the infiltrations in this study to other studies, very little information was found. Initially the shape and appearance of the infiltrations was suggestive of biodegradation but further investigation lead to the conclusion that they were not due to microbial attack. To review the process by which the infiltrations were eliminated as biodegradation, their similarities and differences to the tunnels produced by microbial attack are briefly discussed. The type one infiltrations were qualitatively similar to budded tunnelling produced by bacteria, as they were spherical in shape and appeared to group within the Haversian system extending to fill it (**Fig. 27, p. 62**). The way in which they were distributed appeared to follow the same microstructure as the lamellae (**Fig. 29, p. 63**), however, it did cross the cement line of the Haversian systems (**Fig. 27, p. 62**) which is not common in budded tunnelling (Jans, 2008)**.** The appearance of the infiltrations appeared to constitute the bone matrix itself thereby obscuring the bone, which differed from microbial attack which bore (process of making a hole) through the bone causing clear open spaces to be present within the bone tissue (Trueman and Martill, 2002). The major difference, characteristic of budded tunnelling, is the presence of cuffing (mineral redeposition) identified under polarised light (Jans, 2008), which was not identified in any of these infiltrations. Although the type one infiltration identified in this study had qualitative similarities to budded tunnelling, the major differences of distribution, appearance and the absence of cuffing lead to the conclusion that these infiltrations were not caused by bacterial tunnelling.

The type two infiltrations were qualitatively similar to Wedl type 2 tunnelling produced by fungi as they were long, thin and branching, displayed no cuffing (mineral redeposition) and had a random distribution passing through cement lines (**Fig. 28, p. 63**) (Jans, 2008). The major difference which is characteristic of this type of tunnelling is the formation of borings radiating from the Haversian canal (Trueman and Martill, 2002; Dixon *et al*., 2008). In comparison, these infiltrations did not appear to alter the bone in any way and radiated not only from the Haversian canals but also from the osteocyte lacunae, which is not common in Wedl tunnelling (**Fig. 28, p. 63**).Although the type two infiltrations identified in this study had qualitative similarities to Wedl tunnelling, the major difference of appearance lead to the conclusion that these infiltrations were not caused by fungal tunnelling.

The colours observed for these infiltrations identified included green, blue, brown, orange and red. These colours did not reveal anything about the infiltrations when compared to the literature but as mentioned previously, the red, orange, blue and green coloured inclusions were suspected to be associated with the infiltrations present in the same section. This may be indicative of how the infiltrations penetrate the bone, suggesting that the inclusions present in the osteocyte lacunae and Haversian canals are the point of entry for the infiltrations. In comparison to the literature, this suggestion is confirmed by Bell (1990) and similar results are described by Piepenbrink (1986). Bell (1990) stated that the canals and osteocyte lacunae provided a primary route for diagenesis to begin and extend deeper into the surrounding bone as diagenetic alteration was mainly found in the lamellar bone, closely associated with the vascular canals. This is clearly seen in **Fig. 27, p. 62** where the infiltrations are grouping around the Haversian canals and smaller groupings are occurring in the concentric lamellae in close proximity to the osteocyte lacunae. Similarly, considering that the type one infiltrations are suggestive of non-tunnelling fungi, discussed in the next section, similar results to those identified in the current study were found by Piepenbrink (1986). Piepenbrink (1986) described that once superficial growth of the non-tunnelling fungi had occurred, this lead to the invasion of the fungi through the vascular channels such as Haversian canals and osteocyte lacunae. This was similar to what was observed in this study because in conjunction with the periosteal zone which was severely infiltrated by type one infiltrations (**Fig. 29, p. 63**), infiltrations were also found in the mesosteal zone which had inclusions in the osteocyte lacunae of similar colours (**Fig. 27, p. 62**). It is important to note that during the archaeological and anthropological investigations, a blue discolouration of the cortical surfaces was identified in the skeletons (**Table 2, p. 43**) which is suggested to be as a result of the blue-green type one infiltrations present in the periosteal zone (**Fig. 29, p. 63**). This collection of brightly coloured infiltrations appeared macroscopically as the blue staining of the bone's surface (**Fig. 8**, **p. 9**) and is described in more detail in the staining section below.

2.4. Staining

Initially during the archaeological and anthropological investigations, many of the skeletal remains in the Crown Mines site had a blue discolouration to their surfaces which looked as though they were stained (**Fig. 8, p. 9**). This staining is similar to what was observed by Piepenbrink (1986), Robles *et al*. (2002) and Reiche and Chalmin (2008). Piepenbrink (1986) identified violet-blue stains on human skeletal remains located in several different crypts and walled graves in Switzerland and West Germany which were not related to the period of inhu-

mation. These stains were suggested to be attributed to saprophytic microorganisms or fungal activity as it was established that bone decomposing fungal acid metabolites could excessively colonise the bone surfaces without the formation of tunnels. This caused diagenetic alteration in the form of staining and fluorescing secondary metabolites that impregnated the bone (Piepenbrink, 1986). This is consistent with the results from this study as no biodegradation was present in any of the samples and many infiltrations were found specifically in the periosteal zone in the area from which the macroscopic stain would have originated. Similarly in Robles *et al*. (2002), a blue-green stain was identified on the shafts of bat and rodent bones in a rich fossil deposit cave in Mexico. The origin of the stain was concluded to be "associated with the influence of impurities of transitional trace metal ions produced by the absorptive capacity of the light elements" (Robles *et al*., 2002: 148). It was suggested that a chemical element in sufficient concentrations was not the cause of the staining but rather the chemical impurities which in small quantities could be incorporated into defective structures, transitional metal ions, to intensely absorb the light to give the mineral an intense colour. Elements that could provide intense colourations to the minerals as suggested by Robles *et al*. (2002) are chromium, manganese, iron, cobalt, nickel and copper. In the current study, chromium, manganese and iron were identified but because the skeletal remains at the Crown Mines site were buried deep under the soil, light could not have been intensely absorbed. Furthermore, it is not possible that this intense absorption of light happened after the soil had been removed, during the exhumation, as the blue discolouration was identified at the moment of exhumation which makes this an unlikely scenario. Lastly, Reiche and Chalmin (2008) investigated ancient bone medieval art objects which displayed a blue staining which was concluded to be due to the uptake of manganese, possibly during diagenesis, and subsequent heating to temperatures of 500°C which caused the manganese ions (Mn^{5+}) to form. Although it is known that the bones in the current study had manganese present, there is no evidence at the Crown Mines site to indicate that the remains were ever intentionally or accidently subjected to heat which suggests that the blue staining was not because of the presence of manganese. This, therefore, suggests that the staining observed macroscopically during the exhumation of the remains is most likely due to fungal activity that colonised the bone surfaces. In addition to this, fungi are known to prefer acidic environments (Piepenbrink, 1986) which is consistent with the pH of 3.5 revealed at the Crown Mines site. This indicates that the infiltrations, specifically the type one infiltrations, are most likely due to microorganisms, specifically non-tunnelling fungi.

