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Histomorphometrical and chemical analysis of human and non-human bones

by

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DECLARATION

I, Desiré Marguerita Brits, hereby declare that this dissertation, to be submitted to the University of Pretoria for the degree of Master of Science in Anatomy, is my own work and has not been submitted to this University or any other tertiary institution for any degree.

Signed _____

D M Brits

This _____ day of _____, 2009.

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ABSTRACT

Aside from macroscopic features of bone and retrievable DNA, few methods are available to accurately separate human and non-human remains found in forensic contexts. The aim of this study was to determine whether significant chemical and histological differences between human and non-human bones exist, which could be used to sort them. Bone samples were taken from femora and tibiae of ten cows (*Bos taurus*), ten sheep (*Ovis aries*), five impalas (*Aepyceros melampus*), 12 donkeys (*Equus africanus asinus*), eight cats (*Felix catus*), 12 dogs (*Canis lupus familiaris*), 12 pigs (*Sus scrofa domestica*), seven primates, 27 adult humans and six juveniles (*Homo sapiens sapiens*).

Microelement analysis was conducted with a scanning electron microscope fitted with an electron dispersive spectrometer (SEM/EDS) and included the analyses of calcium (Ca), phosphorus (P), potassium (K), sodium (Na), chloride (Cl), sulphur (S), silicone (Si), aluminium (Al), magnesium (Mg), zinc (Zn), lead (Pb) and strontium (Sr). Statistically significant differences in the long bones of omnivores and herbivores were observed with regard to K ($p=0.0001$), Mg ($p=0.0214$), Cl ($p=0.0001$) and S ($p=0.0012$) levels, while K ($p=0.0001$), Na ($p=0.0192$) and Cl ($p=0.0001$) levels were significantly different between omnivores and carnivores. Subtle differences between femora and tibiae were also identified and warrant further inspection.

Light microscopy was used to evaluate the histomorphology of cortical bone of the various species. Qualitatively the organisation of various bone structures were assessed while quantitative analyses included measurements of the total

number of osteons and non-Haversian canals and minimum and maximum diameters of osteons and Haversian canals. All species contained Haversian bone either in their femora, tibiae or both bones, except for cow and pig bones which contained only plexiform bone. The presence of plexiform bone accurately excluded remains from a human origin. Statistically significant differences between species were found with regards to all quantitative variables, except for the tibial Haversian canal diameters. The current study illustrated that Haversian canal diameters of 60 µm or more and osteon diameters of 300 µm or more are indicative of primate remains and hence additional research is needed to separate bones of various primate species and adult and juvenile humans.

ABSTRAK

Behalwe vir makroskopiese eienskappe en bruikbare DNS, is daar min metodes vir die akkurate onderskeiding van mens- en dierbene wat in forensiese kontekste gevind word, beskikbaar. Die doel van hierdie studie was om vas te stel of betekenisvolle chemiese en histologiese verskille tussen mens- en dierbene bestaan wat buikbaar is in hul onderskeiding. Beenmonsters van femora en tibiae van tien koeie (*Bos taurus*), tien skape (*Ovis aries*), vyf impalas (*Aepyceros melampus*), 12 donkies (*Equus africanus asinus*), agt katte (*Felix catus*), 12 honde (*Canis lupus familiaris*), 12 varke (*Sus scrofa domestica*), sewe primate, 27 volwasse mense en ses kinders (*Homo sapiens sapiens*) is gebruik.

Mikro-element analise is uitgevoer met ‘n skandeer elektronmikroskoop met ‘n gemonteerde energie dispersiewe spektrometer (SEM/EDS) en het die analise van kalsium (Ca), fosfor (P), kalium (K), natrium (Na), chloor (Cl), swael (S), silikon (Si), aluminium (Al), magnesium (Mg), sink (Zn), lood (Pb) en strontium (Sr) ingesluit. Statisties betekenisvolle verskille in die langbene van omnivore and herbivore is gevind met betrekking tot K ($p=0.0001$), Mg ($p=0.0214$), Cl ($p=0.0001$) en S ($p=0.0012$) vlakke, terwyl K ($p=0.0001$), Na ($p=0.0192$) en Cl ($p=0.0001$) vlakke betekenisvol verskil het tussen omnivore en karnivore. Subtiele verskille tussen femora en tibiae is ook gevind en regverdig verdere navorsing.

Ligmikroskopie is gebruik om die histomorfologie van kortikale been van verskillende spesies te evalueer. Kwalitatiewe inspeksie het die organisasie van verskillende strukture ingesluit, terwyl kwantitatiewe inspeksie metings van die

totale hoeveelheid Haverse sisteme en nie-Haverse kanale asook die minimum en maksimum deursnee van Haverse sisteme en kanale ingesluit het. Alle spesies het Haverse been in die femur, tibia of beide bene bevat, behalwe vir koei- en varkbene wat pleksiforme been bevat het. Die teenwoordigheid van pleksiforme been het bene akkuraat van ‘n menslike oorsprong uitgesluit. Statisties betekenisvolle verskille tussen spesies is ook aangedui met betrekking tot alle kwantitatiewe veranderlikes, uitsluitend tibiale Haverse kanaal afmetings. Die huidige studie het aangedui dat ‘n Haverse kanaal deursnit van 60 µm of meer en ‘n Haverse sisteem deursnit van 300 µm of meer ‘n primaat oorsprong aandui en verdure navorsing word dus benodig om bene van verskillende primaatspesies asook volwasse mens en kinderbene te onderskei.

CHAPTER 1

INTRODUCTION

Forensic anthropologists are increasingly consulted to assist in the identification of skeletal material by compiling an osteodemographic profile that generally consists of the determination of age-at-death, sex, stature and ancestry (Fazekas & Kósa, 1978; Scheuer & Black, 2007). With an increase in crime rates and global mass disasters, more pressure has been placed on the forensic scientist to aid in the identification, or exclusion of human remains recovered amongst animal and environmental ruins (Harsányi, 1993; Cattaneo *et al*., 1999; Martiniaková *et al*., 2006a; Scheuer & Black, 2007; Hillier & Bell, 2007). Differentiating between human and animal bones can easily be done if the remains found contain distinctive gross morphological features related to the specific species involved (Harsányi, 1993; Scheuer & Black, 2007; Hillier & Bell, 2007). However, separating commingled, damaged, burnt or fragmented pieces of bone, with no species-specific morphology, can become difficult (Cattaneo *et al*., 1999; Ubelaker *et al*., 2002; Martiniaková *et al*., 2006a). Accurate differentiation techniques are crucial as fragmented or small animal bones can easily be mistaken for human infant or juvenile remains (Fazekas & Kósa, 1978; Harsányi, 1993). Thus, in the absence of distinct anatomical characteristics, additional methods have to be explored to enable the investigator to accurately determine the origin of the remains in question.

Research has shown that it is possible to separate human and animal remains by using mtDNA (Bataille *et al*., 1999; Cattaneo *et al*., 1999;

Martiniaková *et al*., 2006a). DNA analysis is an accurate separation technique; however, the success of this analysis is dependent on the degradation of the DNA, the laboratory's ability to extract DNA and the presence of contaminants (Cattaneo *et al*., 1999; Scheuer & Black, 2007; Cattaneo *et al*., 2009). It is also a time consuming and expensive procedure. Other separation techniques that have been used to distinguish human from animal remains include immunological reactions, immunoelectrophoresis, fluorescent immunohistological procedures, serological examinations as well as light and scanning electron microscopy (Fazekas & Kósa, 1978; Harsányi, 1993).

A popular and relatively cost effective method for the sorting of fragmentary human and animal remains is the examination of the microstructure of compact bone, as species-specific differences in the histology of bone have been well documented (Enlow & Brown, 1958; Harsányi, 1993; Cattaneo *et al*., 1999; Mulhern & Ubelaker, 2001; Martiniaková *et al*., 2006a; Hillier & Bell, 2007). In a case from Louisiana, forensic anthropologists successfully identified the origin of skeletal remains using histological analysis. The body of a murdered female was found in the Mississippi River and during autopsy, at least two gunshot wounds were identified; one to the head and one to the chest, shattering the humerus. Bone fragments were found in the suspect's car and according to the perpetrator was that of a hunted deer. Investigators compared deer bone and human bone (humerus) from the autopsy to the fragments found in the car. They were able to successfully exclude the fragments from being deer remains due the lack of plexiform bone and other comparable quantitative measurements. The autopsy material was, however,

comparable to the fragments found in the car and the suspect ultimately admitted guilt (Owsley *et al*., 1984).

Histological separation is not always successful as illustrated in a case from South Africa. During the Apartheid era in South Africa a number of anti-apartheid activists' bodies were illegally disposed of. These bodies were blown up, time and time again, until only fragments of bone were left. Forensic anthropologists were approached with fragmentary remains and the origin of these remains had to be established. Some skeletal remains retained species-specific morphological characteristics while others were completely devoid of any identifiable features. Histology was implemented but due to the limited knowledge and experience, the results were inconclusive (Steyn M., pers comm.). Therefore, more research is needed to make osteologists and scientists alike aware of identifiable histological differences between human and non-human osseous tissue.

Histological examination is not only implemented in the separation of human and non-human remains, it has also, more recently, been used as a tool to establish the preservation of bone and consequently the amplification probability of DNA, for further DNA analysis (Martiniaková. *et al*., 2006b).

Chemical analysis of various materials is increasingly used to determine whether or not the materials in question are of skeletal, dental or non-biological origin. This forms a 'preliminary test' of questionable debris and can consequently decrease unnecessary DNA analysis on non-skeletal or dental remains (Ubelaker *et al*., 2002). The potential of chemical analysis in forensic anthropology was emphasised in February 2001 in Georgia, USA. A number of urns filled with human cremains, cement and other biological

materials were presented to forensic anthropologists for human identification. Knowing that trace elements are stored in the body and that different materials have different chemical signatures, investigators were able to identify human cremains from other biological materials (Brooks *et al*., 2006). Similarly, differences within the element distribution of various animal bones have been indicated and are mainly attributed to the different diets preferred by various animals (Toots & Voorhies, 1965). Toots and Voorhies (1965) indicated higher strontium content in the skeletal remains of herbivores, compared to carnivores. These differences were attributed to the increased amount of strontium found in vegetation that formed the main diet of herbivores.

As an increased amount of skeletal material is submitted to forensic anthropologists, fast and effective separation techniques need to be set in place that permit the investigator to accurately determine the origin of remains in question. Therefore, the aim of this study was to firstly evaluate the chemical differences amongst the long bones of humans, non-human primates and other quadrapedal mammals that could aid in sorting commingled, damaged or fragmented skeletal elements. It is hypothesized that the dietary differences between herbivores, carnivores and omnivores will lead to differences in the chemical composition of the bones of animals from these feeding groups. It will be assessed whether these differences are of sufficient magnitude and consistency to separate human from non-human skeletal remains.

Secondly, the histomorphometrical differences between the bones of humans and various other South African species will be studied, in order to

determine whether the expected differences can be used to identify the species of origin. This will include both morphological descriptions and measurements of various histological structures such as the size of osteons (Haversian systems) and Haversian canals. Some success with this has been reported by other authors (Jowsey, 1966; Cattaneo *et al*., 1999; Cuijpers, 2006; Martiniaková *et al*., 2006a; Martiniaková *et al*., 2006b; Hillier & Bell, 2007; Martiniaková *et al*., 2007a; Zedda *et al*., 2008), but as each area of the world has its own range of animal species, this needs to be done for southern African fauna as well.

CHAPTER 2

LITERATURE REVIEW

2.1. BONE HISTOLOGY

The difference between bone and cartilage had been illustrated as early as the days of Aristotle (384-322 BC), who was able to distinguish between cartilaginous and bony fish due to the presence of either cartilaginous or bony tissue. Bone only exists in vertebrates and forms the skeletal system of the body (Hall, 2005). It is made up of specialised connective tissue and serves to support the body, protects the organs, gives attachment for muscles and ligaments that therefore allow body movements and it houses the bone marrow that is responsible for the production of blood cells. Bone also stores a number of minerals including phosphate (P) and about 99% of the body's calcium (Ca), making it actively involved in maintaining homeostasis in the body (Francillon-Vieillot *et al*., 1997; Weiner *et al*., 1999; Junqueira & Carneiro, 2003; Hall, 2005; Antonio, 2008).

Macroscopically, mammalian bone can be separated into two types of bone; cortical bone and cancellous bone (Klevezal, 1996; Hillier & Bell, 2007). About 80% of the entire human skeletal is composed of cortical bone compared to the mere 20% contributed by cancellous bone (Ott, 2002; Taicher *et al*., 2003). Cancellous bone, also known as spongy or trabecular bone (*substantia spongiosa*), consists mainly of bony spicules or trabeculae located on the internal surface and at the extremities (epiphyses) of long bones (Schultz, 1997; Hillier & Bell, 2007). Cortical bone is hard bone that surrounds the external surface of bones (*corticalis*) and when present as a

thick layer, it is also referred to as compact bone (*substantia compacta*) (Schultz, 1997).

Microscopically, cortical bone can be subdivided into three zones that are not clearly delimited. These zones are the periosteal (outer surface), mesosteal (central portion) and endosteal (inner surface) zones. The periosteal and endosteal zones are the result of appositional activity of the periosteum and endosteum respectively, while the mesosteal zone is formed as a result of the secondary reconstruction of bone (Klevezal, 1996; Hillier & Bell, 2007).

All bones in the body are lined internally and externally by connective tissue and layers of osteogenic cells (bone forming cells). The internal lining of bone is a thin, single layer of osteoprogenitor cells and is known as the endosteum (Junqueira & Carneiro, 2003). This layer was unknowingly described by Robin (1849) who described a lining layer of osteoblasts along areas of bone formation (Hall, 2005). A connective tissue membrane known as the periosteum lines cortical bone on the outer surface and consists of two layers; the inner and outer periosteal layers (Junqueira & Carneiro, 2003; Antonio, 2008). The outer layer consists of collagen fibres and fibroblasts whilst the inner layer or cambium consists of osteoprogenitor cells (Francillon-Vieillot *et al*., 1997; Junqueira & Carneiro, 2003; Antonio, 2008). These two layers ensure nutrition to the bone as well as a sufficient supply of cells that are capable of bone repair and growth (osteoblasts) (Junqueira & Carneiro, 2003).

Bones are classified into four categories based on the shape and structure of the bone; i.e. long, short, flat and irregular bones (Ross *et al*., 1995).

Long bones (E.g. ulna and metatarsal bones)

These bones consist of a shaft or diaphysis and two extremities or epiphyses. The epiphyseal ends are comprised of cancellous bone surrounded by a thin layer of cortical bone. The diaphysis, on the other hand, is comprised of a thick layer of compact bone surrounding a small amount of cancellous bone that encloses a medullar cavity (*cavitas medullaris*). Less distinctive transitional areas known as the metaphysis are also present and are located between the diaphysis and the two epiphyses (Ross *et al*., 1995; Francillon-Vieillot *et al*., 1997; Junqueira & Carneiro, 2003; Halstead, 2007).

Short bones (E.g. carpal and sesamoid bones)

Short bones are very similar to long bones. They are almost cuboidal in shape and have a spongy core surrounded by cortical bone (Ross *et al*., 1995; Schultz, 1997; Francillon-Vieillot *et al*., 1997).

Flat bones (E.g. bones of the skull vault)

Flat bones do not have epiphyses. These bones are characterised by double layers of cortical bone known as plates that sandwiches a single layer of spongy bone called the diploë (Ross *et al*., 1995; Schultz, 1997; Junqueira & Carneiro, 2003).

Irregular bones (E.g. vertebrae and pneumatic bones)

The complex shapes of irregular bones are not comparable with any of the characteristics observed in long bones, short bones or flat bones (Ross *et al*., 1995). Some irregular bones are filled with air to form cavities known as

paranasal sinuses. These sinuses are present within a number of skull bones such as the maxilla and are also referred to as pneumatic bones (Ross *et al*., 1995; Schultz, 1997).

2.1.1. Microstructure of bone

With the invention of the microscope a whole new world opened to the field of research (Hall, 2005). The microscope allowed researchers such as Havers (1691/1692) and Leeuwenhoek (1693) to describe bone on a histological level (Enlow & Brown, 1956; Francillon-Vieillot *et al*., 1997; Hall, 2005).

2.1.1.1. General bone microscopy

Bone can be classified according to three distinct criteria. Of these criteria, (a) the organisation of the bone matrix and (b) the arrangement of the vascular canals within the compact part of the bone are most frequently used when describing bone. The third criterion revolves around the history of the bone formation patterns and include bone types such as primary and secondary and, periosteal and endosteal bone (Francillon-Vieillot *et al*., 1997).

If one examines the various organizations of the collagen fibres found within the bone matrix, three types of bone can be described and include woven bone, parallel-fibred bone and lamellar bone (Francillon-Vieillot *et al*., 1997).

Woven bone (fibrous bone or non-lamellar bone) initially appears as spicules or trabecules which are characterised by a haphazard organisation of coarse and loosely packed collagen fibres with randomly arranged osteocytes

(Francillon-Vieillot *et al*., 1997; Hillier & Bell, 2007; Antonio, 2008). The collagen fibres are laid down very rapidly, become highly mineralized and form the scaffold needed for lamellar bone formation (Currey, 2003). Woven bone is produced during periods of growth and is commonly observed in foetal and infant bones (Francillon-Vieillot *et al*., 1997; Hillier & Bell, 2007). It is also produced when tissue repair is needed and in response to a number of bone affecting pathologies (Junqueira & Carneiro, 2003).

Parallel-fibered bone (thin-fibered bone or 'pseudo-lamellar' bone) can be described as the transition between woven and lamellar bone. It consists of collagen bundles that are loosely packed that project in similar directions when microscopically observed with polarised light (anisotropic) (Francillon-Vieillot *et al*., 1997).

Bone tissue that exhibits a highly organised collagen arrangement is known as lamellar bone (Francillon-Vieillot *et al*., 1997). It is characterised by circumferentially deposited thin, parallel layers or sheets of bone called lamellae (Enlow D.H. 1966; Junqueira & Carneiro, 2003). Generally, long bones are characterised by outer circumferential lamellae near the periosteum and inner circumferential lamellae that enclose the marrow cavity at the endosteal zone (Junqueira & Carneiro, 2003). Interstitial or intermediate lamellae are also present in long bones and are the remnants of osteons and circumferential lamellae that have been replaced during bone growth or bone remodelling and are enclosed between the inner and outer circumferential lamellae (Cohen & Harris, 1958; Junqueira & Carneiro, 2003). Each lamella consists of minerals and layers of collagen fibres with a few longitudinally arranged vascular canals observed between successive lamellae (Locke,

2004; Hillier & Bell, 2007). Vascular canals surrounded by a few concentric lamellae are known as primary osteons and are initially present in lamellar bone due to the transition between immature and mature bone (Klevezal, 1996; Hillier & Bell, 2007). Lamellar bone is formed by a very slow formation rate that ensures a more precise and organised appearance (Francillon-Vieillot *et al*., 1997; Currey, 2003).

Cortical bone as well as cancellous bone can be comprised of any of the above mentioned bone tissue types (Klevezal, 1996; Mohsin *et al*., 2002).

Describing bone according to vascular canal arrangements provides classification groups such as primary vascular bone, Haversian bone and non-vascular (avascular) bone (Enlow & Brown, 1958; Harsányi, 1993; Francillon-Vieillot *et al*., 1997).

Primary vascular bone

Primary vascular bone consists of vascular canals and primary osteons arranged within the bone matrix (Enlow & Brown, 1958). These canals can be organised in one or more directions (Francillon-Vieillot *et al*., 1997).

Unidirectional classification of bone includes circular (**Figure 2.1**), radial (**Figure 2.2**), reticular (**Figure 2.3**) or longitudinally orientated vascular canals. Bone tissue that exhibits longitudinally orientated vascular canals, are characterised by vascular canals that run parallel to the long axis of bone (Enlow & Brown, 1956). The orientation of these vascular canals can further be divided into circular rows (**Figure 2.4**), radial rows (**Figure 2.5**) or it can be orientated in bundles (**Figure 2.6**) (Francillon-Vieillot *et al*., 1997).

Multidirectional vascular canals give rise to laminar, plexiform, radial or reticular bone (**Figure 2.6-2.10**) (Francillon-Vieillot *et al*., 1997).

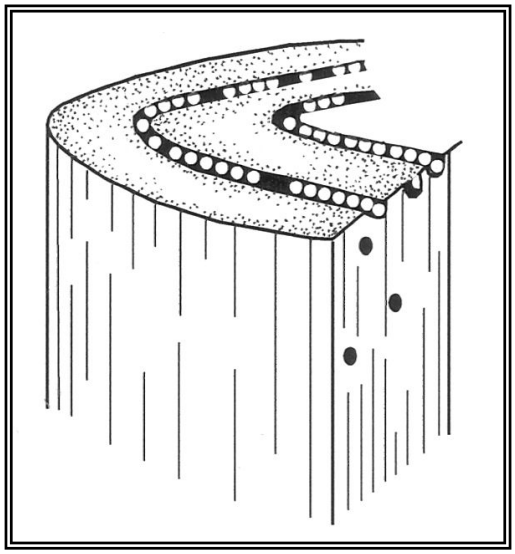


Figure 2.1 *Circularly orientated vascular canals (Redrawn from Francillon-Vieillot et al., 1997).*

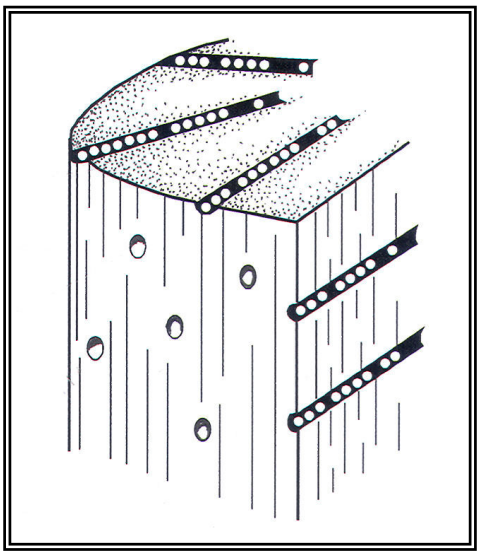


Figure 2.2 *Radially orientated vascular canals (Redrawn from Francillon-Vieillot et al., 1997).*

Primary vascular laminar bone (**Figure 2.7**)

Laminar bone consists of laminae or broad bands of bone that are arranged circumferentially around the medullary cavity of bone (Enlow &

Brown, 1956). These bands are separated from one anther by consecutive circularly and longitudinally orientated vascular canals (Francillon-Vieillot *et al.*, 1997).

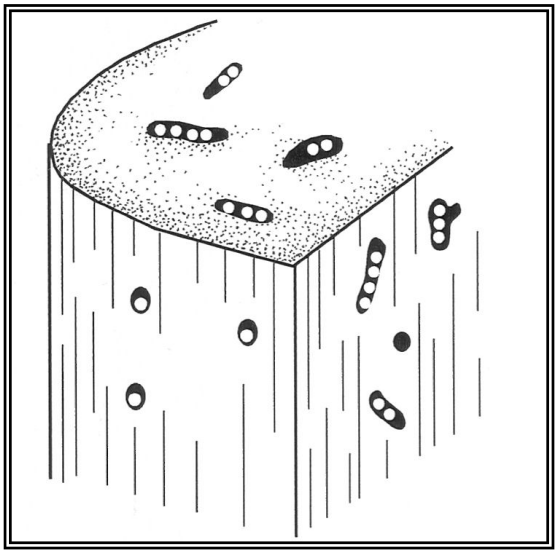


Figure 2.3 *Reticular or irregular orientated vascular canals (Redrawn from Francillon-Vieillot et al., 1997).*

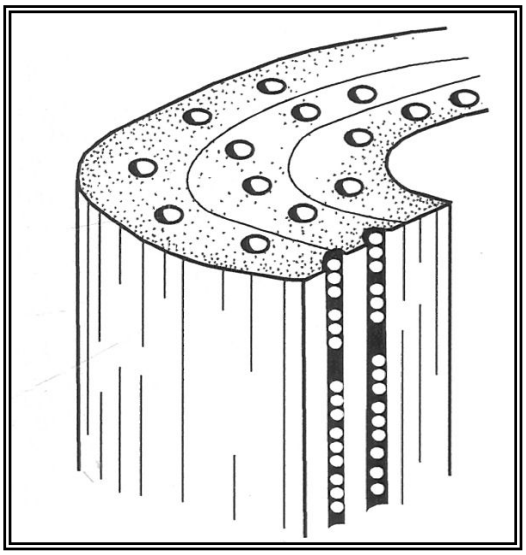


Figure 2.4 *Longitudinal vascular canals arranged in circular rows (Redrawn from Francillon-Vieillot et al., 1997).*

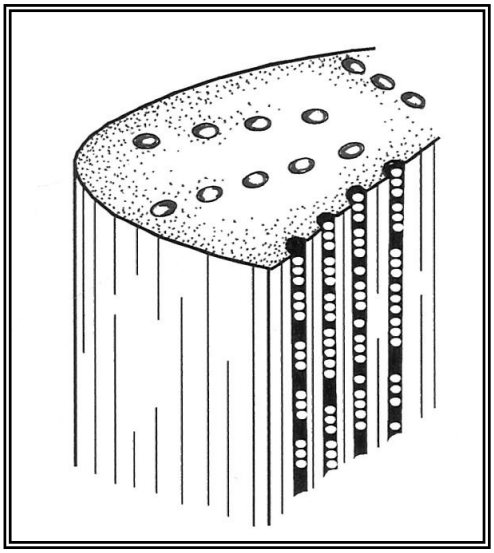


Figure 2.5 *Longitudinal vascular canals arranged in radial rows (Redrawn from Francillon-Vieillot et al., 1997).*

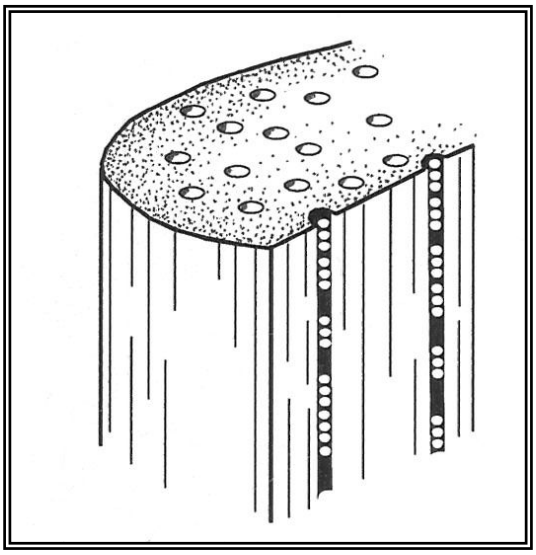


Figure 2.6 *Longitudinal vascular canals arranged in bundles (Redrawn from Francillon-Vieillot et al., 1997).*

Primary vascular plexiform bone (**Figure 2.8**)

This type of bone tissue is very similar to laminar primary vascular bone but differs with regard to the dense vascular system where canals are orientated not only longitudinally and circularly (circumferentially), but also radially (Francillon-Vieillot *et al*., 1997). This highly organised vascular pattern gives

bone a particular “brick wall” appearance (Enlow & Brown, 1956, 1957 and 1958; Enlow, 1966; Pearce *et al*., 2007; Hillier & Bell, 2007).

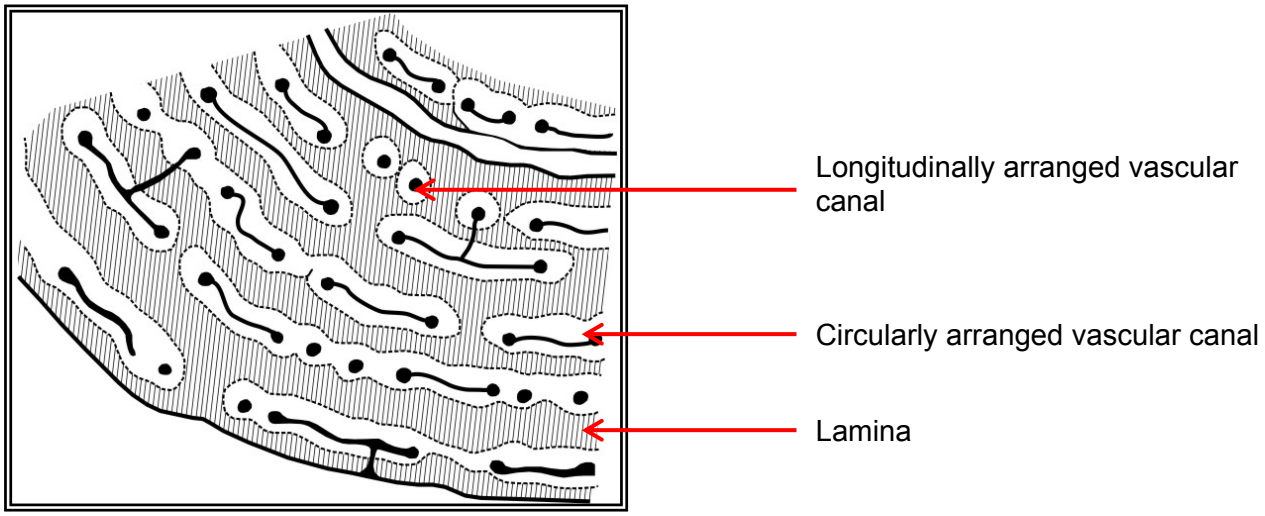


Figure 2.7 *Primary vascular laminar bone (Redrawn from Francillon-Vieillot et al., 1997).*

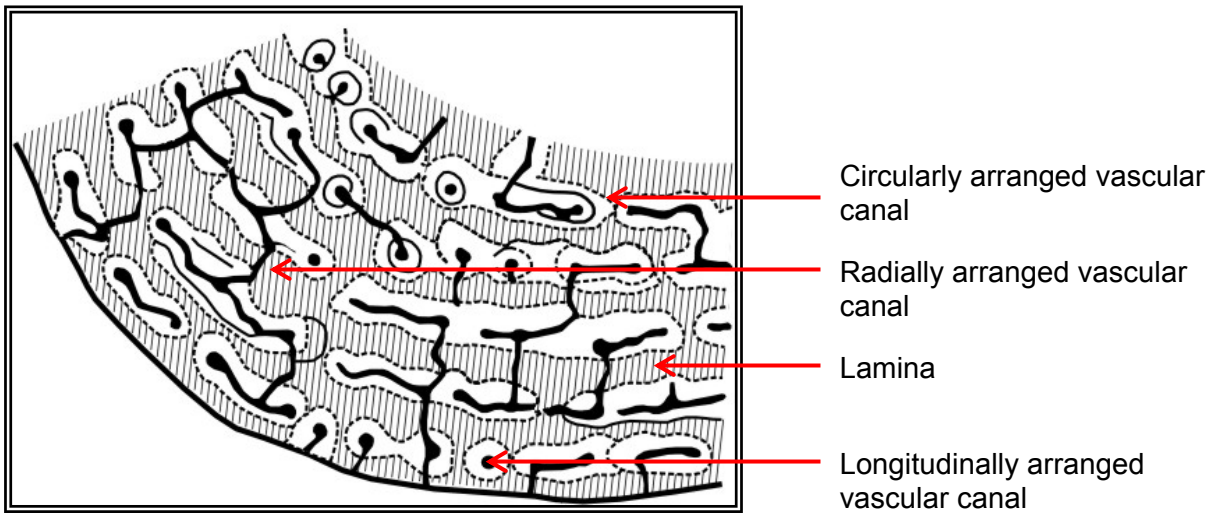


Figure 2.8 *Primary vascular plexiform bone (Redrawn from Francillon-Vieillot et al., 1997).*

Primary vascular radial bone (**Figure 2.9**)

Cortical bone containing branched or unbranched vascular canals that radiate out from either the endosteal or periosteal surfaces are described as radial bone tissue (Enlow & Brown, 1956).

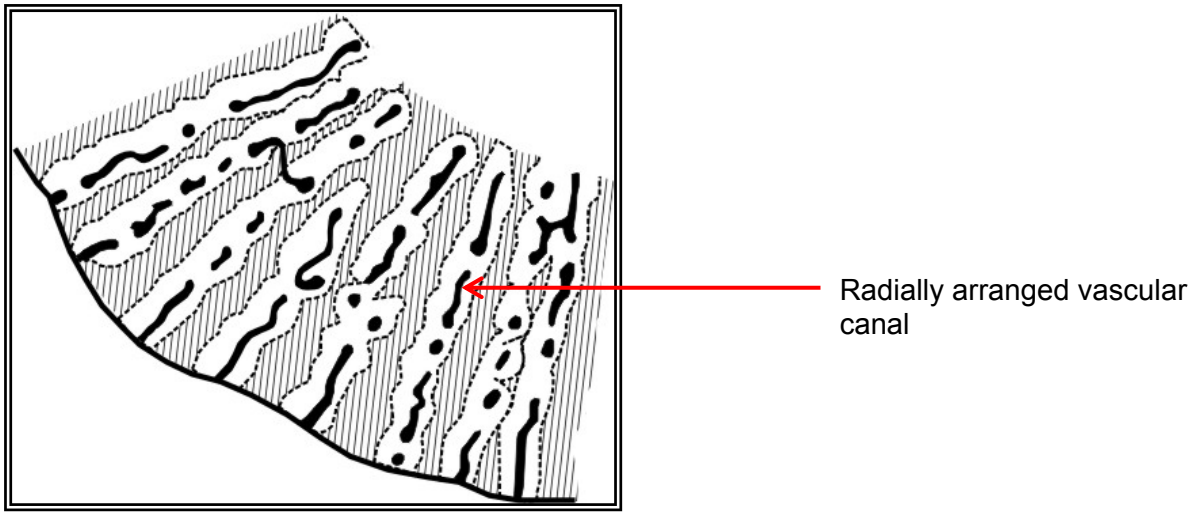


Figure 2.9 *Primary vascular radial bone (Redrawn from Francillon-Vieillot et al., 1997).*

Primary vascular reticular bone (**Figure 2.10**)

In reticular bone tissue the vascular canals appear unorganised and branch out in any direction (Enlow & Brown, 1956; Francillon-Vieillot *et al*., 1997).

Figure 2.10 *Primary vascular reticular bone (Redrawn from Francillon-Vieillot et al., 1997).*

Haversian bone

The presence of secondary osteons forms the basis of this type of bone tissue (Enlow & Brown, 1958). Osteons, secondary osteons or Haversian

systems are round or ellipsoidal in shape and are formed by the successive, inward formation of lamellae (Cohen & Harris, 1958; Mohsin *et al*., 2002; Junqueira & Carneiro, 2003). These concentric lamellae consist of collagen fibres that are arranged in 4-20 lamellae of about 3-7 µm that surround a centrally located vascular canal (Junqueira & Carneiro, 2003; Hillier & Bell, 2007). These canals are known as Haversian canals and were named after Clopton Haver (1665-1702) who first identified them (Hall, 2005). The diameter of Haversian canals differ due to the remodelling of bone and can vary between 100 µm and 400 µm (Mohsin *et al*., 2002; Junqueira & Carneiro, 2003). Newly formed osteons have very big Haversian canals with the latest formed lamellae in direct contact with the canal. The canal houses blood vessels, nerves and connective tissue (Junqueira & Carneiro, 2003). Haversian canals can be 0.001-2.1 mm long and are traditionally believed to run straight and parallel to the long axis of bone (Mohsin *et al*., 2002; Junqueira & Carneiro, 2003). Research by Cohen and Harris (1958) on dog femora has, however, indicated that some osteons give off a number of branches while spiralling around the axis of the bone. The authors noted a clockwise spiralling in the left femur and an anticlockwise spiralling in the right femur (Cohen and Harris, 1958).

Osteons are connected to the periosteum, bone marrow and to one another by perpendicularly orientated Volkmann's canals (Mohsin *et al*., 2002; Junqueira & Carneiro, 2003). These canals are radially orientated vascular canals that are not surrounded by concentric lamellae (Francillon-Vieillot *et al*., 1997; Junqueira & Carneiro, 2003). Depending on the number of osteons present and the arrangement of these osteons, Haversian bone can be further

subdivided into irregular, dense or endosteal Haversian bone (Enlow & Brown, 1956; Hillier & Bell, 2007). Irregular Haversian bone contains a few, scattered osteons, while dense Haversian bone is characterised by numerous, tightly packed osteons with little or no interstitial lamellae in between neighbouring osteons (Francillon-Vieillot *et al*., 1997; Hillier & Bell, 2007). Endosteal Haversian bone contains larger, irregular shaped osteons that are mainly located at the endosteal zone of bone (Pfeiffer, 1998; Hillier & Bell, 2007).

The third criterion for bone classification depends on the ontogenetic patterns of bone formation and, among others, describes bone as either primary or secondary, or periosteal or endosteal (Francillon-Vieillot *et al*., 1997).

Primary bone is bone that is laid down during initial growth and contains primary osteons (Enlow & Brown, 1958; Hillier & Bell, 2007). These osteons develop together with unremodelled primary bone and are thus known as primary osteons (Kerley, 1965). Primary osteons are surrounded by a few concentric layers of bone and are responsible for blood and nutrient supply (Hillier & Bell, 2007). Apart from a few areas in the body such as the areas around the sutures of the skull, all primary bone is replaced by secondary bone in adult humans (Junqueira & Carneiro, 2003).

Secondary bone or Haversian bone is formed after pre-existing bone had been resorbed. It is characterised by the presence of osteons (secondary or tertiary) and has a general appearance as described in Haversian bone above (Hillier & Bell, 2007).

Primary and secondary osteons are very similar in appearance but differ with regards to size and general orientation (Hillier & Bell, 2007). Secondary osteons are generally larger than primary osteons and are delineated by a cement line that is absent in primary osteons (Kerley, 1965; Mohsin *et al*., 2002; Junqueira & Carneiro, 2003; Hillier & Bell, 2007). Also, secondary osteons intersect the circumferential lamellae while primary osteons usually form between the lamellae (**Figure 2.11**) (Hillier & Bell, 2007). The central canal of primary osteons has a diameter of less than 100 µm and contains two or more blood vessels whilst secondary osteons have a larger canal with a single blood vessel (Hall, 2005).

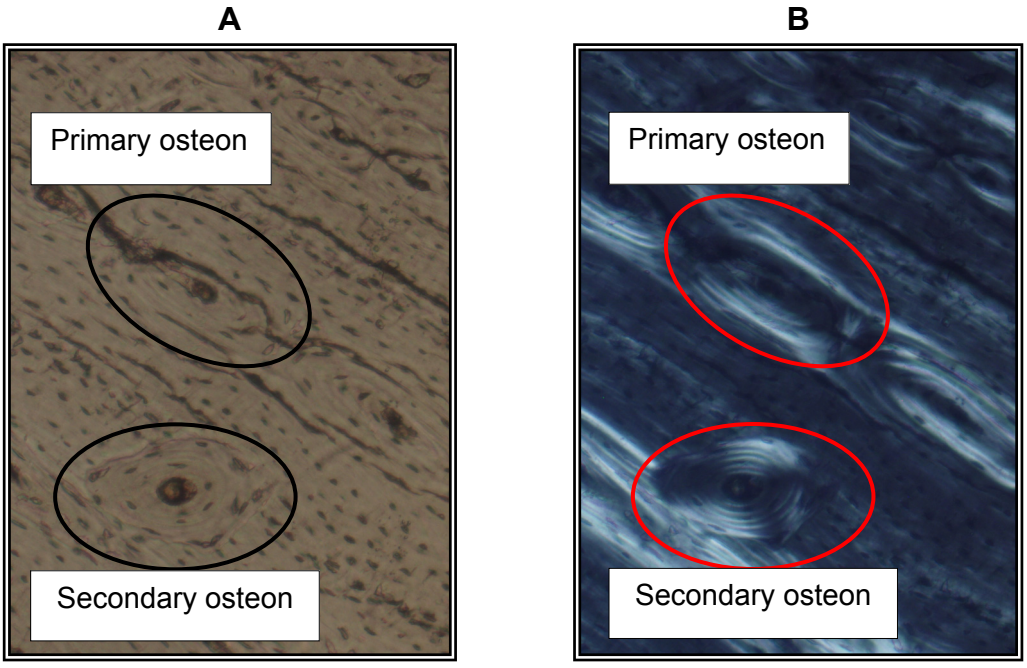


Figure 2.11 *Illustration of primary and secondary osteons as observed in a donkey (Equus africanus asinus) femur. Primary osteons are found between lamellae while secondary osteons intersect the circumferential lamellae. Observation A made with normal bright light and observation B made with polarised light (Magnification x100, photograph from current study).*

Non-vascular bone

Non-vascular bone, as the name implies, is bone tissue that is completely devoid of all vascular canals (Enlow & Brown, 1958).

Bone can exhibit only a single or a combination of any of the different bone types described above, e.g. cortical or cancellous, woven or lamellar, vascular or non-vascular, primary (circular, radial, reticular, longitudinal, laminar, plexiform, etc.) or secondary (irregular, endosteal or dense Haversian bone tissue) (Enlow & Brown, 1956; Francillon-Vieillot *et al*., 1997). An individual may have one bone consisting entirely of one type of tissue and another bone consisting entirely of a different type of bone tissue (Enlow & Brown, 1956). It is of the utmost importance to gain thorough understanding of the different types of bone tissue as various terms, such as laminar bone and Haversian bone and, lamellae and laminae are used and misunderstood throughout the literature (Francillon-Vieillot *et al*., 1997; Locke, 2004; Hillier & Bell, 2007; Zedda *et al*., 2008).

2.1.1.2. Microscopic bone differences in various animals

In 1849, John Quekett initiated research on the bone microstructure of animal species and indicated a number of differences within the micro-architecture of bone among various mammalian species (Enlow & Brown, 1956; Hall, 2005). Similar studies have continued over time and are still of interest to scientists today as most vertebrate species do not exhibit the classical textbook description of Haversian bone tissue (Enlow & Brown, 1958; Enlow, 1966; Singh *et al*., 1974; Hall, 2005).

The histomorphology of bone of adult and juvenile humans (*Homo sapiens sapiens*) as well as that of a few animal species will be discussed below. Images of the different animal bone types, taken from the current study, were also included to supplement various descriptions.

Order: Primate

Juvenile (Homo sapiens sapiens) bone

Immature human skeletons are characterised by a predominance of non-Haversian bone in the periosteal zone, except for areas of muscle attachment where dense Haversian bone is present (**Figure 2.12**). The endosteal surface of growing individuals is characterised by dense Haversian bone while non-Haversian bone can be either one or a combination of the various types of primary bone. With age, primary bone is gradually replaced by Haversian bone during bone remodelling (Enlow, 1966).

Robling and Stout (1999) noted that drifting osteons are commonly observed in juvenile bones. Drifting osteons are described as osteons that drift towards the endosteal zone of bones. These osteons are characterised by continuous bone resorption and bone formation on opposite sides that lead to the formation of elongated osteons with eccentrically located Haversian canals (Robling & Stout, 1999).

Plexiform bone is also occasionally observed in juvenile osseous remains because of its fast production rate that easily accommodates for rapid increase in skeletal length and size (Martiniaková *et al*., 2006b; Cattaneo *et al*., 2009).

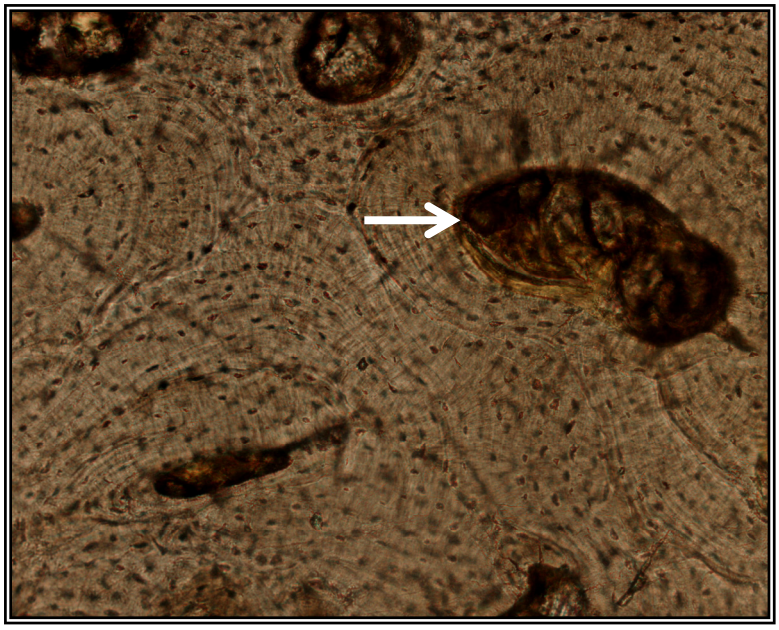


Figure 2.12 *Illustration of dense Haversian bone observed in a juvenile (Homo sapiens sapiens) tibia viewed with normal bright light. White arrow indicates a large Haversian canal within an osteon (Magnification x100).*

Adult (Homo sapiens sapiens) bone

The general microstructure of adult long bones is characterised by dense Haversian bone (**Figure 2.13**), sandwiched between endosteal and periosteal layers of circumferential lamellae (Francillon-Vieillot *et al*., 1997; Hillier & Bell, 2007). There are more circumferential lamellae in the periosteal zone of adult bones than in the endosteal zone (Hillier & Bell, 2007). Some areas of human bone consist of a few scattered secondary osteons and not the dense Haversian bone as generally accepted (Enlow & Brown, 1958). The osteons are mostly round with eccentrically located Haversian canals (Harsányi, 1993). No primary osteons are present in adult compact bone (Martiniaková *et al*., 2006a).

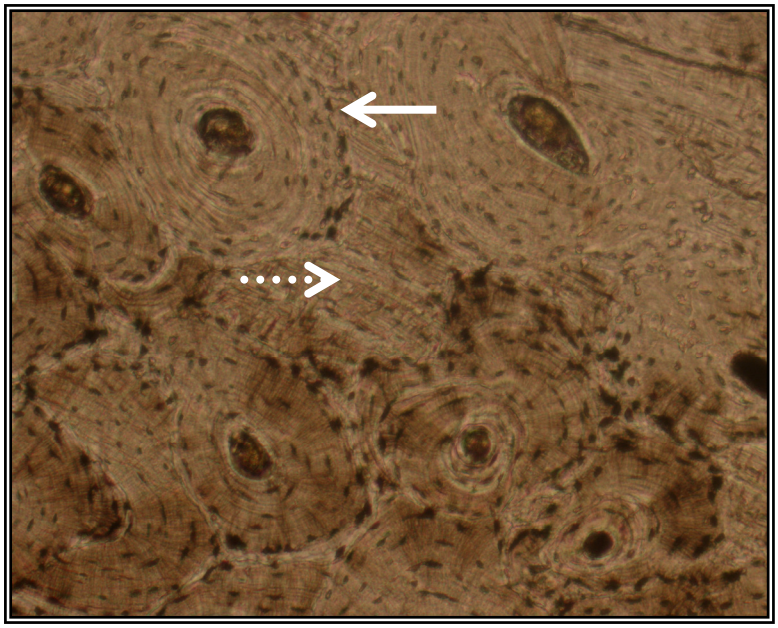


Figure 2.13 *Illustration dense Haversian bone observed in an adult human (Homo sapiens sapiens) femur viewed with normal bright light. A few scattered secondary osteons (white arrow) interrupt the continuous periosteal circumferential lamellae indicated by the white dotted arrow (Magnification x100).*

Old world monkey (Ceropithecidae) bone

Immature primate skeletal remains consist mostly of primary vascular longitudinal bone (Enlow & Brown, 1958; Singh *et al*., 1974; Hillier & Bell, 2007). This primary bone is replaced by secondary bone and thus the skeletal elements of mature primates consist of dense Haversian bone (**Figure 2.14**). This dense Haversian bone is located between thin layers of endosteal and periosteal circumferential lamellae (Hillier & Bell, 2007). Not all primate bones are composed entirely of dense Haversian bone; if the primary canals within the primary bone are numerous, the resulting Haversian bone will be dense, whilst few scattered primary canals will result in irregular Haversian bone (Enlow & Brown, 1958).

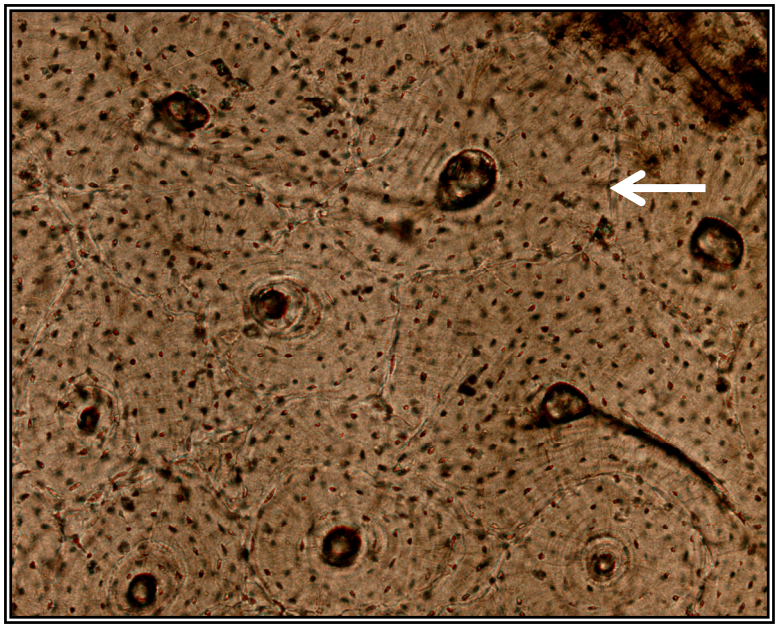


Figure 2.14 *Illustration of dense Haversian bone observed in an old world monkey (Ceropithecidae) tibia viewed with normal bright light. Osteons (white arrow) are closely related to one another with few interstitial lamellae between neighbouring osteons (Magnification x100).*

Order: Artiodactyla

Pig (Sus scrofa domestica) bone

Martiniaková *et al*. (2006a and 2006b) describe the compact bone of pigs as mainly primary vascular plexiform bone. However, primary vascular laminar bone (**Figure 2.15**) has also been recorded in pig long bones and is formed by broad slabs of circumferentially arranged laminae (Martiniaková *et al*., 2007a). Towards the middle third of pig bones the primary bone becomes dense Haversian bone with a large number of resorption lacunae observed especially in antero-medial views of bone slides (Martiniaková *et al*., 2007a).