Histologically in this study, the samples revealed additional staining of the bones besides the macroscopic blue staining discussed above and was qualitatively described by location and col-

our. Stained bone was mainly located in the periosteal and endosteal zones (**Table 6, p. 58**). Similar patterns of staining as observed in the periosteal and endosteal zones of this study were present in Hedges (2002) where staining was found extending into the periosteal surface of the buried bone and Hollund *et al*. (2011) who found staining along the periosteal and endosteal surfaces of bone. Interestingly, no staining was observed in the mesosteal zone in Hollund *et al*. (2011) as compared to the brown staining observed in the Haversian canals of the mesosteal zone in this study (**Fig. 32, p. 66**). This brown staining is discussed at a later stage and its suggestive association with the inclusions found in the osteocyte lacunae could explain why no staining was found in Hollund *et al*. (2011).

The colours portrayed in the samples included green, brown, orange and red stains. (**Fig. 31, Fig. 32** and **Fig. 33, p. 65-67**). Similar stains of red, brown, green and orange were identified in the literature as compared to the colours portrayed in this study. Dark red-brown staining which is the most common staining occurring in archaeological bone was found to result from humic (organic constituents of soil) infiltrations (Hedges, 2002; Jans *et al*., 2002; Farlow and Argast, 2006; Turner-Walker and Jans, 2008; Hollund *et al*., 2011). Considering that the skeletal remains in the current study were buried for a long period of time in the soil, the red and brown stains observed could have resulted from the infiltration of humus into the bones from the surrounding soil. Red and green stains similar to those in the current study were identified by Piepenbrink (1986) and Reiche and Chalmin (2008) whom identified the stains as fungal activity that did not form tunnels and copper ions (Cu^{2+}) respectively. As discussed previously, no biodegradation was present in the samples of this study and therefore it is possible that the red staining may have resulted from fungal activity. The green stain on the other hand could not be as a result of copper ions as no copper was identified in the bone samples. Importantly, because the Crown Mines' skeletons may have been exposed to the polluted paddock water seen in **Fig. 8** (**p. 13**), it must be taken into account that the staining mentioned above may have occurred when the skeletal remains came into contact with the contaminated water. The exact contaminants present in the paddocks are unknown but it is suggested that the brown water seen in **Fig. 8** indicates a heavy iron load and the green water, a metal salt precipitate.

The most abundant stain identified in this study was the orange stain found predominantly in the periosteal and endosteal zones (**Fig. 31, p. 65** and **Fig. 33, p. 67**). Hollund *et al*. (2011) recognised similar orange staining along the periosteal and endosteal surfaces which compared well with the current study. This orange stain was closely associated with the framboidal pyrite inclusions. These framboidal pyrite inclusions were discussed previously in the inclusions section. Hollund *et al*. (2011) indicated that the orange stain identified in the endosteal zone of

bone was as a result of the iron sulphide from the framboidal pyrite becoming partially oxidised to form iron oxides. These framboidal pyrite grains were histologically represented as having an orange translucent edge indicative of the oxidation and formation of the iron oxides. In **Fig. 33, p. 67** this orange translucent edge and associated orange stained bone is present which could indicate that the inclusions are in fact framboidal pyrite as was suggested previously. Similarly, red and brown stains like the ones in the current study were identified by Reiche and Chalmin (2008) which resulted from iron oxides. Considering that a large amount of iron was chemically identified in the bone samples, discussed in the chemical analysis section, and the paddock water was suspected to have a heavy iron load, this could support the presence of iron oxides causing orange, red and brown staining. Overall, the literature has revealed that the red stains identified in the current study are most likely as a result of humic infiltrations, fungal activity or iron oxides, the brown stains as a result of humic infiltrations or iron oxides and the orange stains as result of iron oxides.

This orange stain produced by the framboidal pyrite inclusions described by Hollund *et al*. (2011) demonstrates a relationship between the inclusions present and the staining of the bone. Likewise, in **Fig. 32, p. 66** only the stained Haversian systems had inclusions present in the osteocyte lacunae, thus indicating that the black inclusions may have assisted in the production of the brown stain. On the other hand, **Fig. 35, p. 68** does not support this suggestion as only inclusions are present, with no staining. This may be because of a few factors. With the transformation of iron sulphide to iron oxide in the framboidal pyrite, a process of oxidation had to occur - a process whereby oxygen is chemically combined (Hollund *et al*., 2011). If for some reason no oxygen was present in the environment this process would not occur, possibly resulting in no stained bone. It is also known that there are different inclusions found in diagenetically altered bone, such as insect parts (Garland, 1989) and sand (Jans *et al*., 2002) which are not expected to chemically change in structure, therefore, resulting in no stained bone. Interestingly, in many instances, infiltrations were commonly found in the stained regions, particularly the periosteal zone (**Fig. 29, p. 63**), but did not appear to be associated with the production of the stain as was found with the inclusions. This was clearly demonstrated in the samples where other areas such as the endosteal zone had similar stains to the periosteal zone but did not have any infiltrations present (**Fig. 33, p. 67**). In general, this suggests that some of the inclusions that become incorporated into the bone's open spaces may result in staining of the bone in the areas which are in direct contact, whereas infiltrations do not necessarily result in staining of the bone.

Another characteristic that was noted in this study with regards to the stains was a degree of colour change from the periphery, extending deeper into the bone's surface. For example, the bone displayed a dark red stain towards the periosteal surface becoming more orange and eventually brown towards the mesosteal zone, becoming lighter from its point of origin. Similar results to those acquired in this study were obtained by Hollund *et al*. (2011) where the dark orange stain along the periosteal and endosteal surfaces became a lighter orange/yellow as it infiltrated deeper into the bone's cortex. Furthermore, in a study done by Hedges (2002) a suggestion was made that the brown staining found extending into the periosteal surface was due to some kind of diffusion-reaction effect. This implies that a few different staining materials may have come into contact with the bone's surface at one point or another, causing a diffusion-reaction which allowed the stains to further penetrate the bone. This may be an important aspect to consider when determining the sequence of diagenetic alteration as the most peripheral stain may indicate the most recent associated material that came into contact with the bone.