Plexiform bone is the characteristic bone tissue of hogs, according to descriptions by Enlow and Brown (1958). The authors confirm the presence of laminations, but state that the addition of radial canals gives a more complex appearance to the bony tissue. Bone tissue transforms generally

from plexiform tissue to regions with scattered osteons to dense Haversian bone tissue at the endosteal regions (Enlow & Brown, 1958).

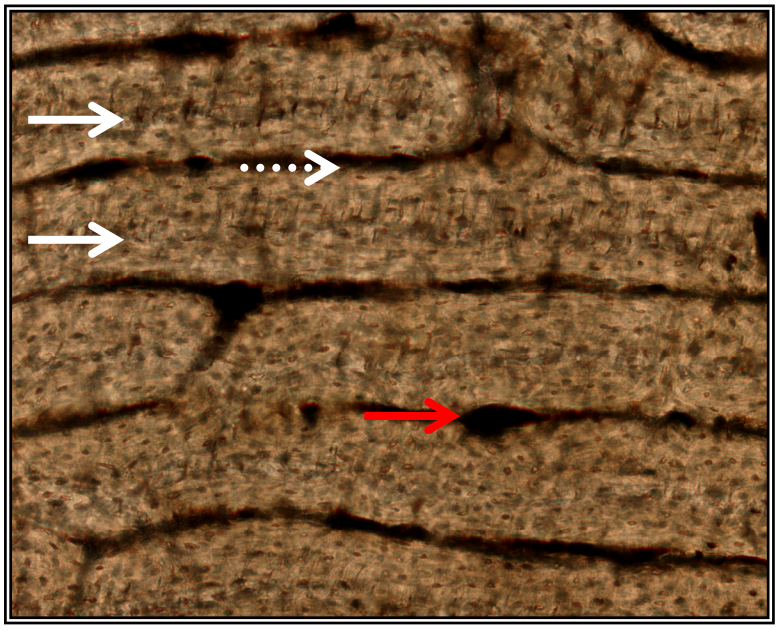


Figure 2.15 *Illustration of primary vascular laminar bone observed in a pig (Sus scrofa domestica) femur viewed with normal bright light. White arrows indicate circumferential laminae separated by circularly orientated vascular canals (white dotted arrow). Red arrow indicates longitudinally orientated vascular canal (Magnification x100).*

Cow (Bos taurus) bone

Plexiform bone (**Figure 2.16**) with numerous primary canals makes up the basic structural pattern of cow long bones. Areas of non-vascular bone are also present around the periosteal and endosteal areas (Martiniaková *et al*., 2007a). In the middle zone irregular to dense Haversian bone is present (Enlow & Brown, 1958; Martiniaková *et al*., 2007a; Zedda *et al*., 2008). Osteons have been described as elliptical in shape with about 5-7 lamellae surrounding the Haversian canals (Zedda *et al*., 2008).

Most histological descriptions of cow bones are based on subadult cow bones rather than adult cow bones due to modern butchering practices that slaughter cows between 13 and 24 months of age (Hillier & Bell, 2007).

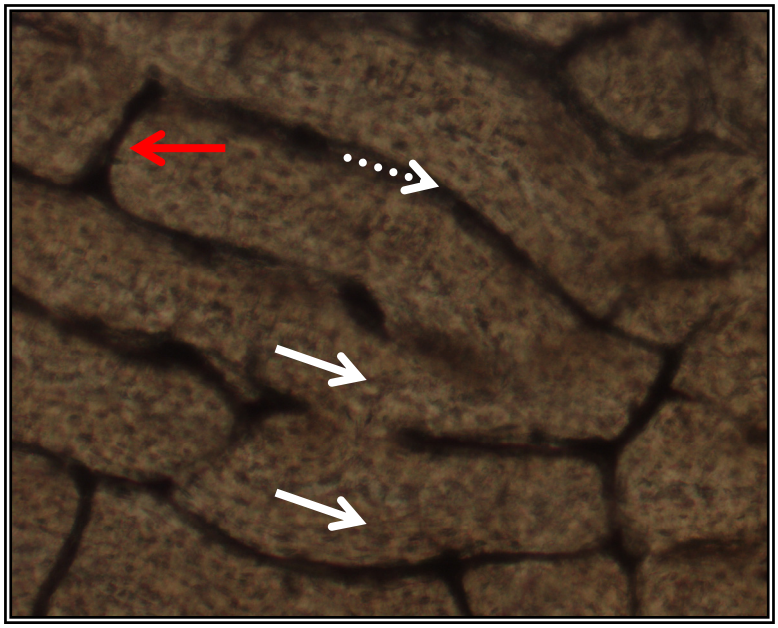


Figure 2.16 *Illustration of primary vascular plexiform bone observed in a cow (Bos taurus) femur viewed with normal bright light. White arrows indicate circumferential laminae separated by circularly orientated vascular canals (white dotted arrow). Red arrow indicates radially orientated vascular canal (Magnification x100).*

Sheep (Ovis aries) bone

Tubular bone of sheep aged 3-4 years is characterised mainly by plexiform bone (**Figure 2.17**), while tubular bones of sheep aged 7-9 years exhibit Haversian bone (Pearce *et al*., 2007).

Few, scattered secondary osteons are present at the periosteal and endosteal antero-lateral surfaces of sheep bones and contribute to the appearance of irregular Haversian bone (Martiniaková *et al*., 2007a; Martiniaková *et al*., 2007b). Dense Haversian bone tissue is commonly encountered in the mesosteal zone (Enlow & Brown, 1958; Martiniaková *et*

al., 2007a; Martiniaková *et al*., 2007b) with irregular shaped Haversian canals (Hillier & Bell, 2007).

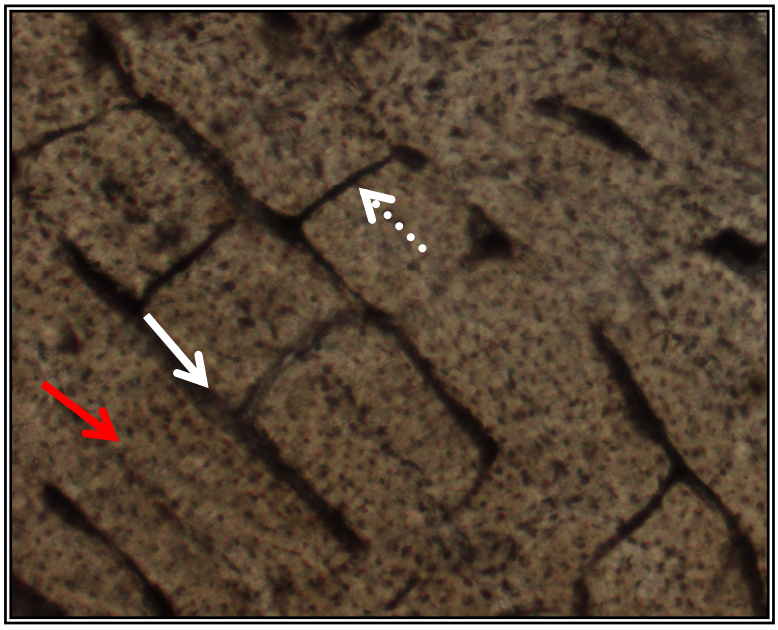


Figure 2.17 *Illustration of primary vascular plexiform bone observed in a sheep (Ovis aries) tibia viewed with normal bright light. Red arrow indicates a circumferential lamina separated from consecutive lamina by a circularly orientated vascular canal indicated by the white arrow. White dotted arrow indicates a radially orientated vascular canal (Magnification x100).*

Mulhern and Ubelaker (2001) indicated plexiform bone with bands of osteons in sheep bones. Osteon bands or osteon banding can basically be described as the linear arrangement of five or more primary or secondary osteons (**Figure 2.18**) (Mulhern & Ubelaker, 2001).

Modern artiodactyls follow a similar structural pattern and plexiform bone tissue is mainly found in these animals (Enlow & Brown, 1957; Francillon-Vieillot *et al*., 1997). In mid-sized to large herbivorous mammals, the plexiform bone will to some extent be replaced by Haversian bone (Martiniaková *et al*., 2007b). The outer layer can exclusively be composed of

plexiform bone, while older regions develop few isolated secondary osteons. The inner, endosteal layer on the other hand can be completely composed of dense Haversian bone (Enlow & Brown, 1958).

A study by Singh *et al*. (1974) examined rib, tibia and/or femora of artiodactyla. They noted primary vascular reticular bone with some areas completely devoid of vascularization in these animals.

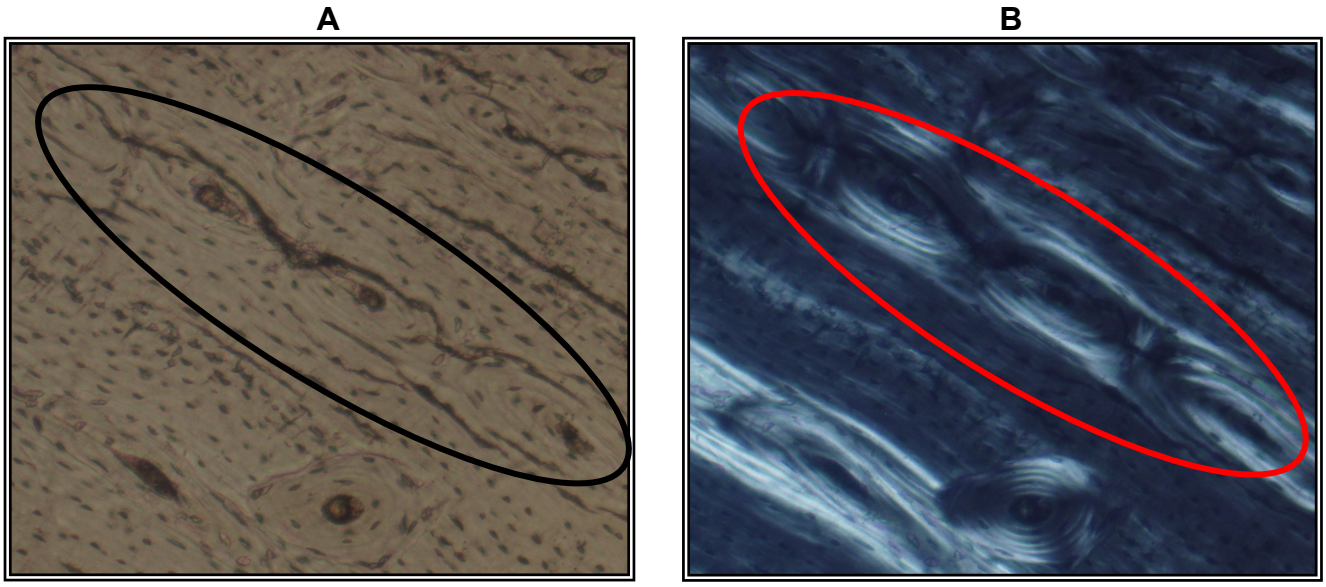


Figure 2.18 *Illustration of osteon banding as observed in a donkey (Equus africanus asinus) femur. Observation A made with normal light and observation B made with polarised light (Magnification x100).*

Order: Perissodactyla

Horse (Equus ferus caballus) bone

The *substantia compacta* of long bones of horses varies in structure and depends upon the location studied. Although the basic pattern of the diaphysis is primary vascular reticular bone, the organised nature of the tissue often approaches primary vascular plexiform bone (**Figure 2.19**). Haversian bone development differs in various areas and in some areas only scattered

secondary osteons are found amongst the reticular tissue, whilst other areas are completely replaced by dense Haversian bone (Enlow & Brown, 1958). According to Zedda *et al*. (2008), horse Haversian canals are circular in shape. The well defined endosteal circumferential lamellae and the periosteal circumferential lamellae are thin and generally interrupted by Haversian bone with numerous resorption spaces present near the endosteal surface (Zedda *et al*., 2008).

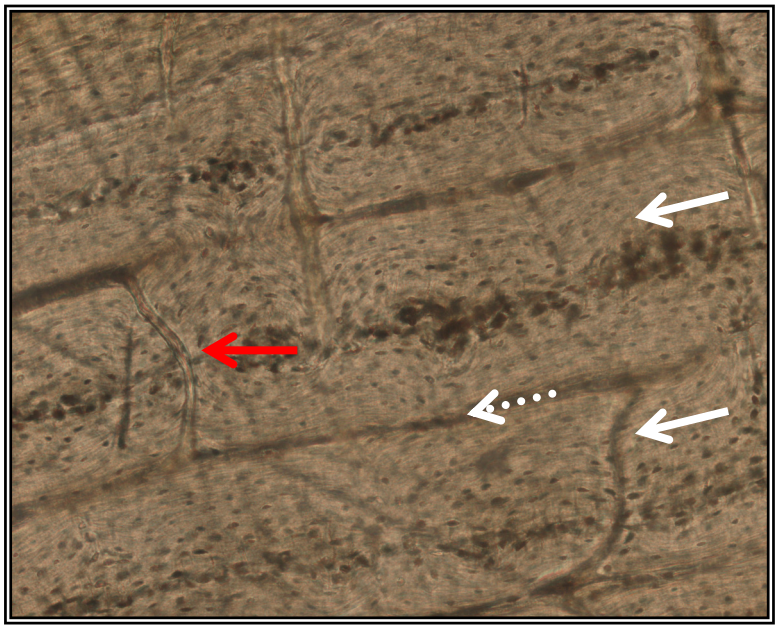


Figure 2.19 *Illustration of primary vascular plexiform bone observed in a donkey (Equus africanus asinus) femur viewed with normal bright light. White arrows indicate circumferential laminae separated by circularly orientated vascular canals indicated by the white dotted arrow. Red arrow indicates a radially orientated vascular canal (Magnification x100).*

Order: Carnivora

Cat (Felix catus) bone

Circumferential lamellae sandwich a compact area that consists mainly of small osteons that contribute to the formation of dense Haversian bone

(**Figure 2.20**). The periosteal circumferential lamellae are thin compared to the thicker endosteal circumferential lamellae. A large number of Volkmann's canals have been recorded in cat long bones (Enlow & Brown, 1958) as well as primary vascular longitudinal bone tissue with areas devoid of vascular canals (Singh *et al*., 1974).

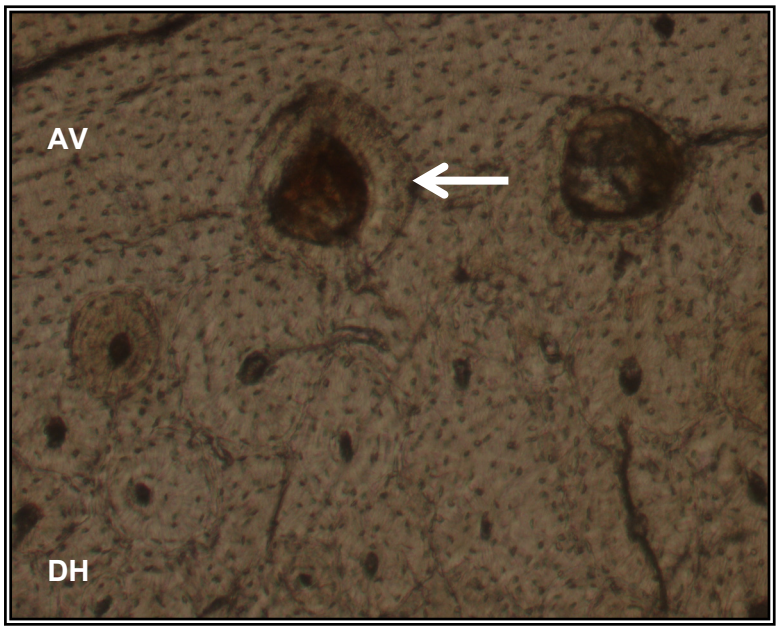


Figure 2.20 *Illustration of avascular bone (AV) combined with dense Haversian bone (DH) observed in a cat (Felix catus) tibia viewed with normal bright light. White arrow indicates an osteon with a large Haversian canal (Magnification x100).*

Dog (Canis familiaris) bone

Remnants of plexiform bone at the periosteal surface of immature dog bones have been documented, whilst the compacta of long bones of mature dogs consist mainly of dense Haversian bone (**Figure 2.21**) (Enlow & Brown, 1958; Pearce *et al*., 2007; Hillier & Bell, 2007). Osteons have various shapes with relatively small Haversian canals (Hillier & Bell, 2007). Circumferential lamellae are present at both the endosteal and periosteal surfaces and are

often interrupted by a few scattered osteons (Enlow & Brown, 1958; Hillier & Bell, 2007).

Interestingly to note, Pearce *et al*. (2007) have suggested that dog bone structure might the most similar to that of human bones.

Depending upon the species as well as the bones examined, carnivore bone can exhibit any of the forms of primary bone; e.g. longitudinal, radial, reticular or plexiform bone that may be replaced by Haversian bone (Enlow & Brown, 1958).

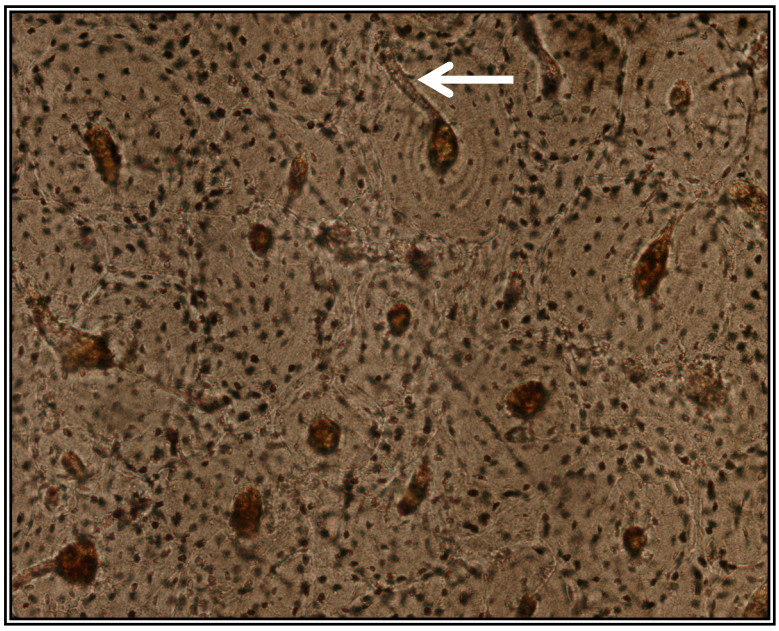


Figure 2.21 *Illustration of dense Haversian bone observed in a dog (Canis familiaris) tibia viewed with normal bright light. Osteons are small and tightly packed together. White arrow indicates radially orientated vascular canal or Volkmann's canal (Magnification x100).*

2.1.1.3. Factors that affect bone microstructure

The microscopic appearance of bone is influenced by a number of factors such as age, sex, population affinity, type of bone used (femur or skull), area

of bone sampled (epiphysis or diaphysis), amount of bone sectioned (ground or wedges), trauma, pathology and diagenesis to name but a few.

Age

The type of bone present in an individual is directly related to age at death. Prenatal skeletons consist predominately of primary bone tissue that remains apparent in the first two to three years after birth (Enlow, 1966). During intrauterine development, mid-gestational compact bone is characterised by a high osteon density with a few wide lamellae and Haversian canals. This bone tissue has been found to differ significantly from late gestational bone tissue which is characterised by narrow, sparse Haversian canals (Baltadjiev, 1995). With advancement in age, primary bone is gradually replaced by secondary bone that brings about a number of age-related changes in the microstructure of bone (Kerley, 1965; Enlow, 1966). These changes ultimately affect the osteons within secondary bone and consequently the measured values obtained for these units (Hall, 2005; Martiniaková *et al*., 2007b). Ageing leads to a decrease in the size of osteons and a general increase, of about 20%, in the number of osteons present in femoral and tibial bones of humans aged 36 to 75 (Hall, 2005).

Kerley (1965) noted a number of age-related differences in the histological appearance of the subperiosteal parts of long bone midshafts. These differences included an increase in the number of osteons and osteon fragments, coupled with a decrease in the percentage of circumferential lamellar bone and the number of non-Haversian canals, from childhood to adolescence. It was also noted that the non-Haversian canals were rarely

seen after 40 and had completely disappeared by the age of 55 (Kerley, 1965). Similar results were obtained in a study conducted by Currey (1964). Singh and Gunberg (1970) also indicated an increased number of osteons with age together with an increase in the number of lamellae per osteon. Additionally the authors also illustrated a decrease in the average Haversian canal diameter. In contrast to this, Thompson (1980) indicated an increase in Haversian canal size and thus an overall increase in Haversian canal area. Similarly Jowsey (1966) indicated significant increases in femoral Haversian canal perimeter with age.

A statistically significant increase in the median number of osteons/area unit with an increase in age was reported by Lynnerup *et al*. (2006). An increase in age has also been linked to an increase in the total area of interstitial lamellae (Cohen & Harris, 1958).

Haversian bone changes in growing animals follow a similar route to that of growing humans (Hillier & Bell, 2007). Mulhern and Ubelaker (2003) examined long bone midshafts of juvenile chimpanzees and found histological age related changes that are similar to that of human juveniles. Their results indicated an increase in the number of osteons and osteon fragments with age and a decrease in the number of non-Haversian canals. A decrease in the amount of circumferential lamellar bone was also linked to ageing (Mulhern & Ubelaker, 2003).

In rhesus macaques, Havill (2004) reported an increase in osteon population density with a decrease in bone formation rate in older animals. Ageing of larger mammals where bone tissue consists mainly of primary vascular bone can be observed in the partial or complete replacement thereof

by Haversian bone (Hillier & Bell, 2007). Likewise, Mori *et al*. (2005) reported an increased number of osteons together with the replacement of primary vascular laminar bone by Haversian bone in young calves, sheep and pigs with age. Foal long bones consist mainly of reticular or primary vascular plexiform bone while adult horse long bones are composed mainly of dense Haversian bone tissue, clearly illustrating the changes that occur with aging (Zedda *et al*., 2008).

Sex

Male and female differences in cortical bone histology have been described. Burr and colleagues (1990) indicated that Pecos Indian women had larger osteons than the Pecos Indian men while modern populations have shown larger Haversian canals in females compared to the increased number recorded in men. Thompson (1980) indicated increased and faster bone loss in females over 40 years of age as compared to bone loss observed in men. For age at death, more accurate results were obtained when sex-specific equations were utilised. This is believed to be linked to sex differences that are observed with relation to the differences in the number of osteons and osteon fragments that are related to age. In males, the number of osteons and their fragments increases over time, while females reach a plateau during the sixth decade of life (Stout, 1998).

Male and female microstructural differences are not found by all and authors such as Enlow D. H. (1966), Singh and Gunberg (1970) and Pfeiffer S. (1998) did not observe any significant differences in their studies of the microstructure of bone.

Mammals that are smaller or equal in size to monkeys, exhibit sexual dimorphism that affects the general size of measurable microscopic units (Hillier & Bell, 2007). Such differences have been indicated in the rhesus macaques which illustrated the statistically significant effect of sex on osteon area and osteon size. Males exhibited larger osteons compared to their female counterparts. These dissimilarities are believed to be related to the general differences in total body size (Havill, 2004). Differences in osteon diameters between male and female raccoon dogs have also been illustrated with larger osteon diameters recorded in males to the smaller, yet numerous osteons, of female raccoon dogs (Hidaka *et al*., 1998).

Population group

Variability between population groups has been reported (Burr *et al*., 1990; Stout, 1998; Cho *et al*., 2002). Burr *et al*. (1990) described the microstructure of bone of an archaic Native American population (Pecos Indians) and modern populations as very similar, although Pecos Indian women had smaller Haversian canals compared to modern women. Haversian canal areas in the Pecos Indian group in general were also found to be almost half the size of that found in modern populations (Burr *et al*., 1990). Cho *et al*. (2002) studied rib samples from European-American and African-Americans and found significant population variations with regards to osteon population density, osteon cross-section area and relative cortical area. Greater osteon sizes in European-Americans compared to African-Americans were also indicated (Cho *et al*., 2002).

On the contrary, not all studies have recorded differences in the histological structures of various populations. Pfeiffer (1998) looked at osteon area and Haversian canal area in ribs and femora of 18th century Huguenots (Spitalfields), 19th century British settlers (St. Thomas) and 20th century South African cadavers and found no significant differences between the population groups. Similarly, Kerley (1960) found no noteworthy differences in the microstructure of bone of various population groups and suggested the use of age-at-death-estimation charts for any skeletal remains, even if sex and race are unknown (Kerley, 1965). However, age estimations for "black" individuals from the Dominican Republic were underestimated, on average, by 11 years with the use Kerley's charts. This is believed to be related to the fact that Kerley's original age estimation charts were derived from work done on Caucasoids (Stout, 1998).

A number of papers examine the microscopic differences between various human populations; however, very little information exists on differences encountered amongst various breeds of animals. Modifications to osteon patterns have been linked to various breeds of animals (Zedda *et al*., 2008) and if one considers the size differences of various dog breeds such as Great Danes (*Canis lupus familiaris*) and chihuahuas (*Canis familiaris*), one will surely encounter size differences in measurable variables when the bone microscopy of these breeds are examined and compared. Such differences have been reported by Jowsey (1968), who illustrated increased values for osteon diameters and Haversian canal perimeters with an increase in size. These increases are however, only continued until a body size is reached that is similar to that of man (Jowsey, 1968). Differences in microstructure of wild

and domesticated animals have also been suggested and deserve further elaborations (Stout & Simmons, 1979).

Bones sampled

Major differences in the general distribution of compact bone, for example between long and flat bones, are well known (Enlow & Brown, 1958; Enlow D.H. 1966; Schultz, 1997). Other differences can been observed in Haversian system and Haversian canal diameters between skeletal elements such as ribs and femora (Hillier & Bell, 2007). Rib and femoral osteon differences have been recorded and indicated smaller rib osteons compared to femoral osteons and subsequent significantly smaller rib osteon areas as compared to osteon areas in femora (Pfeiffer *et al*., 2006).

Evans and Bang (1966) illustrated noticeable differences in the number and size of osteons between human femora and fibulae. The femora exhibited a larger number of smaller sized osteons and osteon fragments with little interstitial lamellae as opposed to the smaller number, yet larger osteons and osteon fragments and increased interstitial lamellae, observed in fibulae. The number of osteons/mm^2 was also significantly greater in the femur than the fibula (Evans & Bang, 1966). Correspondingly, an increased number of osteons has also been indicated in the femora of humans and chimpanzees compared to the numbers of osteons found in tibiae and fibulae (Mulhern & Ubelaker, 2003).

Similar to humans, differences in the histology of bones within a single animal have been illustrated and are believed to be related to different remodelling rates (Hillier & Bell, 2007). Work done by Skedros *et al*. (2003)

on Rocky Mountain mule deer illustrated a general increase in the number of osteons/mm^2 (secondary osteon population density) from proximal to distal in the forelimb bones. This is believed to be the result of increased loading on the more distal bones which is proposed to be linked to increased remodelling (Skedros *et al*., 2003). Large variations in the density of osteons have been found amongst various long bones of a single horse as illustrated by the larger femoral osteons compared to humeral osteons (Zedda *et al*., 2008).

Sample location

The histological appearance of bone also differs within various areas of a bone and even within a single section of bone and as stated by Enlow (1966) "no two areas of any given bone will be quite alike in microstructure". Differences in the histological appearance of the three zones within compact bone, although not very distinct, have been illustrated (Singh & Gunberg, 1970; Klevezal, 1996; Hillier & Bell, 2007). Harsányi (1993) reported such differences, stating that Haversian canals located near the periosteum are narrower compared to the wider Haversian canals located near the endosteum.

Work done by Pfeiffer *et al*., (1995) illustrated variability in bone remodelling in different samples taken from the midshaft of a femur. It was found that the percentage of remodelled bone in the endosteal zones was higher compared to the samples taken from other areas. The lowest remodelling value was found in the anterior surface of the femur but this area exhibited the highest amount of variability (Pfeiffer *et al*., 1995). Variation of osteon areas within an individual bone sample has also been reported

together with increased Haversian canal and osteon size variations (Pfeiffer, 1998).

Chan and colleagues (2007) determined the presence and the effects of intersection (sections from proximal to distal) and intrasection (within a section) variation on histological age estimation. The authors found that even though variation is present between sections, such differences are less significant than the differences observed within a single section. These differences are believed to be related to the remodelling of bone and because muscles attach to the posterior surface of the femur, higher mechanical stresses are imposed on this region and can consequently lead to increased numbers of secondary osteons (Chan *et al*., 2007). Equally, very dense concentrations of osteons have also been illustrated in crests, tubercles and other areas associated with ligament and tendon attachments in both humans and nonhumans (Enlow, 1966). This was also reported by Weiner (1999) who found increased numbers of osteons in smaller animals near areas of tendon or ligament attachments.

The microscopic structure of horse bones has also been illustrated to differ according to the sample location (Enlow & Brown, 1958). Dense Haversian tissue as well as scattered osteons in various regions of the same horse long bones have been demonstrated (Zedda *et al*., 2008).

Pathology

Nutritional deficiencies as well as hormonal influences can lead to the development of various diseases. Many of these diseases can affect the normal formation rate of bone and subsequently the micro-architecture thereof

(Kerley, 1965; Enlow, 1966). Important to note is that not all diseases affect the normal appearance of bone and one must pay close attention to the macroscopic appearance of abnormal bone formation, bone loss, bone shapes and sizes to correctly identify and separate pathological remains from normal skeletal elements (Buikstra & Ubelaker, 1994). A number of these bone affecting diseases do not only affect the morphological composition of the bone, but also the chemical composition of bone. Examples of bone affecting diseases according to Schultz's (2001) classification include the following disease types: skeletal dysplasias (e.g. osteogenesis imperfecta), metabolic bone diseases (e.g. osteomalacia, rickets, scurvy), osteoporosis and bone atrophy, infectious diseases of bone (e.g. osteomyelitis), circulatory diseases, haematological disorders, bone tumours and tumour-like conditions and miscellaneous conditions (e.g. Paget's disease). A few of the more commonly encountered diseases will be briefly discussed.

Mammalian bones are affected similarly to that of human bone by a number of pathological conditions such as metabolic disorders (osteoporosis, osteomalacia), hormonal disturbances, disease and infections (Chaplin, 1971).

Malnutrition

Paine and Barrett (2006) have described enlarged secondary osteons and Haversian canals with reduced cortical bone in bone sections removed from ribs in individuals suffering from poor diets and pellagra (Pain & Brenton, 2006). Three of the most common nutritional deficiencies encountered are

rickets (vitamin D deficiency), scurvy (vitamin C deficiency) and iron deficiency anaemia (Keough, 2007).

Rickets and osteomalacia

Rickets is only encountered in young individuals and develops due to inadequate vitamin D intake (Keough, 2007). It is characterised by abnormal bone calcification and bone distortion of the epiphyseal plates. Insufficient calcium intake has also been associated with incomplete bone calcification and the subsequent development of rickets (Junqueira & Carneiro, 2003).

Osteomalacia is similar to rickets but is characteristically encountered in adults (Chaplin, 1971). This disease is characterised by poor calcification of newly formed bone and partial decalcification of previously calcified bone (Francillon-Vieillot *et al*., 1997; Junqueira & Carneiro, 2003). Similar to rickets, the development of osteomalacia has also been associated with a lack of adequate calcium intake (Chaplin, 1971; Weinstein, 1992; Junqueira & Carneiro, 2003).

Osteoporosis

A lack of balance between bone formation and bone resorption results in osteoporosis (Chaplin, 1971; Teitelbaum, 2000). This is the most common bone disease in the western world. It is related to aging and affects mainly white postmenopausal women (Iskrant & Smith, 1969). The bone resorption leads to the enlargement of various canals and spaces in the bone that ultimately renders the bone extremely fragile (Enlow, 1966; Havill, 2004).

Secondary osteoporosis can also develop and can be related to disorders such as scurvy and diabetes mellitus (Hillier & Bell, 2007).

Paget's disease

This disease affects most bones of the skeleton and is histologically characterised by excessive and rapid resorption and formation of bone (Bell & Jones, 1991). Studies have indicated that the number of osteon fragments is larger than the number of osteons observed in such bone (Kerley, 1965). The number of the giant multinucleated osteoclasts also increases and can exceed the normal observed value by more than 10 times (Weinstein, 1992).

Diagenesis

Diagenetic change can be observed as a number of physical and chemical changes (dissolution, precipitation, mineral replacement and recrystallization) that affect the organic component, inorganic component and microstructural arrangement of bone and teeth, after death (Bell, 1990; Bell & Jones, 1991; Katzenberg & Harrison, 1997; Schultz, 2001). Postmortem alterations to bone have been recognised as early as 1864 and were first described by Wedl (Hackett, 1981; Bell *et al*., 1996; Jans *et al*., 2004).

Diagenesis can be detrimental to the recovery and use of mtDNA. It can be misleading and limits the applicability of histology to interpret and describe various bone pathologies (Hackett, 1981; Bell *et al*., 1996). It can also lead to the interpretation and description of various 'pseudo-pathologies' (Hackett, 1981; Buikstra & Ubelaker, 1994; Schultz, 2001). Diagenesis is brought about mainly by the effects of bacteria and fungi (Bell, 1990; Bell & Jones, 1991;

Bell *et al*., 1996; Jackes *et al*., 2001; Jans *et al*., 2004). Other factors such as plant roots, algae, arthropods and their larvae, worms, protozoa and other mechanical agents such as water have also been known to affect bone and teeth (Schultz, 2001). The development of foci or tunnels is the main destruction caused by bacterial or fungal activity and four types of foci/tunnels have been described: Wedl tunnels, budded tunnels, linear-longitudinal tunnels and lamellated foci (Hackett, 1981). The Wedl tunnels develop as a result of fungal alterations while the other tunnels are caused mainly by bacterial effects (Hackett, 1981; Jans *et al*., 2004). Bacteria as well as plants and soil minerals can also cause colour changes and other alterations to bone surfaces. Insects, worms and some burrowing micro-fauna infestation can produce abnormal postmortem bone resorption (Buikstra & Ubelaker, 1994). Research by Hackett (1981) indicated that invasion of altering organisms is mainly from the surrounding soil. The organisms enter the surface of bone and migrate towards the inner surface through a series of various canals. Thus, extensive diagenetic alterations can be observed in the subperiosteal regions as this is the region of entrance (Bell, 1990).

Research done by Jans *et al*. (2004) on archaeological material illustrated significant differences in the diagenetic alterations of bone between human and animal. A total of about 261 human and nonhuman remains were examined histologically and by mercury intrusion porosimetry. The authors found that 68% of all bones were affected by postmortem changes with statistically significant differences observed between the affected human and non-human bones. In humans 74% of bone was diagenetically altered mainly by bacterial attacks. Linear-longitudinal tunnelling was most commonly

observed followed by budded tunnels. Lamellate tunnels were rarely seen. In animals only 57% of bone was diagenetically altered, mainly by fungi. The most common alterations observed were Wedl tunnels followed by linear-longitudinal tunnelling and similar to what was the case in humans, only rare incidences of lamellate tunnels were recorded (Jans *et al*., 2004).

Burning

In general, burning of bone causes bone cracks, bone splits and a loss of the outer cortical layer by flaking (Bradtmiller *et al*., 1984; Harsányi, 1993; Buikstra & Ubelaker, 1994). Excessive heat has been linked to shrinkage of osteons and blurring of lamellae (Hillier & Bell, 2007).

An investigation by Nelson (1992) compared the histology of fresh and burnt bones and confirmed the shrinkage of osteons with increased heat and also indicated a significant decrease in osteon size related to burning. A similar study by Cattaneo and colleagues (1999) measured osteons and Haversian canal diameters in fresh and burnt human and animal bones. Their results indicated insignificant shrinkage of the variables at temperatures of 800ºC (Cattaneo *et al*., 1999).

Work by Bradtmiller and Buikstra (1984) on burned cortical bones, on the other hand, revealed an increase in osteon size, with even larger osteons observed in bones burned with flesh compared to unburned bones.

Even though the exact effects of burning of bone are debatable, the existence of such alterations is clear.

Freezing

Tersigni (2007) compared frozen and non-frozen human bone sections. Analysis of the frozen sections under a scanning electron microscope indicated some cracking around Haversian canals and osteons, yet no significant alterations to the bone microstructure were observed.

Immobilization

Long term immobilization has been found to affect the integrity of bone (Stout, 1982). A study by Stout (1982) on long bones and ribs of a quadriplegic individual and an individual bound to a wheelchair, revealed histological descriptions similar to that seen in osteopenia. This osteopenia is often also referred to as disuse osteoporosis and develop due to immobilization (Stout, 1982).

In a study conducted on male monkeys, Haversian bone loss especially from the cortex of the proximal tibia together with larger intracortical resorption cavities was observed within 7 months of immobilization. The authors are of the opinion that immobilization results in uncontrolled, uncoordinated and unbalanced bone remodelling (Young *et al*., 1986).

Gestation and Lactation

Premature bone loss in 20-29 year old Sudanese Nubian females has been observed and is believed to be related to child bearing and lactation (Martin & Armelagos, 1979). Similarly very active bone resorption during late gestation and early lactation has been indicated in sheep (Martiniaková *et al*., 2007b).

Other factors

Other less common factors that can also affect normal bone microscopy include drugs and alcoholism (Stout, 1998). For example, significantly reduced cancellous bone has been described in alcoholics (Weinstein, 1992).

2.1.2. Bone cells

Three types of cells are present in bone; osteoblasts, osteocytes and osteoclasts (Junqueira & Carneiro, 2003).

2.1.2.1. Osteoblasts

Osteoblasts are cells located near the surface of bone and are arranged similarly to simple epithelium (Junqueira & Carneiro, 2003). These cells are responsible for the production of the organic matter (type I collagen, proteoglycans and glycoproteins) of the bone matrix (Teitelbaum, 2000; Junqueira & Carneiro, 2003; Antonio, 2008). Osteoblasts are, however, also important in the formation of the inorganic component of bone. These cells are involved in a process known as bone apposition whereby an uncalcified substance called osteoid is secreted from the surface of cells that are related to existing bone (Junqueira & Carneiro, 2003; Antonio, 2008).

These cells were initially recognised as bone forming cells by Carl Gegenbaur (1864) who also named these cells osteoblasts (Hall, 2005).

2.1.2.2. Osteocytes

During the active secretion of osteoid, osteoblasts are enclosed by the secreted bone matrix and become known as osteocytes (Junqueira &

Carneiro, 2003; Halstead, 2007). Purkinje described the empty bone spaces occupied by osteocytes as lacunae, previously known as corpuscles of Purkinje (Enlow & Brown, 1956). These lacunae are found within the concentric lamellae that surround the Haversian canals (Junqueira & Carneiro, 2003). Osteocytes are flat, almond shaped cells with cytoplasmic processes that are embedded in a calcified network referred to as the canaliculi (Junqueira & Carneiro, 2003; Antonio, 2008). These cytoplasmic processes are closely related to one another and aid in the process of nutrient diffusion and cell communication and can connect up to 15 cells with one another. Osteocytes are primarily responsible for the maintenance of the bone matrix and with their death they initiate bone resorption (Junqueira & Carneiro, 2003).

2.1.2.3. Osteoclasts

The very large, multinucleated cells found in bone are the osteoclasts. Osteoclasts are located in cavities within bone known as Howship's lacunae (Junqueira & Carneiro, 2003; Antonio, 2008). These cells resorb and remodel bone and were first named by Kölliker (1873) (Hall, 2005). At the attachment site of active osteoclasts, the cells are folded into irregular projections to form a border, know as the "ruffled or brush border" (Francillon-Vieillot *et al*., 1997; Teitelbaum, 2000; Junqueira & Carneiro, 2003; Antonio, 2008). This border is surrounded by a clear or cytoplasmic zone that contains no organelles. It ensures that the cell attaches to the resorption area while creating a "microenvironment" for bone remodelling and resorption to occur (Teitelbaum, 2000; Junqueira & Carneiro, 2003).

2.1.3. Bone formation

Bone formation occurs through a histocytological process known as osteogenesis. Osteogenesis mediates the secretion of collagen and non-collagen fibres by osteoblasts and also the arrangement of the fibres in the bone matrix. The process also mediates the secretion and arrangement of the inorganic constituent of the bone matrix (Francillon-Vieillot *et al*., 1997). The first bone or osteoid that is deposited is unmineralized and gradually becomes mineralized by the secretion of hydroxyapatites (Hall, 2005). Bone formation can follow one of two routes; intramembraneous ossification or endochondral ossification. Intramembraneous ossification is the direct mineralization of bone matrix from a pre-existing membranous structure, while endochondral ossification is the process whereby bone matrix is laid down on an already established cartilage model (Junqueira & Carneiro, 2003). The initial bone that develops is immature and large quantities of ground substance are present in the bone matrix (Ross *et al*., 1995).

2.1.3.1. Intramembraneous ossification

Mesenchymal cells migrate to areas where bone formation will occur and form a type of membrane. The area becomes increasingly vascularized and a number of cytological changes results in the formation of osteoblasts. Osteoblasts are responsible for the secretion of the organic matter (type I collagen, proteoglycans and glycoproteins) of the bone matrix, known as osteoid (Ross *et al*., 1995). With increased osteoid production the cells become enclosed within the newly secreted bone within openings known as lacunae (Junqueira & Carneiro, 2003). The matrix will gradually become

calcified. The initial bone that is laid down is immature and is characterised by the presence of woven bone (Ross *et al*., 1995). This woven bone resembles spicules or trabeculae that eventually fuse to form thin bony plates (Antonio, 2008). Most of the flat bones of the human skull as well as the clavicle and the mandible are formed by intramembraneous ossification (Ross *et al*., 1995; Junqueira & Carneiro, 2003). This process also contributes to the growth of short bones and the thickening of long bones (Junqueira & Carneiro, 2003).

2.1.3.2. Endochondral ossification

This type of ossification is observed in long bones, short bones and weight bearing bones such as the vertebra, and is characterised by the presence of a hyaline cartilage model that resembles a miniature version of the bone that is to be formed (Ross *et al*., 1995; Junqueira & Carneiro, 2003). Osteoblasts derived from perichondrial cells align at and form the inner osteogenic layer of the periosteum (Ross *et al*., 1995). Endochondral ossification starts at the diaphysis of the cartilage model with the osteogenic layer producing a cylinder of bone known as the bone collar (Ross *et al*., 1995; Junqueira & Carneiro, 2003). Blood vessels pierce through the bony collar allowing the influx of osteoprogenitor cells. Programmed cell death and calcification of the cartilaginous matrix leads to the removal of the cartilage and the subsequent production of osteoid. Calcification of the cartilage matrix creates attachment sites for the osteoblasts for further primary bone production, thus creating the primary ossification centre. At both ends of the cartilage model (epiphyses) secondary ossification centres appear and with the remodelling of bone the

primary and secondary ossification centres are eventually turned into cavities that gradually fill with bone marrow. At the epiphyses, epiphyseal cartilage remains until adulthood and play a crucial role in the lengthening the bones (Junqueira & Carneiro, 2003).

There are five zones present within the epiphyseal cartilage. The first zone is the resting zone (Ross *et al*., 1995; Junqueira & Carneiro, 2003). This zone consists of hyaline cartilage while the second zone or proliferation zone is characterised by dividing chondrocytes that are arranged in columns that are mainly orientated parallel to the long axis of the bone (Junqueira & Carneiro, 2003). These cells actively secrete cartilaginous matrix (Ross *et al*., 1995). The hypertrophic cartilage zone is characterised by large chondrocytes with increased glycogen in the cytoplasm (Ross *et al*., 1995; Junqueira & Carneiro, 2003). The matrix in this area is decreased to a thin layer between the chondrocytes. In the calcified cartilage zone, the thin layer of matrix calcifies and the chondrocytes die. The last zone is the ossification or resorption zone and is characterised by the appearance of newly formed endochondral bone tissue (Junqueira & Carneiro, 2003).

2.1.4. Bone remodeling and growth

Due to the hard nature of bone, specialized processes are needed for bone development (Enlow, 1966). This process is known as remodelling and is very important for bone growth, mineral metabolism and micro-damage repair. It also plays a crucial role in the ability of bone to maintain its shape and adapt to various mechanical stresses (Enlow, 1966; Ott, 2002). The ability of bone to adapt to mechanical stresses is known as Wolff's law. This frequently cited

and often critiqued 'law' infer that bone will be formed where increased stress is imposed upon bone (Pearson & Lieberman, 2004). Bone remodelling thus relies on the balance between bone resorption and bone formation of the endosteal, periosteal and internal surfaces (Enlow, 1966). This balance is regulated by a number of biochemical and mechanical factors (Ott, 2002) and also by cellular and hormonal mechanisms (Pearson & Lieberman, 2004).

Primary bone is the first osseous tissue deposited during the initial phases of bone formation and is gradually replaced by secondary bone (Junqueira & Carneiro, 2003). Primary osteons are vascular canals located within primary bone (Enlow, 1966) and also become gradually replaced by secondary osteons (Antonio, 2008). The lifespan as well as the production time of osteons vary amongst different species. For two year old cats it takes about 50 days to create an osteon while the same process takes a 45 year old man 100 days. The average lifespan of a human osteon is about 15 years, which means that a mere 0.05% of the skeleton is remodelled on a daily basis (Hall, 2005). The time span needed for a remodeling cycle to occur can vary between four to five years. On average ±20% of cancellous bone is in the process of remodeling and that will occur about every two years (Ott, 2002). Remodelling occurs throughout an individual's life span and it has been indicated that about 5% of adult compact bone and 25% of cancellous bone is annually renewed (Scheuer & Black, 2000).

Bone growth and bone remodeling is very active in children (Junqueira & Carneiro, 2003). Large degrees of remodeling have been indicated in primates, carnivores, perissodactyls and artiodactyls compared to the little remodeling observed in smaller mammalian species (Currey, 2003).

Remodelling is believed to be responsible for various microstructural differences observed between different bones of the body, different parts of a single bone and between different species (Enlow, 1966).

2.2. CHEMICAL ANALYSIS

Studies involving the elemental composition of bone can shed light on early lifestyles, environments, diets, health and even migrating patterns of early populations and include either stable isotope or trace element analysis (Keegan, 1989; Aufderheide, 1989; Buikstra & Ubelaker, 1994). Trace element analysis relies on the unique atomic structure of elements (Gabriel, 1985), while stable isotope analysis depends upon different element masses which is directly related to the number of neutrons within the atom (Keegan, 1989). Trace element studies involve a number of methods used to obtain qualitative as well as quantitative information regarding the elements present within a specimen (Goldstein *et al*., 1981; Gabriel, 1985; Ward, 2000; Chandler, 2007). Qualitatively it aims to identify the elements present within a specific sample and quantitatively it strives to give the exact contribution of each of these elements as accurately as possible (Gabriel, 1985; Ward, 2000).

A scanning electron microscope (SEM) fitted with an energy dispersive spectrometer or SEM/EDS is often the only means to characterise small, limited forensic samples (Ward, 2000). It is a fast, affordable and simple method to give accurate information regarding the elements present within any material (Boaz & Hampel, 1978; Brooks *et al*., 2006; Chandler, 2007; Bush *et al*., 2007). The accuracy of the technique is influenced by the

homogeneity of the sample, sample size and preparation techniques (Ward, 2000). It is favoured in areas such as metallurgy, physics, electronics, mineralogy, zoology, pathology and biochemistry to name but a few (Chandler, 2007). It is also used in forensic science to understand structures and chemical compositions of numerous materials received as evidence (Ubelaker *et al*., 2002).

During the 1930's, the basic principles of the scanning electron microscope were discovered and further researched in Germany. In 1965 Cambridge Scientific Instrument Co. introduced the world's first commercial SEM and at the end of the 1940's it was Castaing and Guinier (1949) that first combined X-ray analysis to the scanning electron microscope (SEM). Some alternative uses for the SEM include the observation of a specimen's surface topography, observation of the composition of surfaces, analysis of elements of specimens, observations of the internal structure of specimens, observations of the internal characteristics of a specimen, observations with regards to the crystalline structure of specimens and observations concentrating on the magnetic domain of samples (Invitation to the SEM world: for people who are using the SEM for the first time, JOEL Ltd., unpublished manuscript). Microanalysis permits the examination of systems, enzyme activity, chemical analysis, the relationship between elemental composition and morphology and the general distribution of ions and elements within cells (Goldstein *et al*., 1981).

The first paper to describe the chemistry of bone was published in 1970 (Katzenberg & Harrison, 1997). Such publications are invaluable and by knowing the elemental analysis of bone, one can reconstruct the actual

composition of the diet and complement archaeological information of modern and historic species (Klepinger *et al*., 1986; Buikstra & Ubelaker, 1994).