2.5. Birefringence

The assessment of birefringence illustrates the degree to which the organic and inorganic microstructure of the bone is diagenetically altered. In this study, majority of the samples are well preserved with perfect birefringence in the periosteal, mesosteal and endosteal zones (**Table 6, p. 58**). Considering that birefringence is dependent upon the other diagenetic alterations such as microcracks, inclusions, infiltrations, staining and biodegradation; comparisons cannot be made directly to the literature unless all these diagenetic alterations are found to be consistent. For example, the stained regions found in the current study displayed a perfect birefringence with the exception of severely degraded bone (**Table 5, p. 57**). This was not consistent with Piepenbrink (1986) and Hollund *et al*. (2011) who revealed a distinct reduction in birefringence in the stained areas due to the bone being more diagenetically altered as compared to the stained bone areas in this study's samples. Piepenbrink (1986) had both longitudinal and transverse cracking and torsion of the hard tissue, and extensive appearance of microcrystalline depositions and inclusions whereas Hollund *et al*. (2011) had an entire sample that had a reduced birefringence as a result of the stained areas, coinciding with generalised destruction and 100% cracking. This was only seen in a few samples in the current study which were severely degraded (**Fig. 26, p. 61**), showing loss of bone microstructure and a reduced birefringence in conjunction with stained bone. This loss of birefringence may be indicative of a deterioration of collagen or a loss of orientation of the hydroxyapatite crystals (Jans *et al*., 2002). Other areas may have similarly been affected as it was suspected that staining occurred

in the infiltrated regions too (**Fig. 29, p. 63**). However, the inability to visually assess the bone in these regions for staining and generalised destruction prevented any conclusions being made about its birefringence. Nonetheless, majority of the samples in this study were microscopically well preserved and had very little microstructural disorganisation. This is important to note because it implies that staining occurs early on in diagenesis, before disorganisation of the bone microstructure. This, therefore, may be a factor involved in the initiation of the microstructural destruction.

2.6. Biodegradation

Microbial attack or tunnelling caused by fungi and bacteria were not identified in this study. Microbial attack is known to occur early on in the diagenetic process, less than 500 years (Hedges, 2002), and is dependent on environmental conditions (Jans, 2008). According to Jans *et al*. (2004) bacterial attack is responsible for majority of the biological alteration in human bone while fungal attack is less common. The main bacterial tunnelling occurring in human bone is linear longitudinal and budded tunnelling while lamellate tunnelling is rare. Factors that may have prevented this microbial attack include staining by humic acid or iron and soils that contain low oxygen, have a low pH and high leaching rate of moveable water through the soil (Hanson and Buikstra, 1987; Hedges, 2002; Farlow and Argast, 2006; Gaudry, 2010; Hollund *et al*., 2011). As suggested previously, the red, brown and orange stains identified in this study could be due to humic infiltrations or iron oxides which may have prevented biodegradation. Similarly, the depth to which the individual was buried and the fact that the soil may have been altered due to the presence of gold mine tailings may have also affected the amount of oxygen present, resulting in an anaerobic environment for decomposition (Dent *et al*., 2004). As mentioned previously, there has been a lot of erosion and removal of topsoil by the Crown Gold Recoveries which makes estimating the actual depth of burial difficult, however, it has been suggested that the standard burial depth that the individuals would have been buried at, at that time, would have been 6ft (1.83m) or 4ft (1.22m) to save time and money (pers. comm. Anton Pelser). Dent *et al*. (2004) illustrated that there was an oxygen availability of 21% 1.9 metres into the soil and the literature has revealed that studies are least concerned with oxygen diffusion below a depth of 1 metre as it decreases rapidly regardless of soil porosity. It is not known how long the mine dump tailings were situated above the cemetery or when they first occurred in the area but this additional "waste rock" would have increased the depth (deeper than 1 meter) between the skeleton and the surface for many of the graves, causing very little oxygen to be present at the level at which the remains were buried.

The pH of the soil was acidic at the Crown Mines site which is known to inhibit bacterial growth. A close relationship between a low pH and a high leaching soil such as sandy soil, discussed in the soil analysis section below, is also known to occur. This constant movement of water as described by Hanson and Buikstra (1987) indicates that if chemical elements such as iron, discussed in the chemical analysis section, reach levels in the soil that are toxic to microflora, this may result in the inhibition of microbial growth. Similarly, the high leaching rate of the sandy soil results in a very dry substrate which in conjunction with the 6 months of low rainfall each year (**Fig. 9, p. 16**) provide very little moisture for the fungi and bacteria to thrive.

Besides the diagenetic alteration observed in this study, another histological difference was found between the individuals not associated with diagenesis. One individual, GR79, displayed rather large Haversian canals and holes in the bone which was not common in any of the other samples (**Fig. 21, p. 52** and **Fig. 23, p. 53**). These large Haversian canals could be indicative of the age of the individual while the holes which are known as resorption spaces are thought to occur during bone remodelling (Ortner, 2003). The continuous process of bone remodelling that occurs as an adult gets older begins with the removal of bone by a cutting cone or resorption space which is later replaced or filled with new bone to form a secondary Haversian system. With age, this bone formation begins to lag more and more behind bone resorption, resulting in fewer concentric lamellae surrounding a very large Haversian canal, consistent with what was observed in this study. Furthermore, the abnormal thinning of compact bone, reduced spongy bone and enlarged Haversian canals in the compact bone seen in **Fig. 21, p. 52** and **Fig. 23, p. 53** are thought to be consistent with indicators of the pathological changes such as osteoporosis (Ortner, 2003). Ortner (2003) also indicated that osteoporosis did not usually manifest itself before the fifth decade and was more frequent in females than in males. This indicates that individual GR79 could have been older than the other individuals in the Crown Mines sample and that the individual's sex may have been female. The anthropological report for GR79 was a photographic report and therefore did not give additional information (i.e. stature, estimated age and sex) about the individual but it is known that only male migrant workers were working on the Crown Mines at that time (Callinicos, 1981). Considering that there are no documented records as to the use of this particular site, it is unknown whether the cemetery was used by the mines specifically or in general for the area at that time. It is, therefore, possible that female individuals may have been buried at the Crown Mines cemetery.

3. Soil Analysis

Soil analysis revealed that the Crown Mines had soil that was acidic (pH 3.5) and of the sandy soil type. It is said that the pH of soils found in archaeology range between 3.5 and 8.5, with acidic soils in particular resulting from rainfall and leaching, acidic parent material (underlying geological material -generally bedrock, or a superficial or drift deposit), organic matter decay and harvesting of high yielding crops (Mays, 1998; Johnson and Zhang, 2013). Due to the conditions presented in this study, it is suggested that the resulting acidic pH of 3.5 is because of rainfall and leaching. The study done by Johnson and Zhang (2013) stated that Oklahoma soils are generally acidic if the rainfall is above 30 inches (762mm) per year and if the soil type allows for large amounts of water to pass through the soil rapidly. In comparison, the rainfall at the Crown Mines site had a total average rainfall of 753mm per year (**Fig. 9, p. 16**) which compares well to Johnson and Zhang (2013). Moreover, the soil type in the Crown Mines site was established to be of the sandy soil type which allows for quick drainage of water due to large spaces between the particles, retaining very little water (Bruand *et al*., 2007). This is consistent with Johnson and Zhang (2013). Considering that the Crown Mines site had gold mine tailings present, rainfall and leaching would have also contributed to acid mine drainage in the area, resulting in the alteration of the soil found in close proximity to the buried remains (Akcil and Koldas, 2006). As oxygenated water (rainfall) percolated through the dump, it would have reacted with the pyrite, causing acidification of the water (pH 3.4) and the soil (Naicker *et al*., 2003; Tutu *et al*., 2008). During the reclamation process, paddocks were also formed at the site which may have become contaminated, discussed previously on p.14, resulting in the water containing a heavy iron load or metal precipitate. This may have made the paddock water acidic which could have leached through the underlying soil, also making the soil acidic. Once reclamation of the area was completed, the soil were usually limed to reduce the acidity of the underlying soil (Tutu *et al*., 2008), however, it is unclear whether this was done at the Crown Mines because the soil still remained acidic. Besides the fact that continuous leaching produces a more acidic environment, it is also known to increase the solubility of compounds in the soil (Hanson and Buikstra, 1987), such as iron, which was found to be a major chemical element instead of a trace chemical element in this study.

4. Chemical Analysis

A few bone and soil samples collected from the Crown Mines site were chemically assessed. The chemical elements identified in the bone samples included oxygen (38.66%), calcium

(33.30%), phosphorus (18.21%), iron (10.04%), sulphur (1.50%), molybdenum (0.32%), sodium (0.29%), manganese (0.10%), aluminium (0.08%), silicon (0.06%), fluorine (0.05%), potassium (0.04%) and chromium (0.02%) (**Fig. 37** and **Fig. 38, p. 71**). Compared to the literature: oxygen, calcium, phosphorus, sulphur, molybdenum, manganese, aluminium, silicon, fluorine and chromium are normally present in bone (Grynpas *et al*., 1987; Bocherens, 1997; Sastri *et al*., 2001; Jugdaohsingh, 2007; Izci *et al*., 2013). Oxygen, calcium, phosphorus and sulphur are described as major chemical elements and molybdenum, manganese, aluminium, silicon, fluorine and chromium are described as trace chemical elements which are consistent with the results in this study. Although silicon is known to be present in normal bone due to ingestion through the diet, it is important to note that during the grinding process of the bone samples, sandpaper made of silicon carbide may have further contaminated the sample (Jugdaohsingh, 2007). Furthermore since acid mine drainage may have been present in the area, the bones may have been contaminated (but in very small quantities) by aluminium, manganese, chromium and perhaps sulphur in the form of sulphate (SO_4^{2-}), as these chemical elements are commonly found associated to acid mine drainage (Naicker *et al*., 2003; Tutu *et al*., 2008)

Of the chemical elements identified: sodium, iron, potassium and molybdenum showed variation in their weight percentages in terms of diagenetic alteration and soil contamination. Sodium and iron were displayed as trace and major chemical elements respectively which were inconsistent with normal bone. Potassium and molybdenum, although displayed as trace chemical elements consistent with the literature, showed an increase in their weight percentages compared to normal bone. Sodium which normally constitutes more than 1% of the bone, as it is a major chemical element, was found at percentage levels comparable to the other trace elements. This was consistent with Lambert *et al*. (1983) who observed sodium depletion from the bone into the surrounding soil through leaching. Iron that is meant to be a trace chemical element in normal bone was found to constitute more than 1% of the bone such as often described for major elements such as calcium and phosphorus. This is not surprising as iron is known to contaminate archaeological bone due to soil contamination during diagenetic alteration (Lambert *et al*., 1979; Lambert *et al*., 1983; Hanson and Buikstra, 1987) and is a by-product of acid mine drainage, particularly in surface or ground water in mining areas (Naicker *et al*., 2003) and within polluted paddock water (Tutu *et al*., 2008), which may have filtered through the soil, resulting in an increased concentration of iron. Similarly, potassium is also a contaminative element where "the variable degree of influx of potassium into the bone suggests a sensitivity of the process to specific environmental characteristics of the burial" (Lambert *et al*.,

1983: 415). Finally, molybdenum which is normally incorporated into bone through diet has also been observed in other studies to be as a result of contamination from the soil (Izci *et al*., 2013).

When comparing the quantity of each element present in the periosteal, mesosteal and endosteal zones - iron, aluminium, potassium, manganese, calcium, phosphorus and sodium could be compared to Lambert *et al*. (1983) and Müller *et al*. (2011), however, for the other elements, very little literature was available for comparison. Lambert *et al*. (1983) observed a build-up of iron along the outer and inner surfaces of bone and Müller *et al*. (2011) observed significantly higher values of iron near the bone's surface which were similar to the iron predominantly found in the periosteal and endosteal zones in this study (**Fig. 37, p. 71**). There was a build-up of aluminium along the outer surface of the bone which was consistent with aluminium only being present in the periosteal zone in this study (**Fig. 38, p. 71**) and potassium had a build-up along the outer and inner bone surfaces found similar to being present in the periosteal and endosteal zones (**Fig. 38, p. 71**). Lambert *et al*. (1983) found that manganese was concentrated only in the outer surfaces of bone while Müller *et al*. (2011) found that manganese was only present in the inner surfaces of the bone. This was consistent with these results and show manganese present in the periosteal and endosteal zones, however, a small amount was also found in the mesosteal zone (**Fig. 38, p. 71**). Lambert *et al*. (1983) and Müller *et al*. (2011) reported that calcium had a homogenous distribution throughout the bone which was similar to the results obtained in this study, where calcium was present in the periosteal, mesosteal and endosteal zones, with the exception of a slight decrease occurring in the periosteal and endosteal zones (**Fig. 37, p. 71**). Calcium is known to leach from the bone into the soil (Lambert *et al*., 1983) which may explain this decrease in the periosteal and endosteal zones because those two zones are the first areas expected to be affected by diagenetic alteration based on the results of this study. Similarly, phosphorus also had a homogeneous distribution (Müller *et al*., 2011) which is consistent with this study, where phosphorus was present in the periosteal, mesosteal and endosteal zones, with the exception of the periosteal zone having a higher weight percentage (**Fig. 37, p. 71**). Müller *et al*. (2011) indicated lower concentrations of sodium towards the outer zones of the cross section of bone. The homogeneous distribution found in the samples were similar with regards to the endosteal zone which illustrated sodium depletion but the periosteal zone showed an increase rather than a decrease as compared to Müller *et al*. (2011). This may have resulted from the different environments the bones were subjected to, resulting in different interactions between the bone and soil during diagenesis.

The chemical elements identified in the soil samples included oxygen (46.30%), silicon (31.85%), iron (10.23%), aluminium (8.82%), molybdenum (2.05%), sulphur (1.11%), potassium (0.67%), titanium (0.60%) and phosphorus (0.10%) (**Fig. 39** and **Fig. 40, p. 73**). All these elements are consistent with the chemical elements present in the soil described by Shacklette and Boerngen (1984). Acid mine drainage that may have affected the soil is indicative of high concentrations of iron, aluminium, manganese and sulphate, and low concentrations of toxic heavy metals (Naicker *et al*., 2003; Akcil and Koldas, 2006). No toxic heavy metal concentrations were found in the soil assessed from the Crown Mines, however, it is unclear whether the iron, aluminium and sulphur-oxygen (sulphate) concentrations were affected by acid mine drainage. This does not mean that there were no heavy metals present in the soil prior to this analysis as it is known that the surface and ground water drainage systems in similar mining areas were greatly contaminated by heavy metals which could mean that the heavy metals that had been present at the site had simply washed away (Naicker *et al*., 2003).