More recently elemental analysis proved very useful in the separation of cremains and other common materials (Brooks *et al*., 2006). The Tri-State Crematory incident in Nobel, Georgia in February 2001 forced researchers to explore alternative techniques to determine human ash or cremains form other unknown materials. Only a small number of bodies entrusted to this crematory were cremated, whilst the other bodies were illegally disposed. Urns were filled with concrete, cement, wood ash or mixtures of human and nonhuman components. Brooks and colleagues (2006) used a technique known as Inductive Coupled Plasma-Optical Emission Spectroscopy (ICP-EOS), a technique that allows for element analysis. This method is based on the knowledge that trace elements in humans are stored in the skeleton and that the chemical signature is expected to remain, even after cremation. Their results indicated that elemental analysis paired with statistical analysis provided a successful means for the separation of cremains from other materials. They do, however, note that not all elements are equally useful for separation and therefore it is not possible to correctly identify samples by using only one element. They also reported that elemental analysis is unsuitable for the separation of cremains of different species (human vs. *Canis familiaris*) as no differences are expected in the chemical composition of human and various animal skeletons (Brooks *et al*., 2006).

With the recent development of DNA analysis more skeletal material and dental tissues are submitted for human identification purposes (Ubelaker *et al*., 2002). Taking the cost of DNA analysis into account, a preliminary

method for the determination of human tissue is needed prior to DNA analysis. In accordance to these needs, in 1994, the Federal Bureau of Investigation (FBI) initiated the development of a database (Spectral Library for Identification and Classification Explorer [SLICE]) containing X-ray spectra of various materials. The idea was to compare evidence in question to the database to determine whether or not the material is of dental or skeletal origin and if not, it allows for further comparison of the material in question to other known materials. They indicated that the system does not allow for absolute material identification based solely upon the chemical composition of the material and separating between species did not appear to be possible either. The database contains both human and nonhuman bone and tooth samples. Up to date, the database has about 1800 entries of which 48 are bone samples (34 human and 14 nonhuman), 44 tooth samples (22 human and 22 nonhuman) and 23 other materials of biological origin (Ubelaker *et al*., 2002).

Research has indicated that bones of various mammalian species consist of more or less the same elements; however, distinguishing characteristic chemical compositions of these elements do exist (Blitz & Pellegrino, 1969; Schultz, 1997). A difference in the element distribution of carnivores and herbivores has previously been indicated and is attributed to the major differences found in the diets followed by the various feeding groups (omnivores, carnivores and herbivores) (Toots & Voorhies, 1965; Parker & Toots, 1970; Pingitore *et al*., 2001).

Parker and Toots (1970) and Toots and Voorhies (1965) indicated that the strontium content in bone is a result of the strontium content of the prevalent

food of animals and therefore strontium is regarded as a suitable element to study various animal diets. It has been indicated that the strontium levels in carnivores (such as cats and dogs) are much lower than that of herbivores with differences that appear to be significant ($p < 0.001$). These results correspond to findings by Odum (1957) who noted a continuous decrease in the strontium content from plants to herbivores and from herbivores to carnivores. Higher strontium levels have also been indicated in herbaceous vegetation compared to that of grass and therefore one can expect varying strontium contents between grazers and browsers (Toots & Voorhies, 1965). The available strontium in different food types are represented in bone and depends on the strontium available within the food that has been consumed (Katzenberg & Harrison, 1997).

After analysing skeletal and dental material from seven different species, Boaz and Hampel (1978) were unable to indicate a connection between strontium content and the diet of different animals. In some instances their results indicated higher strontium content in carnivores compared to herbivores. Their result did, however, confirm higher strontium levels in browsers (giraffe) compared to grazers (bovids) (Boaz and Hampel, 1978).

2.2.1. Chemical composition of bone

As early as the 18th century, the 'dual organic-mineral nature' of bone had been noted by Hérissant (1758) (Francillon-Vieillot *et al.*, 1997). Bone consists of a number of cells and calcified material. The calcified material consists of organic and inorganic matter (Junqueira & Carneiro, 2003). If the calcified material or inorganic component of bone is removed, the bone will

maintain its shape but it will be very flexible. When one removes the organic component of bone, the bone will maintain its shape but it will be very brittle (Chaplin, 1971; Fazekas & Kósa, 1978; Francillon-Vieillot *et al*., 1997; Junqueira & Carneiro, 2003).

The organic component of bone consists of collagen (±90-95%) and ground substance. This includes minor organic components such as proteoglycans, glycoproteins, serum proteins and lipids (Francillon-Vieillot *et al*., 1997; Junqueira & Carneiro, 2003).

The inorganic component of bone includes mainly phosphate (P), calcium (Ca), carbonate, citrate, nitrate, magnesium (Mg), sodium (Na), fluoride (F) and strontium (Sr) (Schultz, 1997). Other elements mentioned in the literature that also form part of the inorganic component of bone include potassium (K), silicon (Si), barium (Ba) and manganese (Mn) (Francillon-Vieillot *et al*., 1997; Junqueira & Carneiro, 2003). Klepinger *et al*. (1986) examined the chemical composition of archaeological bones from Sicily. Elements found within the femora of the archaeological remains included cadmium (Cd), barium (Ba), iron (Fe), manganese (Mn), copper (Cu), magnesium (Mg), potassium (K), sodium (Na), calcium (Ca) and strontium (Sr).

Calcium and phosphate are the most abundant elements in bone and teeth, and form hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] crystals in vertebrate osseous tissue (Cassella *et al*., 1995; Francillon-Vieillot *et al*., 1997; Ubelaker *et al*., 2002; Junqueira & Carneiro, 2003). Sodium and magnesium represent only a small portion of the inorganic component of bone, however, this fraction represents ±50% and 65% of the sodium and magnesium content of the entire human body, respectively (Francillon-Vieillot *et al*., 1997).

The strontium concentration of adult bones is between 150-250 parts per million (ppm) (ash) and represents almost 100% of the body's strontium content. The average concentration of lead in skeletal material is 40ppm and represents almost 95% or more of the body's lead content. Zinc concentration in the body averages at about 200ppm and is mainly stored in skeletal muscle and osseous tissue (Aufderheide, 1989).

In an attempt to separate dental and osseous tissue from other materials, Ubelaker *et al*. (2002) recorded calcium/phosphate (Ca/P) ratios with scanning electron microscopy/energy dispersive X-ray spectroscopy for a number of materials. Their results indicated that calcined spongy human bone had a Ca/P ratio of 1.89, burned human skull had a Ca/P ratio of 1.90 and synthetic hydroxyapatite had a ratio of 1.75. They illustrated sufficient differences to successfully separate either dental or osseous tissues from other materials (Ubelaker *et al*., 2002).

Literature on chemical composition of bone proves to be somewhat confusing especially if one looks at the different forms of bones used (e.g., whole bone, powdered bone, wet bone, dry bone and bone ash), and if one takes into account that calculations for percentages can be based on either bone volume or bone weight (Chaplin, 1971).

2.2.1.1. Factors that affect the chemical composition of bone

A number of methods exits that can generate values for elements within bone, but unfortunately the opportunity to utilise this information is often hampered by problems such as diagenesis (Buikstra & Ubelaker, 1994).

Other factors exist that also affect the normal chemical composition of bone and will be briefly discussed below.

Age

Fazekas & Kósa (1978) reported work by Swanson and Iob (1937), who examined epiphyseal cartilages and long bones of human foetuses. The authors noted a significant decrease in the magnesium and chloride components of bone during early embryonic life. The rest of the inorganic elements also decrease during foetal life but at a much slower rate (Fazekas & Kósa, 1978). Likewise Goldman *et al*. (2003) examined the mineral density of midshaft femoral sections and observed a decrease in the mineralization density of bone with advancement of age (Goldman *et al*., 2003). Martin, Burr and Schaffler (1985) observed similar results for female Eskimos. The authors measured amongst other variables, bone mineral content (BMC) of Alaskan Eskimo tibiae and found that it decreased significantly, by about 50%, between the 3rd and 6th decades of life. However, this was not the case for males, as no significant decrease with age was recorded by Martin *et al*. (1985). On the other hand, work bone by Rowland *et al*. (1959) on calcium densities in dog and man, indicated no calcium density changes related to age (Rowland *et al*., 1959).

Human versus animal

Little information regarding the differences in the chemical make up of human and non-human bones are available and only a few studies have indicated such differences (Toots & Voorhies, 1965; Boaz & Hampel, 1978).

Pearce *et al*. (2007) described minor differences in the inorganic component of human and animal bones, while Rowland, Jowsey and Marshall (1957) suggested significant differences in the chemical composition of various animal bones. It is believed that these dissimilarities are due to vast differences observed in the mineral density of bone amongst various species (Jowsey, 1966). This study was published in the Argonne National Laboratory Radiological Physics Division Semi-annual Report, ANL-5829, July-December 1957 and unfortunately is not freely available for appraisal.

Toots and Voorhies (1965) recorded lower strontium levels in carnivores (such as cats and dogs) compared to herbivores and suggested that these variations are due to different feeding patterns. Unfortunately, with examinations of larger groups these results were not be reproduced by Boaz and Hampel (1978) and in some cases higher strontium values were recorded in carnivores compared to herbivores.

Scanning electron microscopy/energy dispersive X-ray spectroscopy (SEM/EDS) has also been implemented in the separation of dental and osseous remains form other unknown materials. Again the success of the technique depended on the characteristic chemical signature of various materials. Although a very successful technique, it was also not able to distinguish skeletal remains form various species (Ubelaker *et al*., 2002).

Bone sampled

The study by Klepinger *et al*. (1986) on archaeological bone illustrated chemical differences among various long bones and the authors suspected that is due to the heterogeneity of bone.

Zinc concentrations of the diaphysis of long bones are lower than that recorded in vertebrae, which can be twice as high as the long bone zinc concentrations (Aufderheide, 1989). Such variations in element accumulation in bone have been linked to different bone types (Martinez-Gracia *et al*., 2005). Other elements show no difference with regard to the bone analysed and strontium contents of ribs and femora remain more or less the same (Aufderheide, 1989).

Sample location

According to Klepinger *et al*., (1986) and Katzenberg and Harrison (1997), Gruppe (1988) indicated a difference in the levels of minor elements present within cortical and cancellous bone of humans. Similarly, higher mineralised material has been recorded in woven bone compared to lamellar bone (Francillon-Vieillot *et al*., 1997). Not only have variations in the mineralised material of bone been indicated among different bone types, variations in elemental composition across the cortex of femora have also been illustrated by Klepinger *et al*. (1986).

Burr (1980) stated that the mineral composition of bone will vary within a single bone and within a single bone section and the author is of the opinion that this is linked to variations caused by mechanical stresses.

Differences between endosteal and periosteal elemental compositions have been found and are also linked to the lack of homogeneity of skeletal material (Klepinger *et al*., 1986). Equally, significant differences within mineral density between the periosteal and mesosteal zones have been indicated in femoral midshaft sections (Goldman *et al*., 2003).

Pathology

A number of diseases exist that affect both the chemical and/or morphological composition of bone and to mention but a few, include osteomalacia, osteopetrosis, osteomas, osteoporosis, periostitis, osteomyelitis, Paget's disease, diabetes mellitus, osteogenesis imperfecta, acromegaly, hyperparathyroidism, leprosy and syphilis (Halstead, 2007; Hillier & Bell, 2007).

Work conducted by Cassella *et al*. (1995) on calcium phosphate ratios (Ca/P ratio) of bone from normal individuals and individuals suffering from osteogenesis imperfecta indicated significantly lower Ca/P ratio (Ca/P ratio = 1.488) in individuals suffering from osteogenesis imperfecta compared to ratios observed in normal individuals (Ca/P ratio = 1.631) Cassella *et al*., 1995).

The numerous effects of pathologies on bone remodeling have been reported to affect the chemical composition of bone and increased serum calcium has been documented in individuals suffering form hyperparathyroidism that is also related to increased bone resorption (Ott, 2002).

Interestingly, Taicher and colleagues (2003) reported that the chemical composition of specific bone tissues in healthy bone and osteoporotic bone is similar.

Diagenesis

The chemical composition of bone is believed to vary between bones of the same skeleton and even within a single bone section. These variations are

not only believed to be linked to the lack of homogeneity of bone, but also to postmortem alterations (Klepinger *et al*., 1986). Mineral re-deposition is characteristic of most forms of diagenetic tunnels and is usually associated with partial or complete loss of minerals and bone matrix (Hackett, 1981).

Elements such as copper and zinc are not accumulated in bone during life and therefore may occur in bone due to postmortem exchanges that occur between the bone and the environment the bone was found in (Buikstra & Ubelaker, 1994). These exchanges can either increase or decrease a particular element (Buikstra & Ubelaker, 1994; Pingitore *et al*., 2001). Equally, Parker and Toots (1970) stated that fluorine is not part of the original apatite of bone and that its presence is probably the result of post-mortem alterations (Parker and Toots, 1970). These alterations are believed to be dependant upon the availability of an element within the environment, the exposure time of the bone to the specific element as well as environmental temperatures. An increase in the fluorine content of bone after death has also been indicated by Jackes *et al*. (2001) and is related to postmortem bacterial alterations. Signs of calcium replacement by strontium have also been indicated (Parker & Toots, 1970). Other elements such as silicon, manganese and iron fill holes in bone by postmortem infiltration. Manganese is more apparent in very small openings such as lacunae, while iron occupies larger openings such as the Haversian canals (Parker & Toots, 1970).

Infiltration of these additional elements affects the true representation of bone elements as observed in the living tissues (Parker & Toots, 1970).

Gestation and lactation

Absorbed strontium competes with calcium for absorption and during pregnancy and lactation strontium has been found to accumulate in bone (Aufderheide, 1989). If the calcium requirements are not met by the individual's calcium stores it will be removed from maternal reserves and ultimately affect the calcium composition of bone (Martin & Armelagos, 1979).

Other factors

Another factor that may affect the micronutrient content of bone is alcoholism. Increased lead absorption and negative zinc balances within bone have been linked to alcohol abuse (Aufderheide, 1989).

The exact effects of gender, population groups, freezing, burning, immobilization and drugs on the chemical composition of bone have not yet been thoroughly described in the literature.

2.3. HISTOMORPHOMETRICAL ANALYSIS

Literature comparing human and animal bone microscopy is voluminous; unfortunately a large number of these papers are not available in English which restricts the understanding of some of the basic bone descriptions and early comparative results.

Histological examinations are more commonly used in forensic anthropology to aid in the estimation of age at death or in the diagnosis of specific bone affecting diseases (Stout & Simmons, 1979; Maat *et al*., 2002). It has become more popular in recent years as a separation tool for the determination of human or nonhuman origin of skeletal material (Martiniaková

et al., 2006a; Hillier & Bell, 2007). Microscopic inspections have also aided in the identification of bone from other unknown materials (Walter *et al*., 2004).

According to Harsányi (1993), Kenyeres and Hegyi (1903) reported that histological sections of the compact bone of long bone shafts could be used to determine the human origin of fragmented remains. Unfortunately this body of work is not available in English. Kenyeres and Hegyi (1903) found that the mean diameter of Haversian canals in humans was significantly larger than that of other vertebrates (Harsányi, 1993). These results have been confirmed by a number of other authors such as Harsányi (1993) and Martiniaková *et al*. (Martiniaková *et al*., 2006a; Martiniaková *et al*., 2006b).

According to Fazekas & Kósa (1978), Demeter and Mátyás (1928) were able to describe qualitative microstructural differences within species by examining the vascular patterns of human and animal bones. Fazekas & Kósa (1978) also reported work on work by Schranz (1933) who also examined the microstructure of human and various animal bones and illustrated differences in the overall microstructure of bone with similar Haversian canal diameters for man and horse. Rämsch and Zerndt (1963) similarly illustrated differences in human and non-human bone microstructure and classified five groups according to Haversian canal diameters. These groups described Haversian canal sizes as either very small, small, moderately large, large and very large. Haversian canals were classified as very small if the Haversian canal diameters were 10 µm or less and as small if the diameters were between 11-20 µm. Canals were identified as moderately large if the diameters were between 21-40 µm, large for diameter values between 41-80 µm and very large for values above 80 µm. The authors

reported that Haversian canal diameters of 40 µm and less were rarely recorded in humans (Fazekas & Kósa, 1978; Harsányi, 1993). According to Harsányi (1993) average Haversian canal diameters are about 60 µm, while other studies have report average human Haversian canal diameters in humans of about 80 µm or above. In 2000, Sauer and Lackey stated that Haversian canal diameters smaller than 50 µm indicated remains of nonhuman origin. However, according to Martiniaková *et al*. (2006a) this statement is still controversial and as illustrated by Albu *et al*. (1990) human Haversian canal diameters can range between 20-300 µm (Albu *et al*., 1990).

In a study comparing burnt and unburnt human and non-human bones, Cattaneo *et al*. (1999) emphasised the accuracy and reliability of histological examinations for the identification of human and nonhuman bones. These authors noted significant differences between human and non-human bones with regards to osteons and Haversian canal measurements. A number of papers published by Martiniaková *et al*. compared the area, perimeter, minimum diameter and maximum diameter measurements of Haversian canals, secondary osteons and primary vascular canals in various species. A number of statistically significant differences were recorded for various parameters between different animals (Martiniaková *et al*., 2006a; Martiniaková *et al*., 2006b; Martiniaková *et al*., 2007a). Discriminant function analyses were also implemented to formulate identification equations with various degrees of accuracy (Martiniaková *et al*., 2006b; Martiniaková *et al*., 2007a).

Work by Martiniaková *et al*. (2006a) also included qualitative descriptions of compact bone of human and animal osseous remains. Plexiform bone was

mainly observed in the long bones of large and medium sized animals. The presence of this bone type can be considered, to some extent, as a nonhuman determinant as it is absent in humans and smaller animals (Martiniaková *et al*., 2006a; Cattaneo *et al*., 2009). It has, however, been observed in individuals with signs of pathology and rarely in foetal bones or fast growing humans (Mulhern & Ubelaker, 2001; Martiniaková *et al*., 2006a; Hillier & Bell, 2007). Several publications have confirmed that the presence of plexiform bone can be very successful in the separation of human and animal fragmented remains (Owsley *et al*., 1984; Dix *et al*., 1991; Mulhern & Ubelaker, 2001; Hillier & Bell, 2007). This type of bone tissue is characteristically found in the periosteal layer but may not always be present as trauma, fire and post-mortem damage may lead to the loss of the periosteal layer (Hillier & Bell, 2007).

The success of histomorphometrical analysis has been illustrated in a case by Dix *et al*. (1991). A female disappeared without a trace. Two years later her car was found, containing multiple bone fragments, glass, shotgun pellets and dried blood. The anthropologist examined both the macroscopic and microscopic appearance of the bone fragments. Macroscopically the anthropologist was able to identify a number of foramina which was thought to be linked to that of a skull. Histological examinations confirmed the presence of osteons and the particular size of the osteons, the cortical bone thickness and absence of plexiform bone, all indicated a human origin. DNA analysis of the dried blood confirmed that is was that of the victim and so the guilt of the perpetrator was proven, even without the victim's body (Dix *et al*., 1991).

In another case a female had been shot and bone fragments found at the scene were identified by the suspect as deer remains. The fragments were taken to an anthropologist who compared human and deer bone sections. The deer bone exhibited mainly plexiform bone with primary osteons that were similar in size and shape, while the human sample exhibited secondary osteons with distinctly larger Haversian canal diameters. The bone fragments were positively identified as human bone due to the lack of plexiform bone and the distinctive shape and size of both osteons and Haversian canals (Owsley *et al*., 1984).

Other studies concentrate on the density of the Haversian bone and results have indicated that human bone exhibited higher densities of Haversian tissue compared to other mammalian animals, but unfortunately this does not always allow for positive identification of human remains (Mulhern & Ubelaker, 2001; Martiniaková *et al*., 2007a).

The presence of osteon banding has also been explored as a possible means to separate skeletal remains. Mulhern and Ubelaker (2001) compared human subadult and adult femoral sections with that of subadult sheep and miniature swine and found significant differences in the osteon banding patterns between humans and animals (Mulhern and Ubelaker, 2001).

A number of histological, qualitative and quantitative, and chemical differences in a variety of mammalian species have been identified and described in the literature. This study aimed to reproduce these differences in a South African sample to determine if sufficient differences exist that ca be utilised in the separation of various animals and human skeletal remains.

CHAPTER 3

MATERIALS AND METHODS

3.1. MATERIALS

3.1.1. The skeletal samples

Bone samples were removed from the femora and tibiae of human (adult and juvenile) and other mammalian (domesticated and non-domesticated) skeletons. Long bones of the lower limbs were selected, due to their relatively good preservation at archaeological and forensic sites, a thick layer of compact bone which permits optimal examination of the bone's microstructure and to make it comparable to previous studies (Martiniaková *et al*., 2007a).

The nonhuman mammalian sample consisted of eight cat (*Felix catus*), 12 dog (*Canis familiaris*), ten cow (*Bos taurus*), ten sheep (*Ovis aries*), 12 donkey (*Equus africanus asinus*), five impala (*Aepyceros melampus*), ten pig (*Sus scrofa domestica*) and seven old world monkey (*Cercopithecinae*) bones. The "old world monkey" group included bony elements removed from baboons (*Papio ursinus*) and vervet monkeys (*Chlorocebus pygerythrus*). Cat, medium-sized dog and donkey bones were collected from the Anatomy and Physiology Department, Faculty of Veterinary Sciences, University of Pretoria at Onderstepoort. This department has an animal bone collection that was started around 1950 which consists mainly of animal skeletal remains that had not been embalmed (De Villiers I. L., pers comm.). However, for the purpose of this study dissected animal material from Onderstepoort was specially macerated and degreased and thus no material from the skeletal collection was used. Animals for dissection purposes are

acquired from Onderstepoort Veterinary Animal Hospital or bought from private farmers or at auctions. These animals are put down and then embalmed at Onderstepoort. Cats, however, are not embalmed but frozen until such time that these remains are needed for dissections. Only healthy animals, with no visible sign of abscesses, cancers, etc. are obtained (De Villiers I. L., pers comm.).

Cow, sheep, impala and pig remains were acquired from local butchers and hunters, and were macerated and degreased at the Department of Anatomy, Faculty of Health Sciences, University of Pretoria. The old world monkeys utilised in this study form part of a small animal bone collection also held in the Department of Anatomy, which are usually used for teaching purposes. The limited numbers of old world monkeys represented in this study is due to the lack of skeletal material available for invasive sampling, while the small number of impala bones included in the study is ascribed to the general lack of impala bones within animal bone collections.

All animal remains were of adult ages, as deducted from the fusion of the epiphyseal plates, except for the subadult cows (20-22 months), sheep (±8 months) and pigs (10-12 months). The subadult ages of these animals are ascribed to modern butchery practices in which subadult animals are slaughtered (Hillier & Bell, 2007). The animal remains did not have any macroscopic indication of bone altering diseases and unfortunately no information regarding sex and dietary intake was available.

Human remains were obtained from the Student Bone Collection of the Department of Anatomy, University of Pretoria. The human sample comprised of 26 adult bones (14 males and 12 females) of known age, sex,

ancestry and cause of death. The adult ages ranged between 22 and 80 years with an average age of 39.15 ± 16.11 yrs. The remains were randomly selected; however, femora and tibiae were excluded when either pathology was visible or the person had died of a known nutritional deficiency, such as malnutrition, pellagra, or kwashiorkor. These deficiencies are known to play an active role in the alteration of bone microstructure (Junqueira & Carneiro, 2003; Pain & Brenton, 2006; Halstead, 2007).

As little has been documented on the microstructure and chemical composition of juvenile bones, three juvenile femora and three juvenile tibiae were also included. Within the Pretoria Bone Collection at least 183 records exist for children aged between one and 15 years of age. For the majority of juvenile material within the Pretoria Bone Collection broncho/pneumonia (15%) and gastroenteritis (13%) were the most common causes of death. Unfortunately these remains do not form part of the Student Bone Collection, therefore limiting the number of bone samples which could be included. Also, no information regarding the cause of death is available for the specific individuals included in this study. Similar to what was the case for adults, specimens were excluded if any visible bone pathology was present. Since the material had not been properly accessioned into the bone collection, age at death had to be estimated. The age of each of these juvenile human bones was determined by measuring the diaphyseal lengths of long bones with an osteometric board and then plotting them on a growth chart for South African children, such as in Steyn and Henneberg (1996). This is not a very accurate method of age determination, but provides a broad indication of the age categories. This method produced juvenile ages ranging between three and

14 years with an average age of 6.83 ± 5.19 yrs. The demographic information of the human sample utilised in this study is summarised in **Table 3.1**.

For the chemical analysis of bone, the entire sample was divided into three groups depending on the feeding preference of the species; i.e. omnivores, carnivores and herbivores (**Table 3.2**). This was done because one would expect broad differences in the chemical composition of bone due to the vast differences found in the food types ingested, assimilated and thus stored within the skeletal elements.

For the histological analysis of bone each of the species was analysed and described individually.

3.2. METHODS

3.2.1. Sampling procedure

Bone samples of approximately 2-4 mm thick were removed from the anterior aspect of the diaphysis of each of the bones, using a small hacksaw fitted with a 228-32-5 P blade. Two transverse parallel cuts, roughly 2-4 mm apart, were made perpendicular to the long axis of the bone extending into the medulla (**Figure 3.1**). Care was taken not to scratch the periosteal layer and also not to saw too quickly, as this can lead to the formation of small cracks.

A thin (50 mm) scraper was inserted into one of the perpendicular cuts and bent towards the second cut, allowing a smooth break of the bone at the base of the section. The sections were removed, labelled and bagged to prevent commingling. The smallest possible samples were removed from the bone,

so as to limit damage and ensure their continuous use for further research and education.

A total of 106 bone sections were removed. These sections were first prepared for chemical analysis and then further filed for histomorphometrical analysis.

Table 3.1 *Demographic information of the adult and juvenile Homo sapiens sapiens samples.*

	Adults (n=26)				Juveniles (n=6)		
	Sample	Age (yrs)	Sex	Cause of death	Sample	Age (yrs)	Sex
Femora	Adult 1	25	♂	Brain tumour	Juvenile 1	13	-
	Adult 2	26	♂	Pulmonary tuberculosis	Juvenile 2	3	-
	Adult 3	35	♂	Coronary complications	Juvenile 3	3	-
	Adult 4	28	♂	Liver tumour			
	Adult 5	45	♀	Broncho-pneumonia			
	Adult 6	22	♀	Pneumonia			
	Adult 7	25	♀	Oesophageal cancer			
	Adult 8	47	♂	Natural causes			
	Adult 9	80	♂	Broncho-pneumonia			
	Adult 10	32	♂	Liver tumour			
	Adult 11	40	♀	Broncho-pneumonia			
	Adult 12	50	♀	Cervical cancer			
	Adult 13	69	♂	Rectal cancer			
	Adult 14	65	♀	Coronary complications			
	Adult 15	22	♀	Coronary complications			
	Adult 16	51	♀	Tuberculosis			
	Adult 17	57	♂	Broncho-pneumonia			
	Adult 18	40	♀	Cervical cancer			
	Adult 19	53	♂	Lung cancer			
Tibiae	Adult 1	25	♂	Brain tumour	Juvenile 1	14	-
	Adult 2	26	♂	Pulmonary tuberculosis	Juvenile 2	4	-
	Adult 3	35	♂	Coronary complications	Juvenile 3	4	-
	Adult 4	28	♂	Liver tumour			
	Adult 5	45	♀	Broncho-pneumonia			
	Adult 6	22	♀	Pneumonia			
	Adult 7	25	♀	Oesophagus cancer			
Mean		**39.15**				**6.83**	
SD		**16.11**				**5.19**	

SD = standard deviation

Table 3.2 *Various species within particular feeding groups (n = 106).*

	Species	Femora (n)	Tibiae (n)	Total (n)
Omnivores	Humans - adult *(Homo sapiens sapiens)*	19	7	26
	Humans -juvenile *(Homo sapiens sapiens)*	3	3	6
	Old world monkeys (*Cercopithecinae*)	3	4	7
	Pigs (*Sus scrofa domestica*)	5	5	10
Carnivores	Cats *(Felix catus)*	4	4	8
	Dogs *(Canis familiaris)*	6	6	12
Herbivores	Cows (*Bos taurus*)	5	5	10
	Sheep (*Ovis aries*)	5	5	10
	Donkeys (*Equus africanus asinus*)	6	6	12
	Impala (*Aepyceros melampus*)	3	2	5
Total		**59**	**47**	**106**

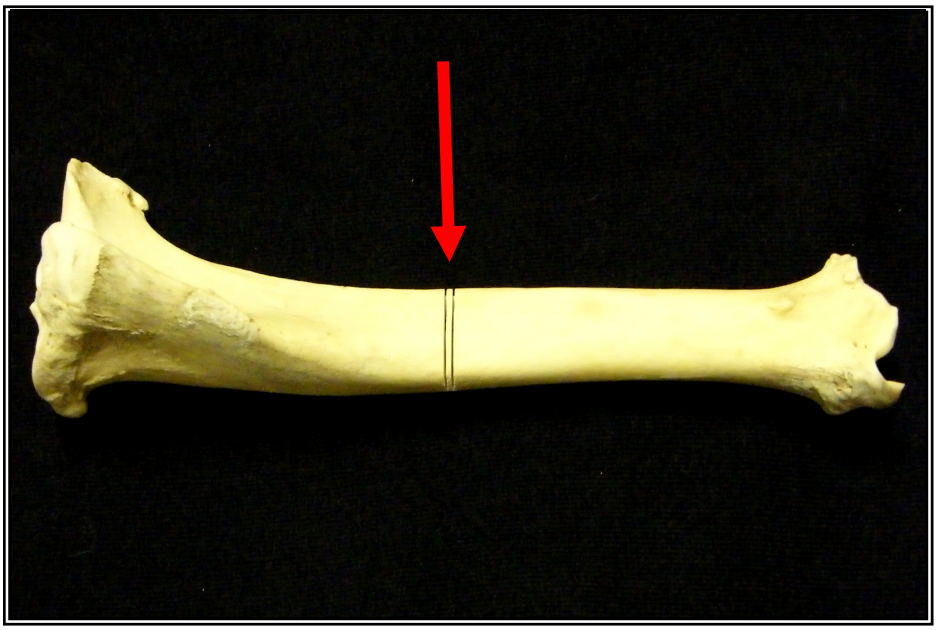


Figure 3.1 *Two parallel cuts approximately 2-4 mm apart on the anterior aspect of the diaphysis of an impala (Aepyceros melampus) long bone.*

3.2.2. Chemical analysis

3.2.2.1. Sample preparation

Frost (1958) realised the need for a manual method to prepare sections for the examination of fresh bone tissue microstructure. With an interest in the mineral content of bone, Frost questioned the reliability of observations made from bone samples that have been exposed to fixation, dehydration, embedding and heat. He developed a preparation technique that did not involve any of these potentially affecting variables (Frost, 1958). This technique has been modified and improved to include sample preparation for poorly preserved and even cremated skeletal material by Maat *et al*. (2002). It is important to note that most preparation methods alter the specimen in some way or another. Such alterations can been accepted so long as one is aware of these changes and understands them (Chandler, 2007).

Chemical analysis requires minimal sample preparation (Bush *et al*., 2007). No chemicals are used and the preparation procedures are generally rapid, easy and affordable (Frost, 1958; Boaz & Hampel, 1978; 2000b; Maat *et al*., 2002). The bone samples were polished according to the stipulations in "The manual for the preparation of ground sections for the microscopy of bone tissue" by Maat *et al*. (2002) (Step1-5).

Step 1: A glass slab of approximately 13.5 x 22.5 cm was covered with a thin, evenly spread layer of petroleum jelly (Vaseline). Half a sheet of P220 waterproof sandpaper was placed, abrasive side up, on top of the glass slab with the edges just overlapping that of the glass slab. This is to prevent water

from seeping between the sheet and the slab and also prevents the Vaseline from contaminating the sandpaper.

Step 2: Sections were removed according to the above description.

Step 3: A few drops of distilled water were dropped in the middle of the sheet and a small piece of abrasive paper was used to grind a starting point in the middle of the sheet. The drops of water prevent large amounts of heat from forming and consequently prevent any possible changes that could occur during the polishing process (Frost, 1958). A section of bone was polished by placing the middle three fingers on the section (one finger for smaller pieces of bone), and applying moderate pressure whilst grinding the piece of bone in a circular or elliptical motion. This procedure was continued until both sides of the bone sample appeared evenly flat and smooth.

Step 4: Thick samples that became too smooth for easy handling were further polished with the aid of “Frost’s gripping device”. A small piece of abrasive paper was folded around the centre of a microscope glass slide with the abrasive side outwards. The free ends of the abrasive paper were used to form the gripping edges, with the index finger on the centre of the slide and the thumb and middle finger supporting it on either side. To use this device, one must ensure that the abrasive paper is slightly larger than the sample to be filed. The piece of bone to be polished is placed below the device and is grounded further in a circular motion (Frost, 1958).

Step 5: To ensure that the section continued to be grounded equally, pressure was alternated with the fingers, applying more pressure to thicker areas. Grinding started in the centre of the sandpaper and continued outwards, avoiding the outer most edges left to overlap that of the glass slides.

For the micro-element analysis the bone sections were polished on both sides only until the surfaces were smooth and no apparent crack or hacks were visible. The samples were repeatedly rinsed in distilled water and then removed with a soft paint brush. The samples were placed on a marked piece of filter paper in a Petri dish and allowed to dry for 2-3 days. To aid in the drying process the Petri dish was placed on top of a metal grid that had been placed over a layer of silica gel within a glass bowl and covered with a glass lid.

Double sided carbon tape was placed on a metallic microscope slide. Three to four polished bone sections were placed on the slide and marked on the back (**Figure 3.2**). Most biological material requires a coating layer to make the sample conductive, to decrease the development of electrostatic charges and inhibit the production of heat, so as to prevent any damage to the sample (Goldstein *et al*., 1981; Chandler, 2007). All the metal slides with the bone samples were coated with a thin layer of approximately 20 nm of gold (Au) for about 10 seconds in a scanning electron microscope autocoating unit E5200 (Polaron equipment Ltd.). This particular coating medium was chosen because it did not interfere with the elemental analysis that was to be performed (Chandler, 2007).

3.2.2.2. Elemental analysis

Analysis was done using a scanning electron microscope (SEM) fitted with an electron dispersive spectrometer (EDS) at the micro-analysis laboratory of the University of Pretoria. A JOEL JSM-5800 LV scanning electron microscope fitted with a Noran Vantage 6 electron dispersive spectrometer (SEM/EDS) was used. Standard analysis conditions included an excitation voltage of 15-20 keV and a dead time of ±20%. SEM/EDS is a simple, accurate and non-destructive method for element analysis (Boaz & Hampel, 1978; Bush *et al*., 2007). An electron beam irradiates a specific area of the sample causing interactions between the electrons from the electron beam and the atoms inside the specimen (Chandler, 2007). Information is emitted by specific characteristic X-rays from the irradiated sample and transformed into an electrical signal. Elements on the periodic table are unique with regards to their atomic structure and therefore emit unique X-rays that can be recognised for each element (Gabriel, 1985). EDS thus produces an identifiable X-ray spectrum, specific to each material (bone, wood, etc.) (Ubelaker *et al*., 2002).

A quantitative approach was taken in this study and the elements selected for analysis included potassium (K), calcium (Ca), phosphorus (P), silicon (Si), aluminium (Al), sodium (Na), magnesium (Mg), chlorine (Cl), sulphur (S), zinc (Zn), lead (Pb) and strontium (Sr). Analyses were performed on three random areas of the compact bone, close to the periosteal surface. Analysis of more than one area provides a better representation of the sample as a whole (Cassella *et al*., 1995). The duration of each analytical cycle was 100 live seconds. Results were obtained in a Microsoft Office Word document and

were transferred to a Microsoft Office Excel 2003 spread sheet to aid in further statistical analysis.

3.2.3. Histomorphometrical analysis

3.2.3.1. Sample preparation

The bone sections used in the above-mentioned chemical examination were also analysed histomorphometrically. The bone sections on the metal slides were removed with a scalpel by inserting the blade underneath the entire section of bone and then carefully lifting it (**Figure 3.2**). For the examination of the microstructure of bone, the sections were further polished according to Maat *et al.'s* (2002) description, repeating Steps 1-5 and continuing with Steps 6-9.

Figure 3.2 *A scalpel bade was used to remove the bone samples from the metal slides.*

Step 6: The bone sections were grounded until it became opaque and reached its final thickness of ±50-100 µm.

Steps 7: The grounded sections were rinsed repeatedly in clean distilled water using a soft paintbrush to ensure that all the dirt and sandpaper debris has been removed from both sides.

Step 8: The cleaned sections were removed with the brush and placed on a marked piece of filter paper in a Petri dish and allowed to dry for one to two days.

Step 9: A piece of dark paper was placed underneath the glass slab to serve as a background. A marked microscope glass slide was cleaned with alcohol and placed on top of the glass slab. A few drops of mounting medium (Entellan) were dropped in the middle of the slide and the bone section was lifted from the filter paper with tweezers. The section was placed on top of the mounting medium and covered with an additional few drops of the medium. To ensure that the section remained in the middle of the slide, a needle tip was used to rectify the position. The glass coverslip was dipped in xylene and excess xylene was removed by tapping a corner of the coverslip on paper towel. The coverslip was placed over the mounting medium by dragging one side of the slip in the medium to its required position and then lowering the rest of the slide carefully over the sample. The mounting medium spread underneath the entire coverslip and by keeping the slide horizontal, microscopic examinations were carried out immediately to ensure that no

bubbles occurred underneath the coverslip. The slide was left to dry one to two days before it was placed in a microscopic slide box for transport and storage.

If air bubbles formed, the entire slide was placed upright in xylene until the section could be removed. Step 9 was repeated whilst the section was still wet with xylene, using a new glass slide. If the slides to be examined were too thick, the section was also placed upright in xylene until it could be removed. The bone section was cleaned, left to dry and polished again continuing from step 6 onwards.

3.2.3.2. Light microscopic analysis

The microscopic architecture of bone was observed with a Nikon transmission light microscope with a 10x Plan DL Ph1 objective lens and a CFW 10x ocular lens, fitted with a Nikon digital camera (DXM1200F) at the micro-analysis laboratory at the University of Pretoria. All observations were made with normal bright light, with a number of additional observations made with polarised light.

A permanent marker was used to mark the anterior side of the bone slide with a straight 1 cm line. This line was divided into five equal intervals, 2.5 mm apart. Areas were marked at 0 mm, 2.5 mm, 5 mm, 7.5 mm and 1 cm and during the histological analysis the microscopic grid was aligned with one such area and a photograph was taken. This study took digital photographs with the aid of a computer program, Nikon ACT-1 for L-1 Version, of the periosteal surface of the compact bone of the areas marked 2.5 mm, 5 mm

and 7.5 mm (**Figure 3.3**). These points ensured repeatable observations of the same bone areas.

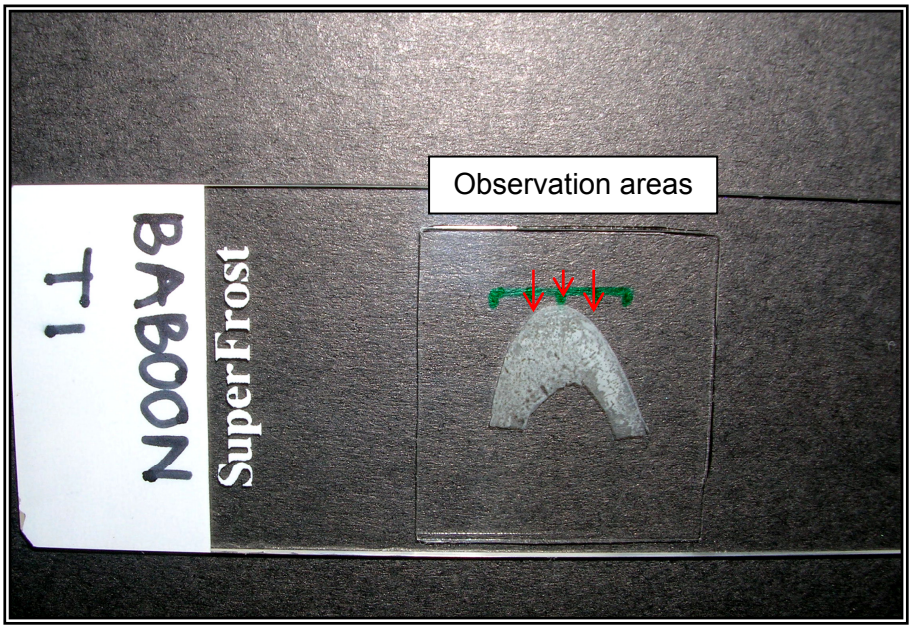


Figure 3.3 *A marked bone slide indicating the specific areas (red arrows) where digital photographs were taken.*

All the images were imported into UTHSCSA Image Tool Windows Version 3.00 (Copyright © 1995-2002) for closer examination and measuring. With the aid of Image Tool the total area of observation was measured as 0.96 mm x 1.2 mm to give a view field of 1.152 mm^2. Three view fields per slide were examined thus giving a total observation area of 3.456 mm^2.

A number of measurable and non-measurable variables were selected to assist with the comparison between human and animal bone. The measurable variables included the number of osteons and non-Haversian canals, as well as the minimum and maximum diameters of osteons and Haversian canals. Several non-measurable variables were also observed in order to describe the general histological appearance of various bones.

Measurable variables

The total number of osteons and non-Haversian canals in each of the three view fields were counted and then added to represent the entire bone section examined (3.456 mm^2). The total number of osteons included all measurable and non-measurable osteons. Osteons were regarded as measurable when the cement line of the osteon was clearly observed on the outer edges of the concentric lamellae and if the Haversian canal was measurable and not obstructed or obscured by Volkmann's canals. Non-measurable osteons included osteons where the cement line was not clearly demarcated, osteons that were located on the edge of photos or osteons where the Haversian canals were obscured or cut off by the edge of photos. Non-measurable osteons located on the edge of a photo were only counted if a complete Haversian canal was identified.

The total number of non-Haversian canals was counted and, as described by Kerley (1965), included vascular canals and primary osteons.

The total number of osteons and non-Haversian canals observed in each animal was added and averaged to obtain a representative value per specie. The minimum and maximum diameters of osteons were measured at the broadest and narrowest areas of the osteon, perpendicular to one another (**Figure 3.5**). The maximum and minimum diameters of the Haversian canals were also measured perpendicular to one another, at the narrowest and broadest areas of the canal (**Figure 3.5**). These values were particularly selected so as to compare these results to those of others in the literature (Cattaneo *et al*., 1999; Martiniaková *et al*., 2006a; Martiniaková *et al*., 2006b; Martiniaková *et al*., 2007a; Martiniaková *et al*., 2007b; Zedda *et al*., 2008).

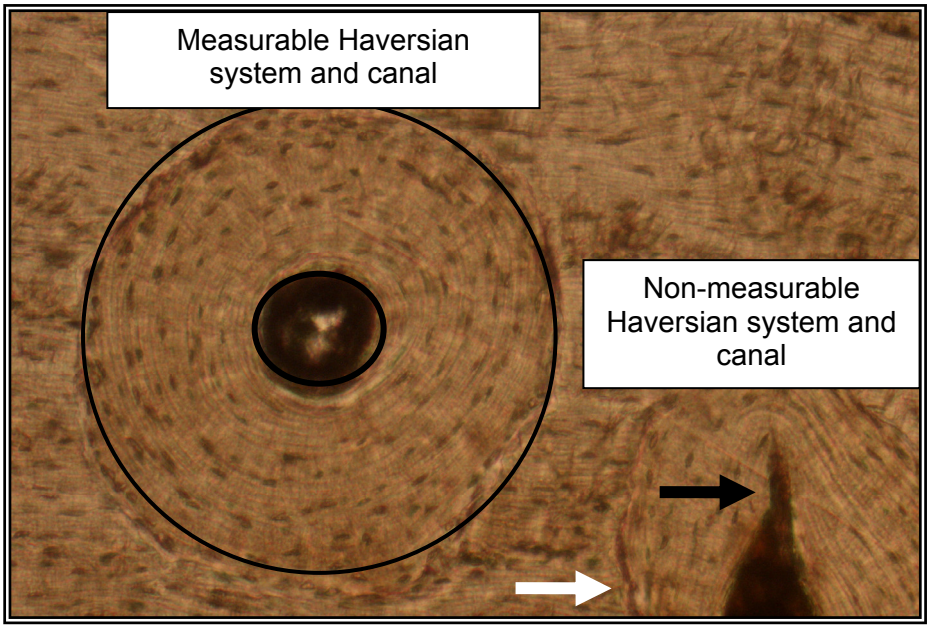


Figure 3.4 *Illustration of a measurable and non-measurable osteon. The white arrow indicates an incomplete osteon and the black arrow indicates an incomplete Haversian canal located at the edge of a photo (Homo sapiens sapiens femur x 100).*

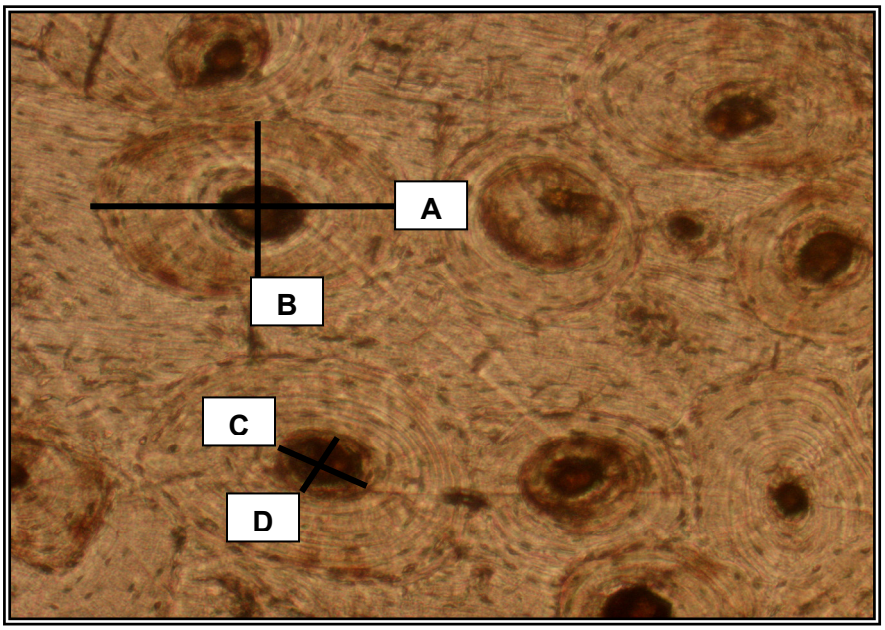


Figure 3.5 *Illustration of measured variables. A) maximum diameter of osteon, B) minimum diameter of osteon, C) maximum diameter of Haversian canal and D) minimum diameter of Haversian canals (Homo sapiens sapiens tibia x 100).*

All measurements were carried out in Image Tool (µm) and the results were documented in a Microsoft Office Excel 2003 worksheet for further analysis.

Measurements were averaged to obtain representative values for all the animal species studied. As differences in the microstructure of various bones of the body have been indicated, results for the measurements were presented separately for femora and tibiae (Evans & Bang, 1966; Hillier & Bell, 2007).

Non-measurable variables

In an attempt to simplify the observed qualitative characteristics of bone microstructure, descriptions of each of the species was made according to the organization of the vascular canals found close to the periosteal surface of the compact part of the bone. The periosteal surface of bone was specifically selected because of the presence of primary bone tissue, particularly plexiform bone, that has been successfully indicated in the separation of human and non-human bones (Cuijpers, 2006; Hillier & Bell, 2007). With increased amounts of secondary bone (Haversian bone), successful separation of human and animal remains become increasingly difficult. This is due to the fact that many mammals have Haversian bone towards the mesosteal and endosteal surfaces that closely resembles that of human Haversian bone (Hillier & Bell, 2007).

To qualitatively describe the bone of the various species studied, attention was given to the organisation of primary vascular bone, Haversian bone and non-vascular bone structures. These arrangements were described in Chapter 2 (Literature Review, section 2.1.1, p.9). Primary vascular bone included descriptions of the general orientation of the vascular canals, i.e. uni- (circular, radial, reticular or longitudinal) and multidirectional (laminar,

plexiform, radial or reticular) canals. Haversian bone included descriptions of the general arrangements of the osteons, i.e. irregular, dense or endosteal. Descriptions of the general shape and sizes of structures found within primary vascular and Haversian bone were also included. Non-vascular bone tissue, as the name suggests, were recorded when no vascular canals were present within the bone.

3.2.4. Statistical interpretation

3.2.4.1. Statistical interpretation of the chemical analysis

The species sampled in this study were pooled together in three groups according to their feeding habits, i.e. herbivores, carnivores and omnivores. These three groups were then compared with regard to their chemical composition, to determine whether any statistically significant differences exist amongst the groups that could aid in the identification of human bones found commingled amongst animal bones or foreign debris.