To summarise, the periosteal zone of majority of the sections in this study was lost during bone sampling which resulted in some of the periosteal zone descriptions being of the superficial mesosteal zone instead. The manual grinding technique was not as effective in the sample preparation of severely diagenetically infiltrated and fragile sections, and although cracks and artefact scratches were present, it did not affect the qualitative interpretations of the bone's microstructure. Histologically, the Crown Mines' bone samples had good preservation with microcracks, inclusions, infiltrations and staining identified. Even though the microcracks associated with diagenetic alteration and sample preparation were not distinguished, the literature does show that the microcracks radiating from the Haversian canals, as observed in the current study, are associated with diagenetic alteration. It is not known how these interstitial lamellae microcracks in the current study were formed, but the microcracks radiating from the Haversian canals are indicative of the sample drying out either during processing or extreme conditions at the Crown Mines site. The inclusions present in this study were similar to those described in the literature in terms of location and colour, and an association was found between the inclusions and infiltrations of the same sample. The blue macroscopic staining observed on the skeletal remains during the exhumation is most likely because of fungal activity excessively colonising the bone surfaces, suggesting that the type one (spherical) infiltrations identified in this study are non-tunnelling fungi. Histologically, the red stains could be due to humic infiltrations, fungal activity or iron oxides, the brown stains may be because of humic infiltrations or iron oxides and the orange stains could be as a result of iron oxides. Staining is suggested to

occur early on in diagenesis, before structural disorganisation, and may be a contributing factor in initiating it. Although the Crown Mines site had been associated with gold mining activity, no heavy metal concentrations were present in the bone or soil samples and thus did not contribute to the blue staining present on the periosteal surface, even though the soil was acidic. Elements such as iron, aluminium, potassium, manganese, calcium, phosphorus, sodium and molybdenum were chemically altered due to diagenetic alteration with some elements such as sodium being transferred to the soil and others such as iron, potassium and molybdenum being transferred from the soil to the bone, causing contamination of the bone samples.

To assess and quantify the microcracks identified in this study using the same manual grinding technique, it is suggested that new sections be made using the descriptions given in Papageorgopoulou *et al*. (2010) for confocal laser scanning microscopy. Further research would also benefit from preparing new bone samples for SEM-EDS to establish the nature and chemical composition for identification of the inclusions, infiltrations and staining found in this study. Moreover, additional research is needed to determine the microbiology of the infiltrations by collecting additional samples from the grave site upon exhumation as suggested by Piepenbrink (1986). This may lead to a better understanding of the relationship between inclusions and staining, and the diffusion mechanisms of inclusions associated with infiltrations and how they penetrate the bone during diagenesis. Overall, because the mesosteal zone in majority of the samples in the current study were well preserved, it may be possible to acquire more accurate estimates of age-at-death, pathology and trauma by assessing the bone histology. However, considering that most research related to age-at-death estimations specifically use the periosteal zone for accurate interpretations, future research would benefit from investigations of the mesosteal zone as this area is generally less diagenetically altered than the other zones. Further research may also benefit from assessing the longitudinal sections of bone in comparison to the cross-sections obtained in this study.

Conclusion

The aim of this study was to describe the overall histological integrity of the skeletal remains from the Crown Mines site. Although initially during the morphological analysis very little information could be obtained from the bones due to the cortical surfaces being poorly preserved, the histology of the bone has revealed more promising results. This study revealed that majority of the samples had diagenetic alterations in the periosteal and endosteal zones with the mesosteal zone remaining relatively intact and displaying good preservation. The macroscopic blue staining of the bones was most likely non-tunnelling fungi excessively colonising the bone surfaces whereas histologically, these areas of staining are illustrated as blue-green type one spherical infiltrations. Other diagenetic alterations identified include: microcracks radiating from the Haversian canals associated with the drying out of the sample either during processing or at the Crown Mines site; type two long and thin infiltrations; inclusions; staining of the bone from humic infiltrations, fungal activity and iron oxides; and no biodegradation was present.

A chemical analysis was done to determine if these diagenetic alterations had affected the bone chemically and whether there had been a transfer of elements between the bone and the surrounding soil or vice versa. Although the Crown Mines site had been associated with gold mining activity, no heavy metal concentrations were present in the bone or soil samples and thus did not contribute to the blue staining present on the periosteal surface, even though the soil was acidic. Elements such as iron, aluminium, potassium, manganese, calcium, phosphorus, sodium and molybdenum were chemically altered due to diagenetic alteration, with some elements such as sodium being transferred to the soil and others such as iron, potassium and molybdenum being transferred from the soil to the bone, causing contamination of the bone samples.

Lastly, after assessing these diagenetic alterations that occurred in the bones, it is suggested that better estimates of age-at-death and descriptions of pathology and trauma may be acquired from the samples due to the well preserved mesosteal zones. Future research would benefit from investigating the mesosteal zone which was less diagenetically altered as age-at-death currently uses the periosteal zone specifically.

References

Adebisi, S.S., 2009. Forensic anthropology in perspective: The current trend. *The Internet Journal of Forensic Science*, 4(1).

Akcil, A. and Koldas, S., 2006. Acid Mine Drainage (AMD): causes, treatment and case studies. *Journal of Cleaner Production*, 14, pp.1139–1145. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0959652605000600 [Accessed March 3, 2013].

Alldredge, B.K. *et al*., 2009. *Applied therapeutics: the clinical use of drugs*, Philadelphia: Lippincott Williams and Wilkins.

Anon, 1997. Information on measurement of pH in soil. *Eutech Instruments*. Available at: www.eutechinst.com/techtips/tech-tips6.htm [Accessed June 25, 2013].

Baxter, K., 2004. Extrinsic factors that effect the preservation of bone. *The Nebraska Anthropologist*, 19, pp.38–45.

Beauchesne, P. and Saunders, S., 2006. A test of the revised Frost's "Rapid Manual Method" for the preparation of bone thin sections. *International Journal of Osteoarchaeology*, 16, pp.82–87.

Bell, L.S., 1990. Palaeopathology and Diagenesis: An SEM evaluation of structural changes using backscattered electron imaging. *Journal of Archaeological Science*, 17, pp.85–102.

Bell, L.S., Skinner, M.F. and Jones, S.J., 1996. The speed of post mortem change to the human skeleton and its taphonomic significance. *Forensic Science International*, 82, pp.129–140.

Bocherens, H., 1997. Chemical composition of dinosaur fossils. In P. Currie and K. Padian, eds. *Encyclopedia of dinosaurs*. Academic Press, pp. 111–117.

De Boer, H., Aarents, M. and Maat, G., 2012. Staining ground sections of natural dry bone tissue for microscopy. *International Journal of Osteoarchaeology*, 22, pp.379–386. Available at: http://doi.wiley.com/10.1002/oa.1208 [Accessed August 15, 2013].

Brand South Africa, South Africa's weather and climate. *Big Media Publishers*. Available at: http://www.southafrica.info/travel/advice/climate.htm [Accessed April 22, 2013].

Brits, D., 2009. *Histomorphometrical and chemical analysis of human and non-human bones*. University of Pretoria.