All the elements measured (K, Ca, P, Si, Al, Na, Mg, Cl, S, Zn, Pb, Sr) in this study were continuous and were summarized using descriptive statistics, i.e. range, mean and standard deviation. Chemical analysis was carried out on femora and tibiae of each of the species. Average values for each of the elements for each of the bones of the particular animals were calculated and summarised in table format. Thereafter femoral and tibial values were added together to get an overall presentation of the chemical composition of bone for each of the animals. Finally the chemical compositions of the various animals were pooled together in the above-mentioned feeding groups. To visually aid in the illustration of the chemical composition of bone, all elements that

yielded statistically significant differences were plotted on separate graphs, with the various feeding groups on the x-axis and the percentage on the y-axis.

The elemental results for femora, tibiae and femora and tibiae pooled were compared in a one-way analysis of variance (ANOVA) for each of the parameters. ANOVA is indicated when multiple comparisons are needed and describes the variability as observed both amongst and within groups (Samuels & Witmar, 2003). However, when the assumption of equal variances between the groups were violated (Bartlett's test for equal variance), a one-way ANOVA for ranks was done (Becker P.J., pers comm.). To interpret significant differences between groups, pair-wise comparisons were done using the Bonferroni adjustment. For example, in any of the three pair-wise comparisons the particular groups of the pairs were considered significant if $p \leq 0.05$ (Becker P.J., pers comm.). The Bonferroni method was the method of choice because this method does not rely on equal sample sizes for comparisons (Samuels & Witmar, 2003).

3.2.4.2. Statistical interpretation of the histomorphometrical analysis

To quantitatively describe bone, descriptive statistics were used and included ranges, means and standard deviations of a number of characteristics as outlined below. All of these variables were given separately for femora and tibiae for each of the species studied.

The total number of osteons and non-Haversian canals and the minimum and maximum diameters of osteons and Haversian canals were summarised in tables. The minimum and maximum osteon and Haversian canal diameter

measurements were also summed and averaged to obtain general osteon and Haversian canal sizes.

The results obtained for each of the measurable variables were compared with a Kruskal-Wallis test. This nonparametric test was indicated as the sample size was small and the assumption of equal variances was violated. This test determines if there are differences amongst the groups, but does not indicate between which groups these differences are found (Zar, 1999). Due to the limited sample sizes no further statistical tests were conducted because the small sample sizes will jeopardise conclusions drawn from such tests (Becker P.J., pers comm.).

Intra-observer and inter-observer error

To ensure repeatability and comparability of the methods used, intra-observer and inter-observer error tests were conducted.

Intra-observer error testing was done to assess the ability of the original investigator to reproduce the results, while the inter-observer error testing was done to assess whether the results can be reproduced by other individuals using the same method. For this purpose, a number of slides were randomly selected and various measurements were repeated.

The repeatability of the total number of osteons and non-Haversian canals counted was determined in 16 slides. For the quantitative analysis, the total number of measurable and non-measurable osteons was also assessed in these slides. In these slides, about 140 osteons and non-Haversian canals were counted by an external investigator and re-counted by the original investigator.

To assess the repeatability of the measurements, six slides were randomly selected and previously identified measurable osteons were marked. This was done to ensure that the measurements obtained for an osteon was comparable between the observers. A total of 64 measurements of the minimum and maximum diameters of osteons and Haversian canals were measured and repeated by the original investigator and by two external investigators, both with previous experience in the field of histology.

The repeated measurements, as well as the external investigators' measurements, were recorded in a Microsoft Office Excel 2003 worksheet and were statistically compared with a one-way analysis of variance (ANOVA).

CHAPTER 4

RESULTS

4.1. CHEMICAL ANALYSIS

Micronutrient analysis was done with a scanning electron microscope fitted with an energy dispersive spectrometer (SEM/EDS). Three random areas on the periosteal bone surface were examined and the results were averaged. A total of 318 chemical analyses were conducted and these results were tabulated per species examined.

Animals with similar feeding habits were grouped together, i.e. herbivores, carnivores and omnivores, and the chemical analysis of these groups were compared to determine whether or not statistically significant differences existed amongst these groups. Elements that varied significantly from one another were plotted on histograms, so as to emphasise the areas of notable difference.

4.1.1. Chemical composition of bone of various animal species

The mean values (%) and standard deviations (SD) of the basic elements that contributed to the formation of both femora and tibiae of all human and nonhuman groups studied were summarised, per species, in **Tables 4.1 – 4.10**. The data for femora and tibiae were initially reported separately because differences in the chemical composition of various long bones have previously been indicated by Klepinger and colleagues (1986). However, the element percentages of these bones were later pooled so as to give an overall chemical composition for each of the animals studied. These values

were averaged values and only serve to provide an overall estimation of the chemical composition of bone and were therefore not discussed separately.

Tables summarising the chemical composition of various individual animal bones were included in **Appendix A** (femora) (p.212) and **Appendix B** (tibiae) (p.215).

In **Table 4.1**, the elemental composition of cat (*Felix catus*) bones is shown. *Felix catus* consisted of an average of 71.53 ± 3.09% calcium in the femur and 72.45 ± 3.15% in the tibia, followed by phosphorus which contributed 23.92 ± 2.73% and 23.94 ± 0.88% respectively. The elements with the lowest contributions were aluminium (0.01 ± 0.01 %) in the femur and silicon (0.01 ± 0.01%) in the tibia. Lead content in both bones were highly variable between individual cats. In the femur, it ranged between 0 and 10.14%, with a mean of 3.2 ± 4.71%; whereas a more moderate lead range between 0 and 5.2%, with an average of 2.19 ± 2.24 was documented in the tibiae.

Calcium contributed 71.90 ± 0.05% of the femora and 72.81 ± 1.06% of the tibiae in dogs (*Canis familiaris*) as shown in **Table 4.2**. The second most common element observed was phosphorus which contributed 23.67 ± 2.11% and 23.91 ± 1.89% in the two bones, respectively. In the femur, strontium and aluminium each contributed only 0.03% (±0.03; ±0.05 respectively) while in the tibia, strontium contributed only 0.01 ± 0.02%. The distribution range of the lead content in dog femora was similar to that of cat tibiae. The lead content in the femur ranged between 0 and 5.65% with an average of 3.08 ± 2.23%.

Table 4.1 *Mean values (%) and standard deviations (SD) of the elements present in cat (Felix catus) bones (n=8).*

Elements	Femur		Tibia		Pooled	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Ca	71.53	3.09	72.45	3.15	71.99	2.41
P	23.92	2.73	23.94	0.88	23.93	2.40
Pb	3.20	4.71	2.19	3.96	2.70	3.32
Mg	0.37	0.04	0.34	0.03	0.35	0.07
Na	0.30	0.05	0.40	0.05	0.35	0.04
Zn	0.20	0.16	0.17	0.23	0.19	0.05
Cl	0.14	0.09	0.12	0.03	0.13	0.07
S	0.12	0.07	0.11	0.07	0.12	0.10
K	0.10	0.08	0.12	0.02	0.11	0.08
Sr	0.07	0.11	0.06	0.12	0.07	0.11
Si	0.03	0.04	0.01	0.01	0.02	0.03
Al	0.01	0.01	0.09	0.19	0.05	0.05
Total	100.00		100.00		100.00	

Table 4.2 *Mean values (%) and standard deviations (SD) of the elements present in dog (Canis familiaris) bones (n=12).*

Elements	Femur		Tibia		Pooled	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Ca	71.90	0.50	72.81	1.06	72.35	0.73
P	23.67	2.11	23.91	1.89	23.79	1.99
Pb	3.08	2.23	1.72	1.33	2.40	1.71
Na	0.31	0.08	0.29	0.10	0.30	0.09
Mg	0.26	0.10	0.25	0.11	0.25	0.10
S	0.25	0.08	0.28	0.05	0.27	0.06
Zn	0.23	0.24	0.48	0.30	0.35	0.24
Cl	0.11	0.06	0.11	0.08	0.11	0.02
Si	0.08	0.05	0.05	0.05	0.06	0.04
K	0.05	0.03	0.03	0.02	0.04	0.02
Sr	0.03	0.03	0.01	0.02	0.02	0.02
Al	0.03	0.05	0.06	0.10	0.04	0.05
Total	100.00		100.00		100.00	

Table 4.3 illustrates the chemical composition of cow (*Bos taurus*) femora and tibiae. Cow femora consisted of an average of 69.99 ± 6.59% calcium and 22.55 ± 2.24% phosphorus. The calcium content of the cow femur ranged from 58.40% to 74.30%, and the lead content between 0.78% and 21.79% with an average of 6.19 ± 8.88%; clearly illustrating the high variability of the femoral lead content observed amongst the various cows in the sample. Silicone contributed the least to the chemical composition of cow femora, namely 0.02 ± 0.03%. In the tibiae of cows calcium formed 72.45 ± 1.37% of the mineral mass followed by 23.17 ± 0.28% phosphorus. A very small fraction of only 0.01 ± 0.02% was contributed by both silicone and strontium.

Table 4.3 *Mean values (%) and standard deviations (SD) of the elements present in cow (Bos taurus) bones (n=10).*

Elements	Femur		Tibia		Pooled	
	Mean	SD	Mean	SD	Mean	SD
Ca	69.99	6.59	72.79	1.37	71.39	2.90
P	22.55	2.24	23.17	0.28	22.86	1.06
Pb	6.19	8.88	2.91	1.63	4.55	3.93
Mg	0.39	0.06	0.35	0.05	0.37	0.05
Na	0.33	0.06	0.34	0.03	0.34	0.04
Zn	0.18	0.22	0.16	0.12	0.17	0.12
S	0.16	0.07	0.13	0.09	0.14	0.05
Cl	0.08	0.04	0.08	0.03	0.08	0.03
K	0.05	0.04	0.04	0.04	0.04	0.02
Al	0.04	0.04	0.02	0.03	0.03	0.02
Sr	0.03	0.03	0.01	0.02	0.02	0.01
Si	0.02	0.03	0.01	0.02	0.01	0.02
Total	100.00		100.00		100.00	

Table 4.4 summarises the chemical composition of the bones of sheep (*Ovis aries*). The highest contributing element was calcium with 71.77 ± 0.31% followed by 26.46 ± 0.19% phosphorus in femora. No aluminium was

recorded in sheep femora and only 0.04 ± 0.10% was detected in sheep tibiae. Strontium formed the lowest fraction of sheep tibiae and only 0.03 ± 0.03% were recorded. Calcium formed 71.21 ± 0.23% of sheep tibiae and phosphorus provided 26.36 ± 0.36%. Comparatively high values for sodium and magnesium were documented in both sheep femora (0.51 ± 0.13% and 0.48 ± 0.07%, respectively) and tibiae (0.53 ± 0.05% and 0.45 ± 0.06%, respectively).

Table 4.4 *Mean values (%) and standard deviations (SD) of the elements present in sheep (Ovis aries) bones (n=10).*

Elements	Femur		Tibia		Pooled	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Ca	71.77	0.31	71.21	0.23	71.49	0.27
P	26.46	0.19	26.36	0.36	26.41	0.16
Na	0.51	0.13	0.53	0.05	0.52	0.09
Mg	0.48	0.07	0.45	0.06	0.46	0.05
S	0.22	0.09	0.23	0.06	0.22	0.06
Pb	0.15	0.10	0.79	0.36	0.47	0.14
Zn	0.12	0.06	0.15	0.09	0.13	0.07
K	0.09	0.04	0.05	0.04	0.07	0.03
Si	0.08	0.07	0.06	0.05	0.07	0.04
Sr	0.05	0.06	0.03	0.03	0.04	0.04
Cl	0.07	0.02	0.09	0.03	0.08	0.02
Al	0.00	0.00	0.04	0.10	0.02	0.05
Total	100.00		100.00		100.00	

The elemental composition of donkey (*Equus africanus asinus*) femora and tibiae are summarized in **Table 4.5**. Calcium formed 73.27 ± 1.55% of the general composition of tibiae and 74.12 ± 1.18% of femora. Phosphorus formed 23.84 ± 1.98% of tibiae and 22.86 ± 0.44% of femora. Silicone was not detected in donkey tibiae, and only contributed 0.02 ± 0.03% in femora. The aluminium portion in femora was also low, namely 0.02 ± 0.01. Similar to

the lead content in cat, dog and cow femora, donkey femora also illustrated highly variable lead contents. Lead ranged between 0.70% and 4.52%, with a mean femoral content of 1.88 ± 1.46%.

Table 4.5 *Mean values (%) and standard deviations (SD) of the elements present in donkey (Equus africanus asinus) bones (n=12).*

Elements	Femur		Tibia		Pooled	
	Mean	SD	Mean	SD	Mean	SD
Ca	74.12	1.18	73.27	1.55	73.70	0.53
P	22.86	0.44	23.84	1.98	23.35	1.13
Pb	1.88	1.46	1.44	1.34	1.66	1.09
Na	0.30	0.03	0.35	0.13	0.32	0.06
Mg	0.27	0.04	0.26	0.04	0.27	0.02
Zn	0.21	0.32	0.37	0.20	0.29	0.22
S	0.12	0.11	0.08	0.07	0.10	0.08
Cl	0.10	0.05	0.14	0.05	0.12	0.03
Sr	0.06	0.05	0.18	0.26	0.12	0.11
K	0.05	0.04	0.03	0.02	0.04	0.03
Si	0.02	0.03	0.00	0.00	0.01	0.02
Al	0.02	0.01	0.05	0.05	0.03	0.02
Total	100.00		100.00		100.00	

Table 4.6 demonstrates the chemical composition of bones from impala (*Aepyceros melampus*). No traces of silicone, aluminium or strontium were observed in the tibiae, and no aluminium or strontium was reported in the femora. Similarly to the above descriptions, calcium was the most abundant element and contributed 71.10 ± 0.37% and 71.31 ± 0.30% in femora and tibiae respectively, followed by the phosphorus which contributed 26.93 ± 0.55% and 27.00 ± 0.02% respectively. Surprisingly, high values for sodium and magnesium were documented in impala bone with 0.59 ± 0.09% sodium in femora and 0.56 ± 0.02% in tibiae. The highest magnesium percentages

were recorded in impala remains, namely 0.65 ± 0.07% in femora and 0.56 ± 0.02% in tibiae.

Table 4.6 *Mean values (%) and standard deviations (SD) of the elements present in impala (Aepyceros melampus) bones (n=5).*

Elements	Femur		Tibia		Pooled	
	Mean	SD	Mean	SD	Mean	SD
Ca	71.10	0.37	71.31	0.30	71.15	0.33
P	26.93	0.55	27.00	0.02	26.93	0.30
Mg	0.65	0.07	0.56	0.02	0.63	0.07
Na	0.59	0.09	0.56	0.02	0.59	0.08
Pb	0.45	0.24	0.27	0.09	0.41	0.17
Cl	0.12	0.01	0.13	0.03	0.12	0.01
K	0.06	0.07	0.02	0.03	0.06	0.07
Zn	0.05	0.07	0.10	0.14	0.06	0.05
S	0.03	0.02	0.05	0.00	0.03	0.02
Si	0.01	0.01	0.00	0.00	0.01	0.00
Sr	0.00	0.01	0.00	0.00	0.00	0.00
Al	0.00	0.00	0.00	0.00	0.00	0.00
Total	100.00		99.99		100.00	

The data obtained for the chemical composition of pig (*Sus scrofa domestica*) bones are tabulated in **Table 4.7**. Similar to impala, no traces of aluminium were reported in either the femora or the tibiae and no traces of strontium were reported in the tibiae. The pig femora consisted mainly of calcium, 71.92 ± 1.90% and 26.12 ± 1.48% phosphorus with 71.45 ± 0.48% calcium and 25.51 ± 1.70% phosphorus in the tibiae. High sodium and magnesium levels were also recorded in pig bones with 0.48 ± 0.13% magnesium in femora and 0.46 ± 0.06 in tibiae. Sodium percentages in the femora were 0.50 ± 0.09% and 0.48 ± 0.10% in tibiae.

Table 4.7 *Mean values (%) and standard deviations (SD) of the elements present in pig (Sus scrofa domestica) bones (n=10).*

Elements	Femur		Tibia		Pooled	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Ca	71.92	1.90	71.45	0.48	71.68	1.04
P	26.12	1.48	25.51	1.70	25.81	1.58
Na	0.50	0.09	0.48	0.10	0.49	0.09
Mg	0.48	0.13	0.46	0.06	0.47	0.07
Pb	0.37	0.28	1.50	1.87	0.94	0.87
S	0.36	0.17	0.32	0.12	0.34	0.14
Zn	0.08	0.09	0.09	0.10	0.08	0.06
Cl	0.07	0.06	0.07	0.05	0.07	0.05
K	0.06	0.03	0.10	0.03	0.08	0.02
Si	0.04	0.02	0.02	0.02	0.03	0.01
Sr	0.01	0.01	0.00	0.00	0.00	0.01
Al	0.00	0.00	0.00	0.00	0.00	0.00
Total	100.00		100.00		100.00	

Data on the elemental analysis of the bones of primates are reported in **Table 4.8**. Trace amounts of strontium were reported in the femora of old world monkeys (*Cercopithecinae*), 0.02 ± 0.04%, and little silicone was found in the tibiae, 0.04 ± 0.02%. Calcium and phosphorus contributed 72.07 ± 3.35% and 22.36 ± 0.75% to the tibiae. In the femora, calcium ranged between 67.48% and 74.81% and on average represented 72.27 ± 4.15% of all elements. The minimum value was observed in an individual femur that had a high lead content of 9.05%. Lead varied across a range of 0-9.05% with an average of 3.57 ± 4.82%, illustrating the high variability of the element between individual bones of the particular species. The lead content in tibiae also illustrated high variability and ranged between 0 and 6.54%, with an average of 2.75 ± 3.29%. A higher than normal chlorine percentage was documented in the tibiae, 0.21 ± 0.01%.

Table 4.8 *Mean values (%) and standard deviations (SD) of the elements present in old world monkey (Cercopithecinae) bones (n=7).*

Elements	Femur		Tibia		Pooled	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Ca	72.27	4.15	72.07	3.35	71.87	2.17
P	22.47	0.90	22.36	0.75	22.36	0.50
Pb	3.57	4.82	2.75	3.29	3.27	2.19
Zn	0.44	0.33	1.47	1.03	1.22	0.93
Na	0.32	0.16	0.36	0.09	0.33	0.08
Mg	0.27	0.04	0.26	0.12	0.25	0.09
Cl	0.21	0.01	0.15	0.06	0.18	0.03
K	0.18	0.11	0.12	0.03	0.14	0.04
S	0.15	0.09	0.31	0.16	0.24	0.05
Si	0.06	0.04	0.04	0.02	0.05	0.02
Al	0.05	0.08	0.06	0.06	0.06	0.05
Sr	0.02	0.04	0.05	0.04	0.04	0.03
Total	100.00		100.00		100.00	

Table 4.9 illustrates the elemental representation of tibiae and femora in adult *Homo sapiens sapiens*. Human tibiae had little contribution from elements such as silicone and aluminium and aluminium only contributed 0.04% (±0.05 and ±0.04 respectively) and silicone only 0.02% (±0.02) to femora. Calcium, as expected, had the greatest representation in both femora (70.77 ± 1.59%) and tibiae (73.06 ± 3.44%). The calcium content of tibiae ranged between 65.69% and 75.67%, with an average of 73.06 ± 3.44%. The lowest calcium value was partly due to a large concentration of lead (12.75%) in one particular bone. The lead content ranged between 0.90% and 12.75% with an average of 3.35 ± 4.50%. Phosphorus contributed 24.96 ± 2.32% in femora and 21.98 ± 1.32% in tibiae. The highest sodium percentage recorded was for adult femora with an average of 0.62 ± 0.16%. Higher than normal chlorine percentages were also observed in adult bones,

with average percentages of 0.26 ± 0.12% and 0.30 ± 0.17% in the femora and tibiae respectively.

Table 4.9 *Mean values (%) and standard deviations (SD) of the elements present in adult human (Homo sapiens sapiens) bones (n=26).*

Elements	Femur		Tibia		Pooled	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Ca	71.00	1.25	73.06	3.44	71.31	1.62
P	25.22	2.07	21.98	1.32	24.66	2.09
Pb	1.84	2.95	3.35	4.50	2.13	3.12
Na	0.62	0.16	0.33	0.17	0.57	0.17
Mg	0.32	0.07	0.17	0.07	0.30	0.08
Cl	0.26	0.12	0.30	0.17	0.27	0.12
Zn	0.24	0.31	0.28	0.27	0.24	0.30
S	0.21	0.13	0.22	0.05	0.20	0.11
K	0.12	0.07	0.13	0.09	0.12	0.06
Sr	0.11	0.15	0.12	0.10	0.11	0.14
Al	0.06	0.06	0.04	0.05	0.06	0.06
Si	0.02	0.02	0.04	0.04	0.02	0.02
Total	100.00		100.00		100.00	

Table 4.10 describes the elemental composition of juvenile *Homo sapiens sapiens*. As expected, calcium was the most abundant element in the bones of juveniles, contributing 68.55 ± 3.70% to femora and 69.24 ± 2.68% to tibiae. These values are lower when compared to the other species analyzed, including adult humans. In the femora, calcium ranged between 64.67% and 72.03%, with an average of 68.55 ± 3.70%. Again, the lowest calcium value was observed in a femur with a considerably high lead content (13.78%). In juvenile bones, strontium contributed the least; 0.01 ± 0.01% and 0.03 ± 0.03% for the femora and tibia respectively. Juvenile bones had the highest lead content for both the femora (8.89 ± 4.42%) and tibiae (8.77 ± 2.57%). In the femora the lead content ranged between 5.19% and 13.78% and between

5.81% and 10.48% for tibiae. The highest chlorine percentage was also recorded in juveniles and juvenile tibiae consisted of a chlorine average of 0.34 ± 0.20%.

Table 4.10 *Mean values (%) and standard deviations (SD) of the elements present in human juvenile (Homo sapiens sapiens) bones (n=6).*

Elements	Femur		Tibia		Pooled	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Ca	68.55	3.70	69.24	2.68	68.68	2.85
P	20.97	0.86	20.11	0.24	20.50	0.53
Pb	8.89	4.42	8.77	2.57	9.08	3.11
Zn	0.36	0.35	0.30	0.28	0.30	0.29
Mg	0.34	0.10	0.18	0.10	0.27	0.13
Na	0.30	0.02	0.34	0.10	0.31	0.05
S	0.24	0.17	0.45	0.32	0.38	0.26
Cl	0.17	0.08	0.34	0.20	0.23	0.17
K	0.10	0.10	0.11	0.07	0.11	0.07
Al	0.04	0.06	0.06	0.11	0.06	0.09
Si	0.03	0.04	0.08	0.04	0.06	0.02
Sr	0.01	0.01	0.03	0.03	0.02	0.02
Total	100.00		100.00		100.00	

From the above tables, calcium was shown to be the highest represented element and ranged between 58.40% and 75.40% in femora and 65.96% and 75.67% in tibiae. The highest calcium percentage was recorded in an adult human tibia and the lowest percentage in a cow femur. The second most common element was phosphorus which ranged from 18.16% to 27.54% in femora and 19.38% to 27.01% in tibiae. An impala had the highest phosphorus percentage and a cow femur the lowest. Lead contributed between 0 and 21.79% in femora and between 0 and 12.75% in tibiae. Silicone contributed the smallest fraction in both femora (0-0.17%) and tibiae

(0-0.15%). The remaining elements contributed on average, between 0 and 2.58% to the elemental composition of bone.

Due to the limited sample size, a large variation and overlap was observed between the various species and no attempt was made to determine intra- or interspecies variation, or to compare the statistical significance of the mean differences between them. Species were grouped together according to feeding habits not only to increase the sample sizes, but also to provide for a more meaningful assessment of differences between species and between the certain chemical elements discussed above. Also, researchers have suggested that differences due to the various food types ingested among animals may have an effect on the element composition presented in their bones (Toots & Voorhies, 1965; Parker & Toots, 1970; Pingitore *et al*., 2001).

4.1.2. Chemical composition of bone of various feeding groups

The human and nonhuman samples were grouped together according to their feeding preference, namely herbivores (n=37), carnivores (n=20) and omnivores (n=49). The herbivorous group included cow (*Bos taurus*), sheep (*Ovis aries*), donkey (*Equus africanus asinus*) and impala (*Aepyceros melampus*) bones. The carnivorous group included cat (*Felix catus*) and dog (*Canis familiaris*) bones, while pig (*Sus scrofa domestica*), old world monkey (*Cercopithecinae*) and human juvenile and adult (*Homo sapiens sapiens*) bones formed the omnivorous group.

The chemical composition of the three groups was compared with a one-way analysis of variance (ANOVA) and the mean percentages (%) and standard deviations (SD) of the femora, tibiae and pooled bones of the

various feeding groups are compared in **Tables 4.11**, **4.12** and **4.13**. Due to the limited sample size the chemical compositions of femora and tibiae were pooled together and also compared between the various feeding groups. All elements that indicated significant differences were highlighted and shown in histograms.

4.1.2.1. Chemical composition of femora

Descriptive statistics for the femora and the degree of statistically significant differences between the three feeding groups are summarised in **Table 4.11**.

The composition of phosphorus was similar amongst the three groups and contributed in the following manner; 24.33 ± 2.301% in herbivores, 23.77 ± 2.229% in carnivores and 24.67 ± 2.355% in omnivores. In general, silicone, aluminium and strontium contributed only minimally to the chemical composition of femora of the feeding groups. Aluminium contributed least to the composition of femora. In herbivores this element only contributed 0.016 ± 0.025% and in carnivores only 0.018 ± 0.037%. In omnivore femora, silicone's contribution was the smallest, namely 0.029 ± 0.029. Strontium percentages in omnivores (0.073 ± 0.129%) were slightly larger than those of herbivores (0.041 ± 0.046%) and carnivores (0.045 ± 0.074%). Recorded percentages for zinc contributions were somewhat lower in herbivores (0.168 ± 0.217%) compared to that of carnivores (0.22 ± 0.198%) and omnivores (0.246 ± 0.291%).

Table 4.11 *Mean elemental composition (%), standard deviations (SD) and statistically significant differences of the various elements between the femora of herbivores, carnivores and omnivores.*

Elements	Femora						One-way ANOVA*	
	Herbivores (n=19)		Carnivores (n=10)		Omnivores (n=30)		p-Value	Statistically significant differences** observed between:
	Mean	SD	Mean	SD	Mean	SD		
Ca	71.871	3.753	71.753	1.832	71.033	2.129	**0.0263**	Omnivores and herbivores
P	24.328	2.301	23.772	2.229	24.666	2.355	0.5674	-
Pb	2.445	5.201	3.127	3.186	2.472	3.713	0.8990	-
Mg	0.408	0.131	0.304	0.095	0.343	0.100	**0.0469**	Carnivores and herbivores
Na	0.399	0.128	0.304	0.068	0.534	0.181	**0.0001**	Omnivores and herbivores Omnivores and carnivores
Zn	0.168	0.217	0.22	0.198	0.246	0.291	0.6126	-
S	0.147	0.103	0.2	0.103	0.230	0.144	0.1051	-
Cl	0.086	0.038	0.122	0.070	0.211	0.124	**0.0001**	Omnivores and herbivores Omnivores and carnivores
K	0.052	0.043	0.071	0.059	0.116	0.077	**0.0029**	Omnivores and herbivores
Sr	0.041	0.046	0.045	0.074	0.073	0.129	0.9597	-
Si	0.039	0.052	0.06	0.050	0.029	0.029	0.2905	-
Al	0.016	0.025	0.018	0.037	0.045	0.056	0.2467	-

**If Bartlett's test for equal variances was significant, i.e. assumption of equal variances violated, the ANOVA for ranks was preformed*

***Particular differences were established using pair-wise comparisons at the Bonferroni adjusted level of significance*

The exceptionally large standard deviations (SD) observed in the lead content of the groups indicate the high variability of this element within the feeding groups. Large standard deviations (SD) were also observed for the elements that contributed least to the chemical composition of bone and are also indicative of the high variability of these elements within the bones of the various animal species.

Statistically significant differences were observed in potassium, calcium, sodium, magnesium and chlorine values. These differences were mainly amongst omnivores and herbivores, with the exception of magnesium that indicated significant differences between carnivores and herbivores. Sodium and chlorine were also significantly different between carnivores and omnivores. Histograms comparing the mean values (%) and standard deviations (SD) of the elements that indicated statistically significant differences between herbivores, omnivores and carnivores are shown in **Figures 4.1 – 4.5**.

Figure 4.1 illustrates the mean values (%) and standard deviations of potassium. The mean values are depicted by the bars while the vertical lines indicate standard deviations (SD). Potassium levels were significantly higher in the femora of omnivores compared to those of herbivores ($p=0.0029$), with the highest values recorded in the nonhuman primates. However, it can be seen that the values varied considerably between the various species within a particular feeding group, with no clear pattern emerging among them.

The average percentage for potassium in omnivores, herbivores and carnivores are indicated by the last bars of each series. Potassium formed an average contribution of 0.052 ± 0.043% of herbivore, 0.071 ± 0.059% of

carnivore and 0.116 ± 0.077% of omnivore femora, clearly indicating a larger contribution to omnivores as compared to both herbivores and carnivores.

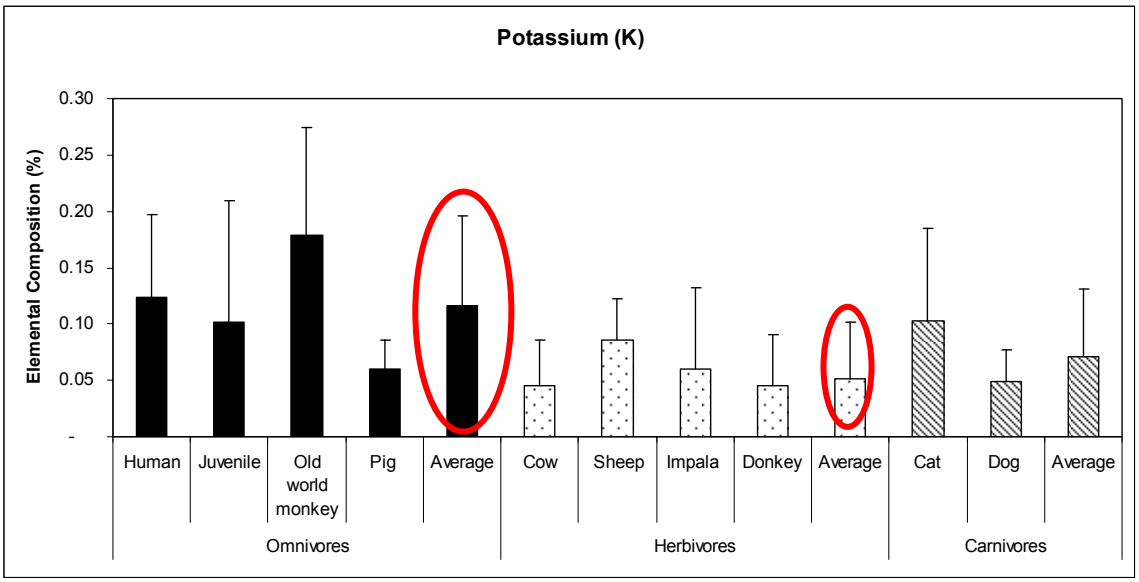


Figure 4.1 *The mean values (%) and standard deviations for potassium (K) in omnivore, herbivore and carnivore femora. Circles indicate statistically significant different groups. Juvenile in this figure refers to subadult Homo sapiens sapiens.*

Calcium contributed an average of 71.87 ± 3.753% in herbivore, 71.75 ± 1.832% in carnivore and 71.03 ± 2.129% in omnivore (**Figure 4.2**) femora, and the difference was statistically significant between omnivores and herbivores ($p = 0.0263$). The highest calcium percentage was recorded in donkey (herbivore) femora and the lowest percentage in human juveniles. It is interesting to note the differences in calcium content between adult (71.00%) and juvenile *Homo sapiens sapiens* (68.55%). These differences might be related to age differences between these two groups and also regarding bone remodelling patterns.

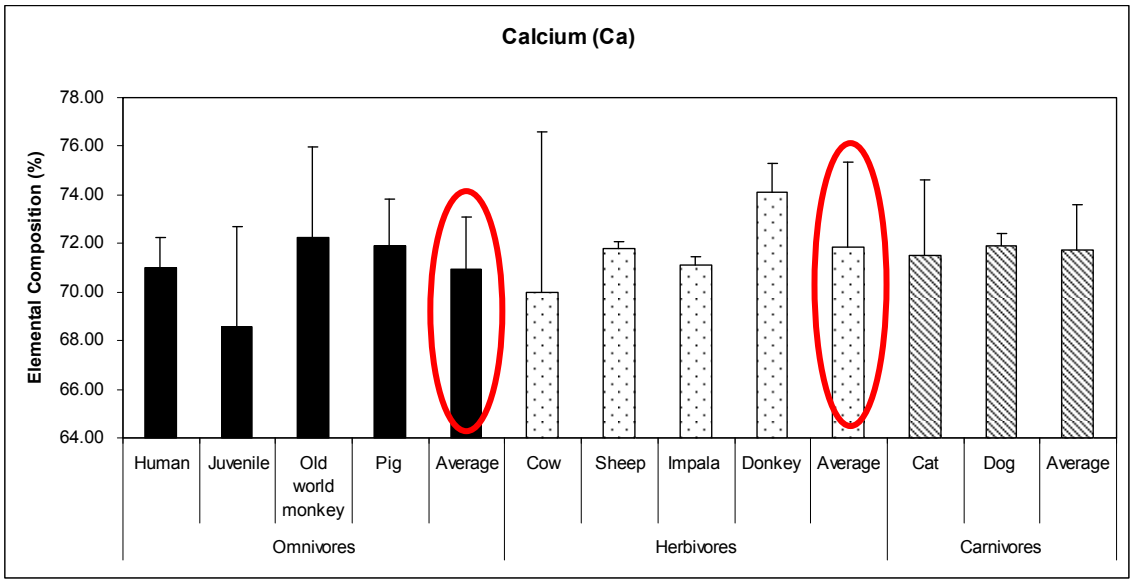


Figure 4.2 *The mean values (%) and standard deviations of calcium (Ca) in omnivore, herbivore and carnivore femora. Circles indicate statistically significant different groups. Juvenile in this figure refers to subadult Homo sapiens sapiens.*

Sodium (**Figure 4.3**) was statistically significantly different between omnivores and herbivores and between omnivores and carnivores (p=0.0001), with the highest levels recorded in omnivores (0.534 ± 0.181%), followed by herbivores (0.399 ± 0.128%) and then carnivores (0.304 ± 0.068%). The sodium content in carnivores was similar to their calcium content, in that there was less variation between the animals within this feeding group, than compared to herbivores and omnivores. Once again, a difference in the sodium content of adult and the juvenile *Homo sapiens sapiens* was present and might be related to dietary differences between the two groups.

Variation in the mean value (%) of the magnesium content of femora in herbivores, carnivore and omnivores is illustrated in **Figure 4.4**. Magnesium constituted 0.408 ± 0.131% of herbivore, 0.304 ± 0.095% of carnivore and 0.343 ± 0.100% of omnivore femora. High magnesium values were recorded

in pig, sheep and adult human femora and statistically significant differences with regards to this element were observed between herbivores and carnivores (p=0.0469).

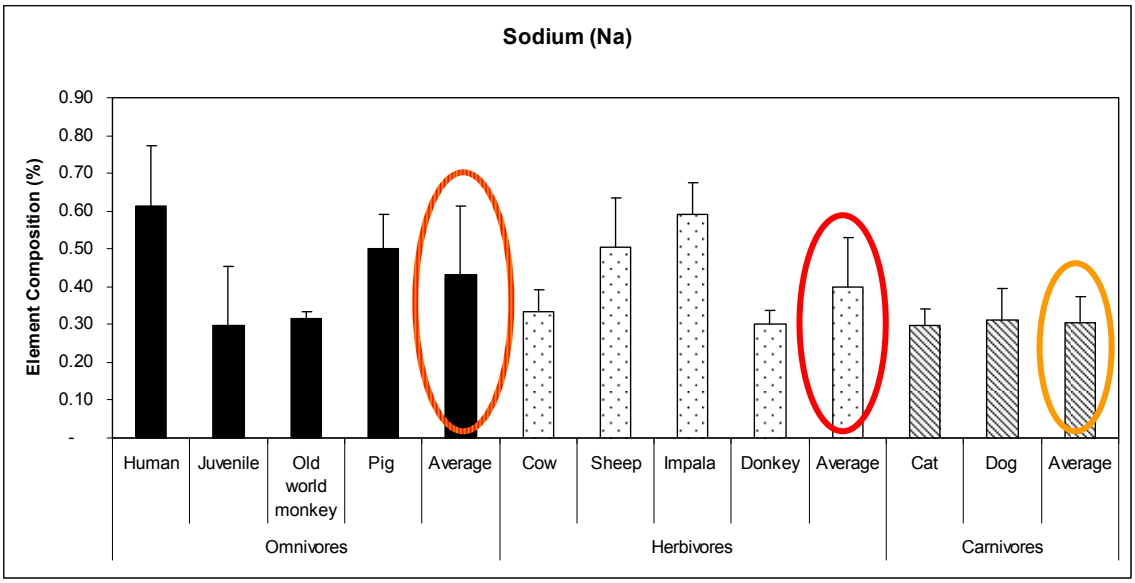


Figure 4.3 *The mean values (%) and standard deviations of sodium (Na) in omnivore, herbivore and carnivore femora. Circles indicate statistically significant different groups. Juvenile in this figure refers to subadult Homo sapiens sapiens.*

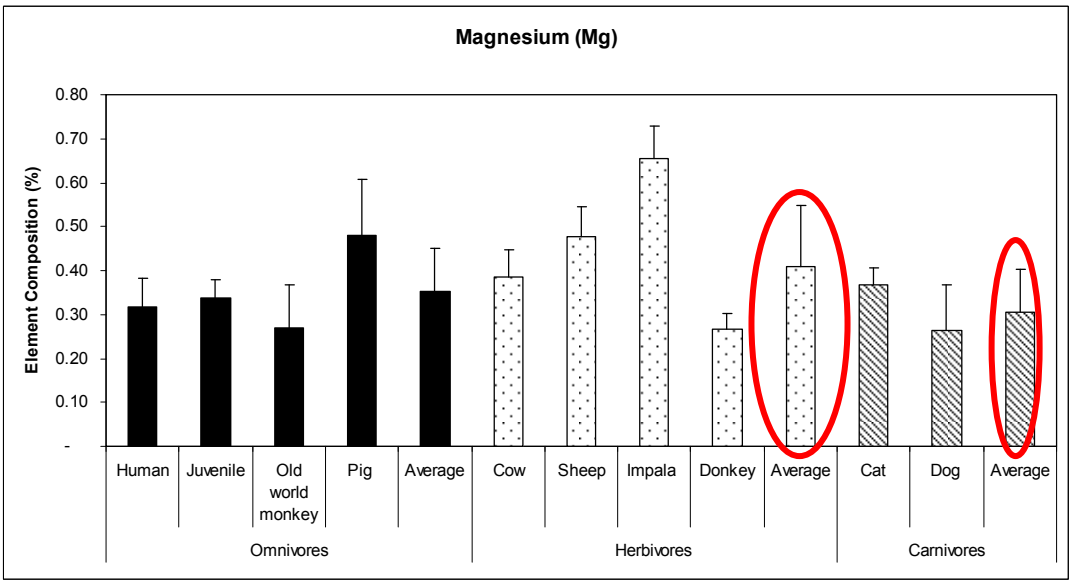


Figure 4.4 *The mean values (%) and standard deviations of magnesium (Mg) in omnivore, herbivore and carnivore femora. Circles indicate statistically significant different groups. Juvenile in this figure refers to subadult Homo sapiens sapiens.*

Mean values and standard deviations (SD) for chlorine are depicted in **Figure 4.5**. The average percentages for chlorine (Cl) in femora were 0.086 ± 0.038% in herbivores, 0.122 ± 0.070% in carnivores and 0.21 ± 0.124% in omnivores. These values were statistically significant (p=0.0001) between omnivores and herbivores and between omnivores and carnivores. High values for chlorine were observed in the adult human and old world monkey femora. This trace element showed variation within the omnivorous and herbivorous groups, while it appeared more consistent in the carnivorous groups.

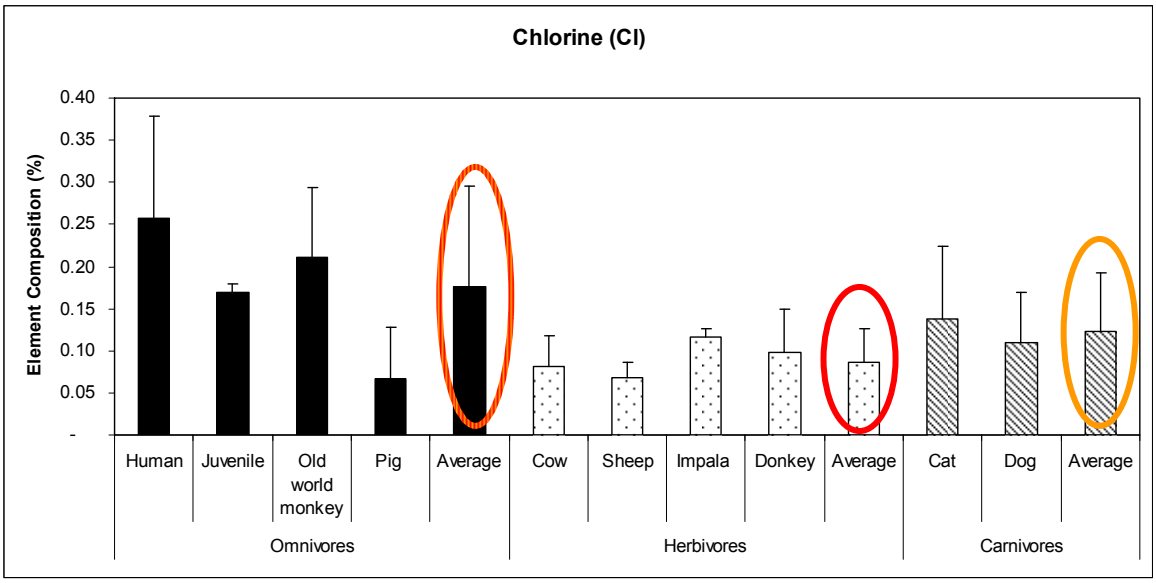


Figure 4.5 *The mean values (%) and standard deviations of chlorine (Cl) in omnivore, herbivore and carnivore femora. Circles indicate statistically significant different groups. Juvenile in this figure refers to subadult Homo sapiens sapiens.*

4.1.2.2. Chemical composition of tibiae

Descriptive statistics for the tibiae, indicating the degree of statistical significance between the three feeding groups, are summarised in **Table 4.12**.

Table 4.12 *Mean elemental composition (%), standard deviations (SD) and statistically significant differences of the various elements between the tibiae of herbivores, carnivores and omnivores.*

Elements	Tibia						One-way ANOVA*	
	Herbivores (n=18)		Carnivores (n=10)		Omnivores (n=19)		p-Value	Statistically significant differences** observed between:
	Mean	SD	Mean	SD	Mean	SD		
Ca	72.346	1.426	72.666	1.447	71.822	2.901	0.7427	-
P	24.707	1.894	23.924	1.870	22.696	2.205	**0.0150**	Omnivores and herbivores
Pb	1.538	1.450	1.907	1.646	3.594	3.993	0.5394	-
Na	0.419	0.125	0.334	0.109	0.375	0.134	0.2273	-
Mg	0.369	0.110	0.284	0.110	0.267	0.148	**0.0475**	Omnivores and herbivores
Zn	0.222	1.174	0.354	0.286	0.480	0.705	0.6037	-
S	0.130	0.094	0.213	0.123	0.303	0.163	**0.0011**	Omnivores and herbivores
Cl	0.111	0.048	0.113	0.073	0.213	0.167	0.1243	-
Sr	0.068	0.161	0.032	0.065	0.058	0.080	0.6444	-
K	0.036	0.031	0.068	0.071	0.117	0.061	**0.0001**	Omnivores and herbivores Omnivores and carnivores
Al	0.033	0.059	0.070	0.100	0.037	0.058	0.3633	-
Si	0.020	0.037	0.033	0.045	0.039	0.037	0.3159	-

**If Bartlett's test for equal variances was significant, i.e. assumption of equal variances violated, the ANOVA for ranks was preformed*

***Particular differences were established using pair-wise comparisons at the Bonferroni adjusted level of significance*

Unlike the differences observed in femoral sodium and chlorine content, no statistically significant variations were recorded for these elements in the tibiae. The chlorine content did, however, remain higher in omnivores compared to the other two groups. The zinc content in omnivores (0.480 ± 0.705%) was also higher than that of herbivores (0.222 ± 1.174%) and carnivores (0.354 ± 0.286%). Similar to what was the case in femora, high standard deviations for the lead content in tibiae were also observed and reflect the inconsistency of this element within bone. Silicon, aluminium and strontium remained the smallest contributors. Aluminium and strontium contributed 0.033 ± 0.059% and 0.020 ± 0.037% in herbivores, 0.070 ± 0.100% and 0.033 ± 0.045% in carnivores and 0.037 ± 0.058% and 0.039 ± 0.037% in omnivores. No mentionable observations were recorded for strontium other than its small contribution to all feeding groups. As expected, the calcium contribution exceeded that of all the other elements and was 72.346 ± 1.426% in herbivores, 72.666 ± 1.447% in carnivores and 71.822 ± 2.901% in omnivores. Unlike the calcium content in femora, these percentages were not significantly different between the feeding groups.

Statistically significant differences were observed in potassium, phosphorus, magnesium and sulphur. Again, histograms were used to assist in the visual assessment of the statistically significant elements and are shown in **Figures 4.6 – 4.9**.

Mean values (%) and the standard deviations for potassium can be seen in **Figure 4.6**. Similar to the femora, potassium was also significantly different between the feeding groups for tibiae. Statistically significant differences were indicated between omnivores and herbivores and between omnivores

and carnivores (p=0.0001). The average potassium component was 0.036 ± 0.031% in herbivores, 0.068 ± 0.071% in carnivores and 0.117 ± 0.061% in omnivores and is illustrated by the last bar in each series of the histogram. The highest potassium values were recorded in adult *Homo sapiens sapiens*. High potassium levels were also recorded in cat tibiae compared to the much smaller amount that was recorded in dog tibiae, contributing to the high amounts of variation found among the carnivores.

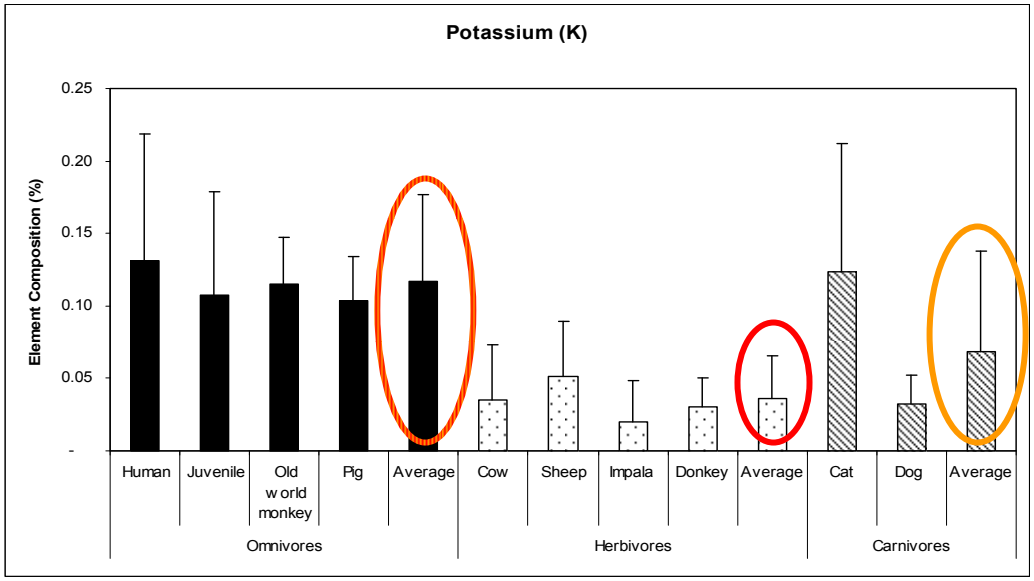


Figure 4.6 *The mean values (%) and standard deviations of potassium (K) in omnivore, herbivore and carnivore tibiae. Juvenile in this figure refers to subadult Homo sapiens sapiens.*

High phosphorus values have been recorded for impala tibiae and can be seen in the summary of the means (%) and standard deviations of phosphorus in **Figure 4.7**. Phosphorus percentages were consistent in carnivores, with little variation noted in the herbivore and omnivore groups. Phosphorus contributed 24.707 ± 1.894% in herbivores, 23.924 ± 1.870% in carnivores and 22.696 ± 2.205% in omnivores and statistically significant differences were illustrated between omnivores and herbivores (p=0.0150).

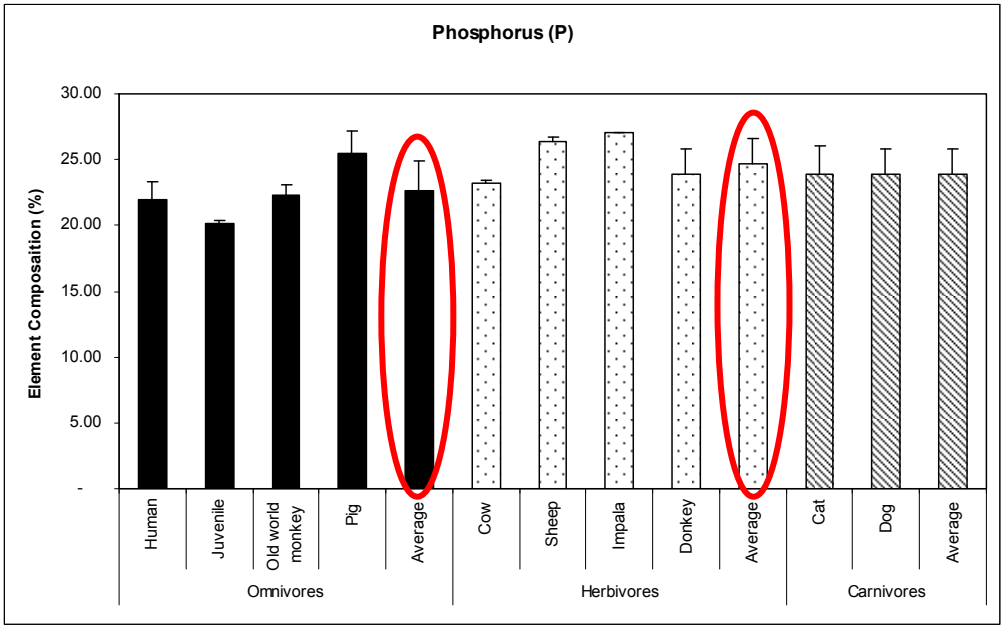


Figure 4.7 *The mean values (%) and standard deviations of phosphorus (P) in omnivore, herbivore and carnivore tibiae. Juvenile in this figure refers to subadult Homo sapiens sapiens.*

The histogram in **Figure 4.8** summarises the mean (%) and standard deviations for magnesium. This element also illustrated statistically significant differences in femora of herbivores and omnivores (p=0.0475). Magnesium contributed 0.369 ± 0.110% in herbivores, 0.284 ± 0.110% in carnivores and 0.267 ± 0.148% in omnivores with the highest value having been recorded in impala tibiae. Variation in and between the various groups are apparent with no clear pattern emerging.

Figure 4.9 illustrates the high variability of mean sulphur values, especially within herbivores. Sulphur contributed 0.130 ± 0.094% to herbivores, 0.214 ± 0.123% to carnivores and 0.303 ± 0.163% to omnivores with statistically significant differences observed between omnivores and herbivores (p=0.0011). The highest value recorded for sulphur was in human juvenile which was noticeably less than the value of adult humans. These differences

are probably best understood in relation to the age differences observed between the two groups.

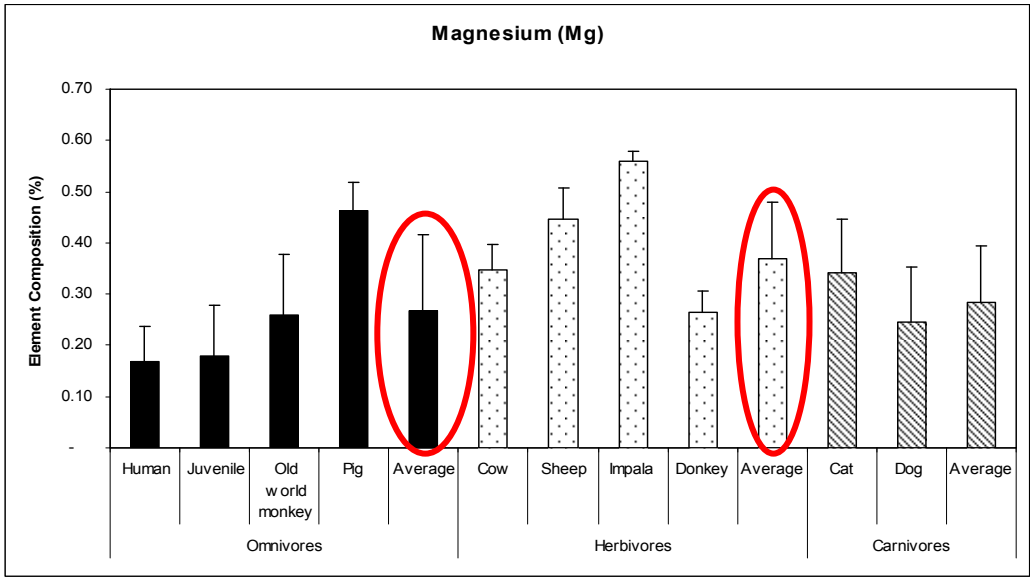


Figure 4.8 *The mean values (%) and standard deviations of magnesium (Mg) in omnivore, herbivore and carnivore tibiae. Juvenile in this figure refers to subadult Homo sapiens sapiens.*

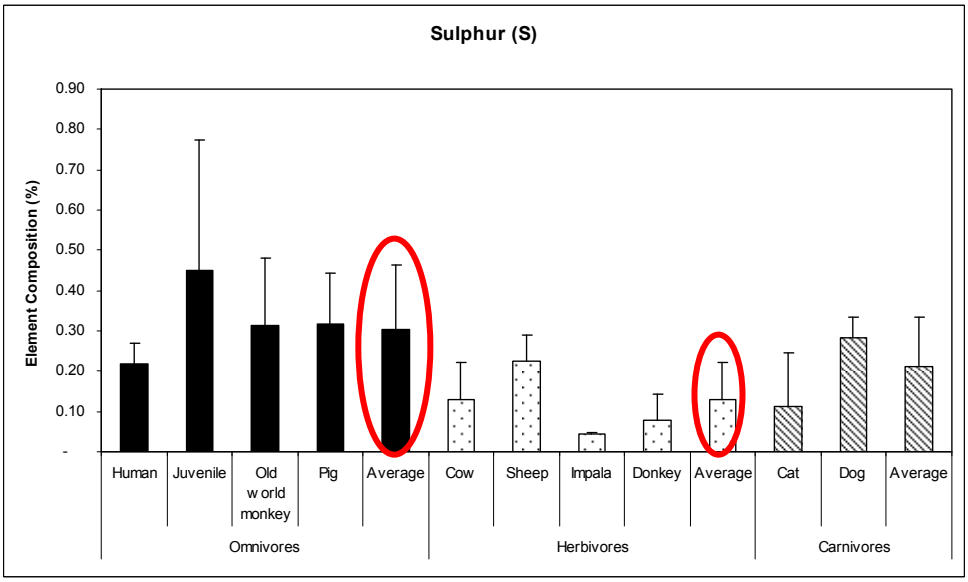


Figure 4.9 *The mean values (%) and standard deviations of sulphur (S) in omnivore, herbivore and carnivore tibiae. Juvenile in this figure refers to subadult Homo sapiens sapiens.*

4.1.2.3. Chemical composition of femora and tibiae pooled

Femora and tibiae were pooled together and descriptive statistics, also indicating degree of significance between the three feeding groups, are shown in **Table 4.13**.

The largest contributing elements to bone were calcium and phosphorus. The composition of these elements was similar amongst the three groups with the highest calcium percentage recorded in carnivores (72.21 ± 1.507%) and the lowest in omnivores (71.038 ± 2.032%). High phosphorus values were recorded in herbivores (24.596 ± 1.950%) with lower levels observed in carnivores (23.848 ± 2.034%).

In general, silicone, aluminium and strontium contributed only minimally to the chemical composition of long bones of the various feeding groups. Aluminium contributed least, followed by silicone. Strontium percentages decreased from omnivores (0.103 ± 0.250%), to herbivores (0.054 ± 0.791%) to carnivores (0.04 ± 0.069%). The zinc content was somewhat lower in herbivores (0.181 ± 0.158%) compared to that of carnivores (0.289 ± 0.205%) and omnivores (0.351 ± 0.505%).

High lead percentages were recorded in omnivores (3.100 ± 3.784%), followed by carnivores (2.517 ± 2.308%) and herbivores (1.912 ± 2.582%) and similar to what was the case for both femora and tibiae, large standard deviations (SD) were observed in the lead content of the bones of the various groups, indicating the high variability of this element within the particular feeding crowds.

Statistically significant differences were observed in potassium, sodium, magnesium, chlorine and sulphur levels. These differences were mainly

amongst omnivores and herbivores, with the exception of sodium that indicated significant differences between omnivores and carnivores. Potassium and chlorine were also significantly different between omnivores and carnivores. Histograms comparing the mean values (%) and standard deviations (SD) of the elements that indicated statistically significant differences are shown in **Figures 4.10 – 4.14**.

Figure 4.10 illustrates the mean values (%) and standard deviations of potassium. The average potassium percentages in omnivores were 0.118 ± 0.057%, 0.052 ± 0.035% in herbivores and 0.071 ± 0.062% in carnivores. As was the case for femora, a larger contribution of potassium is observed in omnivores compared to both herbivores and carnivores. This clearly illustrates differences in the feeding behaviour of the various groups. Potassium levels were significantly higher in omnivores compared to those of herbivores and carnivores ($p=0.0001$), with the highest values recorded in the nonhuman primates. Low potassium percentages were recorded for cow, donkeys and dogs with considerable variations noted between carnivores.

Sodium levels were significantly different between omnivores and carnivores ($p=0.0192$) (**Figure 4.11**). These levels were consistent between carnivores but varied considerably between omnivorous animals. Sodium contributed 0.481 ± 0.190% in omnivores, 0.423 ± 0.130% in herbivores and 0.322 ± 0.071% in carnivores. Some variation with regard to this element was also evident between the herbivorous animals, especially between human adults and juveniles. These dissimilarities might be linked to dietary differences.

Table 4.13 *Mean elemental composition (%), standard deviations (SD) and statistically significant differences of the various elements of long bones of herbivores, carnivores and omnivores.*

Elements	Pooled						One-way ANOVA*	
	Herbivores (n=37)		Carnivores (n=20)		Omnivores (n=49)		p-Value	Statistically significant differences** observed between:
	Mean	SD	Mean	SD	Mean	SD		
Ca	72.108	1.791	72.21	1.507	71.038	2.032	0.0788	-
P	24.596	1.950	23.848	2.034	23.926	2.418	0.5343	-
Pb	1.912	2.584	2.517	2.308	3.100	3.784	0.4495	-
Na	0.423	0.130	0.322	0.071	0.481	0.190	**0.0192**	Omnivores and carnivores
Mg	0.402	0.134	0.296	0.098	0.318	0.107	**0.0214**	Omnivores and herbivores
Zn	0.181	0.158	0.289	0.205	0.351	0.505	0.3407	-
S	0.132	0.086	0.208	0.107	0.260	0.150	**0.0012**	Omnivores and herbivores
Cl	0.101	0.032	0.117	0.044	0.22	0.129	**0.0001**	Omnivores and herbivores Omnivores and carnivores
Sr	0.054	0.791	0.04	0.069	0.103	0.250	0.8569	-
K	0.052	0.035	0.071	0.062	0.118	0.057	**0.0001**	Omnivores and herbivores Omnivores and carnivores
Si	0.028	0.038	0.049	0.043	0.032	0.026	0.2496	-
Al	0.023	0.030	0.044	0.048	0.052	0.058	0.2468	-

**If Bartlett's test for equal variances was significant, i.e. assumption of equal variances violated, the ANOVA for ranks was preformed*

***Particular differences were established using pair-wise comparisons at the Bonferroni adjusted level of significance*

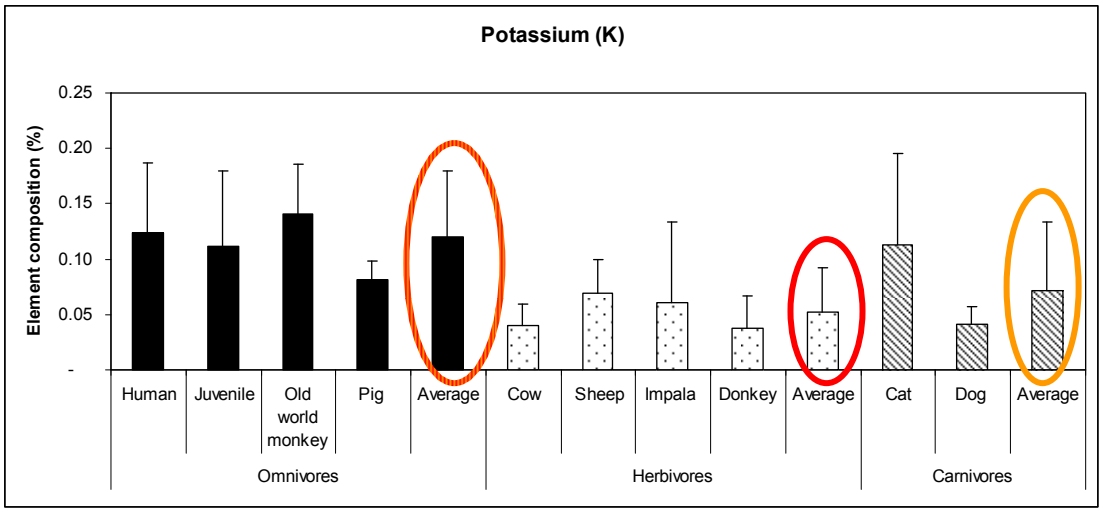


Figure 4.10 *The mean values (%) and standard deviations of potassium (K) in omnivore, herbivore and carnivore long bones. Juvenile in this figure refers to subadult Homo sapiens sapiens.*

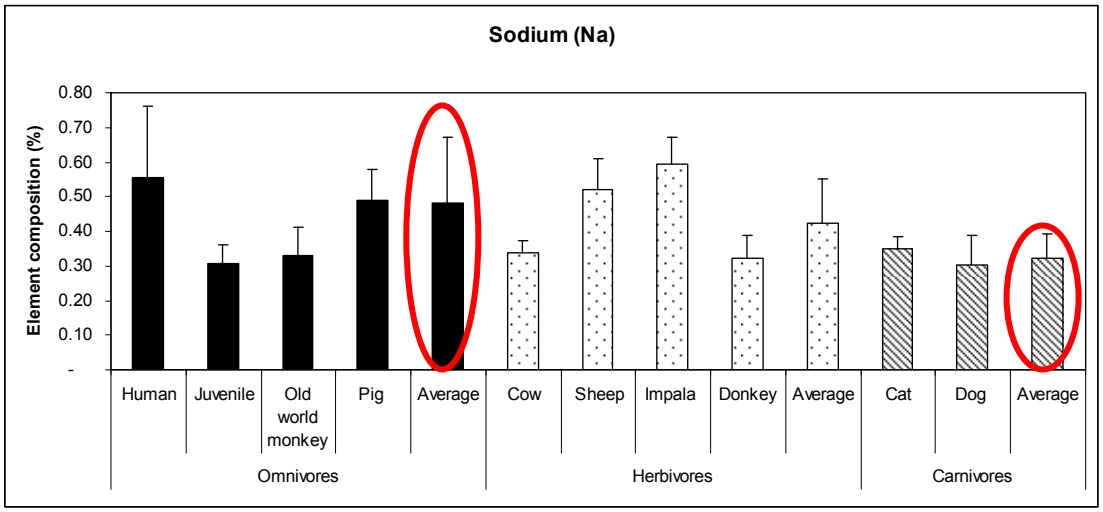


Figure 4.11 *The mean values (%) and standard deviations of sodium (Na) in omnivore, herbivore and carnivore long bones. Juvenile in this figure refers to subadult Homo sapiens sapiens.*

The contribution of magnesium to long bones is summarised in **Figure 4.12**. Variation concerning this element is clearly observed between all feeding groups. Omnivore bones consisted of 0.318 ± 0.107%, carnivores of 0.296 ± 0.098% and herbivores of 0.402 ± 0.134% magnesium with

statistically significant differences observed between omnivores and herbivores (p=0.0214).

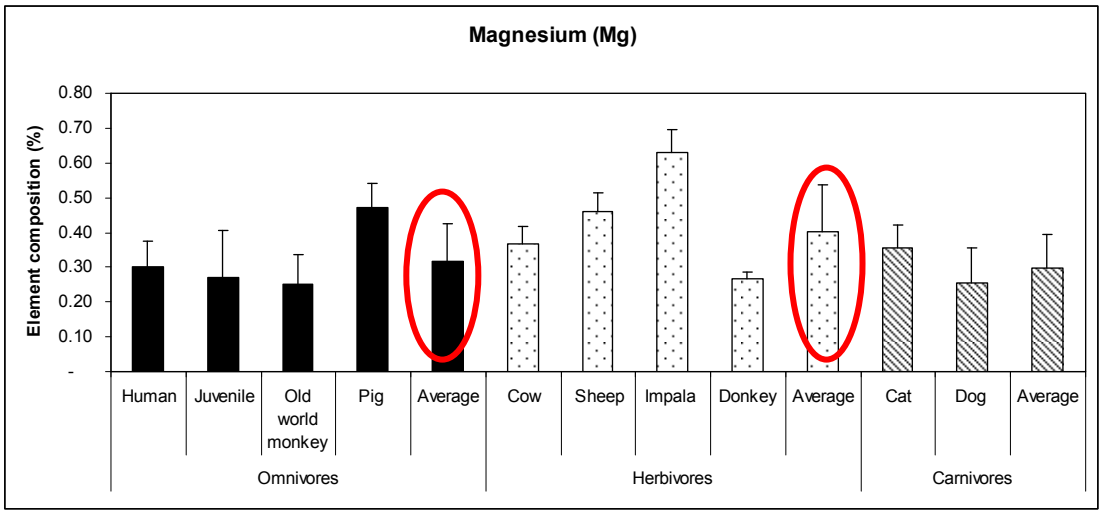


Figure 4.12 *The mean values (%) and standard deviations of magnesium (Mg) in omnivore, herbivore and carnivore long bones. Juvenile in this figure refers to subadult Homo sapiens sapiens.*

Chlorine percentages were significantly different between omnivores and herbivores and omnivores and carnivores (p=0.0001), as illustrated in **Figure 4.13**. Low chlorine contributions were recorded in herbivores and carnivores with varying values recorded for omnivorous animals. Omnivores contained 0.22 ± 0.129% chlorine, while carnivores and herbivores contained 0.117 ± 0.044% and 0.101 ± 0.032% respectively.

Sulphur values varied considerably between all feeding groups (**Figure 4.14**) with statistically significant differences recorded between omnivores and herbivores (p=0.0012). Sulphur contributed 0.260 ± 0.150% to omnivores, 0.208 ± 0.107% to carnivores and 0.132 ± 0.086% to herbivores. Low levels of sulphur were documented for impala (0.03 ± 0.02%) with high levels recorded for human juveniles (0.38 ± 0.26%). Similar to what was the case

for the sulphur content in tibiae, sulphur levels in human juveniles were noticeably higher than that of adult humans. These differences may also be related to age differences observed between the two groups.

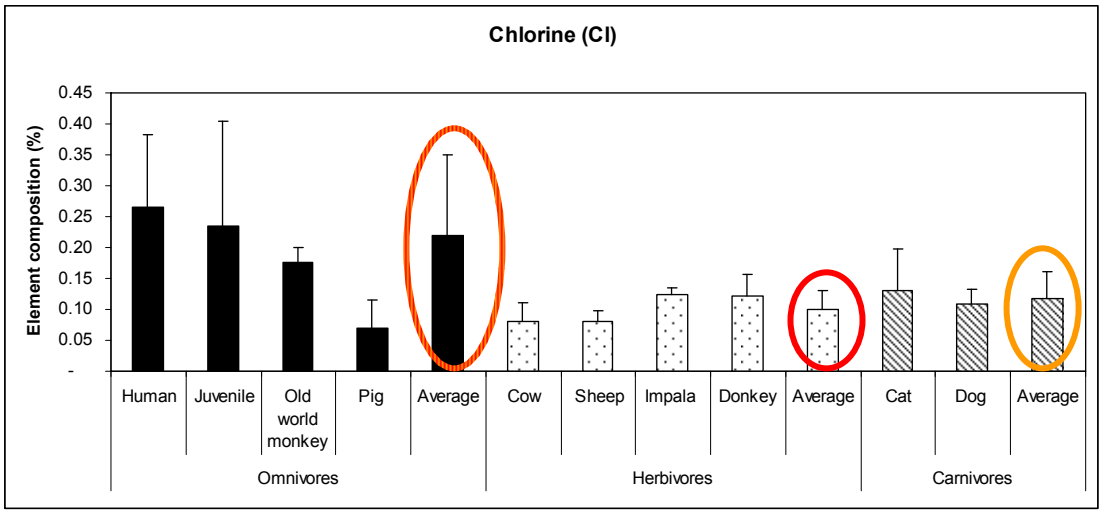


Figure 4.13 *The mean values (%) and standard deviations of chlorine (Cl) in omnivore, herbivore and carnivore long bones. Juvenile in this figure refers to subadult Homo sapiens sapiens.*

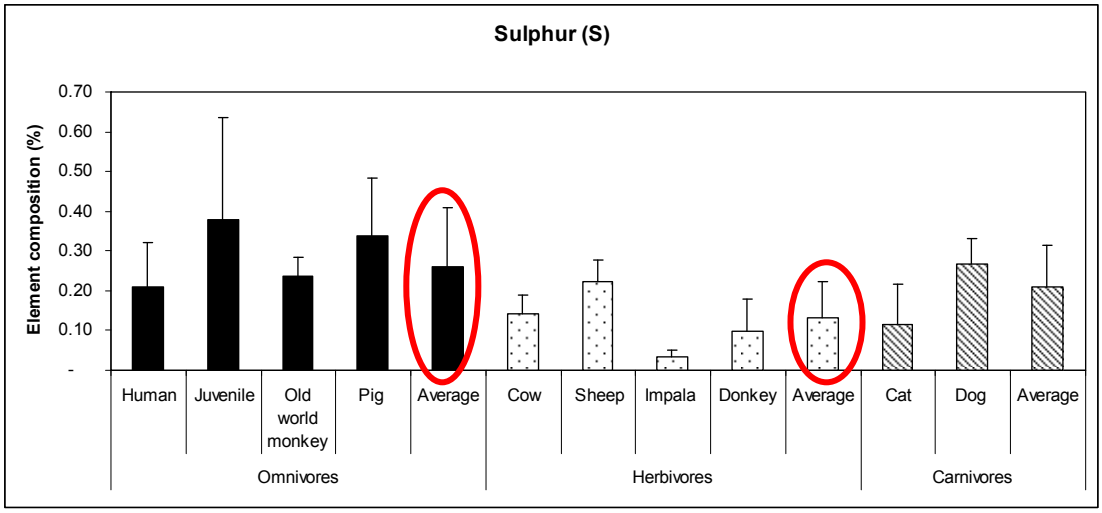


Figure 4.14 *The mean values (%) and standard deviations of sulphur (S) in omnivore, herbivore and carnivore long bones. Juvenile in this figure refers to subadult Homo sapiens sapiens.*

4.2. HISTOMORPHOMETRICAL ANALYSIS

Histological analysis was done with a Nikon transmission light microscope with a 10x objective and a 10x ocular lens, fitted with a digital camera. Three areas close at the periosteal surface of each bone sample were examined and a total of 318 digital photographs were taken. Qualitative and quantative analyses were performed on the microstructure of the compact bone of humans and animals. Qualitative examinations (histomorphoscopy) were performed to determine the presence of any morphological differences that could assist in the separation of human and non-human remains. Quantitative analysis was conducted to assess whether statistically significant differences existed between these groups which, in turn, could also be used for separating them.

4.2.1. Qualitative analysis

The morphological appearance of the anterior margin of the long bones was described separately for each group. The periosteal surface and its underlying bone was assessed because of the presence of plexiform bone, a type of primary vascular tissue, that has successfully been used to separate human and non-human remains (Hillier & Bell, 2007). Descriptions of the primary vascular bone, the Haversian bone and the non-vascular bone tissue were given for each species, as was described in Chapter 3 (Materials and Methods, section 3.2.3.2, p.81). This study did not elaborate on collagen orientation, but rather concentrated on vascular canal arrangements and osteon organisation.

Cat (Felix catus)

Femur

As illustrated by **Figure 4.15**, the bone near the outer periosteal surface of cat femora consisted of a combination of avascular bone and dense Haversian bone tissue. A few areas of primary vascular longitudinal bone tissue were also observed. Primary osteons that ran parallel to the long axis of the bone were characteristic of this tissue. These primary osteons were small, arranged in rows and orientated circularly around the medullary cavity. Towards the endosteal surface, Haversian bone tissue replaced the primary bone found in the periosteum. Small, oval secondary osteons, delineated by distinct cement lines, were organised in rows and were closely related to one another. Few interstitial lamellae were present between the neighbouring osteons. Small Haversian canals, surrounded by a few concentric lamellae, were either eccentrically or centrally located within the osteons. A few Volkmann's canals were observed within the bone tissue with no resorption spaces.

Tibia

A combination of avascular bone and dense Haversian bone tissue also characterised the tibiae of *Felix catus* (**Figure 4.16**). Few interstitial lamellae were observed between the tightly packed osteons within the dense Haversian bone tissue. The small to large osteons were arranged in rows and housed small to large Haversian canals. The Haversian canals were located in either eccentric or central positions and were encircled by a large number of concentric lamellae. Similar to cat femora, only a few Volkmann's canals were recorded.

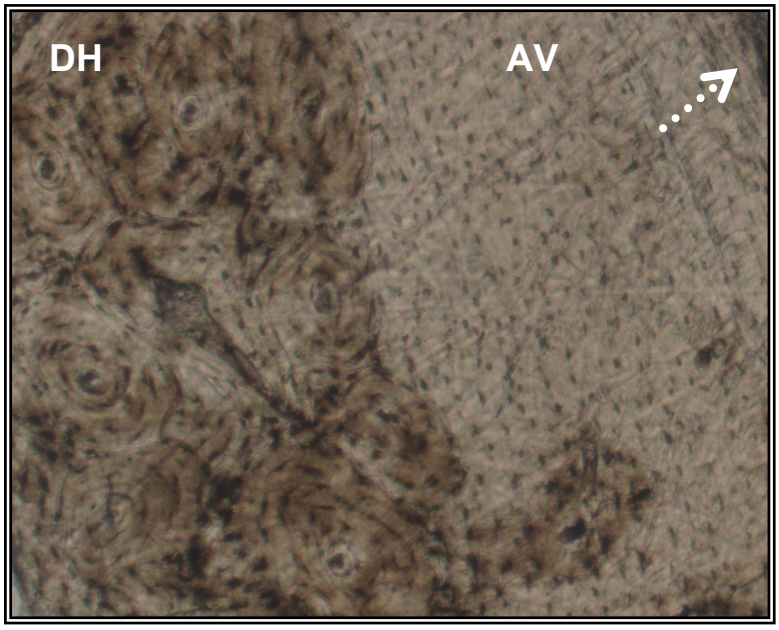


Figure 4.15 *Avascular (AV) bone with a combination with dense Haversian (DH) bone tissue as observed in cat (Felix catus) femora, viewed with normal bright light. White dotted arrow indicates the direction of the periosteal surface (Magnification x100).*

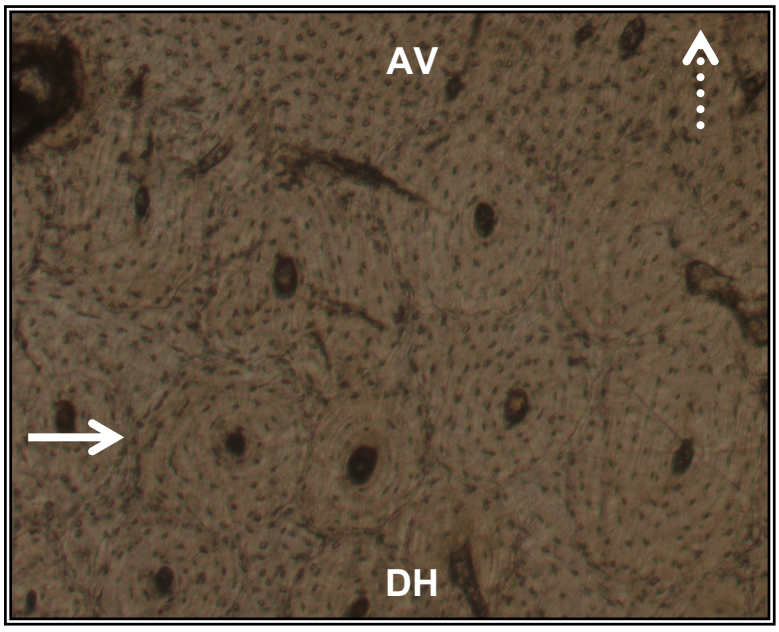


Figure 4.16 *Avascular (AV) bone in combination with dense Haversian (DH) bone tissue as observed in cat (Felix catus) tibiae, viewed with normal bright light. Note the linear organisation of the osteons as indicated by the white arrow. White dotted arrow indicates the direction of the periosteal surface (Magnification x100).*

Dog (Canis familiaris)

Femur

A combination of primary vascular laminar and irregular Haversian bone was observed in the femora of dogs. As seen in **Figure 4.17**, areas of avascular bone tissue were also observed. The primary vascular bone contained small primary vascular canals which were either longitudinally (parallel to the long axis of the bone) or circularly arranged. The circularly orientated vascular canals were arranged circularly around the medullary cavity. These canals were located between consecutive moderate sized laminae and were connected by a few radially orientated vascular canals. A small number of reticular canals were also noted. In the Haversian bone the osteon arrangement varied between scattered osteons, organised rows of osteons and densely packed osteons. Some interstitial lamellae filling the spaces between adjacent osteons were also present. Osteons were mostly small and oval in shape. A few concentric lamellae surrounded large, oval, centrally located Haversian canals.

Tibia

Dense Haversian bone was found in the tibiae of dogs. Medium sized osteons were closely packed and left little to no space for interstitial lamellae. The osteons were generally elliptical to round with a small amount of concentric lamellae surrounding a small to medium sized Haversian canal. The Haversian canals were centrally located and interconnected by a few Volkmann's canals. Irregular shaped resorption lacunae were documented and as illustrated in **Figure 4.18**, were similar in size to that of the osteons.

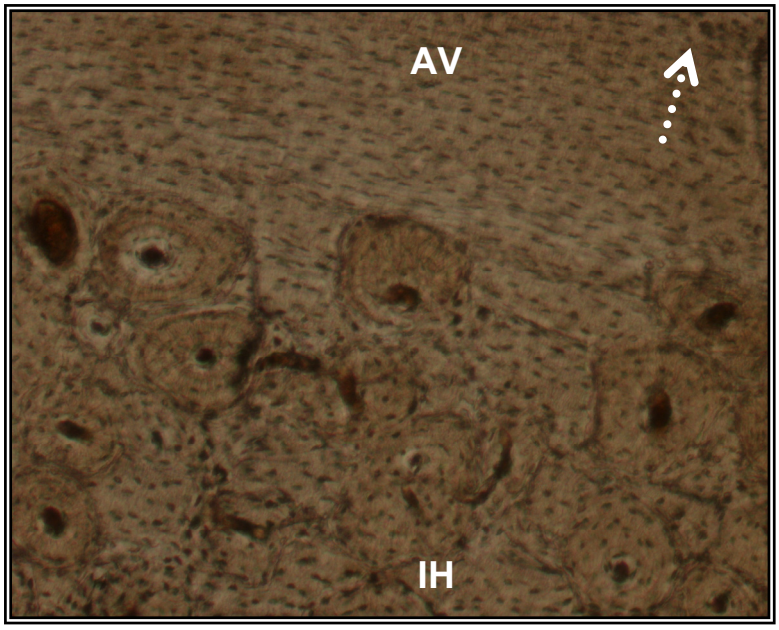


Figure 4.17 *Avascular bone (AV) in combination with irregular Haversian bone tissue (IH) as observed in dog (Canis familiaris) femora, viewed with normal bright light. White dotted arrow indicates the direction of the periosteal surface (Magnification x100).*

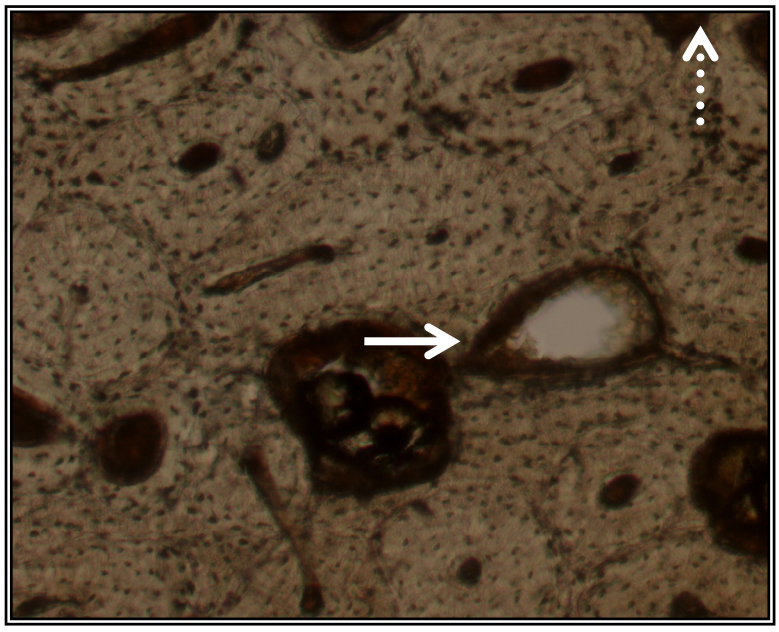


Figure 4.18 *Dense Haversian bone as observed in dog (Canis familiaris) tibiae, viewed with normal bright light. Note the irregularly shaped resorption lacuna, indicated by the white arrow, situated between tightly packed osteons. White dotted arrow indicates the direction of the periosteal surface (Magnification x100).*

Cow (Bos taurus)

Femur

Primary vascular plexiform bone was observed in the femora of cows. Broad sheets of bone were separated from one another by circularly orientated vascular canals (**Figure 4.19**). These canals were connected by a number of radially and/or reticular vascular canals. Situated next to the circularly arranged vascular canals were a number of longitudinally arranged vascular canals that ran parallel to the long axis of the bone. No primary or secondary osteons or resorption spaces were noted.

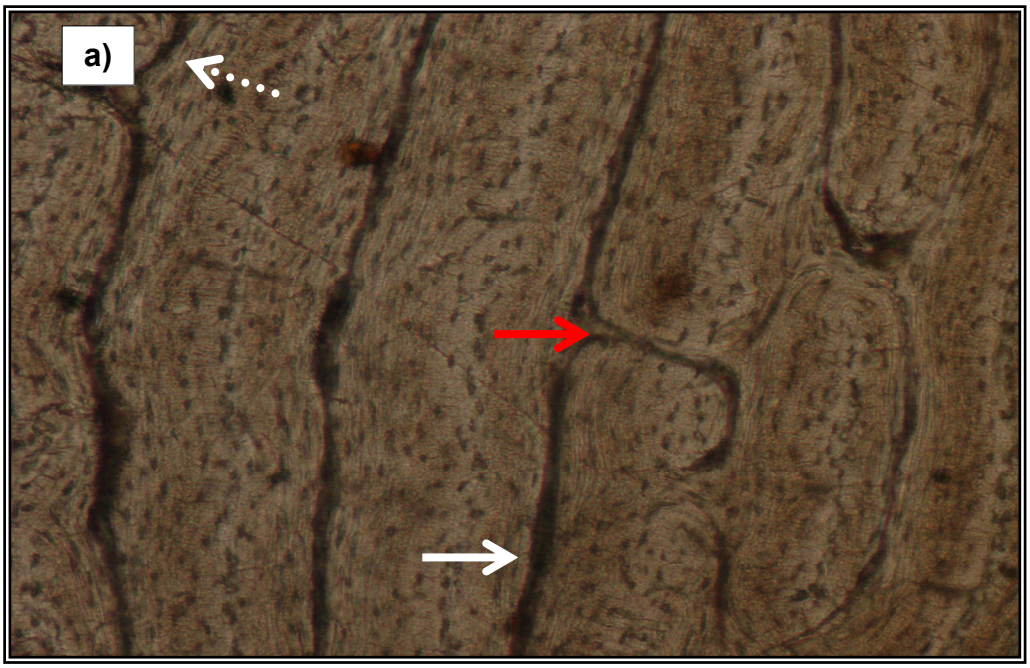
Tibia

Bos taurus tibiae consisted of a combination of primary vascular laminar and plexiform bone tissue (**Figure 4.20**). Broad laminae were separated by circular vascular canals, with a number of longitudinally orientated vascular canals scattered between these circular canals. These laminae surrounded the medullary cavity found within the centre of the diaphysis. A number of radially and/or reticular vascular canals were recorded, which connected successive circularly orientated vascular canals. Similar to cow femora, no osteons or resorption lacunae were found.

Sheep (Ovis aries)

Femur

Primary vascular longitudinal bone tissue was found in the femora of sheep. Longitudinally orientated primary osteons ran parallel to the long axis of bone and were arranged in circular rows around the medullary cavity within the bone shaft (**Figure 4.21**).



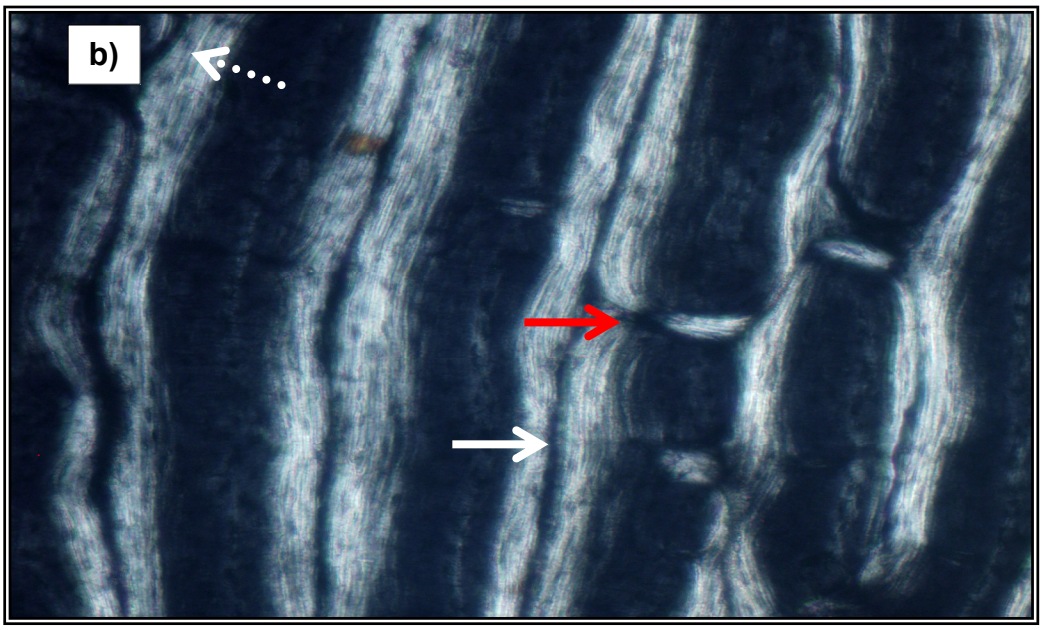


Figure 4.19 *Primary vascular plexiform bone as observed in cow (Bos taurus) femora, viewed with a) normal bright light and b) polarised light. White arrows indicate circumferentially orientated vascular canals and red arrows indicate radially orientated vascular canals. White dotted arrows indicate the direction of the periosteal surface (Magnification x100).*

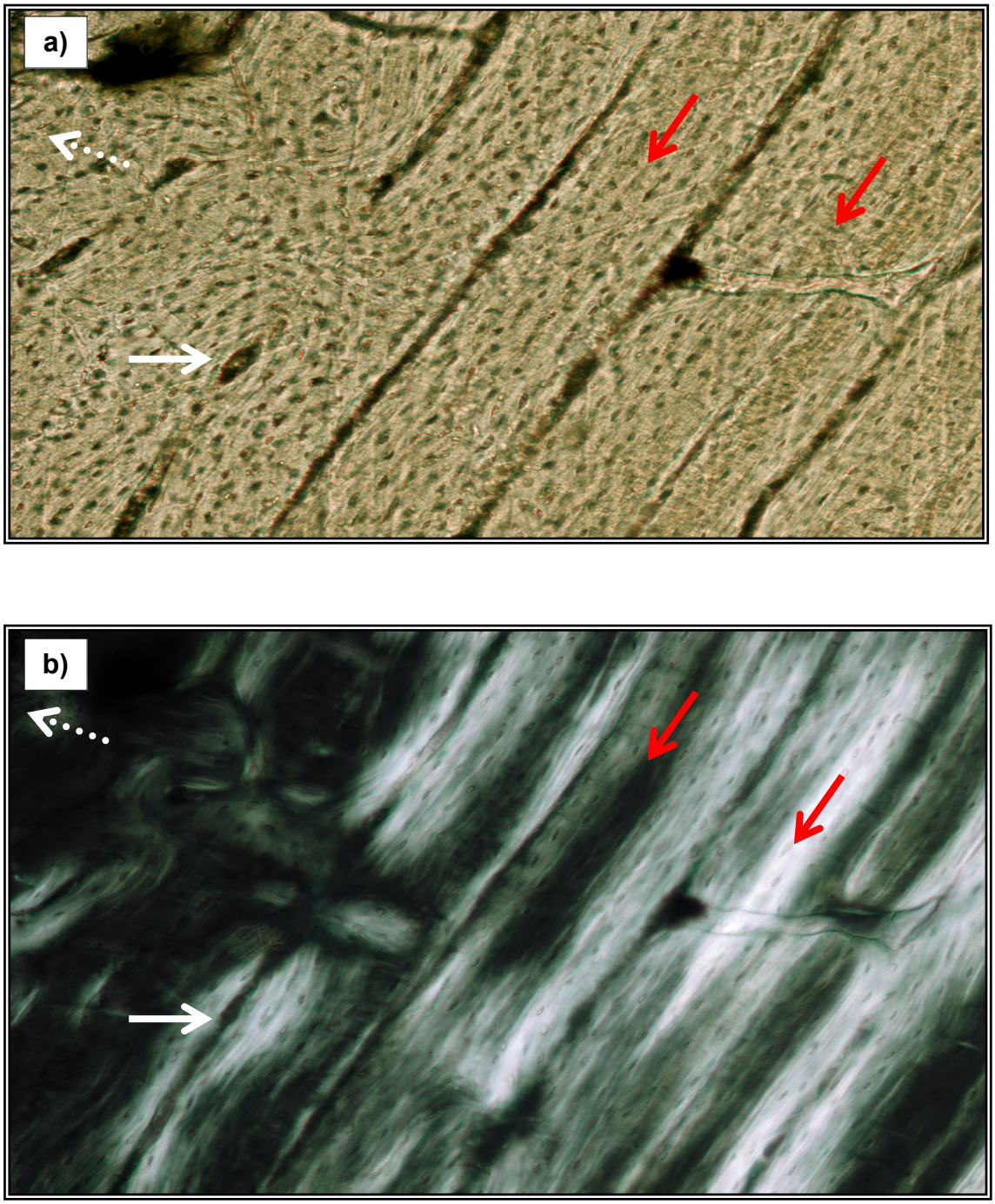


Figure 4.20 *Primary vascular laminar bone as observed in cow (Bos taurus) tibiae, viewed with a) normal bright light and b) polarised light. Red arrows indicate consecutive laminae separated by circularly orientated vascular canals. White arrows illustrate longitudinally orientated vascular canals. White dotted arrows indicate the direction of the periosteal surface (Magnification x100).*

These osteons were aligned in rows known as osteon bands. The exact number of primary osteons per band could not be established as the bands were cut off at the edge of the view field. The primary osteons were oval in shape and were relatively small. A few lamellae surrounded the vascular canals and a large number of short radially orientated vascular canals were also documented.

Tibia

Primary vascular plexiform as well as irregular Haversian bone was observed in the tibiae of sheep. The primary vascular plexiform bone consisted of a number of longitudinally and circularly arranged vascular canals. The circularly orientated vascular canals were located between successive broad bands of laminae. A number of radial canals were present, which connected the circularly orientated vascular canals. These connecting radial canals produced the distinct brick wall appearance commonly observed in plexiform bone.

As illustrated in **Figure 4.22**, irregular Haversian bone was characterised by a number of medium sized osteons that were scattered throughout the bone. These osteons were mainly round to oval in shape and contained large elliptical Haversian canals. These Haversian canals were positioned in the middle of the osteons and were connected to each other by a large number of Volkmann's canals that, in some instances, contributed to a bone tissue appearance similar to that seen in primary vascular reticular bone. A few irregular resorption spaces were also documented.

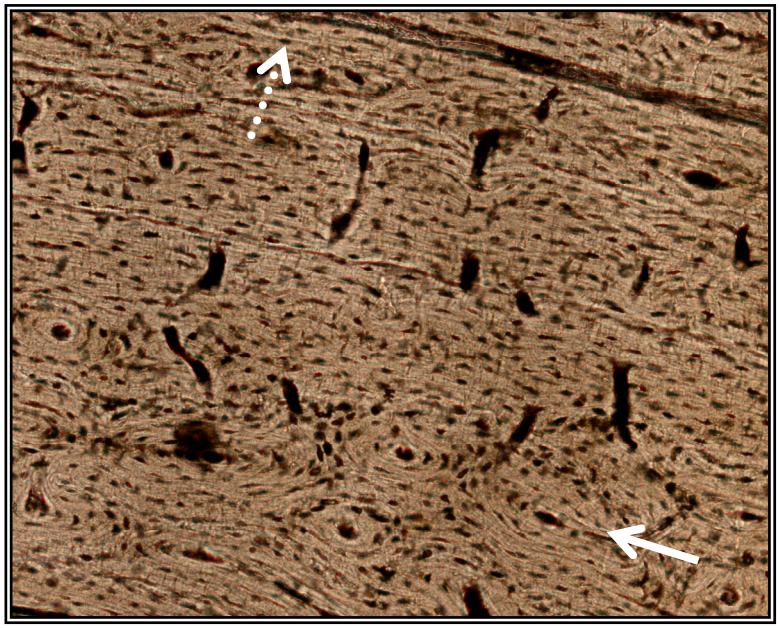


Figure 4.21 *Primary vascular longitudinal bone as observed in sheep (Ovis aries) femora, viewed with normal bright light. White arrow illustrates linear arrangement of primary osteons and white dotted arrow indicates the direction of the periosteal surface (Magnification x100).*

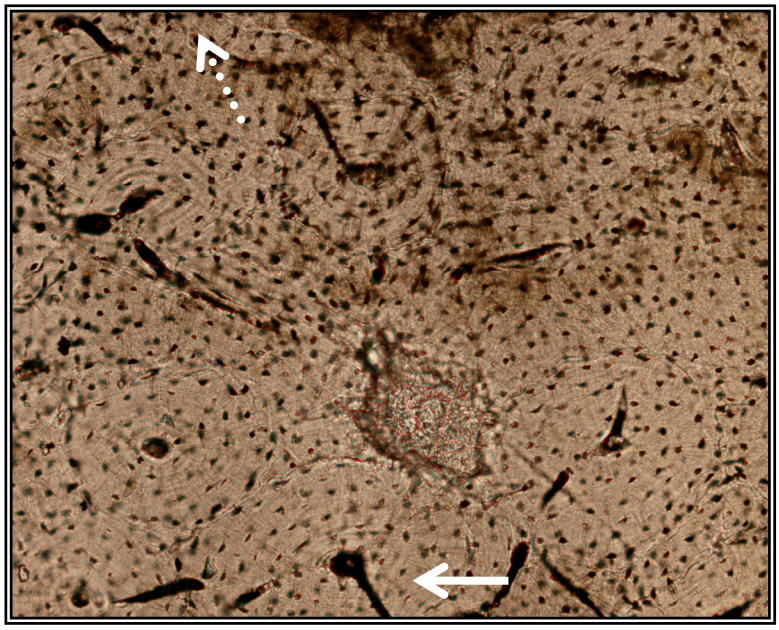


Figure 4.22 *Irregular Haversian bone as observed in sheep (Ovis aries) tibiae, viewed with normal bright light. Secondary osteons were mostly scattered. White arrow illustrates a radially orientated vascular canal or Volkmann's canal. White dotted arrow indicates the direction of the periosteal surface (Magnification x100).*

Donkey (Equus africanus asinus)

Femur

Figure 4.23 illustrates the combination of primary vascular longitudinal and irregular Haversian bone that was found in the femora of donkeys. Primary vascular laminar bone was also observed with laminae separated from one another by circularly arranged vascular canals that were connected by a few, short radially orientated vascular canals. The primary vascular longitudinal bone tissue consisted of a number of circularly arranged longitudinal vascular canals. These canals were small, oval and arranged in rows.

The irregular Haversian bone consisted of secondary osteons that followed a linear arrangement, similar to that of the longitudinal vascular canals. A few osteon bands were noted and were readily identified by the particular string-like appearance they create. Osteons were medium in size, oval in shape and contained centrally located Haversian canals. No resorption spaces were documented.

Tibia

No primary vascular canals were present in the tibiae of donkeys as illustrated in **Figure 4.24**; as the donkey tibiae consisted of dense Haversian bone tissue. Very little interstitial lamellae were recorded between neighbouring osteons. Osteons were large with small to medium sized centrally located Haversian canals. The osteons ran parallel to the long axis of the bone and their arrangements included closely packed rows or osteon bands. A number of short connecting Volkmann's canals were also recorded.