Bromage, T. *et al*., 2003. Circularly polarized light standards for investigations of collagen fiber orientation in bone. *The Anatomical Record*, 274B, pp.157–168.

Bruand, A., Hartmann, C. and Lesturgez, G., 2007. Physical properties of tropical sandy soils: A large range of behaviours. *FAO Corporate Document Repository*. Available at: http://www.fao.org/docrep/010/ag125e/ag125e00.htm [Accessed May 3, 2013].

Burr, D.B., Ruff, C.B. and Thompson, D.D., 1990. Patterns of skeletal histologic change through time: comparison of an archaic native American population with modern populations. *The Anatomical record*, 226, pp.307–313. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2327603.

Byers, S.N., 2002a. Estimating Time Since Death. In K. Hanson, ed. *Introduction to Forensic Anthropology: A Textbook*. USA: Allyn and Bacon, pp. 103–121.

Byers, S.N., 2002b. Postmortem changes to bone. In K. Hanson, ed. *Introduction to Forensic Anthropology: A Textbook*. USA: Allyn and Bacon, pp. 352–373.

Callinicos, L., 1981. *A people's history of South Africa: Gold and workers 1886 - 1924* Volume 1., Johannesburg: Ravan Press.

Cembrowicz, S. and Allain, T., 2007. Osteoporosis: What is it and how is it diagnosed? In G. Clarke, ed. *Osteoporosis*. London: Class Publishing Ltd, pp. 1–20.

Chamberlain, A., 1994. Chemical and biomolecular evidence. In *Interpreting the past: Human remains*. Great Britain: University of California Press, pp. 22–25.

Chan, A.H.W., Crowder, C.M. and Rogers, T.L., 2007. Variation in cortical bone histology within the human femur and its impact on estimating age at death. *American Journal of Physical Anthropology*, 132, pp.80–88.

Cheng, L., 1973. Collection of airborne dust by watersprays. *Industrial and Engineering Chemistry Process Design and Development*. 12(3), pp. 221-225.

Col, J., 2001. Soil layers. *Enchanted Learning*. Available at: http://www.enchantedlearning.com/geology/soil/ [Accessed October 1, 2013].

Collins, D., 1956. Paget's disease of bone. *Lancet*, 2, pp.51–57.

Collins, M. *et al*., 2002. The survival of organic matter in bone: a review. *Archaeometry*, 44(3), pp.383–394. Available at: http://www.blackwell-synergy.com/links/doi/10.1111%2F1475-4754.t01-1-00071.

Cook, S.F., Brooks, S.T. and Ezra-Cohn, H.E., 1962. Histological studies on fossil bone. *Journal of Paleontology*, 36(3), pp.483–494.

Cranfield University, 2013. Soils: Advanced. *Soil-Net project*. Available at: http://www.soil-net.com [Accessed October 2, 2013].

Currey, J., 2003. The many adaptations of bone. *Journal of Biomechanics*, (36), pp.1487–1495.

Curtis, J.M. and Nawrocki, S.P., 2010. Skeletal Aging using Frontal Bone Histomorphometrics. In K. E. Latham and M. Finnigan, eds. *Age Estimation of the Human Skeleton*. Springfield, Illinois: Charles C. Thomas Publisher Ltd, pp. 216–231.

Davis, P.G., 1997. The bioerosion of bird bones. *International Journal of Osteoarchaeology*, 7, pp.388–401.

Dent, B.B., Forbes, S.L. and Stuart, B.H., 2004. Review of human decomposition processes in soil. *Environmental Geology*, 45, pp.576–585. Available at: http://www.springerlink.com/openurl.asp?genre=article&id=doi:10.1007/s00254-003-0913-z [Accessed October 24, 2012].

DiGangi, E. and Moore, M., 2013. *Research methods in human skeletal biology* E. DiGangi and M. Moore, eds., Amsterdam: Academic Press.

Dixon, R.A., Dawson, L. and Taylor, D., 2008. The experimental degradation of archaeological human bone by anaerobic bacteria and the implications for recovery of ancient DNA. In *The 9th International Conference on Ancient DNA and Associated Biomolecules*. Available at: http://eprints.lincoln.ac.uk/2660/ [Accessed March 22, 2012].

Dupras, T.L. *et al*., 2006. An Introduction to Forensic Anthropology and Forensic Archaeology. In *Forensic Recovery of Human Remains*. Florida, USA: CRC Press.

Von Endt, D.W. and Ortner, D.J., 1984. Experimental effects of bone size and temperature on bone diagenesis. *Journal of Archaeological Science*, 11, pp.247–253.

Enlow, D., 1966. An evaluation of the use of bone histology in forensic medicine and anthropology. In F. Evans, ed. *IIIrd International Congress of Anatomists: Symposium on Joints and Bones*. Berlin: Springer Verlag, pp. 92–112.

Evans, F. and Bang, S., 1967. Differences and relationships between the physical properties and the microscopic structure of human femoral, tibial and fibular cortical bone. *American Journal of Anatomy*, 120, pp.79–88.

Farlow, J. and Argast, A., 2006. Preservation of fossil bone from the Pipe Creek sinkhole (Late Neogene, Grant County, Indiana, U.S.A.). *Journal of the Paleontological Society of Korea*, 22(1), pp.51–75.

Fiedler, S. and Graw, M., 2003. Decomposition of buried corpses, with special reference to the formation of adipocere. *Naturwissenschaften*, 90, pp.291–300.

Forbes, S., 2008. Decomposition chemistry in a burial environment. In M. Tibbett and D. O. Carter, eds. *Soil analysis in forensic taphonomy*. Florida, USA: Taylor and Francis Group, pp. 203–223.

Garland, A.N., 1989. Microscopical Analysis of Fossil Bone. *Applied Geochemistry*, 4, pp.215–229.

Gaudry, E., 2010. The Insects Colonisation of Buried Remains. In *Current Concepts in Forensic Entomology*. pp. 273–311.

Giraud-Guille, M.M., 1988. Twisted plywood architecture of collagen fibrils in human compact bone osteons. *Calcified Tissue International*, 42, pp.167–180.

Google Earth, 2014. Satellite image of the Crown Mine dump. *Google Earth*. Available at: earth.google.com [Accessed March 7, 2014].

Google Maps, 2013. Satellite image of the Crown Mines. *Google*. Available at: https://maps.google.co.za/maps [Accessed January 15, 2013].

Grupe, G. and Dreser-Werringloer, U., 1993. Decomposition phenomena in thin-sections of excavated human bones. In G Grupe and A. N. Garland, eds. *Histology of Ancient Human Bone: Methods and Diagnosis*. Berlin-Heidelberg: Springer-Verlag.

Grynpas, M., Pritzker, K. and Hancock, R., 1987. Neutron activation analysis of bulk and selected trace elements in bones using a low flux SLOWPOKE reactor. *Biological Trace Element Research*, 13, pp.333–344. Available at: http://link.springer.com/10.1007/BF02796644.

Hackett, C.J., 1981. Microscopical Focal Destruction (Tunnels) in Exhumed Human Bones. *Medicine Science and the Law*, 21(4), pp.243–265.

Hanson, D.B. and Buikstra, J.E., 1987. Histomorphological Alteration in Buried Human Bone from the Lower Illinois Valley: Implications for Palaeodietary Research. *Journal of Archaeological Science*, 14, pp.549–563.

Hedges, R.E.M., 2002. Bone Diagenesis: An overview of processes. *Archaeometry*, 44(3), pp.319–328.

Hedges, R.E.M., Millard, A.R. and Pike, A.W.G., 1995. Measurements and Relationships of Diagenetic Alteration of Bone from Three Archaeological Sites. *Journal of Archaeological Science*, 22, pp.201–209.

Hillier, M.L. and Bell, L.S., 2007. Differentiating Human Bone from Animal Bone: A Review of Histological Methods. *Journal of forensic sciences*, 52(2), pp.249–263. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17316219 [Accessed June 20, 2011].

Hollund, H.I. *et al*., 2011. What Happened Here? Bone Histology as a Tool in Decoding the Postmortem Histories of Archaeological Bone from Castricum, The Netherlands. *International Journal of Osteoarchaeology*.

Izci, Y. *et al*., 2013. Paleodietary analysis of human remains from a Hellenistic-Roman cemetery at Camihöyük, Turkey. *Journal of Anthropology*, pp.1–7.

Jans, M.M.E. *et al*., 2004. Characterisation of microbial attack on archaeological bone. *Journal of Archaeological Science*, 31, pp.87–95.

Jans, M.M.E., 2005. *Histological Characterisation of Diagenetic Alteration of Archaeological Bone*. Vrije Universiteit: Amsterdam.

Jans, M.M.E. *et al*., 2002. In situ preservation of archaelogical bone: A histological study within a multidisciplinary approach. *Archaeometry*, 44(3), pp.343–352.

Jans, M.M.E., 2008. Microbial bioerosion of bone – a review. In M. Wisshak and L. Tapanila, eds. *Current Developments in Bioerosion*. Berlin Heidelberg: Springer-Verlag, pp. 197–413.

Johnson, G. V and Zhang, H., Cause and effects of soil acidity. *Oklahoma Cooperative Extension Service*. Available at: http://www.soiltesting.okstate.edu/Extn_Pub/F-2239web.pdf [Accessed May 3, 2013].

Jugdaohsingh, R., 2007. Silicon and bone health. *Journal of Nutrition Health and Aging*, 11(2), pp.99–110. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2658806/.

Keough, N., 2007. *Estimation of age at death from the microscopic structure of the femur*.

Keough, N., L'Abbé, E.N. and Steyn, M., 2009. The evaluation of age-related histomorphometric variables in a cadaver sample of lower socioeconomic status: implications for estimating age at death. *Forensic science international*, 191(1-3), pp.114.e1–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19679414 [Accessed June 6, 2013].

Kerley, E., 1965. The microscopic determination of age in human bone. *American Journal of Physical Anthropology*, 23, pp.149–164. Available at: http://www.ncbi.nlm.nih.gov/pubmed/4305670.

Kramer, B. and Allan, J.C., 2005. The Structure of Bone and Cartilage. In *Fundamentals of Human Osteology*. Durban: Lexisnexis Butterworths, pp. 3–17.

Lambert, J. *et al*., 1983. Electron microprobe analysis of elemental distribution in excavated human femurs. *American Journal of Physical Anthropology*, 62, pp.409–423. Available at: http://www.ncbi.nlm.nih.gov/pubmed/6666771.

Lambert, J., Szpunar, C. and Buikstra, J., 1979. Chemical analysis of excavated human bone from Middle and Late Woodland sites. *Archaeometry*, 21(2), pp.115–129. Available at: http://doi.wiley.com/10.1111/j.1475-4754.1979.tb00248.x.

Lorenzo, J., Canalis, E. and Raisz, L., 2011. Metabolic bone disease. In H. Kronenberg *et al*., eds. *Williams Textbook of Endocrinology*. Philadelphia, Pa: Saunders Elsevier.

Maat, G.J.R., Van Den Bos, R.P.M. and Aarents, M.J., 2001. Manual Preparation of Ground Sections for the Microscopy of Natural Bone Tissue: Update and Modification of Frost's "Rapid Manual Method." *International Journal of Osteoarchaeology*, 11, pp.366–374.

Maggiano, C. *et al*., 2009. Confocal laser scanning microscopy: a flexible tool for simultaneous polarization and three-dimensional fluorescence imaging of archaeological compact bone. *Journal of Archaeological Science*, 36, pp.2392–2401. Available at: http://dx.doi.org/10.1016/j.jas.2009.06.021.

Maples, W.R. and Browning, M., 1994. *Dead Men Do Tell Tales. The Strange and Fascinating Cases of a Forensic Anthropologist.*, New York: Doubleday.

Marotti, G., 1993. A new theory of bone lamellation. *Calcified Tissue International*, 53(Suppl 1), pp.S47–S56.

Mays, S., 1998. *The archaeology of human bones*, London: Routledge.

McCalden, R. *et al*., 1993. Age-related changes in the tensile properties of cortical bone. The relative importance of changes in porosity, mineralization, and microstructure. *Journal of Bone and Joint Surgery*, 75(8), pp.1193–1205.

Molleson, T., 1990. The accumulation of trace metals in bone during fossilization. In N. D. Priest and F. L. Van der Vyver, eds. *Trace metals and fluoride in bone and teeth*. Boca Raton, Florida: CRC Press, pp. 341–365.

Mulhern, D., 2000. Rib remodeling dynamics in a skeletal population from Kulubnarti, Nubia. *American Journal of Physical Anthropology*, 111(4), pp.519–530.

Mulhern, D. and Van Gerven, D., 1997. Patterns of femoral bone remodeling dynamics in a Medieval Nubian population. *American Journal of Physical Anthropology*, 104(1), pp.133–146.

Müller, K. *et al*., 2011. Microbial attack of archaeological bones versus high concentrations of heavy metals in the burial environment. A case study of animal bones from a mediaeval copper workshop in Paris. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 310(1-2), pp.39–51. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0031018211001477 [Accessed September 13, 2013].

Naicker, K., Cukrowska, E. and McCarthy, T.S., 2003. Acid mine drainage arising from gold mining activity in Johannesburg, South Africa and environs. *Environmental pollution*, 122, pp.29–40. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12535593.

Nawrocki, S.P., 1995. Taphonomic processes in historic cemeteries. In A. Grauer, ed. *Bodies of evidence: Reconstructing history through skeletal analysis*. New York: Wiley-Liss Inc, pp. 49–66.

Nicholson, R.A., 1996. Bone Degradation, Burial Medium and Species Representation: Debunking the myths, an experiment-based approach. *Journal of Archaeological Science*, 23, pp.513–534.

Nielsen-Marsh, C.M. and Hedges, R.E.M., 2000. Patterns of diagenesis in bone I: The effects of site environments. *Journal of Archaeological Science*, 27, pp.1139–1150.