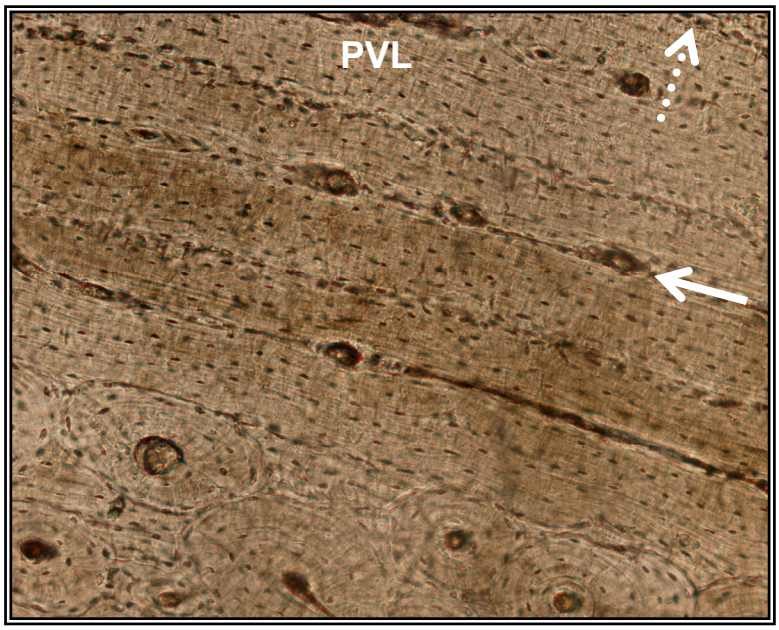


Figure 4.23 *Primary vascular longitudinal (PVL) bone in combination with irregular Haversian (IH) bone as observed in donkey (Equus africanus asinus) femora, viewed with normal bright light. White arrow indicates circularly arranged longitudinal vascular canals. White dotted white arrow indicates the direction of the periosteal surface (Magnification x100).*

Impala (Aepyceros melampus)

Femur

Few impala bones were available for histological analysis, thus making qualitative descriptions difficult. Primary vascular longitudinal bone was noted in impala femora. This bone tissue was characterised by longitudinally orientated primary osteons that ran parallel to the long axis of the bone. The primary osteons were circularly arranged around the medullary cavity and were linearly aligned as illustrated in **Figure 4.25**. These linearly arranged primary osteons bands were similar to those observed in sheep femora. Primary osteons were small to medium and oval in shape. A number of reticular vascular canals were also present and functioned to connect the various primary osteons with each other.

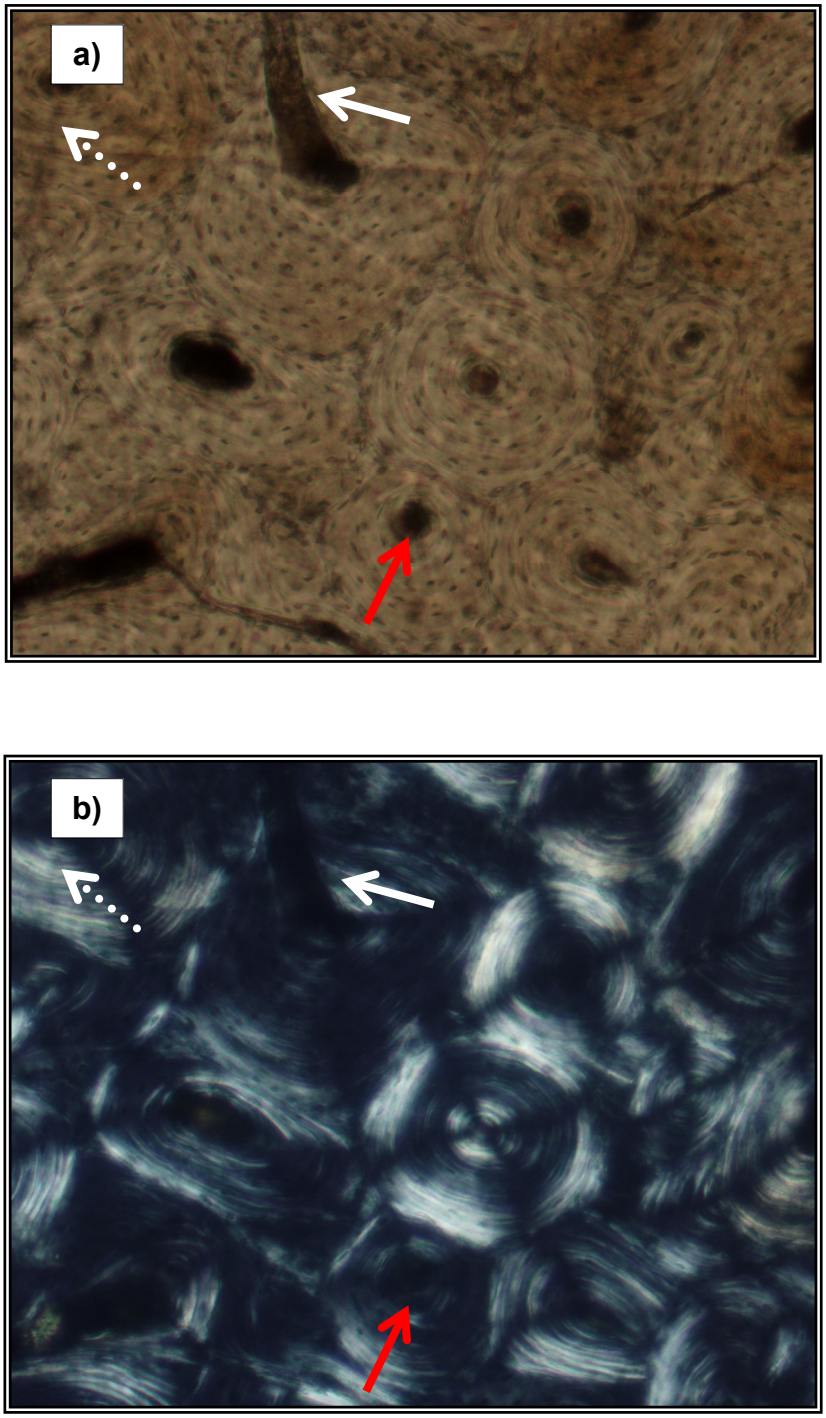


Figure 4.24 *Dense Haversian bone as observed in donkey (Equus africanus asinus) tibiae, viewed with a) normal bright light and b) polarised light. White arrows indicate radially orientated Volkmann's canal. Red arrows indicate linearly arranged, closely packed osteons. White dotted arrows indicate the direction of the periosteal surface (Magnification x100).*

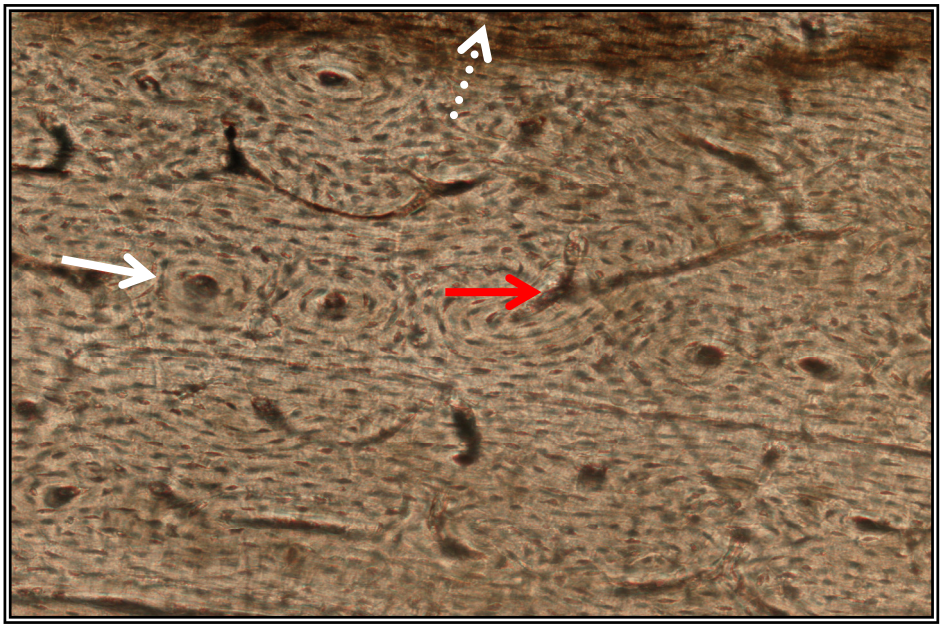


Figure 4.25 *Primary vascular longitudinal bone as observed in impala (Aepyceros melampus) femora, viewed with normal bright light. Red arrow indicates a reticular vascular canal. White arrow illustrates arrow of organised primary osteons or an osteon band. White dotted arrow indicates the direction of the periosteal surface (Magnification x100).*

Tibia

Bone tissue of impala tibiae consisted of either primary vascular longitudinal bone or dense Haversian bone tissue.

The primary vascular longitudinal bone was similar to that found in impala femora with circularly arranged primary osteons. The dense Haversian bone consisted of medium sized round osteons as seen in **Figure 4.26**. These osteons created osteons bands with little interstitial lamellae between adjacent osteons. The Haversian canals were small, round and located within the centre of the osteon. A large number of Volkmann's canals were present and contributed to an appearance of primary vascular reticular bone tissue.

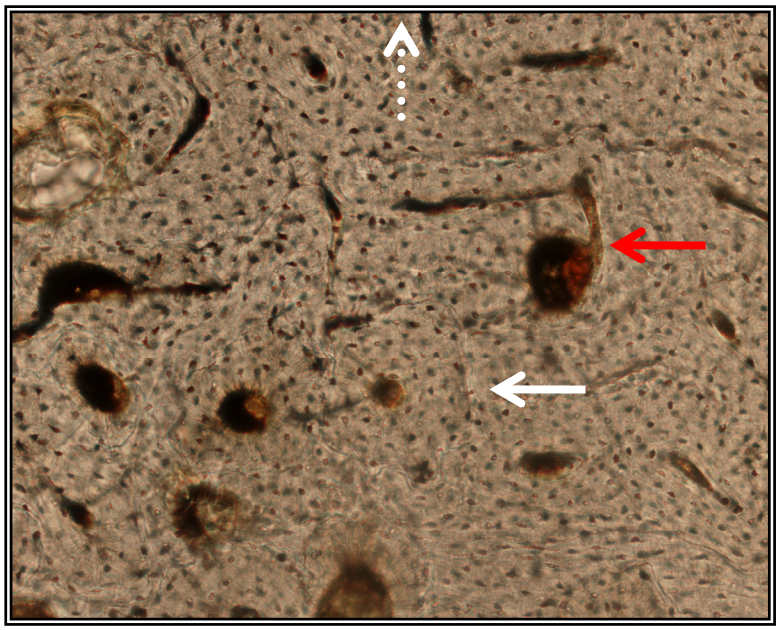


Figure 4.26 *Dense Haversian bone as observed in impala (Aepyceros melampus) tibiae, viewed with normal bright light. Red arrow indicates a Volkmann's canal. White arrow indicates tightly packed, linearly arranged osteons. White dotted arrow illustrates the direction of the periosteal surface (Magnification x100).*

Pig (Sus scrofa domestica)

Femur

All pig femora examined exhibited primary vascular plexiform bone (**Figure 4.27**). This bone tissue was characterised by moderate bands of bone or laminae separated from one another by circularly arranged vascular canals. These laminae surrounded the medullary cavity of the bone. A small number of longitudinally orientated vascular canals situated within the circularly orientated vascular canals were also documented. Radial and/or reticular vascular canals were present and connected successive circular canals. No osteons or resorption lacunae were observed in pig femora.

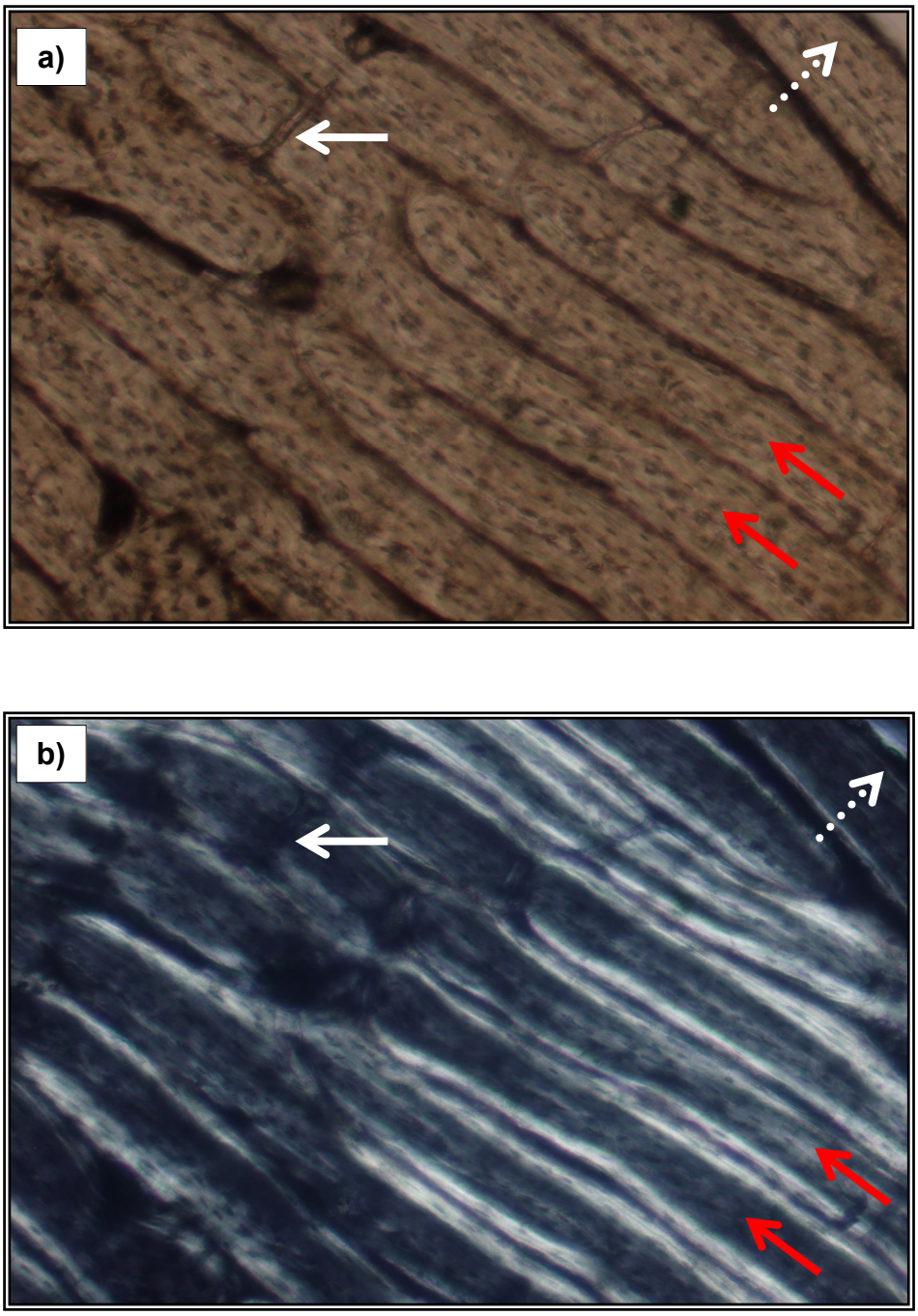


Figure 4.27 *Primary vascular plexiform bone as observed in pig (Sus scrofa domestica) femora, viewed with a) normal bright light and b) polarised light. Circumferentially orientated laminae are indicated by red arrows. Radially orientated vascular canals are pointed out by white arrows. White dotted arrows indicate the direction of the periosteal surface (Magnification x100).*

Tibia

Primary vascular plexiform bone tissue was also the dominant bone type observed in pig tibiae (**Figure 4.28**). Circularly orientated vascular canals were present between adjacent laminae. In the tibiae these laminae were broad and more radial and/or reticular vascular canals were present, connecting circular canals. Scattered within the circularly orientated vascular canals were a few small longitudinally arranged vascular canals. These canals ran parallel to the long axis of the bone and were also connected to one another by radial and/or reticular vascular canals. No osteons or resorption lacunae were noted in pig tibiae, similar to what was found in the femora.

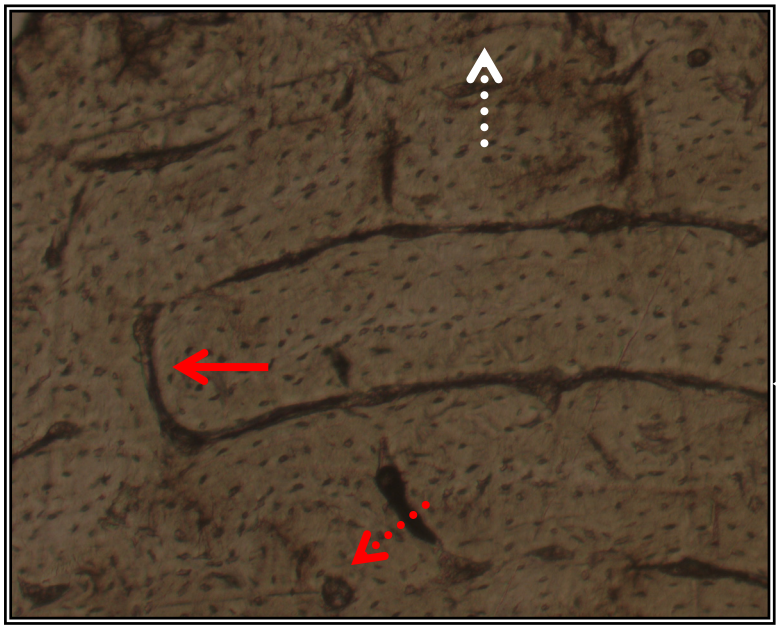


Figure 4.28 *Primary vascular plexiform bone as observed in pig (Sus scrofa domestica) tibiae, viewed with normal bright light. White arrow illustrates lamina located between two successive circularly arranged vascular canals. Red arrow points to a radially orientated vascular canal and red dotted arrow indicates a longitudinally orientated vascular canal. White dotted arrow indicates the direction of the periosteal surface (Magnification x100).*

Old world monkey (Ceropithecidae)

Femur

Old word monkey femora exhibited large areas of avascular bone (**Figure 4.29**). However, very few longitudinally arranged vascular canals were scattered throughout the tissue.

Irregular Haversian bone tissue was also observed and was characterised by scattered osteons. These osteons were mostly medium and in size and were separated from one another by large quantities of unremodelled bone tissue. The osteons were oval in shape and contain large, centrally located Haversian canals.

Tibia

Completely different to old world monkey femora which contained mostly avascular bone, old world monkey tibiae consisted of dense Haversian bone tissue as illustrated in **Figure 4.30**. The dense Haversian bone tissue were organised and characterised by linearly arranged, tightly packed secondary osteons. Few interstitial lamellae were found between the neighbouring osteons. These osteons were large, round to oval in shape and contained large, centrally located Haversian canals. A few Volkmann's canals and large resorption lacunae were also present. The difference between the femora and tibiae might be related to muscle attachments or differences in the loading stressors between the two bones.

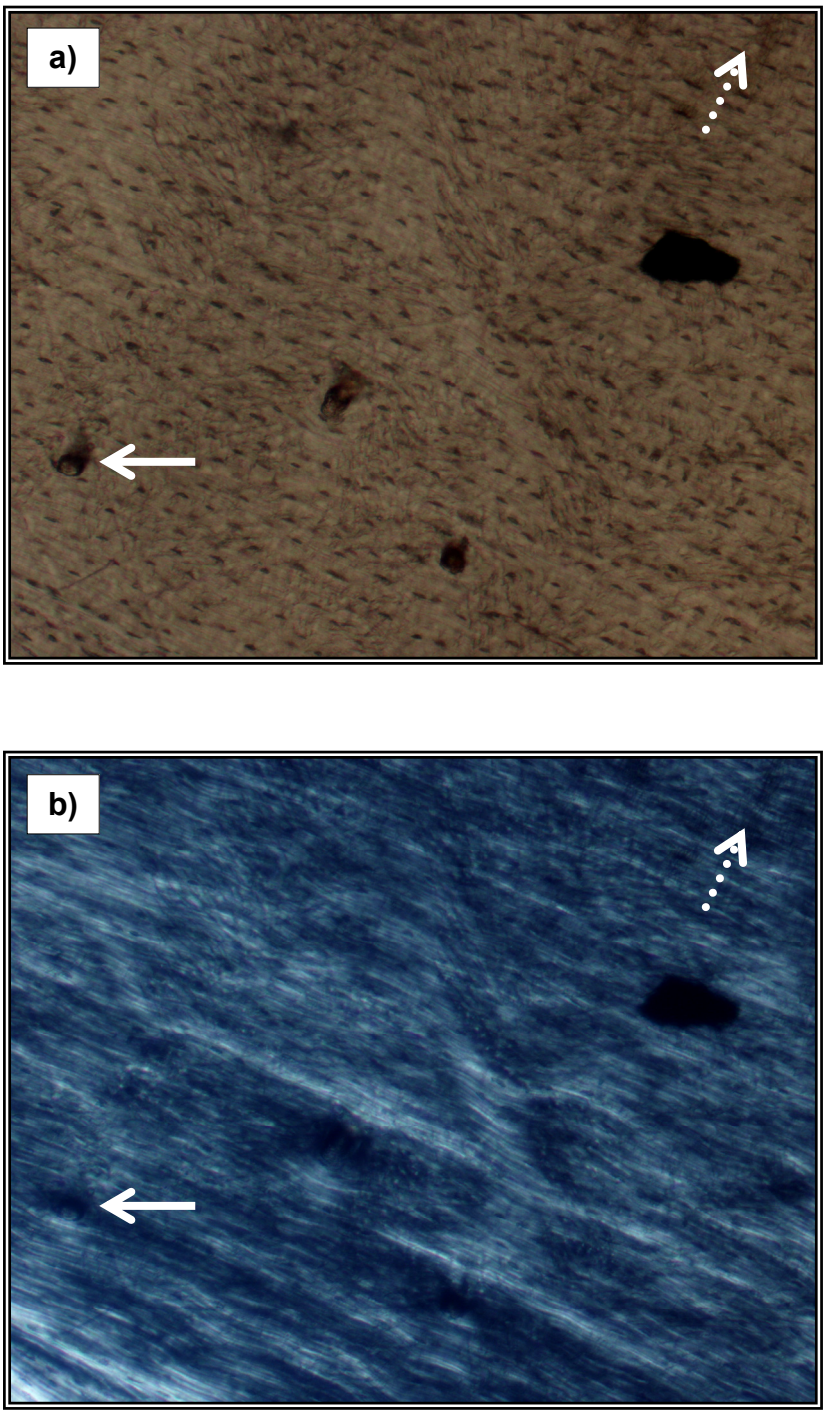


Figure 4.29 *Avascular bone with isolated, scattered vascular canals observed in old world monkey (Ceropithecidae) femora, viewed with a) normal bright light and b) polarised light. White arrows indicate longitudinal vascular canal. White dotted arrows indicate the direction of the periosteal surface (Magnification x100).*

Adult human (Homo sapiens sapiens)

Femur

The general microscopic appearance of adult human femora (**Figure 4.31**) varied between irregular and dense Haversian bone. However, a combination of primary vascular longitudinal bone and irregular Haversian bone tissue was commonly observed. Osteon arrangements subsequently varied from scattered to tightly packed osteons with the number of interstitial lamellae varying accordingly. The medium to large osteons were mainly round and contained centrally located Haversian canals, which were round and medium in size. Small numbers of thin periosteal circumferential lamellae were noted. These circumferential lamellae were occasionally interrupted with a scatter of secondary osteons. A few small, scattered vascular canals were recorded. These canals ran parallel to the long axis of the bone and were arranged in a circular pattern. A few short Volkmann's canals and resorption spaces were also observed.

Tibia

Large, round, tightly packed osteons were abundant throughout the tibiae of adult humans, as illustrated in **Figure 4.32**. This dense Haversian bone consisted of densely packed osteons that appeared to be linearly arranged with few interstitial lamellae present between the adjoining osteons. Medium sized Haversian canals were located in the middle of the osteons. Similar to adult femora, a few short Volkmann's canals and resorption spaces were noted. These resorption spaces were irregular in shape and relatively large. Thin bands of periosteal circumferential lamellae were observed but were not continuous, as they were often interrupted by a scatter of secondary osteons.

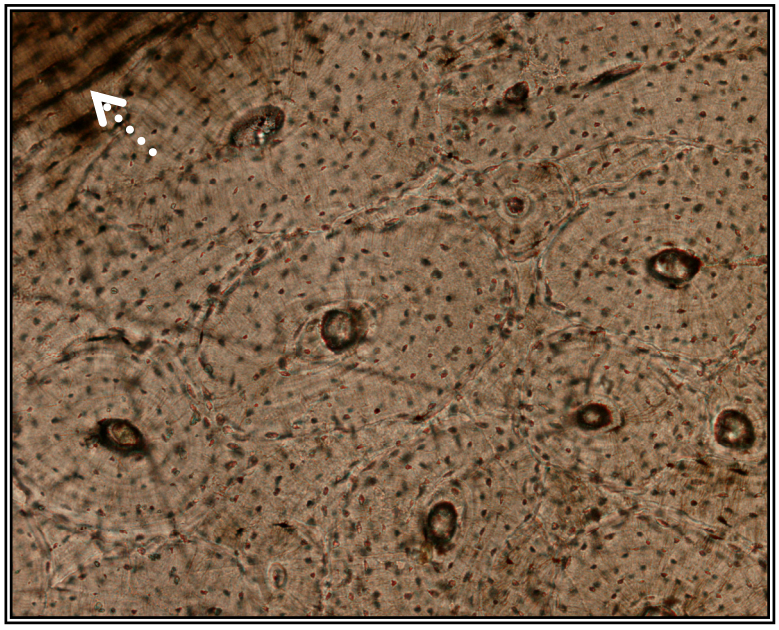


Figure 4.30 *Dense Haversian bone as observed in old world monkey (Ceropithecidae) tibiae, viewed with normal bright light. The osteons were closely related to one another and appeared to be arranged in rows. White dotted arrow indicates a number of circumferential lamellae located at the periosteal surface (Magnification x100).*

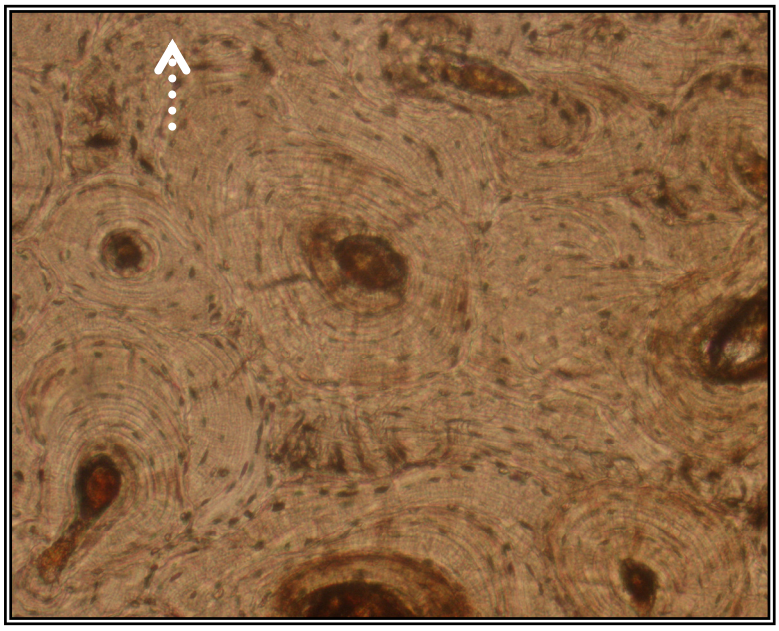


Figure 4.31 *Dense Haversian bone as observed in adult human (Homo sapiens sapiens) femora, viewed with normal bright light. Osteons were closely packed with a few interstitial lamellae in between. White dotted arrow indicates the direction of the periosteal surface (Magnification x100).*

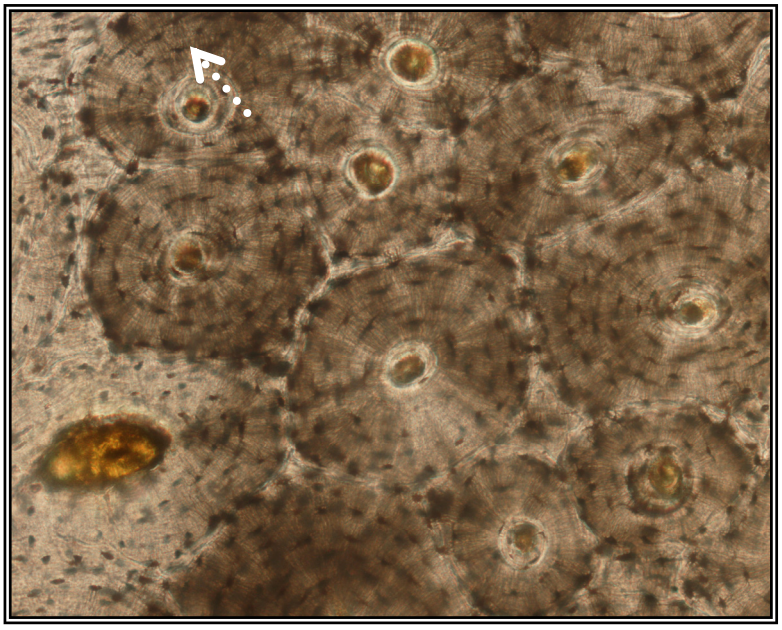


Figure 4.32 *Dense Haversian bone as observed in adult human (Homo sapiens sapiens) tibiae, viewed with normal bright light. Osteons were tightly packed and almost no interstitial lamellae were observed. White dotted arrow indicates the direction of the periosteal surface (Magnification x100).*

Juvenile (Homo sapiens sapiens)

Femur

Only a few juvenile specimens were available for histological analysis, making the qualitative bone descriptions difficult. Irregular Haversian bone tissue was most often observed (**Figure 4.33**). Isolated, scattered secondary osteons characterised this bone tissue. The osteons were oval in shape and ranged from small to large. Between neighbouring osteons, large amounts of unremodelled bone tissue were present. The Haversian canals were large, round and centrally located within the osteons. A few Volkmann's canals were observed together with large irregularly shaped resorption lacunae. A number of longitudinally arranged primary osteons were also recorded in some areas.

Tibia

Similar to juvenile femora, juvenile tibiae were characterised by irregular Haversian bone (**Figure 4.34**). Secondary osteons were separated from one another by large amounts of unremodelled bone. Large secondary osteons were mainly oval in shape and housed large Haversian canals. Connecting these canals with one another were a few short Volkmann's canals. In some bone sections, thin bands of periosteal circumferential lamellae containing a few scattered, longitudinally orientated primary osteons were observed. No resorption spaces were found.

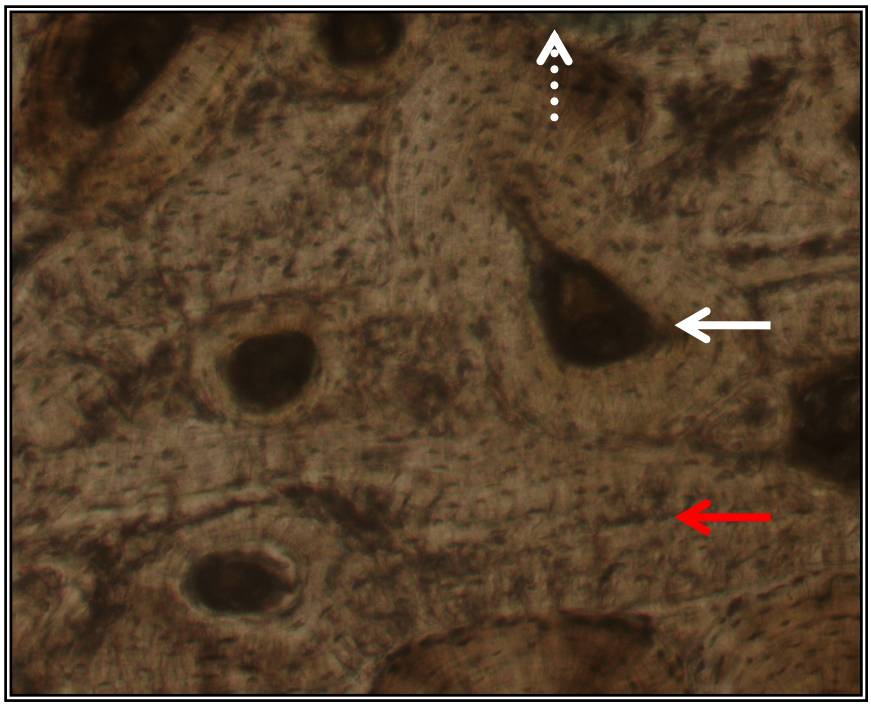


Figure 4.33 *Irregular Haversian bone as observed in juvenile (Homo sapiens sapiens) femora, viewed with normal bright light. Neighbouring osteons were separated from one another by areas of unremodelled bone indicated by red arrow. White arrow illustrates large Haversian canals housed within a drifting osteon. White dotted arrow indicates the direction of the periosteal surface (Magnification x100).*

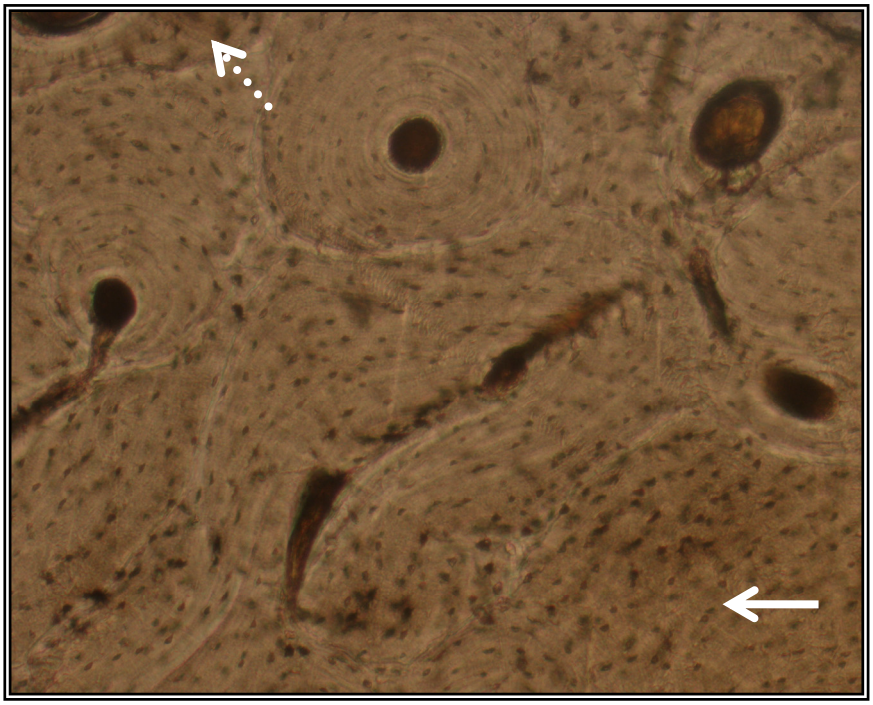


Figure 4.34 *Irregular Haversian bone as observed in juvenile (Homo sapiens sapiens) tibiae, viewed with normal bright light. Scattered osteons were separated by areas of unremodelled bone indicated by white arrow. White dotted arrow indicates the direction of the periosteal surface (Magnification x100).*

4.2.2. Quantitative analysis

The quantitative characteristics of human and non-human bones were also examined and compared. The total number of osteons and non-Haversian canals (primary vascular canals and primary osteons) was documented for the total observation area of 3.456 mm^2. This was done by counting these variables in the photographs taken of each species and then calculating an average. Minimum and maximum diameter (µm) measurements of osteons and Haversian canals were also carried out. These variables were particularly selected to ensure comparisons with other studies. The results for femoral and tibial measurements were reported separately as known histological differences exist between these bones (Enlow & Brown, 1958; Evans & Bang, 1966; Enlow, 1966; Pfeiffer *et al*., 1995; Pfeiffer, 1998).

Each of the parameters examined were compared between the species with a Kruskal-Wallis test. These results together with the means and standard deviations for each of the variables examined were summarised in **Table 4.14** for femora and **Table 4.15** for tibiae. To visually assist in the comparisons of these values between the different animals, bar charts (**Figure 4.35** and **Figure 4.39**) and stock charts were also included (**Figure 4.36-37** and **Figure 4.38-40**). Tables which present a summary of the histological measurements of the femora and tibiae of the different species are included in **Appendix C** (femora) (p.217) and **Appendix D** (tibiae) p.220).

A total of 1194 osteons was counted for all species of which only 238 were measurable (98 in femora and 139 in tibiae). Fewer osteons were measured, because the measurement criteria as described in Chapter 3 (Materials and methods, section 3.2.3, p.79) excluded many of them.

In **Table 4.14**, the means and standard deviations (SD) for the total number of non-Haversian canals and osteons for femora of the various species can be seen. Non-Haversian canals were found in all species; however, no secondary osteons were observed in cow, sheep, impala or pig femora. The largest average number of osteons recorded in femora was found in dogs (6.94 ± 4.92) and adult humans (4.32 ± 2.71) with only a few osteons counted in old world monkeys (1.00 ± 1.73) and cats (1.33 ± 2.50). Variations amongst the species are believed to be linked to different bone types (primary vascular, Haversian and avascular bone) present within the femora of these animals. Different arrangement patterns (irregular and dense Haversian bone) within these various types of bone may also be related to the dissimilarities between species. For example, dog has either irregular or

dense Haversian bone, whereas pig and cow only have plexiform bone and thus no secondary osteons.

On average, impala had the largest number of non-Haversian canals (6.78 ± 2.91), followed by human juveniles (5.44 ± 6.77). The least number of non-Haversian canals were observed in cows (0.47 ± 0.99) followed by pigs (1.07 ± 1.79). To visually assist in the comparisons of these values, a number of graphs were included (**Figure 4.35-40**).

Figure 4.35 clearly indicates the variation of the total number of osteons and non-Haversian canals amongst the femora of these animal species. The differences with regard to the number of non-Haversian canals and osteons between adults and juveniles (*Homo sapiens sapiens*) were also illustrated. A larger number of non-Haversian canals were documented in human juveniles, compared to the decreased number noted in adults. Also, more osteons were documented in human adults compared to juveniles. Such differences are to be expected and could be indicative of the differences in bone remodeling known to exist between the two *Homo sapiens sapiens* age groups. This bone remodeling is responsible for the transition of primary bone to secondary bone, commonly seen in adult humans.

The mean and standard deviations (SD) of the minimum and maximum diameters of osteons are also summarized in **Table 4.14**. The smallest osteon diameters (minimum diameter of osteon) were documented in dogs (192.77 ± 28.93 µm), followed by donkeys (201.33 ± 49.38 µm) with the largest osteon diameters (maximum diameter of osteon) recorded in adult humans (382.67 ± 120.27 µm) followed by old world monkeys (366.45 ± 61.00 µm).

Table 4.14 *Table indicating significant and non-significant differences observed within the femora for total number of osteons (Total_ost), total number of non-Haversian canals (Tot_vasc), minimum diameter of osteons (Min_ost), maximum diameter of osteons (Max_ost), average osteon size (Ave_ost), minimum diameter of Haversian canals (Min_can), maximum diameter of Haversian canals (Max_can) and average Haversian canal size (Ave_can).*

	Sample	Total_ost		Tot_vasc		Min_ost (µm)		Max_ost (µm)		Ave_ost (µm)		Min_can (µm)		Max_ can (µm)		Ave_can (µm)	
Sample	**size**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Cat	4	1.33	2.50	2.42	3.99	214.98	23.01	275.73	25.52	245.36	40.29	30.36	21.06	39.59	19.34	34.97	17.35
Dog	6	6.94	4.92	2.17	2.46	192.77	28.93	246.39	33.75	219.58	41.23	29.77	11.63	46.83	16.55	38.30	16.55
Cow	5	-	-	0.47	0.99	-	-	-	-	-	-	-	-	-	-	-	-
Sheep	5	-	-	4.64	2.90	-	-	-	-	-	-	-	-	-	-	-	-
Donkey	6	3.06	3.75	3.50	2.81	201.33	49.38	307.99	85.61	254.66	87.35	39.66	12.08	53.55	16.55	46.61	15.83
Impala	3	-	-	6.78	2.91	-	-	-	-	-	-	-	-	-	-	-	-
Pig	5	-	-	1.07	1.79	-	-	-	-	-	-	-	-	-	-	-	-
Monkey*	3	1.00	1.73	2.00	1.87	277.28	23.53	366.45	61.00	321.86	63.84	92.61	38.67	132.77	51.16	112.69	43.69
Juvenile**	3	2.56	3.09	5.44	6.77	290.73	58.67	365.32	81.97	328.02	78.33	82.55	29.55	110.23	26.37	96.39	30.36
Adult**	19	4.32	2.71	1.88	3.09	279.18	81.89	381.47	117.21	330.33	116.65	75.55	31.79	109.67	49.07	92.61	44.58
p-Value		**0.0001**		**0.021**		**0.02**		**0.044**				**0.002**		**0.002**			

* = *Old world monkeys*

** = *Homo sapiens sapiens*

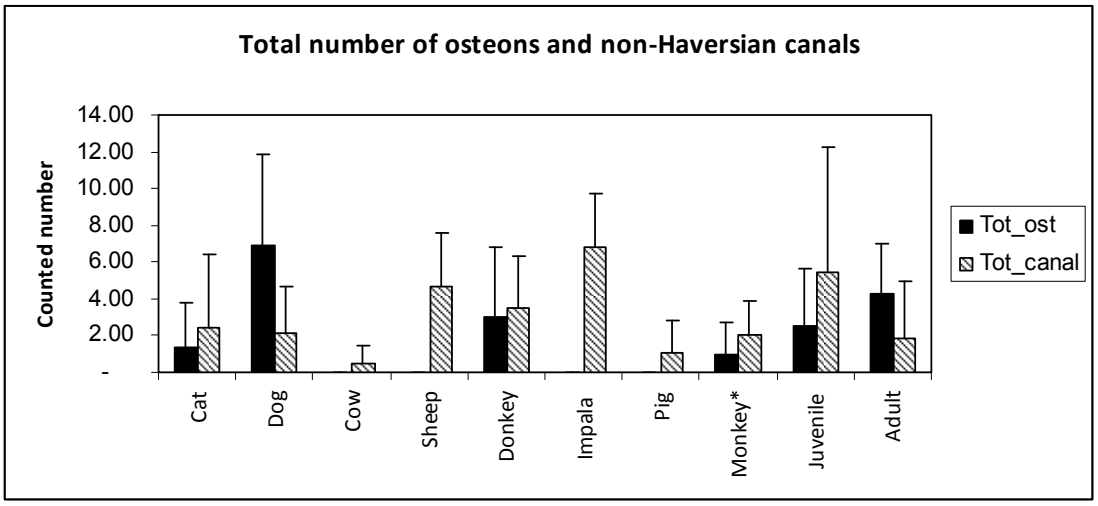


Figure 4.35 *Means and standard deviations of the total number of osteons and total number of non-Haversian canals observed in femora of various species. Juvenile and adult in this figure refers to Homo sapiens sapiens and monkey* refers to old world monkeys.*

Small diameters for the Haversian canals (minimum diameter of Haversian canals) were recorded in dogs (29.77 ± 11.63 µm) and cats (30.36 ± 21.06 µm) while the largest Haversian canal diameters (maximum diameter of Haversian canals) were noted in old world monkeys (132.77 ± 51.16 µm) and juveniles (110.23 ± 26.37 µm).

The minimum and maximum osteon and Haversian canal diameters were pooled together to establish overall osteon and Haversian canal sizes. Sizes are illustrated in **Figures 4.36** (osteon) and **Figure 4.37** (Haversian canal). Both these figures can be used to compare the overlap of osteon and Haversian canal values between species. Osteons and Haversian canals are larger in adults, juveniles and old world monkeys than those of cat, dog or donkey. The mean values for osteon and Haversian canal sizes for humans (adults and juveniles) and monkeys are also in close proximity to one another, whereas cat, dog and donkey values appear to be more variable in mean

size. However, the largest variation in mean osteon and Haversian canal sizes were found in adult humans which overlaps almost completely with all other species.

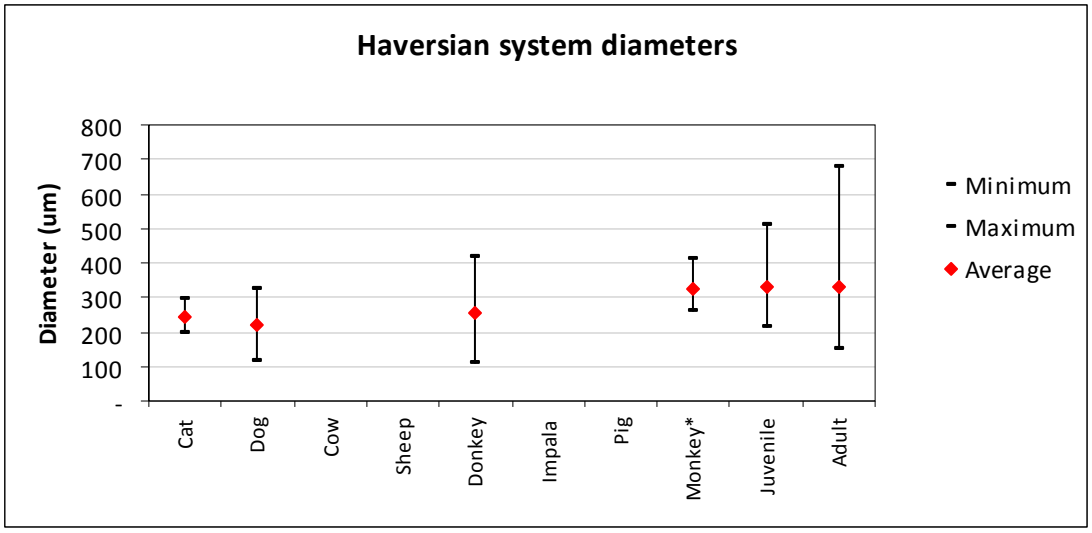


Figure 4.36 *Minimum (µm), maximum (µm) and mean (µm) values for femoral Haversian system diameters in human and non-human samples. Juvenile and adult in this figure refers to Homo sapiens sapiens and monkey* refers to old world monkeys.*

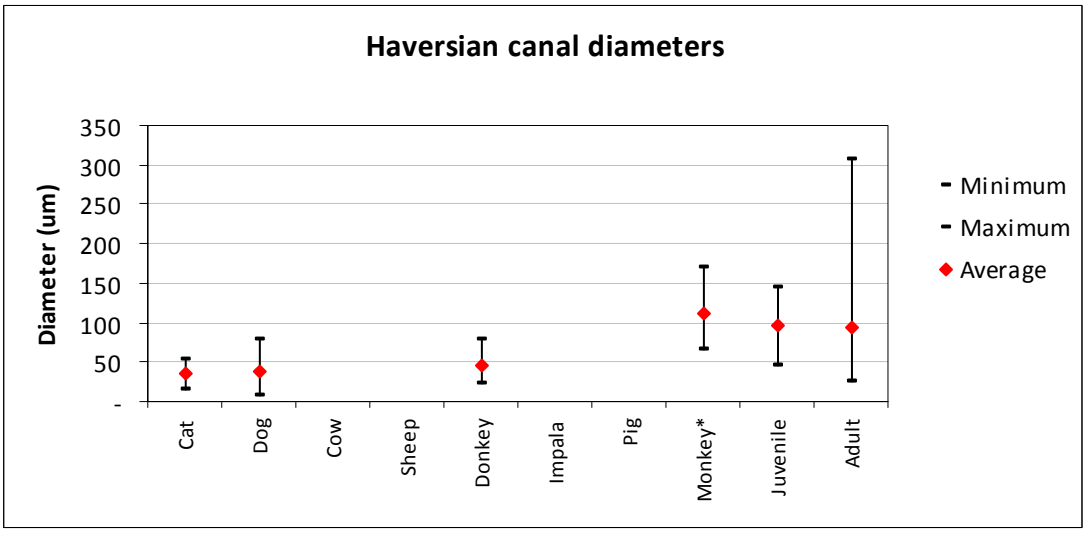


Figure 4.37 *Minimum (µm), maximum (µm) and mean (µm) values for femoral Haversian canal diameters in human and non-human samples. Juvenile and adult in this figure refers to Homo sapiens sapiens and monkey* refers to old world monkeys.*

In **Table 4.15** the means and standard deviations (SD) for the total number of osteons and non-Haversian canals and the diameter measurements of osteons and Haversian canals for the tibiae of the various species can be observed.

No osteons were recorded in cow and pig tibiae, while no non-Haversian canals were documented in cat, dog, donkey, old world monkeys and adults humans. Only a few osteons were observed in juveniles (2.78 ± 1.86) while the largest number of osteons were recorded in dogs (9.94 ± 2.21), followed by donkeys (9.17 ± 2.75). Non-Haversian canals were not recorded in human adults, with only a few observed in juveniles. This may be related to the age differences between the two *Homo sapiens sapiens* groups and to the transition between immature and mature bone. The average number of non-Haversian canals in juveniles (0.22 ± 0.67) and cows (0.87 ± 1.13) were very small while the largest number of non-Haversian canals was found in impala (6.17 ± 7.36).

In **Figure 4.38**, a visual comparison can be made between the differences observed in each of these animals. Interestingly, impala tibiae consisted of more non-Haversian canals than osteons. Similar to what was the case for femora, this figure demonstrates differences between the various animals that may be associated with the different bone types present in each animal. Variations may also be related to differences in muscle attachment and loading of the bones of the various animals. Differences between the femora and tibiae were also noted and could be indicative of variations related to the different bone types present within each of the examined bones.

Table 4.15 *Table indicating significant and non-significant differences observed within the tibiae for total number of osteons (Total_ost), total number of non-Haversian canals (Tot_vasc), minimum diameter of osteons (Min_ost), maximum diameter of osteons (Max_ost), average osteon size (Ave_ost), minimum diameter of Haversian canals (Min_can), maximum diameter of Haversian canals (Max_can) and average Haversian canal size (Ave_can).*

	Sample	Total_ost		Tot_vasc		Min_ost (µm)		Max_ost (µm)		Ave_ost (µm)		Min_can (µm)		Max_ can (µm)		Ave_can (µm)	
Sample	**size**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Cat	4	7.25	3.74	0.00	0.00	223.36	55.43	301.91	74.55	262.64	76.17	40.65	35.75	59.87	38.84	50.26	38.20
Dog	6	9.94	2.21	0.00	0.00	194.82	50.91	269.13	92.53	231.98	82.94	30.12	13.67	46.52	18.02	38.32	17.87
Cow	5	-	-	0.87	1.13	-	-	-	-	-	-	-	-	-	-	-	-
Sheep	5	3.47	3.20	1.27	2.89	225.82	76.38	281.27	97.97	253.55	90.59	35.52	14.49	62.30	29.69	48.91	26.65
Donkey	6	9.17	2.75	0.00	0.00	257.11	57.00	324.77	69.68	290.94	71.71	40.69	12.76	58.94	19.00	49.81	18.49
Impala	2	4.33	6.41	6.17	7.36	212.20	44.90	275.82	48.86	244.01	54.55	42.52	23.14	56.29	24.30	49.41	22.52
Pig	5	-	-	1.53	1.46	-	-	-	-	-	-	-	-	-	-	-	-
Monkey*	4	4.83	3.49	0.00	0.00	245.94	63.49	354.54	124.04	300.24	111.19	62.89	27.69	88.96	40.78	75.92	36.60
Juvenile	3	2.78	1.86	0.22	0.67	309.47	35.31	440.42	19.91	374.94	76.17	99.35	8.02	150.10	8.92	124.73	28.81
Adult	7	6.10	2.19	0.00	0.00	298.18	79.82	389.50	102.43	343.84	102.01	59.87	24.80	83.00	38.67	71.44	34.24
p-Value		**0.0001**		**0.001**		**0.016**		**0.029**				**0.71**		**0.095**			

* = *Old world monkeys*

** = *Homo sapiens sapiens*

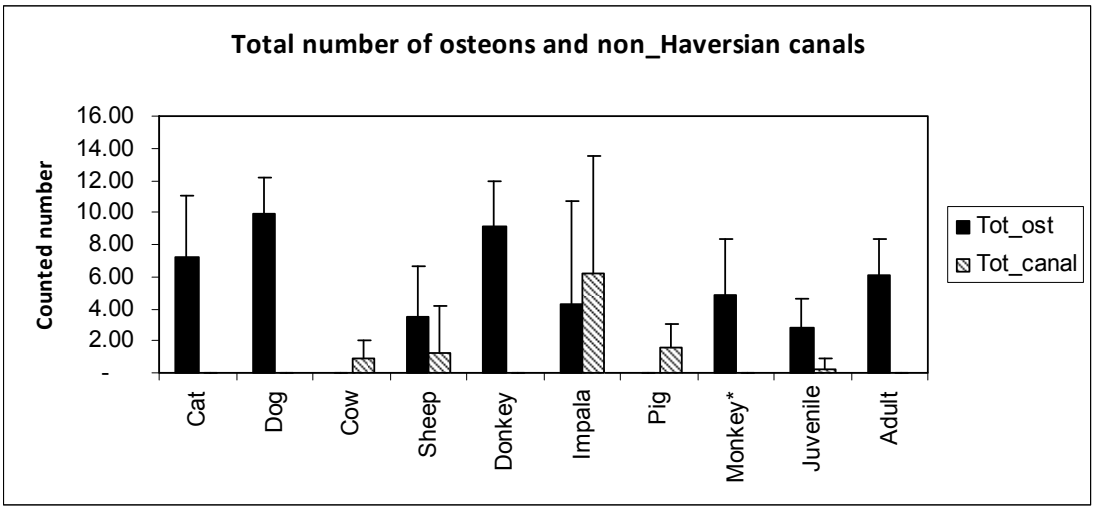


Figure 4.38 *Means and standard deviations of the total number of osteons and total number of non-Haversian canals observed in tibiae of various species. Juvenile and adult in this figure refers to Homo sapiens sapiens and monkey* refers to old world monkeys.*

The means and standard deviations (SD) of the minimum and maximum diameters of osteons and Haversian canals are also summarized in **Table 4.15**. The smallest minimum osteon diameters were documented in dogs (194.82 ± 50.91 µm), followed by impala (212.20 ± 44.90 µm). The greatest maximum osteon diameters were recorded in human juveniles (440.42 ± 19.91 µm) followed by human adults (389.50 ± 102.43 µm).

Not only did dogs have the smallest maximum diameter for secondary osteons, they also had the smallest minimum diameter of the Haversian canal (30.12 ± 13.67 µm). The maximum Haversian canal diameters recorded in human juveniles (150.10 ± 8.92 µm) and adults (83.00 ± 38.67 µm) were large.

Similar to femora, mean osteon and Haversian canal sizes were calculated and were plotted on stock charts to allow for comparisons between the species.

Osteon diameters (**Figure 4.39**) measured in tibiae illustrated wide ranges with closely related averages for cats, dogs, sheep, donkey, impala and old world monkeys. Average measurements for human adults and juveniles were larger, with the largest averages having been recorded in the juveniles.

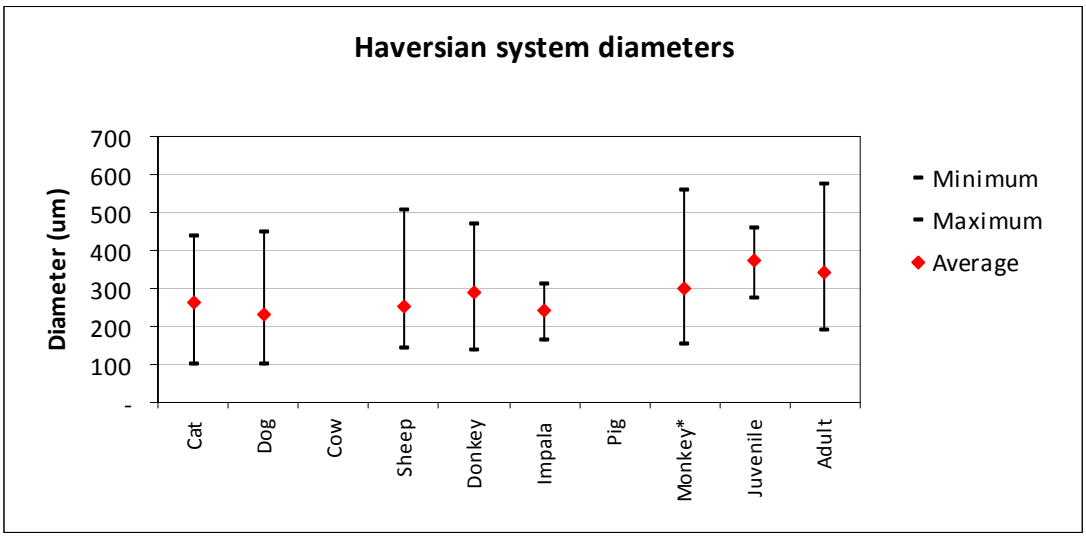


Figure 4.39 *Minimum (µm), maximum (µm) and mean (µm) values for tibial Haversian system diameters in human and non-human samples. Juvenile and adult in this figure refers to Homo sapiens sapiens and monkey* refers to old world monkeys.*

Similar to the Haversian canal diameters in femora, cat, dog, sheep, donkey and impala averages were below the large averages reported for the primates (**Figure 4.40**). As observed in Figure 4.37, old world monkeys had the largest average femoral Haversian canal diameter, while in the tibiae human juveniles had the largest Haversian canal diameter. As was the case with femoral osteon and Haversian canal measurements, all the measured values overlapped between the species. Although the average values in many instances are distinct from each other, the degree of overlap makes

accurate and “clear-cut” decisions regarding the origin of remains complicated especially if these measurements are used alone.

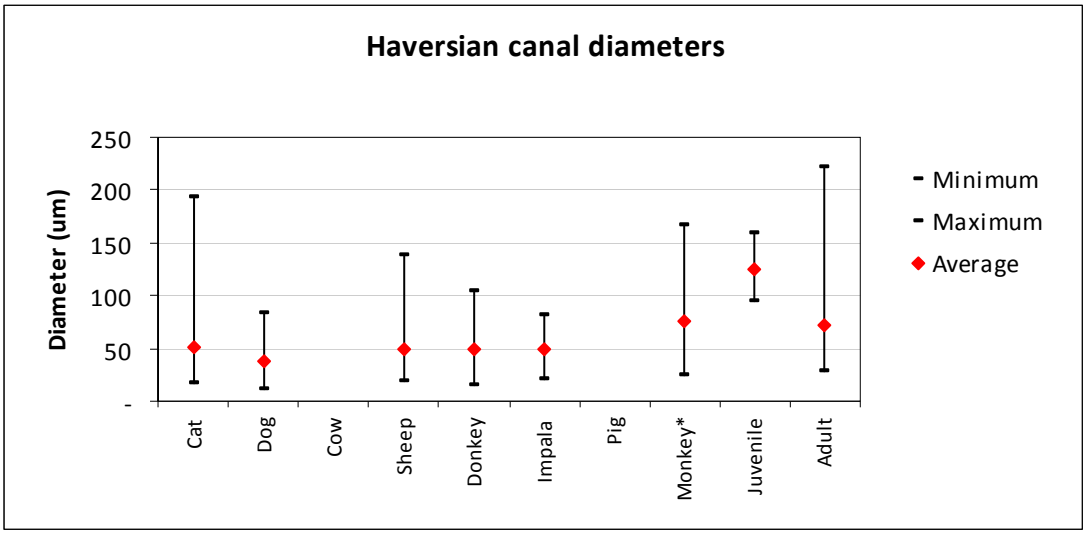


Figure 4.40 *Minimum (µm), maximum (µm) and mean (µm) values for tibial Haversian canal diameters in human and non-human samples Juvenile and adult in this figure refers to Homo sapiens sapiens and monkey* refers to old world monkeys.*

The Kruskal-Wallis test was done to determine differences between the animals with regard to the examined parameters (**Table 4.14-15**). For femora, the test indicated statistically significant differences amongst the study groups for each of the measured variables. Similarly for tibiae, significant differences were noted between groups for all the measured variables except for the minimum and maximum diameters of Haversian canals. The sample size for the quantitative analysis was limited. It illustrated large standard deviations and hence a great overlap was observed for each parameter between the various groups. Therefore further statistical testing was deemed superfluous (Becker P.J., pers comm.). Future research will require a much larger sample size and the results will need to be subjected to multivariate statistical analysis.

4.2.3. Intra-observer and inter-observer repeatability

Intra- and inter-observer error was assessed with interclass correlation coefficients using least squares estimation (LSE). This test strives to obtain a value of one, which is indicative of 100% repeatability. Thus, the closer the recorded values are to one, the better the repeatability of the method used to assess the data.

The intra- and inter-observer repeatability results for the total number of osteons, total number of measurable and non-measurable osteons and the total number of non-Haversian canals are shown in **Table 4.16**. All variables appear to be repeatable and highly reliable. The total number of non-Haversian canals had the highest correlation coefficient between the original and the repeated counts (0.987), while the total number of osteons had the highest inter-observer correlation coefficient (0.961). The total number of measurable osteons yielded the lowest correlation coefficient within (0.952) and between (0.797) observers. In general the total number of osteons, total number of non-measurable osteons and total number of non-Haversian canals were the most reliable parameters.

Table 4.17 summarises the intra- and inter-observer repeatability results for the minimum and maximum osteon and Haversian canal diameters. The highest correlation for the intra-observer agreement was observed in the maximum diameter of osteon measurement (0.967). A lower correlation coefficient was observed in the maximum and minimum diameters of Haversian canal (0.930), although the repeatability for these parameters was still high. The inter-observer repeatability tests compare the results of the initial observer to that of the external investigators. Once again the highest

correlation coefficient was observed in the maximum diameter of osteon, for both external examiners 1 and 2 (0.958 and 0.991, respectively). A lower correlation coefficient for the minimum diameter of osteons between the initial investigator and the first external observer was noted (0.9) while a lower correlation coefficient for the minimum diameter of Haversian canals (0.894) was noted between the original investigator and the second external observer.

In general the minimum diameter of osteons, the maximum diameter of osteons, and the maximum diameter of Haversian canals were the most reliable variables to measure when quantitatively examining histological bone structures.

The documented data for measurements by the original investigator along with that of the external investigators can be viewed in **Appendix E** (p.222) and **Appendix F** (p.223).

Table 4.16 *Intra-observer and inter-observer errors for the total number of osteons (Total_ost), total number of measurable osteons (Meas), total number of non-measurable osteons (Non_meas) and the total number of non-Haversian canals (Tot_vasc).*

Parameters	Intra-observer error LSE*	Inter-observer error LSE*
Total_ost	0.978	0.961
Meas	0.952	0.797
Non-Meas	0.970	0.935
Tot_vasc	0.987	0.938

LSE = Least squares estimation*

Table 4.17 *Intra-observer and inter-observer errors for maximum diameter of osteons (Max_ost), minimum diameter of osteons (Min_ost), maximum diameter of Haversian canals (Max_can) and minimum diameter of Haversian canals (Min_can).*

	Intra-observer error	**Inter-observer error**	
Parameters	**Initial observer**	**External observer 1**	**External observer 2**
	LSE*	**LSE***	**LSE***
Max_ost	0.967	0.958	0.991
Min_ost	0.961	0.9	0.956
Max_can	0.93	0.909	0.925
Min_can	0.93	0.904	0.894

LSE = Least squares estimation*

CHAPTER 5

DISCUSSION

The aim of this research was to examine the chemical composition as well as the qualitative and quantitative histological characteristics of bone to determine whether sufficient differences within these variables exist that could assist the forensic anthropologist in the identification or exclusion of human skeletal remains found fragmented amongst faunal and environmental ruins. Analyses were conducted on femora and tibiae of human adults and juveniles (*Homo sapiens sapiens*), cats (*Felix catus*), dogs (*Canis familiaris*), cows (*Bos taurus*), sheep (*Ovis aries*), donkeys (*Equus africanus asinus*), impala (*Aepyceros melampus*), pigs (*Sus scrofa domestica*) and old world monkeys (*Cercopithecinae*), all of whom are represented in a South African context.

A number of species-specific differences in both the chemical and histomorphometric characteristics of bone have previously been indicated in the literature (Enlow & Brown, 1958; Toots & Voorhies, 1965; Jowsey, 1966; Blitz & Pellegrino, 1969; Martiniaková *et al*., 2006a; Martiniaková *et al*., 2006b; Martiniaková *et al*., 2007a). The focus of this discussion is to interpret the findings regarding the chemical composition and histological characteristics of this study, and also to compare it with results obtained for various species from other geographical areas.

5.1. CHEMICAL ANALYSIS

Chemical analysis is favoured in studies involving lifestyles, environments, migrating patterns, diets and health. This assists scientists in the

understanding of ancient times and allows for comparisons with modern societies (Aufderheide, 1989; Buikstra & Ubelaker, 1994). More recently it was used in forensic science as a possible separation and/or preliminary screening tool for human and non-human elements, with great success (Ubelaker *et al*., 2002; Brooks *et al*., 2006). Using elemental analysis, Brooks and colleagues (2006) separated human cremains found mixed with concrete and other materials; however, using this method to separate the human cremains from *Canis familiaris* was not successful. These results were anticipated by the authors as no differences in the chemical composition of human and animal bones were expected (Brooks *et al*., 2006), despite the fact that different chemical compositions for various mammalian species have been documented together with a number of different mechanisms of mineral uptake associated with various animal species (Blitz & Pellegrino, 1969; Schultz, 1997; Martiniaková *et al*., 2006a).

In this study, the aim of the chemical analysis was to determine the chemical composition of bone of various animals grouped according to their preferred diets, which are omnivores, herbivores and carnivores. The presence of any differences were determined and assessed to see whether these differences are of sufficient magnitude and consistency to distinguish human from non-human skeletal remains.

Quantitative analysis was preformed on potassium (K), calcium (Ca), phosphorus (P), silicon (Si), aluminium (Al), sodium (Na), magnesium (Mg), chlorine (Cl), sulphur (S), zinc (Zn), lead (Pb) and strontium (Sr). Statistically significant differences were observed for calcium (Ca), sodium (Na), chlorine

(Cl), phosphorus (P), sulphur (S), potassium (K) and magnesium (Mg) among the various human and non-human species.

5.1.1. Limitations of chemical analysis

It has been noted that almost all chemical elements within a sample are altered during sample preparation (Goldstein *et al*., 1981). These alterations are observed either by an increase or decrease of a particular element. This was most probably also the case in the current study and a number of factors have been identified that may have affected the outcome.

A number of fleshed bones were received that had to be degreased and macerated before sampling. The affects of degreasing and macerations on the chemical composition of bone is yet to be determined. The effects of water, utilised during these processes are suspected to decrease the percentage of water soluble elements, such as sodium (Na) and chlorine (Cl). Similarly, during the filing process, samples were grinded in water to reduce heat production.

Bone sections were filed with waterproof sandpaper that is known to contain traces of silicone (Si) (Botha A., pers comm.) and as postmortem silicone infiltration is known, the effects on the chemical composition of bone needs to be further explored (Parker & Toots, 1970).

Comparisons of this study to others were difficult as the current study did not express elemental percentages in terms of weight or volume of the bone sample analysed, but rather as percentages of selected elements within bone. Therefore it is recommended that a qualitative analysis first be conducted to

determine which elements are present within the sample, followed by quantitative analysis to determine the percentile contribution of each.

Additionally, small sample sizes made the interpretation of the variations observed for chemical analysis, within and between animals, difficult and as little are know about the history of these animals, understanding such differences becomes complicated.

A limited number of publications deals with the effects of factors such as age, sex, species, type of bones and sample location with regard to the chemical composition of bone and this subsequently limit the credibility of chemical analysis as a possible tool for separation between human and nonhuman bone.

5.1.2. Chemical composition of bone

Chemical equilibrium within the body is maintained by continuous in- and out-flow of elements to and from bony reservoirs that ultimately determines the chemical composition of bone (Bergstrom & Wallace, 1954). In this study, the elemental contribution of adult human bone was found to be 71.31% calcium (Ca), 24.66% phosphorus (P), 2.13% lead (Pb), 0.57% sodium (Na), 0.30% magnesium (Mg), 0.27% chlorine (Cl), 0.24% zinc (Zn), 0.20% sulphur (S), 0.12% potassium (K), 0.11% strontium (Sr), 0.06% aluminium (Al) and 0.02% silicone (Si).

Large values of calcium and phosphorus are to be expected as the hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] of bone, in human or non-human bones, is mainly composed of calcium and phosphorus compounds (Cassella *et al*., 1995; Francillon-Vieillot *et al*., 1997; Ubelaker *et al*., 2002; Junqueira &

Carneiro, 2003). These results are similar to those of Swanson and Iob (1937) who studied human foetal long bones (femora, tibiae and humeri). They measured the elements in millimol of a 100 grams of dry bone, and found that human foetal long bones consisted of 471.7-627.6 mmol calcium, 286.4-366.1 mmol phosphate, 22.1-48.6 mmol sodium, 14.4-21.5 mmol magnesium, 0-11.3 mmol chlorine, 0-7.1 mmol potassium and 0-6.3 mmol sulphur (Fazekas & Kósa, 1978). Although these results were obtained from foetal skeletal remains, it appears to follow a similar pattern to elemental compositions documented in the current study, with high calcium and phosphorus values, followed by the remaining elements.

Klepinger *et al*. (1986) recorded calcium (Ca) percentages for human femora collected from various historical ages from Sicily. Calcium percentages for femora from Iron Ages were 58.8 ± 2.4%, 54.9 ± 2.9% for archaic periods and 52.4 ± 0.9% for the Hellenistic period. According to these authors these calcium percentages are due to postmortem diagenetic alterations in which the calcium percentages increased over a period of time (Klepinger *et al*., 1986). The values reported by these authors are lower than values recorded in the current study and are due to a number of factors. Firstly, one must consider the fact that these remains are of archaeological origin and have been buried for some time. Secondly, the percentages in the study by Klepinger *et al*. (1986) were given in terms of concentrations per ashed weight of bone.

Interestingly, the calcium (Ca) percentages reported for juvenile femora and tibiae of humans were somewhat lower than that observed in adults. Unfortunately, it could not be determined whether these differences were

statistically significant because the small sample size utilised for juveniles can produce misleading results. Recorded calcium percentages in the adult groups were 71.00 ± 1.25% in femora and 73.06 ± 3.44% in tibiae compared to 68.55 ± 3.70% and 69.24 ± 2.68% in juveniles respectively. Likewise, Chan (1991) recorded low calcium values in the radii of juveniles and according to the author this is due to a decrease in the dietary intake of calcium. These differences may also be attributed to the age difference found between the two groups as significant differences in the chemical composition of bone have been previously been linked to age and sex differences (Blitz & Pellegrino, 1969). Age related changes with regard to bone minerals have also been described by Martin *et al*. (1985), Martinex-Gracia *et al*. (2005). The adult ages in this study ranged between 22 and 80 years with an average age of 39.15 ± 16.11 years. Ages for the juvenile material in this study were not known but estimation methods produced an age range between 3 and 14 years with a mean age of 6.83 ± 5.19 years. Little information regarding the background of the juvenile bones used is available and as illustrated in the literature factors such as pathology and diagenesis can also alter the chemical composition of bone (Klepinger *et al*., 1986; Cassella *et al*., 1995; Pingitore *et al*., 2001; Ott, 2002; Halstead, 2007). Therefore, it seems that calcium levels may be lower in juveniles than adults but this need to be confirmed.

For non-humans, calcium (Ca) was the most abundant element recorded, followed by phosphorus (P) for both femora and tibiae of all species. The highest calcium percentage recorded was 74.12 ± 1.18% in donkey femora (*Equus africanus asinus*) with the lowest calcium percentage recorded in cow

femora, 69.99 ± 6.59%. These variations could be related to the different bone types observed in the skeletal elements of the two species, as a relation between various bone types and calcium metabolism has previously been indicated (Jowsey, 1968). Donkey femora consists of a combination of primary vascular longitudinal bone and irregular Haversian bone compared to the primary vascular plexiform bone that make up the osseous tissue of cow remains.

High lead (Pb) contents were observed in cow femora and in both the femora and tibiae of human juveniles (*Homo sapiens sapiens*). Cow femora had an average lead content of 6.19 ± 8.88%, while juveniles had lead percentages of 8.89 ± 4.42% in femora and 8.77 ± 2.57% in tibiae. Similar to juvenile bones, little is known about the history of the animals used in this study. This makes interpretations of observed percentages difficult and one can only speculate possible reasons for this. High lead percentages in cows may be due to the feeding environment these animals were kept in. Higher lead (Pb) concentrations in grazing areas along major highways have been illustrated (Othman *et al*., 1997; Bacon *et al*., 2005). Grass along highways have higher lead (Pb) concentrations in response to increased atmospheric lead (Pb) concentrations due to lead containing petrol utilized by motor vehicles (Othman *et al*., 1997).

Similar to calcium, higher lead (Pb) percentages were recorded in juveniles compared to that of adult *Homo sapiens sapiens.* This may be related to the differences in the intestinal absorption of lead between adults and juveniles. It has been shown that about 50% of ingested lead (Pb) is absorbed by juveniles as apposed to the mere 10% absorbed in adults. Of this, about 95%

is stored in skeletal remains (Aufderheide, 1989). From this it follows that the lead exposure of adult and juvenile humans may have been the same, but that the increased absorption in juveniles may have led to the higher observed lead levels in the younger individuals. As already stated, dissimilarities in the chemical composition of skeletal remains have been linked to age and type of bone analysed (Jowsey, 1968; Blitz & Pellegrino, 1969; Martin *et al*., 1985). Lead content in vertebrae has been indicated to be greater than the lead content observed for in skeletal elements such as tibiae, rib and skull bones (Martinez-Gracia *et al*., 2005). Higher lead (Pb) concentrations can also be linked to a low calcium diets. This is because the body treats and stores calcium and lead ions similarly (Aufderheide, 1989). Thus, the low calcium percentages recorded in the juvenile skeletal material in the current study may be related to the high lead levels observed. As with any element within bone, increased or decreased percentages can also be linked to postmortem diagenetic alterations (Aufderheide, 1989).

Silicone (Si) was one of the smallest contributing elements to the overall chemical composition of bone and the presence of this element is mainly linked to postmortem alteration, as the postmortem infiltration of this element into bone is known (Parker & Toots, 1970). This infiltration can be as a result of the preparation procedures and can occur during any of the preparation steps. These alterations can include the redistribution, reduction, or increase of the particular element within the sample (Goldstein *et al*., 1981).

The highest sulphur values recorded were for juvenile *Homo sapiens sapiens*. Interestingly, these percentages were twice that observed in adult human tibiae. These differences are difficult to explain as they could also be

the result of diagenetic alterations of the juvenile bones. The health and nutritional status of these individuals may also have an effect on the chemical composition as chemical and morphological alterations to bone have been linked to a number of diseases such as osteomalacia, diabetes mellitus and syphilis to name but a few (Halstead, 2007; Hillier & Bell, 2007).

Apart from calcium (Ca) and phosphorus (P), all elements stored within bone are present in extremely small quantities (Aufderheide, 1989). These small, variable quantities of the elements together with the small sample size make intra- and interspecies comparisons quite difficult. Interpretation of the various element percentages is also difficult as little is known about the effects of age, sex and ancestry on the chemical composition of bone.

5.1.3. Chemical composition of bone of various feeding groups

All animals examined within the current study were pooled together into three groups, herbivores, carnivores and omnivores, according to their feeding prevalence. The carnivore group included cats and dogs while cows, sheep, donkeys and impala formed the herbivore group. Pigs, old world monkeys and juvenile and adult humans formed the omnivore group.

Differences in the chemical composition of bone of various animals have been demonstrated and are linked to the various food types ingested (Toots & Voorhies, 1965; Parker & Toots, 1970; Pingitore *et al*., 2001). Similarly, a number of differences between the various feeding groups were recorded in this study and as was the case in the study by Brooks *et al*. (2006), large standard deviations were found. Varying chemical compositions have also

been linked to different bone types, area of bone studied and degree of ossification (Martinez-Gracia *et al*., 2005).

To compare the chemical composition of bone of various feeding groups to other similar studies, femoral and tibial values were combined and will be discussed below.

The chemical composition of herbivore, carnivore and omnivore bone was similar, but several statistically significant differences were found with regard to potassium (K), sodium (Na), chlorine (Cl), magnesium (Mg) and sulphur (S) content (**Table 4.13**, p.116). These variations were mainly between omnivores and herbivores, but significant differences between omnivores and carnivores were also illustrated. Most of these dissimilarities are thought to be related to the various food sources preferred by the feeding groups.

A difference with regard to the potassium (K) content of bone was noted amongst the various feeding groups. These differences were statistically significant between the omnivores and herbivores and omnivores and carnivores (p=0.0001). The highest potassium percentages recorded were for omnivores (0.118 ± 0.057%) followed by carnivores (0.071 ± 0.062%) and then by herbivores (0.052 ± 0.035%). The highest value was recorded in old world monkeys (0.14 ± 0.04%) and as this element is found in various fruits and vegetables (Pereira, 1843; Tucker *et al*., 1999), which mainly forms part of an omnivorous diet, this high value is understandable.

Differences in the sodium (Na) and chlorine (Cl) percentages between the groups were also recorded. These differences were statistically significant for both sodium (p=0.0192) and chlorine (p=0.0001) and were between omnivores and carnivores. Chlorine contributions were also statistically

significant between omnivores and herbivores (p=0.0001). Sodium (Na) levels recorded in omnivores (0.481 ± 0.190%) were substantially higher than that of carnivores (0.322 ± 0.071%). Similarly, high chlorine (Cl) levels were recorded in omnivores (0.22 ± 0.129%), followed by carnivores (0.117 ± 0.044%) and then by herbivores (0.101 ± 0.032%). Again the diets of the various groups can be used to explain the degree of accumulation of these elements among the various species. Both sodium and chlorine are most commonly introduced into the body by the consumption of table salt which consists of sodium and chlorine elements (Pereira, 1843). With modern diets that contain increased amounts of salt, increased percentages of these elements in bone can be expected. This is also illustrated in the highest chlorine percentages recorded in adult *Homo sapiens sapiens* (0.26 ± 0.12%). Equally high sodium values were also recorded in the adult humans (0.56 ± 0.20%) and chlorine. Compared to adult *Homo sapiens sapiens,* lower percentages for both sodium and chlorine were recorded in juveniles (0.31 ± 0.05% and 0.23 ± 0.17%, respectively).

The magnesium (Mg) content decreased from herbivores (0.402 ± 0.134%) to omnivores (0.318 ± 0.107%) to carnivores (0.296 ± 0.098%) and statistically significant differences were indicated between omnivores and herbivores (p=0.0214). Magnesium (Mg) is present in various fruits and vegetables (Pereira, 1843; Tucker *et al*., 1999). These food sources are linked to an herbivorous diet and are not generally included in the diet of carnivores. Therefore, the subsequent decrease of the element from one trophic level to the next is explainable.

Differences with regard to sulphur contribution were also statistically significant between omnivores and herbivores (p=0.0012). High levels were recorded in omnivores (0.260 ± 0.150%), followed by carnivores (0.208 ± 0.107%) and then herbivores (0.132 ± 0.086%). Various vegetables and protein sources such as egg white contain sulphur (Pereira, 1843) and as these food constituents form part of an omnivorous diet, the high recorded values in omnivores are understandable. Interestingly human juveniles (0.38 ± 0.26%) had higher sulphur percentages compared to human adults (0.21 ± 0.11%). Similar to chlorine such differences are assigned to various bone types and age related changes (Jowsey, 1968; Blitz & Pellegrino, 1969; Martin *et al*., 1985).

The particular strontium (Sr) percentages were of interest as almost all of the strontium in the body is stored within bones (Aufderheide, 1989). Toots and Voorhies (1965) indicated significant differences in the strontium content of herbivore and carnivore bones and attributed these differences to the different food sources ingested by these animals. These authors reported that higher strontium contents in herbivore bones are due to the increased amount of strontium (Sr) found in grass and vegetation (Toots & Voorhies, 1965). Significant differences in this regard were not reproduced by the current study (p=0.8569), however, differences within the strontium content of herbivores (0.054 ± 0.791%) and carnivores (0.04 ± 0.069%) were observed. Higher percentages were observed in omnivores (0.103 ± 0.250%) compared to both herbivores and carnivores with the highest recorded value found in human adults (0.17 ± 0.32%). No strontium was recorded in impala and therefore results of the current study supports work by Boaz and Hampel who

were unable to illustrate any relation between the particular diet of the animal and strontium content (Boaz & Hampel, 1978).

This study illustrated differences in the chemical composition of bone with significant differences mainly found between omnivores and herbivores. Unfortunately, no consistent differences were identified, as element contributions for femora and tibiae of a particular animal did not correspond. Some elements were statistically significant between omnivores and herbivores, some between herbivores and carnivores and others between carnivores and omnivores. This is important as a combination of such elements might be potentially useful to determine the feeding group the remains originated from and warrants further research.

Subtle differences in the elemental composition of femora and tibiae were also illustrated as well as differences between animals within a particular feeding group. Future research is needed in this regard to further describe these differences.

5.2. HISTOMORPHOMETRICAL ANALYSIS

Osteoarchaeoloigsts and forensic anthropologists used to avoid histological analysis as they believed sample preparation to be a hard and time consuming process which offered little gain over more easily applied macroscopic methods. However, in recent years the histological structure of bone has become a popular and highly diagnostic research tool in both forensic anthropology and archaeology and is partly due to the ease with which histological bone slides can be prepared and analyzed (Maat *et al*., 2002).

Histological analysis of cortical bone is commonly employed in methods to estimate age at death, in the diagnosis of various diseases affecting bone and in sorting human and faunal remains (Kerley, 1965; Enlow, 1966; Bell & Jones, 1991; Maat *et al*., 2002). The separation of human and nonhuman bone elements is important, as the presence of human remains may validate further criminal investigations (Scheuer & Black, 2007). Accurate separation techniques are necessary as small animal bones such as that of cat and dog and even frog, have been mistaken for human foetal or juvenile remains (Fazekas & Kósa, 1978). Separating human from non-human skeletal remains has successfully been done with DNA analysis (Bataille *et al*., 1999). The success of DNA analysis, however, is dependant upon the amplification ability of DNA as DNA does not survive high temperatures and can degrade easily (Cattaneo *et al*., 1999; Scheuer & Black, 2007). Additional methodologies need to be explored so as to improve and create alternative separation techniques for human and non human skeletal elements.

The aim of this study was to add to the qualitative and quantitative descriptions found in the current literature and to determine whether statistically significant differences are present between human and animal remains from South Africa. Analyses were conducted on ground bone sections of femora and tibiae of human adults and juveniles (*Homo sapiens sapiens*), cats (*Felix catus*), dogs (*Canis familiaris*), cows (*Bos taurus*), sheep (*Ovis aries*), donkeys (*Equus africanus asinus*), impala (*Aepyceros melampus*), pigs (*Sus scrofa domestica*) and old world monkeys (*Cercopithecinae*). Bone sections were removed from the mid-shaft of the diaphyses and the periosteal surface of each histological section was

examined mainly for the presence of primary vascular bone and Haversian bone. The histological structure of the skeleton of impala has not been previously described and therefore this study adds important information concerning the qualitative and quantitative characteristics of this species.

5.2.1. Limitations of histological analysis

The sample preparation method employed in this study is relatively easy, fast and affordable. Unfortunately a number of factors affected the histological appearance of the bone slides. For example, during the grinding of bone sections uneven pressure resulted in laminae of both primary vascular laminar and plexiform bone, "peeling" off as illustrated in **Figure 5.1**.

Some sections had visible file marks (**Figure 5.2**). These marks can be misleading and can ultimately jeopardise the accuracy of the qualitative recording and quantitative measurements. Similarly, fine artificial cracks were also visible in some sections (**Figure 5.3**) and one must be aware of these as they can be misleading in qualitatively descriptions, especially in descriptions pertaining to primary vascular laminar and/or plexiform bone. Unevenly filed bone sections caused distortions observed in photos as smudgy and blurry areas that were ultimately not examinable.

Other photos contained debris that affected the quantitative analysis of the slides (**Figure 5.3**).

Several problems in identifying primary and secondary osteons as well as vascular canals were experienced and it is therefore recommended that photographs taken with normal bright light be supplemented by photos taken with a polarised lens. Examining histological bone slides with a polarizer has

also been proven invaluable when describing collagen fibre orientation and arrangements.

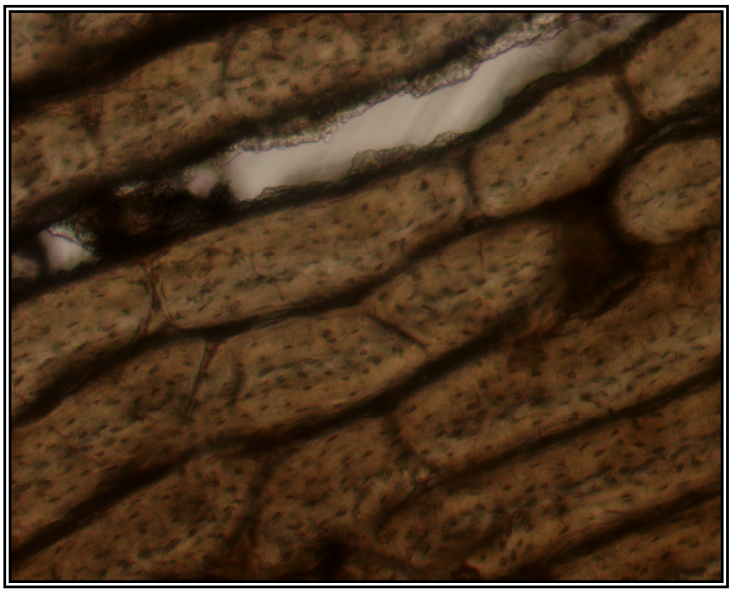


Figure 5.1 *Illustration of laminae 'peeling of' due to uneven pressure during bone grinding.*

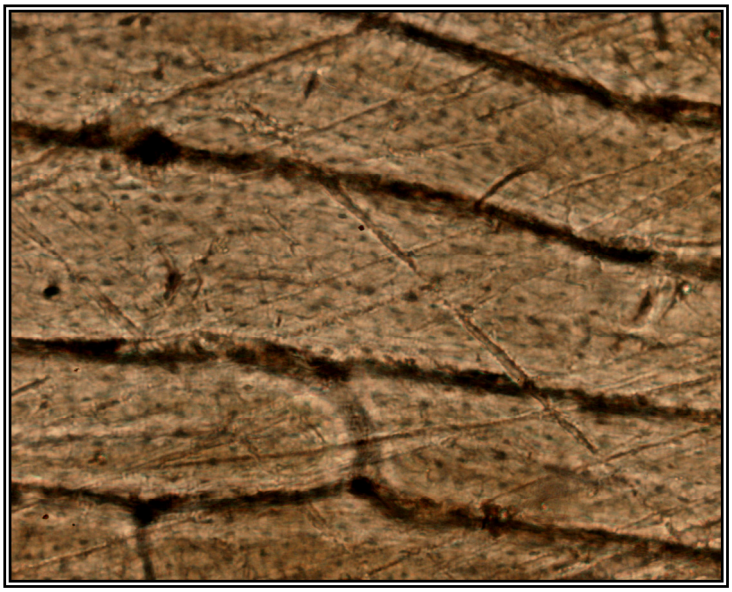


Figure 5.2 *Illustration of 'file marks' produced by bone grinding.*

In general it is recommended that continuous sections of bone be examined, including periosteal, mesosteal and endosteal surfaces to gain an

overall understanding of the structural arrangements and measurements for bone.

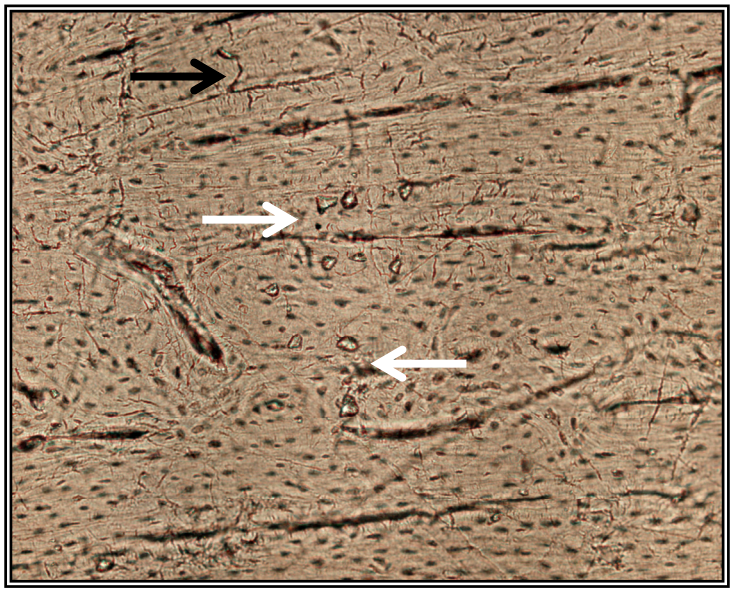


Figure 5.3 *Illustration of fine cracks (black arrow) and debris (white arrows).*

5.2.2. Qualitative analysis

The qualitative characteristics of compact bone of various animal species have been described and classified by Enlow and Brown (1956, 1957 and 1958). Studies that focus on assessing qualitative characteristics of human and animal bone often refer to this earlier classification system to assist in the separation of human remains found amongst other faunal debris (Singh *et al*., 1974; Martiniaková *et al*., 2006a; Martiniaková *et al*., 2006b; Martiniaková *et al*., 2007a; Martiniaková *et al*., 2007b). In general, the qualitative description and classification of bone is highly complex and this study, similar to the work of Locke (2004), did not pay attention to collagen fibre orientation and arrangement. Classification was based on the presence of primary vascular bone, Haversian bone or avascular bone as described in Chapter 3 (Materials and Methods, section 3.2.3.2, p.81). Thus, the general arrangements of

vascular canals and primary and secondary osteons dispersed throughout these bone types were assessed and described.

Most histological textbooks provide only a description of dense Haversian bone which is most often found in adult humans (Enlow, 1966). However, this bone type is not found in all mammalian species, with primary vascular plexiform bone being the most common in large and fast growing mammals such as cows and pigs (Enlow & Brown, 1956; Martiniaková *et al*., 2006a).

Similar to what was found in other studies, Haversian bone was noted in this study in cats, dogs, equid species (donkeys), old world monkeys, human adults and juveniles, but was not found in cows and pigs (Enlow & Brown, 1958; Enlow, 1966; Francillon-Vieillot *et al*., 1997; Hillier & Bell, 2007).

Adult human tibiae consisted entirely of dense Haversian bone, while the bone tissue in adult femora varied between irregular and dense Haversian bone. Similarly, Enlow and Brown (1958) also noted areas containing scattered secondary osteons in adult human skeletal remains. The Haversian bone noted in adult humans in this study contained a number of osteons that were linearly arranged, however, no osteon bands were recorded in either femora or tibiae. Osteon bands as described by Mulhern and Ubelaker (2001) are rows of five or more linearly arranged primary or secondary osteons. The authors recorded frequent, long bands of osteons in some non-human bones, with a single osteon band recorded in an eight and 64-year old human femur. The researchers suggested that with an increase in sample sizes, differences in the number of these bands may assist in the separation of human and non-human bone (Mulhern & Ubelaker, 2001). In the current study osteon bands were, however, recorded in sheep femora, donkey tibiae and in both femora

and tibiae of impalas with linearly arranged primary or secondary osteons observed in donkey femora, old world monkey tibiae, dog femora and both cat femora and tibiae. These linear arrangements are, however, not related to osteon bands.

Each osteon contains a Haversian canal and according to Harsányi (1993) these canals are eccentrically positioned within the secondary osteons of humans. In the current study, it was noted that the Haversian canals were centrally located in both adult human femora and tibiae. These discrepancies might be related to the sampling procedure and to the angles at which these sections were cut. In combination with secondary osteons a number of non-Haversian canals were also recorded. Similar observations were also made by Enlow and Brown (1958), but no primary osteons were found by Martiniaková *et al*. (2006a). This could be related to sex and age differences between the current study and that of Martiniaková *et al*. (2006a) in which only male cadaver femora aged between 36 and 51 years were used. The current study examined both male and female skeletal remains with ages ranging between 22 and 80 years of age. Differences in the microstructure of male and female skeletal remains as well as differences with regard to age have been documented (Kerley, 1965; Thompson, 1980; Burr *et al*., 1990).

Only a few differences in the qualitative appearance of adult and juvenile *Homo sapiens sapiens* bones were observed. Similarly Cuijpers (2006) also reported little differences in the diaphyseal bones of adult and juvenile humans. The variations observed in this study are probably associated with differences in age at death between the adults (39.15 ± 16.11 yrs) and juveniles (6.83 ± 5.19 yrs) which in turn are associated with bone remodelling.

Juvenile bone characteristically contained large Haversian canals as opposed to the medium sized canals recorded in adults. Interstitial lamellae in adults varied between small and large quantities, depending on the type of bone tissue present, i.e. dense or irregular Haversian bone tissue, while juvenile bone contained large quantities of unremodelled bone. This is to be expected based on the manner in which the bone is laid down and remodelled. These remodelling differences are the basis for all studies which seek to estimate age at death from the histological structure of the skeleton.

Throughout the literature, plexiform bone has been documented in horses, pigs, sheep, cows and dogs (Enlow & Brown, 1958; Mulhern & Ubelaker, 2001; Cuijpers, 2006; Martiniaková *et al*., 2006a; Martiniaková *et al*., 2006b; Martiniaková *et al*., 2007a; Martiniaková *et al*., 2007b). This study recorded plexiform bone in both the femora and tibiae of pigs and cows, but not in sheep, horses or dogs. These differences in the presence or absence of plexiform bone may be associated with the age at death of the animals examined (10-12 month old pigs and 20-22 month old cows). Primary bone is the main bone tissue observed in younger animals but with aging, some or all of this bone tissue may become completely replaced by Haversian bone (Martiniaková *et al*., 2007a). As no plexiform bone was observed in either human adult or juvenile remains in this study, similar to what has been stated in the literature, it is proposed that the presence of this bone type can be accurately used to separate at least pig and cow remains from human skeletal elements (Dix *et al*., 1991; Mulhern & Ubelaker, 2001; Locke, 2004; Cuijpers, 2006; Martiniaková *et al*., 2006a; Hillier & Bell, 2007; Martiniaková *et al*., 2007a).

Martiniaková and colleagues (2006a, 2006b and 2007a) indicated a number of resorption lacunae located between secondary osteons in pig femora and associated these spaces with the possible identification of pig remains. Resorption spaces in pig femora were not found in this study but were observed in dog tibiae, sheep tibiae, old world monkey tibiae, juvenile tibiae and adult human femora and tibiae. This was to be expected, considering resorption spaces are found in all cortical bone due to continued bone remodelling. Therefore, in this study it was not possible to link the presence of resorption spaces to pig remains and the presence thereof in adult and juvenile humans thus does not allow distinction between human and pig bones (Martiniaková *et al*., 2006a; Martiniaková *et al*., 2006b; Martiniaková *et al*., 2007a).

Areas of avascular bone at both the periosteal and endosteal surfaces in cow femora have been documented and is considered a possible tool to use for the separation, and hence identification, of cow remains (Martiniaková *et al*., 2006a; Martiniaková *et al*., 2006b; Martiniaková *et al*., 2007a). Avascular or nonvascular bone was not found in either cow femora or tibiae in this study. It was, however, noted in both the femora and tibiae of cats and in the femora of dogs and old world monkeys. Similarly, areas devoid of vascular canals were reported in cat bones by Enlow and Brown (1958) and Singh and colleagues (1974). These discrepancies may be related to the size of the observation areas examined and also to the specific area of bone the sample was taken from. Avascular bone tissue may be recorded for large areas of unremodelled bone or interstitial lamellae. Therefore the presence or absence of avascular bone may not be useful in the identification of cow remains but

could still be considered as a possible means of excluding human skeletal elements as no areas devoid of vascular canals have been recorded in *Homo sapiens sapiens*.

No descriptions of impala bone have previously been recorded. Impala, which is indigenous to the African continent, belongs to the order artiodactyla. The skeletal material of these animals follows the general structural pattern of artiodactyla, with their primary bone being replaced by Haversian bone with advancing age. Their femora contain primary vascular longitudinal bone, whereas the tibiae consisted of primary vascular plexiform bone and dense Haversian bone tissue.

Different bone types have been associated with a variety of mammalian species, most of which contain either Haversian or primary (plexiform bone) bone, with combinations of these also being frequently observed. Similar to other research, this study also associated plexiform bone with non-human remains; however plexiform bone is often restricted to the periosteal surface and destruction of this region is common during the processing techniques (Hillier & Bell, 2007).

Using qualitative analysis for the exclusion of human remains can be employed with relative accuracy. However, there are really no unique qualitative characteristics associated with any specific species, and therefore differentiation between various species based on histological assessment should be approached with caution. If possible, several areas of a bone should be sampled and more than one section assessed.

Unfortunately with the ambiguity of terminology in the literature, understanding and comparing the various bone types can be become

extremely difficult (Francillon-Vieillot *et al*., 1997; Locke, 2004; Zedda *et al*., 2008). Therefore it is important that extensive samples of various bone types with clearly illustrated images should be available, probably in atlas format, in order to consistently and clearly describe and distinguish various species of animals.

5.2.3. Quantitative analysis

As the separation of human and non-human remains qualitatively rely on the presence of plexiform bone, quantitative analyses are needed to assist in sorting remains amongst species which exhibit Haversian bone. To date, osteon and Haversian canal diameters are considered to be the most useful variables to examine when sorting skeletal remains into species (Hillier & Bell, 2007).

The minimum and maximum diameters of osteons and Haversian canals were measured in the current study, as described in Chapter 3 (Materials and Methods, section 3.2.3.2, p.81). These variables were also examined by a number of other studies which allows for comparison between the samples (Albu *et al*., 1990; Cattaneo *et al*., 1999; Martiniaková *et al*., 2006a; Martiniaková *et al*., 2006b; Martiniaková *et al*., 2007a; Martiniaková *et al*., 2007b; Zedda *et al*., 2008; Cattaneo *et al*., 2009).

The quantitative parameters recorded in this study illustrated great variability within and between species (**Figures 4.30-35**). Most of the histomorphometric variables decreased in size from primates to ungulates to carnivores and with the aid of a Kruskal-Wallis test, statistically significant

differences were indicated between the groups, except for the minimum and maximum Haversian canal diameter measurements of tibiae.