Ortner, D., 2003. *Identification of pathological conditions in human skeletal remains* second., California, USA: Academic Press.

Papageorgopoulou, C. *et al*., 2010. Diagnostic morphometric applicability of confocal laser scanning microscopy in Osteoarchaeology. *International Journal of Osteoarchaeology*, 20, pp.708–718. Available at: http://doi.wiley.com/10.1002/oa.1078 [Accessed August 19, 2013].

Pelser, A. and van Vollenhoven, A., 2011. *A report on the historical-archaeological excavation of a late 19th/early 20th century midden on the farm Langlaagte 224IQ, Crown mines, Crownwood road, Johannesburg, Gauteng.*,

Pfeiffer, S. *et al*., 2006. Secondary osteon and haversian canal dimensions as behavioral indicators. *American Journal of Physical Anthropology*, 131, pp.460–468.

Pfeiffer, S., 1998. Variability in osteon size in recent human populations. *American Journal of Physical Anthropology*, (106), pp.219–227.

Pfretzschner, H.-U. and Tütken, T., 2011. Rolling bones – Taphonomy of Jurassic dinosaur bones inferred from diagenetic microcracks and mineral infillings. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 310, pp.117–123. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0031018211000411 [Accessed August 23, 2013].

Piepenbrink, H., 1989. Examples of chemical changes during fossilisation. *Applied Geochemistry*, 4, pp.273–280.

Piepenbrink, H., 1986. Two examples of biogenous dead bone decomposition and their consequences for taphonomic interpretation. *Journal of Archaeological Science*, 13, pp.417–430.

Pollard, A. and Heron, C., 2008. The chemistry of human bone: Diet, nutrition, status and mobility. In *Archaeological chemistry*. Cambridge, UK: RSC Publishing, pp. 346–382.

Price, T.D. *et al*., 1992. Diagenesis in prehistoric bone: problems and solutions. *Journal of Archaeological Science*, 19, pp.513–529.

Rasband, W.S., ImageJ. *U. S. National Institutes of Health*. Available at: http://rsb.info.nih.gov/ij/.

Reiche, I. and Chalmin, E., 2008. Synchrotron radiation and cultural heritage: combined XANES/XRF study at Mn K-edge of blue, grey or black coloured palaeological and archaeological bone material. *Journal of Analytical Atomic Spectrometry*, 23, pp.799–806.

Robles, J. *et al*., 2002. Blue bone analysis as a contribution to the study of bone taphonomy in San Josecito cave, Nuevo Leon, Mexico. *Journal of Cave and Karst Studies*, 64(2), pp.145–149.

Ross, M.H. and Pawlina, W., 2006. *Histology. A text and atlas* Fifth Edit., Lippincott Williams and Wilkins.

Sastri, C. *et al*., 2001. Fluorine determination in human and animal bones by particle-induced gamma-ray emission. *Fresenius' Journal of Analytical Chemistry*, 370, pp.924–929. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11569877.

Schoeninger, M.J. *et al*., 1989. Detection of bone preservation in archaeological and fossil samples. *Applied Geochemistry*, 4, pp.281–292.

Shacklette, H. and Boerngen, J., 1984. Element concentrations in soils and other surficial materials of the conterminous United States. *U.S. Geological Survey Professional Paper 1270*.

Sheridan, S., 2001. Morbidity and mortality in a classic-period Hohokam community. In D. Mitchell and J. Brunson-Hadley, eds. *Ancient burial practices in the American Southwest: Archaeology, Physical Anthropology and Native American perspectives*. University of New Mexico Press, pp. 191–222.

Sillen, A. and Parkington, J., 1996. Diagenesis of Bones from Eland ' s Bay Cave. *Journal of Archaeological Science*, (January 1995), pp.535–542.

Sillen, A. and Sealy, J.C., 1995. Diagenesis of Strontium in Fossil Bone: A Reconsideration of Nelson *et al*. (1986). *Journal of Archaeological Science*, 22(2), pp.313–320.

Singh, I.J. and Gunberg, D.L., 1970. Estimation o f Age at Death in Human Males from Quantitative Histology o f Bone Fragments '. *American Journal of Physical Anthropology*, 33, pp.373–382.

Smith, C.I. *et al*., 2002. The strange case of Apigliano: Early "fossilization" of medieval bone in southern Italy. *Archaeometry*, 44(3), pp.405–415. Available at: http://doi.wiley.com/10.1111/1475-4754.t01-1-00073.

Stout, S.D. and Teitelbaum, S., 1976. Histological analysis of undecalcified thin sections of archaeological bone. *American Journal of Physical Anthropology*, 44, pp.263–269.

Strydom, H. and King, N., 2009. Terrestrial Minerals. In *Fuggle and Rabie's Environmental Management in South Africa*. Cape Town: JUTA Law, pp. 513-576.

Trueman, C. and Martill, D., 2002. The long-term survival of bone: The role of bioerosion. *Archaeometry*, 44(3), pp.371–382.

Turner-Walker, G. and Jans, M., 2008. Reconstructing taphonomic histories using histological analysis. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 266(3-4), pp.227–235. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0031018208002472 [Accessed November 6, 2012].

Tutu, H. *et al*., 2008. The chemical characteristics of acid mine drainage with particular reference to sources, distribution and remediation: The Witwatersrand Basin, South Africa as a case study. *Applied Geochemistry*, 23(12), pp.3666-3684.

Tutu, H. *et al*., 2009. Radioactive disequilibrium and geochemical modelling as evidence of uranium leaching from gold tailings dumps in the Witwatersrand Basin. *International Journal of Environmental Analytical Chemistry*, 89, pp.687-703.

Vass, A.A., 2001. Beyond the grave- understanding human decomposition. *Microbiology Today*, 28, pp.190–192.

Wang, X. *et al*., 2003. Age-related changes of noncalcified collagen in human cortical bone. *Annals of Biomedical Engineering*, 31(11), pp.1365–1371.

Waters, M.R., 1992. *Principles of geoarchaeology: A north american perspective*, Tucson: The University of Arizona Press.

Wedl, C., 1864. Ueber einen Zahnbein und Knochen keinenden. *Pilz Sber Akad Wiss Wein*, 50(1), pp.171–193.

White, E. and Hannus, L., 1983. Chemical weathering of bone in archaeological soils. *American Antiquity*, 48(2), pp.316–322.

White, T. and Folkens, P., 2005. Bone Biology and Variation. In *The Human Bone Manual*. USA: Elsevier Inc., pp. 31–48.

Wilson, L. and Pollard, M., 2002. Here Today, gone Tomorrow? Integrated Experimentation and Geochemical Modeling in Studies of Archaeological Diagenetic Change. *Accounts of Chemical Research*, 35(8), pp.644–651.

Zernicke, R.F. and Loitz-Ramage, B., 2003. Exercise-Related Adaptations in Connective Tissue. In P. V Komi, ed. *Strength and Power in Sport*. Oxford, UK: Blackwell Science, pp. 96–100.