The total number of osteons and non-Haversian canals was counted and represented an observation area of 3.456 mm^2. Non-Haversian canals (vascular canals and primary osteons) were observed in the femora of all species examined, while secondary osteons were not recorded in cow, sheep, impala and pig, as the femora of these animals did not exhibit Haversian bone. The largest number of non-Haversian canals was found in the impala femora (6.78 ± 2.91/3.456 mm^2) and is directly related to the fact that the femora of impala consist of primary vascular longitudinal bone.

Adult *Homo sapiens sapiens* femora consisted primarily of Haversian bone and therefore exhibited few non-Haversian canals (1.88 ± 3.09/3.456 mm^2) when compared to the number of osteons (4.32 ± 2.71/3.456 mm^2). In contrast, human juveniles had larger amounts of non-Haversian canals (5.44 ± 6.77/3.456 mm^2) compared to secondary osteons (2.56 ± 3.09/3.456 mm^2) in their femora. This was to be expected as the cortical bone of juveniles contained large quantities of unremodelled bone. Kerley (1965) also noted a decrease in the number of non-Haversian canals, from childhood to adolescence in humans. A larger number of osteons were present in juvenile tibiae (2.78 ± 1.86/3.456 mm^2) compared to juvenile femora. Similar results were obtained by Mulhern and Ubelaker (2003) in chimpanzees and by Evans and Bang (1966) for various skeletal elements. Equally, a larger number of osteons were recorded in adult tibiae (6.10 ± 2.19/3.456 mm^2) when compared to femora. For both adults and juveniles this can be linked to the bone types present in femora and tibiae. The difference between femoral and

tibial osteons for juveniles is more subtle than for adults. Both juvenile long bones consisted of irregular Haversian bone while adult femora consisted of a combination of irregular and dense Haversian bone. Adult tibiae solely contained dense Haversian bone and hence more secondary osteons were noted.

Dog bones exhibited the largest number of osteons for both femora and tibiae (6.94 ± 4.92/3.456 mm^2 and 9.94 ± 2.21/3.456 mm^2 respectively). The skeletal elements of dogs consisted of dense Haversian bone and as illustrated in the quantitative analysis, these osteons were small. The large number of osteons is thus directly related to the size of these structures as well as the type of Haversian bone present.

Non-Haversian canals were not observed in cat, dog, donkey, old world monkey and adult human tibiae because of the absence of primary bone and hence primary structures. Also, no osteons were observed cow and pig remains as no Haversian bone were noted in the skeletal elements of these animals.

Results of the minimum and maximum diameter measurements from this study and that of previous publications are presented in Table 5.1 and will be discussed below.

Table 5.1 *Histomorphometrical measurements (µm) of Haversian canals and osteons recorded by a number of researchers.*

Researcher	Sample	Min_canal	Max_canal	Ave_canal	Min_ost	Max_ost	Ave_ost
Current study, 2009	Cat (femur)	30.66 ± 21.06	39.59 ± 19.34		214.98 ± 23.01	275.73 ± 25.52	
Femora and tibiae	Cat (tibia)	40.65 ± 35.75	59.87 ± 38.84		223.36 ± 55.43	301.91 ± 74.55	
	Dog (femur)	29.77 ± 11.63	46.83 ± 16.55		192.77 ± 28.93	246.39 ± 33.75	
	Dog (tibia)	30.12 ± 3.67	46.52 ± 18.02		194.82 ± 50.91	269.13 ± 92.53	
	Cow (femur)	-	-		-	-	
	Cow (tibia)	-	-		-	-	
	Sheep (femur)	-	-		-	-	
	Sheep (tibia)	35.52 ± 14.49	62.30 ± 29.69		225.82 ± 76.38	281.27 ± 97.97	
	Donkey (femur)	39.66 ± 12.08	53.55 ± 16.55		201.33 ± 49.38	307.99 ± 85.61	
	Donkey (tibia)	40.69 ± 12.76	58.94 ± 19.00		257.11 ± 57.00	324.77 ± 69.68	
	Pig (femur)	-	-		-	-	
	Pig (tibia)	-	-		-	-	
	Old world monkey (femur)	92.61 ± 38.67	132.77 ± 51.16		277.28 ± 23.53	366.45 ± 61.00	
	Old world monkey (tibia)	62.89 ± 27.69	88.96 ± 40.78		245.94 ± 63.49	354.54 ± 124.04	
	Humans – juvenile (femur)	82.55 ± 29.5	110.23 ± 26.37		290.73 ± 58.67	365.32 ± 81.97	
	Humans – juvenile (tibia)	99.35 ± 8.02	150.10 ± 8.92		309.47 ± 35.31	440.42 ± 19.91	
	Humans – adult (femur)	75.55 ± 31.79	109.67 ± 49.07		280.66 ± 87.71	382.67 ± 120.27	
	Humans – adult (tibia)	59.87 ± 24.80	83.00 ± 38.67		298.18 ± 79.82	389.50 ± 102.43	
Zedda *et al*., 2008	Horse (femur)	33.4 ± 21.2	50.0 ± 27.0	21	156.2 ± 64.6	211.6 ± 88.5	190
Femora and tibiae	Horse (tibia)	41.1 ± 16.6	54.4 ± 19.6		183.1 ± 76.5	236.0 ± 109.8	
	Cow (femur)	20.7 ± 6.1	36.5 ± 16.7	11	128.8 ± 41.5	195.3 ± 73.5	140
	Cow (tibia)	24.3 ± 8.4	39.6 ± 14.2		118.3 ± 40.7	149.0 ± 55.0	
Martiniaková *et al*., 2007	Sheep	11.60 ± 3.36	33.62 ± 8.97		66.57 ± 17.49	207.94 ± 69.37	
Femora	Pig	15.53 ± 5.27	40.53 ± 14.16		84.54 ± 17.15	216.68 ± 52.11	
	Cow	15.60 ± 4.38	47.47 ± 14.98		77.01 ± 15.95	258.45 ± 66.06	

Table 5.1 *Histomorphometrical measurements (µm) of Haversian canals and osteons recorded by a number of researchers (continue).*

Researcher	Sample	Min_canal	Max_canal	Ave_canal	Min_ost	Max_ost	Ave_ost
Martiniaková *et al*., 2006	Cow	15.58 ± 4.32	48.76 ± 15.59		76.22 ± 14.63	269.63 ± 69.15	
Femora	Pig	15.61 ± 5.18	40.60 ± 14.55		83.15 ± 17.24	211.07 ± 55.42	
	Sheep	11.46 ± 3.07	33.63 ± 8.65	22.54 ± 5.86	65.11 ± 17.31	206.27 ± 66.87	
	Human	32.26 ± 7.23	59.99 ± 21.59	46.13 ± 14.41	90.20 ± 19.19	263.76 ± 60.08	
Cattaneo *et al*., 1999	Humans	58.37 ± 1.54	76.86 ± 2.21		277.0 ± 5.21	352.6 ± 7.02	
Burnt humeri and femora	Animals	33.54 ± 0.79	44.51 ± 1.03		223.07 ± 3.58	296.72 ± 5.06	
Albu *et al*., 1990	Bovine	39.81	70.94	55.38			
Femora	Horse	36.21	58.78	47.50			
	Pig	28.73	39.85	34.29			
	Dog	26.30	69.09	48.50			
Owsley *et al*., 1984	Human	63	312	175			
Humeri	Deer	31	250	71			
Jowsey, 1966	Dog						154 ± 38
Femora	Cat						163 ± 30
	Monkey						216 ± 52
	Human (+ rib)						223 ± 50
	Cow						250 ± 40

Min_canal - minimum diameter of Haversian canal

Max_canal - maximum diameter of Haversian canal

Ave_canal - average Haversian canal diameters

Range_canal - range of Haversian canal diameters

Min_ost - minimum diameter of osteon

Max_ost maximum diameter of osteon

Ave_ost - average osteon diameter

The minimum and maximum Haversian canal diameters measured in adult human (*Homo sapiens sapiens*) femora in this study were 75.55 ± 31.79 µm and 109.67 ± 49.07 µm; and 59.87± 24.80 µm and 83.00 ± 38.67 µm in the tibiae. These values are larger than those recorded by Martiniaková *et al.* (2006a) but the tibial measurements from the current study are comparable to values reported by Cattaneo and colleagues (1999). The values recorded by Cattaneo *et al*. (1999) are somewhat smaller than values observed in the current study. This can be due to shrinkage of bone structures related to increased temperatures as the authors examined burned bones sections compared to the fresh ground bone sections analysed in the current study (Nelson, 1992; Cattaneo *et al*., 1999). Also, Cattaneo *et al*. (1999) combined observations made of human long bones (humeri and femora) while the current study separated values for tibiae and femora. This was done because research such as that by Evans and Bang (1966) has indicated differences in the compact bone of various skeletal elements. The smaller values indicated by Martiniaková *et al.* (2006a) compared to the current study may be related to the different age and sexes utilised between the two studies. Martiniaková *et al.* examined only male femora with ages ranging between 36 and 51 years (Martiniaková *et al*., 2006a). The current study examined and combined male and female femora aged between 22 and 80 years. Age related changes have been illustrated by a number of publications and researchers such as Jowsey (1966) recorded a significant increase in femoral Haversian canal perimeter with age. As older individuals were utilised in the currents study the larger measurements compared to Martiniaková *et al.* (2006a) may thus be related to age associated differences. Some variations in skeletal structures

have also been related to sex and as described by Burr and colleagues (1990) females have larger Haversian canals compared to males (Burr *et al*., 1990). As Martiniaková *et al.* (2006a) only examined male femora, the larger values observed in the current study can thus also be linked to increased values associated with female structures. Population differences may also play a role. Comparing histological bone structures can be difficult as measured Haversian canal diameters for human long bones can range between 20 and 300 µm (Albu *et al*., 1990). Similarly measured Haversian canal values in the current study ranged between 25.72 µm and 307.73 µm.

Minimum and maximum osteon measurements for adult *Homo sapiens sapiens* were again larger than those recorded by others (Jowsey, 1966; Martiniaková *et al*., 2006a). Differences compared to Martiniaková *et al.* (2006a) have already been described above while dissimilarities compared to Jowsey (1966) are proposed to be linked to the different skeletal elements examined. Jowsey analysed and combined human rib and femoral measurements while the current study presented femoral and tibial observations separately (Jowsey, 1966). Osteon measurements recorded for femora in the current study were again close to values documented by Cattaneo *et al*. (1999). The authors reported minimum and maximum osteon diameters of 277.0 ± 5.21 µm and 352.6 ± 7.02 µm respectively, compared to 279.18 ± 81.89 µm and 381.47 ± 117.21 µm observed in the current study.

Interesting to note was the relation between osteon and Haversian canal diameters in adult *Homo sapiens sapiens*. Femora had larger Haversian canal diameters compared to tibiae, while tibiae had larger osteons compared to femora. Differences in femoral and tibial osteon sizes have previously

been indicated by Evans and Bang (1966) and were attributed to differences in the tensile strength related to the various bones.

No quantitative bone measurements for juveniles *Homo sapiens sapiens* were found in the literature. Measurements form the current study are summarised in **Table 5.1** (p.182). Minimum and maximum Haversian canal diameters in femora and tibiae were larger in juveniles compared to adults. This is proposed to be related to age and as described by Singh and Gunberg (1970) new osteons are characterised by large Haversian canals which are related to the transition of primary bone to secondary bone. Newly formed osteons have large Haversian canal diameters because with the formation of osteon, concentric lamellae are deposited inward.

Tibial osteon diameters in juvenile *Homo sapiens sapiens* were larger than adult osteon diameters. The tibial osteon diameters in juveniles were also larger than the femoral osteon measurements. Similar to what was the case with juvenile Haversian canal diameters, this is proposed to be linked to bone remodelling associated with muscle attachments (Enlow, 1966).

Jowsey (1966), Albu *et al*. (1990), Martiniaková *et al*. (2006a 2006b and 2007a) and Zedda *et al*. (2008) measured osteons and Haversian canals in cow and pig long bones. No secondary osteons and thus no Haversian canals were observed in the either femora or tibiae of cows and pigs in this study. Compared to work by Martiniaková *et al*. (2006a, 2006b and 2007a) and Zedda *et al*. (2008), these discrepancies may be due to age differences. This study used cow and pig remains younger than that utilised by Martiniaková *et al*. (2006a and 2007a), while Zedda and colleagues (2008) analysed cow bones of about 46 months. The present study observed

primary vascular plexiform bone in cows and pigs with no Haversian structures while the other studies recorded Haversian bone in addition to primary bone. The additional observation of Haversian bone is related to the part of the bone section examined as primary bone is apparent at the periosteal surface as opposed to Haversian tissue that is mainly associated with the mesosteal and endosteal zones (Cuijpers, 2006; Hillier & Bell, 2007). Examinations by Martiniaková *et al*. included observations made not only at the periosteal surface, but also mesosteal area. Similar to this study the authors also observed primary vascular plexiform bone at the periosteal surfaces of cows and pigs (Martiniaková *et al*., 2006a; Martiniaková *et al*., 2007a).

Secondary osteons were recorded in sheep tibiae but not in the femora, where primary vascular longitudinal bone was mainly observed. Similar to what was the case for cow and pig bones, Martiniaková *et al*. (2007a) recorded osteons in femora, as the middle zone of the compact bone was also examined. Measurements recorded in the current study were larger than those noted by the authors and again age differences are proposed to be linked to these dissimilarities. The current study utilised skeletal remains form sheep aged ±8 months compared to those aged 12-15 months at death, examined by Martiniaková *et al*. (2007b).

Haversian canal diameters of donkey femora and tibiae were comparable to values obtained form horses by Albu *et al*. (1990) and Zedda *et al*. (2008) although osteon measurements were somewhat larger than those reported by Zedda *et al*.

Not many publications include quantitative analysis of monkeys and cats. Jowsey (1966) recorded average cat femoral osteon diameters as 163 ± 30 µm, while femoral osteon diameters in the current study were 245.36 ± 40.29 µm. Similarly, osteon diameters of monkeys and dogs in this study were larger than those recorded by Jowsey (Jowsey, 1966). These discrepancies may be attributed to the fact that Jowsey only excluded ellipsoidal osteons, unlike the current study where ellipsoidal osteons were measured. Osteons were only excluded in the current study if they were incomplete, not clearly demarcated by a cement line or if the Haversian canals were not measurable.

Haversian canal measurements of dog femora were comparable to values published by Albu *et al*. (1990). Albu and colleagues reported minimum Haversian canal diameters of 26.30 µm and maximum canal diameters of 69.09 µm, compared to minimum and maximum femoral canal diameters of 29.77 ± 11.63 µm and 46.83 ± 16.55 µm reported in the current study.

This study corroborates findings described by Kenyeres and Hegyi (1903), who recorded larger Haversian canal diameters in humans compared to animals (Harsányi, 1993; Martiniaková *et al*., 2006a). Harsányi (1993) reported that the average Haversian canal diameters for humans are about 60 µm (Harsányi, 1993), while Schranz (1968) recorded average values of 80 µm (Fazekas & Kósa, 1978). The present study could not confirm either of these reports as Haversian canal diameters of 60 µm or above were recorded for old world monkeys and *Homo sapiens sapiens*. Harsányi (1993) also reported that Haversian canal diameters of 40 µm or less are not often found in humans (Harsányi, 1993). Likewise Sauer and Lackey (2000) stated that

Haversian canal diameters smaller than 50 µm indicate nonhuman remains (Martiniaková *et al*., 2006a; Martiniaková *et al*., 2006b).

A number of factors have been identified that alter the general appearance, shape and size of histological structures. Thus, combing qualitative and quantitative analysis may increase accuracy and ease of separation (Martiniaková *et al*., 2006a).

5.2.4. Histological separation of human and non-human remains

Various bones types and histological structure sizes have been recorded in the different species examined in the current study. These variations are often confusing and complicate the separation of human and non-human remains. To assist in the identification or exclusion of human remains a table (**Table 5.2**) summaring observations made in the current study was included.

Primary vascular plexiform bone was mostly observed in non-human remains, however, primary vascular bone was mostly observed in combination with Haversian bone and thus emphasise the importance of quantitative measurements. Results from the current study suggest that osteon diameters less than 300 µm and Haversian canal diameters less than 60 µm are indicative of non-primate osseous tissue as osteon and Haversian canal diameters recored, were rarely below 250 µm and 60 µm.

In general, this study identified quantifiable differences between primates and other mammalian species and similar to what was proposed by other authors combining qualitative and quantitative examinations is recommended (Martiniaková *et al*., 2006a; Hillier & Bell, 2007; Zedda *et al*., 2008).

Table 5.2 *Summary of qualitative and quantitative characteristics of human and non-human bones (current study).*

	Qualitative characteristics		Quantitative characteristics			
			Haversian canal sizes		Osteon sizes	
Animal	**Femora**	**Tibiae**	**Femora**	**Tibiae**	**Femora**	**Tibiae**
Cats *(Felix catus)*	Dense Haversian bone; also primary vascular longitudinal; areas of avascular bone	Dense Haversian bone; areas of avascular bone	<40 µm	<60 µm	<250 µm	>250 µm
Dogs *(Canis familiaris)*	Irregular Haversian bone; also primary vascular laminar; areas of avascular bone	Dense Haversian bone	<40 µm	<40 µm	<250 µm	<250 µm
Cows (*Bos taurus*)	Primary vascular plexiform bone	Primary vascular plexiform bone	-	-	-	-
Sheep (*Ovis aries*)	Primary vascular longitudinal bone	Primary vascular plexiform; irregular Haversian bone	-	<60 µm	-	>250 µm
Donkeys (*Equus africanus asinus*)	Primary vascular longitudinal; irregular Haversian bone	Dense Haversian bone tissue	<60 µm	<60 µm	>250 µm	>250 µm
Impala (*Aepyceros melampus*)	Primary vascular longitudinal bone	Primary vascular longitudinal bone; dense Haversian bone	-	<60 µm	-	<250 µm
Pigs (*Sus scrofa domestica*)	Primary vascular plexiform bone	Primary vascular plexiform bone	-	-	-	-
Old world monkeys (*Cercopithecinae*)	Avascular bone; Irregular Haversian bone	Dense Haversian bone	>80 µm	>60 µm	>300 µm	>300 µm
Humans - adult *(Homo sapiens sapiens)*	Irregular and dense Haversian bone	Dense Haversian bone	>80 µm	>80 µm	>300 µm	>300 µm
Humans -juvenile *(Homo sapiens sapiens)*	Irregular Haversian bone	Irregular Haversian bone	>80 µm	>60 µm	>300 µm	>300 µm

CHAPTER 6

CONCLUSION

1. Microelement analysis illustrated some statistically significant differences in the chemical composition of the long bones of the various feeding groups. This included significant differences between omnivores and herbivores with regard to potassium, magnesium, chlorine and sulphur levels. Statistically significant differences with regard to potassium, sodium and chlorine levels were also observed between omnivores and carnivores.

2. Subtle differences with regards to chemical composition between femora and tibiae were also noted. For femora significant differences were illustrated between omnivores and herbivores for potassium, calcium, sodium and chlorine levels. Sodium and chlorine levels were also significantly different between omnivores and carnivores while magnesium levels were significantly different between herbivores and carnivores. Tibial potassium, phosphorus, magnesium and sulphur levels were significantly different between omnivores and herbivores while tibial potassium levels were also significantly different between omnivores and carnivores. This illustrates that all bones in the body may not have exactly the same chemical composition.

3. A combined assessment of elements that yielded significant differences between omnivores and herbivores and omnivores and carnivores can potentially be used to separate skeletal elements of omnivores from that of herbivores and carnivores and warrants further research.

4. Some species-specific qualitative characteristics of bone were found. As expected some of these differences, such as the presence of primary bone, particularly primary vascular plexiform bone, may be accurately deployed to separate human long bones from that of animals. Cows and pigs predominantly displayed this bone type, whereas humans had irregular or dense Haversian bone with no primary vascular plexiform bone.

5. Skeletons of animal species that consisted of Haversian bone (irregular or scattered) or a combination of primary and Haversian bone can not be accurately separated from human skeletal remains using qualitative descriptions alone and quantitative examinations are needed.

6. Statistically significant quantitative differences with regard to measured variables were illustrated between all species examined, particularly femoral osteon and Haversian canals diameters. The largest osteon and Haversian canal diameters were recorded in human adults, juveniles and old world monkeys, however a considerable amount of overlap was found between all species examined. The current study illustrated that Haversian canal diameters of 60 µm or more and osteon diameters of 300 µm or more are indicative of primate remains. Further research is thus needed to separate bones from adult and juvenile *Homo sapiens sapiens* and human and other primate species.

7. Unfortunately, due to the small sample size, results remain tentative but indicate future potential for both chemical and histological analysis to

distinguish human from non-human skeletal elements. There is much scope for development in this field, as little is known about the effects of age, sex, health and diet, to name but a few, on both the chemical and histological compositions of human and non-human bones. These and other factors need to be explored further using larger sample sizes and incorporating different skeletal remains from a variety of animal groups.

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APPENDIX A

Data summarizing the chemical compositions (%) of various animal femora

Sample	Feeding group	K	Ca	P	Si	Al	Na	Mg	Cl	S	Zn	Pb	Sr
Cat 1	Carnivores	0.17	67.71	20.89	0.07	0.02	0.26	0.35	0.15	0.13	0.09	10.14	0.03
Cat 2	Carnivores	0.17	74.16	24.18	0.06	0.01	0.33	0.38	0.08	0.22	0.40	0.00	0.00
Cat 3	Carnivores	0.08	70.34	27.47	0.00	0.00	0.25	0.41	0.26	0.09	0.26	0.60	0.24
Cat 4	Carnivores	0.00	73.92	23.16	0.00	0.00	0.34	0.32	0.07	0.04	0.07	2.07	0.00
Dog 1	Carnivores	0.03	71.97	22.22	0.14	0.01	0.24	0.25	0.10	0.22	0.23	4.57	0.03
Dog 2	Carnivores	0.06	71.42	26.75	0.10	0.12	0.43	0.43	0.20	0.28	0.11	0.00	0.08
Dog 3	Carnivores	0.03	72.17	25.91	0.02	0.02	0.39	0.33	0.11	0.18	0.08	0.75	0.00
Dog 4	Carnivores	0.04	71.57	21.81	0.10	0.00	0.29	0.18	0.11	0.21	0.03	5.65	0.00
Dog 5	Carnivores	0.03	72.73	22.81	0.01	0.00	0.29	0.15	0.13	0.22	0.25	3.38	0.01
Dog 6	Carnivores	0.10	71.54	22.52	0.10	0.00	0.22	0.24	0.01	0.41	0.68	4.11	0.06
Cow 1	Herbivores	0.04	58.40	18.61	0.06	0.10	0.32	0.32	0.09	0.27	0.00	21.79	0.00
Cow 2	Herbivores	0.11	74.30	23.68	0.04	0.01	0.36	0.45	0.11	0.12	0.00	0.78	0.04
Cow 3	Herbivores	0.01	71.08	22.87	0.00	0.00	0.29	0.37	0.11	0.08	0.07	5.11	0.00
Cow 4	Herbivores	0.03	72.73	23.87	0.00	0.04	0.27	0.45	0.03	0.18	0.50	1.83	0.05
Cow 5	Herbivores	0.03	73.46	23.71	0.00	0.03	0.42	0.35	0.06	0.13	0.32	1.44	0.05
Sheep 1	Herbivores	0.09	71.88	26.38	0.17	0.00	0.43	0.44	0.07	0.24	0.06	0.23	0.00
Sheep 2	Herbivores	0.07	71.48	26.51	0.12	0.00	0.73	0.48	0.08	0.17	0.12	0.10	0.13
Sheep 3	Herbivores	0.15	71.42	26.62	0.11	0.00	0.50	0.59	0.06	0.12	0.15	0.21	0.08
Sheep 4	Herbivores	0.07	72.14	26.17	0.00	0.00	0.42	0.45	0.09	0.37	0.07	0.23	0.00
Sheep 5	Herbivores	0.05	71.95	26.64	0.01	0.00	0.45	0.42	0.04	0.19	0.19	0.00	0.05
Donkey 1	Herbivores	0.00	75.40	23.17	0.00	0.02	0.31	0.29	0.09	0.00	0.03	0.70	0.00
Donkey 2	Herbivores	0.00	73.33	23.13	0.00	0.01	0.25	0.28	0.11	0.00	0.78	2.12	0.00
Donkey 3	Herbivores	0.04	74.54	23.12	0.07	0.02	0.29	0.22	0.06	0.20	0.41	0.96	0.07
Donkey 4	Herbivores	0.04	72.24	22.05	0.06	0.03	0.30	0.32	0.05	0.27	0.00	4.52	0.14
Donkey 5	Herbivores	0.11	75.12	23.04	0.00	0.01	0.36	0.23	0.19	0.07	0.02	0.77	0.08
Donkey 6	Herbivores	0.09	74.10	22.67	0.00	0.01	0.31	0.25	0.09	0.16	0.02	2.23	0.07

APPENDIX A
(continue)

Sample	Feeding group	K	Ca	P	Si	Al	Na	Mg	Cl	S	Zn	Pb	Sr
Impala 1	Herbivores	0.03	71.52	26.46	0.02	0.00	0.50	0.57	0.11	0.04	0.12	0.61	0.01
Impala 2	Herbivores	0.01	70.82	27.54	0.00	0.00	0.59	0.70	0.11	0.05	0.02	0.17	0.00
Impala 3	Herbivores	0.14	70.96	26.80	0.01	0.00	0.68	0.69	0.13	0.01	0.00	0.58	0.00
Pig 1	Omnivores	0.07	70.79	26.98	0.04	0.00	0.54	0.62	0.02	0.32	0.18	0.42	0.02
Pig 2	Omnivores	0.09	71.21	26.72	0.04	0.00	0.54	0.36	0.00	0.61	0.03	0.38	0.00
Pig 3	Omnivores	0.05	70.77	26.77	0.02	0.00	0.50	0.54	0.11	0.43	0.02	0.78	0.02
Pig 4	Omnivores	0.02	71.55	26.61	0.05	0.00	0.58	0.56	0.15	0.21	0.00	0.27	0.00
Pig 5	Omnivores	0.07	75.27	23.49	0.08	0.00	0.35	0.33	0.05	0.22	0.16	0.00	0.00
Old world monkey 2	Omnivores	0.29	67.48	21.54	0.11	0.00	0.14	0.25	0.22	0.25	0.65	9.05	0.00
Old world monkey 3	Omnivores	0.08	74.52	22.52	0.04	0.14	0.41	0.24	0.21	0.08	0.06	1.65	0.06
Old world monkey 4	Omnivores	0.16	74.81	23.34	0.04	0.00	0.41	0.32	0.20	0.12	0.60	0.00	0.00
Juvenile 1	Omnivores	0.00	72.03	21.64	0.00	0.00	0.29	0.23	0.27	0.04	0.30	5.19	0.00
Juvenile 3	Omnivores	0.19	64.67	20.00	0.08	0.00	0.32	0.42	0.13	0.33	0.05	13.78	0.02
Juvenile 4	Omnivores	0.11	68.94	21.27	0.02	0.11	0.28	0.37	0.11	0.34	0.73	7.71	0.00
Human 1534	Omnivores	0.12	71.93	26.04	0.00	0.00	0.71	0.44	0.33	0.09	0.04	0.26	0.04
Human 1815	Omnivores	0.10	72.46	22.37	0.09	0.01	0.39	0.28	0.18	0.44	0.59	2.77	0.34
Human 1821	Omnivores	0.09	70.81	26.04	0.02	0.09	0.82	0.37	0.35	0.40	0.23	0.77	0.00
Human 2167	Omnivores	0.03	70.95	25.90	0.03	0.02	0.49	0.37	0.21	0.08	0.01	1.56	0.34
Human 2183	Omnivores	0.14	70.28	26.51	0.00	0.01	0.67	0.36	0.55	0.26	0.21	1.02	0.00
Human 2193	Omnivores	0.06	71.37	26.46	0.05	0.00	0.62	0.32	0.24	0.27	0.31	0.31	0.00
Human 2340	Omnivores	0.06	71.53	26.42	0.00	0.08	0.70	0.29	0.14	0.20	0.00	0.58	0.00
Human 2403	Omnivores	0.06	71.61	26.32	0.00	0.03	0.63	0.33	0.26	0.16	0.00	0.48	0.12
Human 2646	Omnivores	0.14	71.11	26.00	0.00	0.20	0.77	0.36	0.44	0.34	0.40	0.23	0.00
Human 2829	Omnivores	0.14	69.33	21.42	0.01	0.07	0.35	0.26	0.16	0.33	1.25	6.63	0.07
Human 2864	Omnivores	0.15	66.77	19.95	0.01	0.14	0.40	0.26	0.23	0.13	0.10	11.80	0.06
Human 3011	Omnivores	0.08	72.01	21.96	0.03	0.04	0.38	0.22	0.12	0.27	0.13	4.74	0.01

APPENDIX A
(continue)

Sample	Feeding group	K	Ca	P	Si	Al	Na	Mg	Cl	S	Zn	Pb	Sr
Human 3051	Omnivores	0.08	70.76	26.83	0.03	0.05	0.80	0.36	0.31	0.00	0.24	0.14	0.40
Human 3095	Omnivores	0.22	71.05	25.93	0.00	0.10	0.71	0.36	0.40	0.08	0.10	0.96	0.11
Human 3543	Omnivores	0.10	72.05	26.14	0.01	0.14	0.71	0.30	0.27	0.25	0.02	0.00	0.00
Human 3566	Omnivores	0.10	71.32	26.12	0.03	0.01	0.84	0.29	0.24	0.29	0.18	0.59	0.00
Human 3713	Omnivores	0.34	70.59	26.33	0.01	0.07	0.57	0.19	0.06	0.05	0.62	1.00	0.15
Human 3727	Omnivores	0.11	71.78	25.84	0.01	0.02	0.60	0.26	0.26	0.29	0.08	0.75	0.00
Human 3931	Omnivores	0.24	71.25	26.53	0.03	0.01	0.51	0.42	0.12	0.03	0.08	0.34	0.43

APPENDIX B

Data summarizing the chemical compositions (%) of various animal tibiae

Sample	Feeding group	K	Ca	P	Si	Al	Na	Mg	Cl	S	Zn	Pb	Sr
Cat 1	Carnivores	0.23	71.36	22.07	0.05	0.04	0.38	0.21	0.03	0.07	0.31	5.20	0.04
Cat 2	Carnivores	0.16	72.91	23.20	0.00	0.07	0.34	0.37	0.12	0.30	0.10	2.43	0.00
Cat 3	Carnivores	0.05	70.38	27.00	0.00	0.00	0.55	0.46	0.21	0.00	0.01	1.12	0.21
Cat 4	Carnivores	0.05	75.14	23.48	0.00	0.24	0.34	0.32	0.12	0.07	0.25	0.00	0.00
Dog 1	Carnivores	0.03	72.83	22.73	0.03	0.26	0.27	0.27	0.08	0.26	0.51	2.71	0.04
Dog 2	Carnivores	0.06	71.66	26.19	0.01	0.00	0.32	0.37	0.05	0.35	0.16	0.82	0.00
Dog 3	Carnivores	0.05	72.02	26.39	0.02	0.00	0.44	0.37	0.17	0.22	0.08	0.23	0.00
Dog 4	Carnivores	0.03	73.23	21.99	0.05	0.00	0.28	0.17	0.05	0.24	0.64	3.30	0.02
Dog 5	Carnivores	0.01	74.65	23.32	0.02	0.00	0.28	0.16	0.06	0.29	0.63	0.56	0.01
Dog 6	Carnivores	0.01	72.48	22.87	0.15	0.09	0.14	0.14	0.24	0.33	0.85	2.70	0.00
Cow 1	Herbivores	0.08	74.42	23.40	0.04	0.00	0.35	0.35	0.13	0.13	0.05	1.02	0.04
Cow 2	Herbivores	0.00	73.24	23.11	0.00	0.00	0.35	0.35	0.07	0.08	0.15	2.64	0.00
Cow 3	Herbivores	0.07	73.17	23.08	0.00	0.00	0.28	0.31	0.09	0.29	0.32	2.39	0.00
Cow 4	Herbivores	0.00	70.71	22.78	0.00	0.03	0.37	0.43	0.04	0.07	0.06	5.50	0.00
Cow 5	Herbivores	0.03	72.41	23.48	0.00	0.06	0.35	0.29	0.06	0.07	0.25	3.01	0.00
Sheep 1	Herbivores	0.02	71.33	26.58	0.05	0.00	0.52	0.49	0.13	0.20	0.08	0.60	0.00
Sheep 2	Herbivores	0.09	70.96	25.97	0.06	0.22	0.62	0.39	0.11	0.13	0.21	1.21	0.04
Sheep 3	Herbivores	0.07	71.03	26.67	0.09	0.00	0.52	0.48	0.08	0.27	0.18	0.52	0.08
Sheep 4	Herbivores	0.07	71.54	26.62	0.00	0.00	0.47	0.50	0.06	0.24	0.03	0.47	0.00
Sheep 5	Herbivores	0.00	71.19	25.98	0.12	0.00	0.53	0.37	0.09	0.29	0.25	1.17	0.02
Donkey 1	Herbivores	0.00	71.59	26.11	0.00	0.03	0.38	0.27	0.10	0.00	0.15	0.71	0.65
Donkey 2	Herbivores	0.01	75.54	23.01	0.00	0.02	0.20	0.25	0.17	0.06	0.62	0.00	0.12
Donkey 3	Herbivores	0.06	71.55	26.64	0.00	0.02	0.56	0.34	0.24	0.03	0.17	0.12	0.28
Donkey 4	Herbivores	0.04	74.21	22.33	0.00	0.06	0.35	0.24	0.09	0.18	0.30	2.21	0.00
Donkey 5	Herbivores	0.04	73.04	22.39	0.00	0.01	0.23	0.22	0.13	0.08	0.59	3.27	0.00
Donkey 6	Herbivores	0.03	73.69	22.58	0.00	0.14	0.35	0.26	0.14	0.13	0.38	2.32	0.00

APPENDIX B
(continue)

Sample	Feeding group	K	Ca	P	Si	Al	Na	Mg	Cl	S	Zn	Pb	Sr
Impala 1	Herbivores	0.04	71.52	26.99	0.00	0.00	0.54	0.54	0.11	0.04	0.00	0.20	0.00
Impala 2	Herbivores	0.00	71.09	27.01	0.00	0.00	0.57	0.57	0.16	0.05	0.20	0.33	0.00
Pig 1	Omnivores	0.14	71.37	26.71	0.00	0.00	0.59	0.44	0.05	0.27	0.06	0.36	0.00
Pig 2	Omnivores	0.07	72.07	26.05	0.01	0.00	0.49	0.54	0.03	0.48	0.25	0.01	0.00
Pig 3	Omnivores	0.13	70.74	26.12	0.06	0.00	0.45	0.48	0.02	0.42	0.00	1.60	0.00
Pig 4	Omnivores	0.10	71.46	26.17	0.00	0.00	0.52	0.48	0.12	0.19	0.09	0.87	0.00
Pig 5	Omnivores	0.08	71.59	22.52	0.02	0.00	0.33	0.39	0.13	0.24	0.03	4.68	0.00
Old world monkey 1	Omnivores	0.13	69.85	22.04	0.06	0.12	0.24	0.17	0.18	0.19	2.58	4.45	0.00
Old world monkey 2	Omnivores	0.09	74.79	23.12	0.03	0.11	0.43	0.30	0.07	0.16	0.83	0.00	0.08
Old world monkey 3	Omnivores	0.09	68.57	21.45	0.01	0.00	0.40	0.16	0.14	0.48	2.09	6.54	0.06
Old world monkey 4	Omnivores	0.15	75.07	22.82	0.06	0.02	0.38	0.41	0.21	0.43	0.38	0.00	0.07
Juvenile 1	Omnivores	0.14	67.88	19.90	0.13	0.00	0.43	0.17	0.44	0.30	0.60	10.02	0.00
Juvenile 2	Omnivores	0.03	72.33	20.07	0.06	0.19	0.23	0.08	0.10	0.82	0.24	5.81	0.02
Juvenile 5	Omnivores	0.16	67.50	20.37	0.06	0.00	0.34	0.28	0.47	0.23	0.04	10.48	0.06
Human 1815	Omnivores	0.14	71.46	21.10	0.07	0.04	0.15	0.19	0.29	0.21	0.65	5.49	0.21
Human 2167	Omnivores	0.12	74.86	22.50	0.09	0.03	0.14	0.07	0.38	0.29	0.14	1.16	0.22
Human 2183	Omnivores	0.08	73.43	22.94	0.00	0.00	0.43	0.22	0.53	0.13	0.13	2.11	0.00
Human 3011	Omnivores	0.29	74.67	22.48	0.00	0.02	0.61	0.28	0.15	0.21	0.00	1.05	0.24
Human 3566	Omnivores	0.09	75.67	23.15	0.00	0.14	0.40	0.14	0.11	0.22	0.07	0.00	0.00
Human 3713	Omnivores	0.19	75.34	22.33	0.02	0.02	0.34	0.13	0.14	0.23	0.30	0.90	0.08
Human 3727	Omnivores	0.01	65.96	19.38	0.07	0.01	0.23	0.15	0.49	0.26	0.64	12.75	0.06

APPENDIX C

Data for total number of osteons (Tot_ost), non-measurable osteons (Non_meas_ost), measurable osteons (Meas_ost), minimum osteon diameter (Min_ost), maximum osteon diameter (Max_ost), minimum Haversian canal diameter (Min_canal), maximum Haversian canals diameter (Max_canal) and total number of non-Haversian canals (Tot_vasc) taken in various animal femora (µm)

Sample	Total_ost	Non_mea_ost	Meas_ost	Min_ost	Max_ost	Min_canal	Max_canal	Tot_vasc
Cat 1	0.33	0.33	0	231.26	257.68	45.25	53.26	1
Cat 2	0	0	0	0	0	0	0	0
Cat 3	0	0	0	0	0	0	0	8.67
Cat 4	5	4.67	0.33	198.71	293.77	15.46	25.91	0
Dog 1	4	3	1	167.19	250.04	36.15	62.6	6.33
Dog 2	0	0	0	0	0	0	0	3
Dog 3	10.67	9	1.67	192.14	239.55	31.04	49.68	1
Dog 4	9	7.33	1.67	192.87	259.18	23.11	36.94	1
Dog 5	6.33	5.33	1	209.16	229.24	17.06	26.88	1.33
Dog 6	11.67	10.33	1.33	200.34	249.11	41.26	58.73	0.33
Cow 1	0	0	0	0	0	0	0	0
Cow 2	0	0	0	0	0	0	0	0
Cow 3	0	0	0	0	0	0	0	0
Cow 4	0	0	0	0	0	0	0	1.67
Cow 5	0	0	0	0	0	0	0	0.67
Sheep 1	0	0	0	0	0	0	0	7
Sheep 2	0	0	0	0	0	0	0	0.67
Sheep 3	0	0	0	0	0	0	0	5.33
Sheep 4	0	0	0	0	0	0	0	5
Sheep 5	0	0	0	0	0	0	0	6
Donkey 1	3.67	3	0.67	238.73	384.87	41.27	53.06	2
Donkey 2	6.33	5.67	0.67	230.67	342.08	48.28	71.55	1.33
Donkey 3	0	0	0	0	0	0	0	2.33
Donkey 4	0.67	0.33	0.33	152.31	241.7	36.2	59.73	6.67
Donkey 5	7.67	5.67	2	187.26	282.06	36.83	46.69	4.33
Donkey 6	0	0	0	0	0	0	0	4.33

APPENDIX C
(continue)

Sample	Total_ost	Non_mea_ost	Meas_ost	Min_ost	Max_ost	Min_canal	Max_canal	Tot_vasc
Impala 1	0	0	0	0	0	0	0	7.33
Impala 2	0	0	0	0	0	0	0	4.33
Impala 3	0	0	0	0	0	0	0	8.67
Pig 1	0	0	0	0	0	0	0	0
Pig 2	0	0	0	0	0	0	0	1
Pig 3	0	0	0	0	0	0	0	0
Pig 4	0	0	0	0	0	0	0	0
Pig 5	0	0	0	0	0	0	0	4.33
Old world monkey 2	0	0	0	0	0	0	0	2.33
Old world monkey 3	0	0	0	0	0	0	0	0.33
Old world monkey 4	3	2.33	0.67	277.28	366.45	92.61	132.77	3.33
Juvenile 1	3	2	1	261.2	316.24	83.76	113.5	0.33
Juvenile 2	3	2	1	320.26	414.39	81.35	106.95	10.33
Juvenile 4	1.67	1.67	0	0	0	0	0	5.67
Adult 1543	6.67	5	1.67	262.8	348.2	64.3	100.52	0
Adult 1815	2.67	2.67	0	0	0	0	0	7.33
Adult 1821	5	3.67	1.33	295.4	444.14	66.63	105.18	0
Adult 2167	7.33	6.33	1	207.79	269.52	61.55	94.84	3.33
Adult 2183	3.33	1.33	2	276.28	363.97	89.05	121.66	0.67
Adult 2193	1.67	0.67	1	415.03	538.46	116.99	156.49	0.67
Adult 2340	2	2	0	0	0	0	0	4.33
Adult 2403	6.67	5	1.67	267.06	379.46	71.18	98.75	0
Adult 2646	6.33	4.67	1.67	234.98	309.96	82.76	115.19	2.67
Adult 2829	6	4.33	1.67	247.67	372.75	54.6	97.05	0
Adult 2864	6.33	4.33	2	300.71	409.42	68.59	88.16	0
Adult 3011	1.67	1.33	0.33	243.22	275.7	105.44	129.78	1.67

APPENDIX C
(continue)

Sample	Total_ost	Non_mea_ost	Meas_ost	Min_ost	Max_ost	Min_canal	Max_canal	Tot_vasc
Adult 3051	5	5	0	0	0	0	0	1
Adult 3095	6	5	1	228.13	283.83	37	53.56	0
Adult 3543	5.67	4.67	1	233.44	319.88	72.56	94.58	0
Adult 3566	6	4.33	1.67	353.63	473.28	94.69	142.24	0
Adult 3713	0	0	0	0	0	0	0	9.33
Adult 3727	2.67	2	0.67	358.79	560.48	101.18	180.6	0.33
Adult 3931	1	0.67	0.33	150.59	185.26	65.26	80.94	4.33

APPENDIX D

Data for total number of osteons (Tot_ost), non-measurable osteons (Non_meas_ost), measurable osteons (Meas_ost), minimum osteon diameter (Min_ost), maximum osteon diameter (Max_ost), minimum Haversian canal diameter (Min_canal), maximum Haversian canals diameter (Max_canal) and total number of non-Haversian canals (Tot_vasc) taken in various animal femora (µm)

Sample	Total_ost	Non_mea_ost	Meas_ost	Min_ost	Max_ost	Min_canal	Max_canal	Tot_vasc
Cat 1	5	4	1	167.7	239.5	29.62	46.95	0
Cat 2	3.33	2.67	0.67	273.33	365.37	110.32	129.72	0
Cat 3	10.67	7.33	3.33	228.75	284.83	45.13	65.68	0
Cat 4	10	6.67	3.33	224.67	325.03	25.54	43.96	0
Dog 1	10.67	7.33	3.33	210.89	305.05	35.76	57.51	0
Dog 2	10	8.67	1.33	144.63	185.41	25.36	44.52	0
Dog 3	12.33	11.67	0.67	222.25	344.75	39.52	56.09	0
Dog 4	10.33	6.67	3.67	193.48	253.19	25.02	35.51	0
Dog 5	6.33	6.33	0	0	0	0	0	0
Dog 6	10	10	0	0	0	0	0	0
Cow 1	0	0	0	0	0	0	0	0
Cow 2	0	0	0	0	0	0	0	1
Cow 3	0	0	0	0	0	0	0	0.33
Cow 4	0	0	0	0	0	0	0	2
Cow 5	0	0	0	0	0	0	0	1
Sheep 1	0	0	0	0	0	0	0	3
Sheep 2	6.33	5.33	1	211.78	283.89	53.36	102.29	0
Sheep 3	0	0	0	0	0	0	0	0
Sheep 4	5	3.33	1.67	214.29	251.63	30.78	51.58	3.33
Sheep 5	6	4.33	1.67	245.79	309.33	29.56	49.03	0
Donkey 1	9	6	3	250.11	312.12	38.29	58.47	0
Donkey 2	11.33	9.33	2	253.97	289.13	39.62	48.11	0
Donkey 3	6.33	6	0.33	368.73	401.75	26.72	52.71	0
Donkey 4	11	9	2	239.11	344.31	38.93	57.86	0
Donkey 5	8.67	8	0.67	302.9	327.94	56.14	68.18	0
Donkey 6	8.67	7.67	1	252.68	367.19	47.86	80.11	0

APPENDIX D
(continue)

Sample	Total_ost	Non_mea_ost	Meas_ost	Min_ost	Max_ost	Min_canal	Max_canal	Tot_vasc
Impala 1	1.00	1.00	0.00	0	0	0	0	12.33
Impala 2	7.67	6.67	1.00	212.20	275.82	42.52	56.29	0
Pig 1	0	0	0	0	0	0	0	0.33
Pig 2	0	0	0	0	0	0	0	3.67
Pig 3	0	0	0	0	0	0	0	1.33
Pig 4	0	0	0	0	0	0	0	1.33
Pig 5	0	0	0	0	0	0	0	1.00
Old world monkey 1	6.67	5.00	1.67	209.64	285.85	67.43	100.59	0
Old world monkey 2	7.33	6.00	1.33	267.06	399.58	50.06	67.92	0
Old world monkey 3	1.67	1.33	0.33	247.91	346.54	80.12	105.97	0
Old world monkey 4	3.67	3.00	0.67	293.44	440.19	68.60	93.45	0
Juvenile 1	2.67	2.33	0.33	276.28	419.49	94.74	141.47	0
Juvenile 2	4.00	3.33	0.67	326.06	450.88	101.66	154.42	0
Juvenile 4	1.67	1.67	0	0	0	0	0	0.67
Adult 1543	7.33	6.00	1.33	287.94	326.92	63.08	85.36	0
Adult 1815	6.33	4.33	2.00	215.24	313.91	74.55	96.55	0
Adult 1821	6.00	5.33	0.67	284.22	377.26	53.12	64.10	0
Adult 2167	8.67	4.33	4.33	294.31	401.63	46.80	66.54	0
Adult 2183	5.67	4.33	1.33	387.53	471.37	92.07	138.38	0
Adult 2193	5.33	5.00	0.33	474.64	557.24	28.27	36.20	0
Adult 2340	3.33	3.33	0	0	0	0	0	0

APPENDIX E

Data summarising the total number of osteons (Tot_ost), total number of non-measurable osteons (Non_meas), total number of measurable osteons (Meas) and total number of non-Haversian canals (Tot_vasc) by the original investigator along with that of the external investigators (µm)

	Primary investigator											
	Initial observations				Repeated observations				External observer			
Sample	Total_ost	Non_meas	Meas	Tot_vasc	Total_ost	Non_meas	Meas	Tot_vasc	Total_ost	Non_meas	Meas	Tot_vasc
2193A	3	2	1	0	3	2	1	0	3	2	1	0
2340B	1	1	0	6	1	1	0	4	1	1	0	5
2646B	10	6	4	2	7	5	2	1	8	6	2	1
2864C	10	6	4	1	8	5	3	0	8	5	3	0
3713A	0	0	0	13	0	0	0	7	0	0	0	9
Donkey Femur 1 A	0	0	0	4	0	0	0	2	0	0	0	3
Donkey Femur 2 B	6	4	2	4	7	6	1	3	8	8	0	4
Donkey Femur 3 B	0	0	0	4	0	0	0	5	0	0	0	5
Donkey Femur 4 C	2	0	2	7	2	1	1	10	2	1	1	11
Donkey Femur 5 A	5	1	4	4	6	2	4	4	5	2	3	3
Impala Tibia 1 B	0	0	0	18	0	0	0	15	3	3	0	15
Impala Tibia 1 C	0	0	0	15	0	0	0	14	0	0	0	15
Impala Tibia 2 A	19	19	0	0	18	17	1	0	17	16	1	0
Juvenile Tibia 1 A	3	2	1	0	3	2	1	0	4	3	1	0
Juvenile Tibia 2 C	3	3	0	1	4	4	0	0	5	5	0	0
Juvenile Tibia 5 A	1	1	0	5	1	1	0	3	1	1	0	2

APPENDIX F

Data summarising the maximum diameter of osteons (Max_ost), minimum diameter of osteons (Min_ost), maximum diameter of Haversian canals (Max_can) and minimum diameter of Haversian canals (Min_can) by the original investigator and external investigators (µm)

	Primary investigator							
	Initial observations				Repeated observations			
Sample	Min_ost	Max_ost	Min_can	Max_can	Min_ost	Max_ost	Min_can	Max_can
Horse T 1 B	231.70	258.98	32.63	57.52	242.54	263.39	36.20	59.73
	278.52	392.16	43.29	71.72	280.63	399.05	47.51	88.74
	251.66	275.49	33.67	45.25	276.28	311.65	40.19	54.00
	322.36	375.65	67.33	97.30	332.76	388.65	76.10	103.67
	192.27	298.91	27.21	45.82	193.08	286.03	30.77	38.05
Horse T 1 C	285.34	298.93	47.20	61.35	283.59	312.07	55.13	69.65
	345.06	390.99	37.31	49.24	364.34	398.33	45.25	52.52
	138.60	180.75	21.05	25.60	163.45	245.47	34.15	57.61
Horse T 2 A	254.54	310.23	47.37	57.61	261.62	307.59	48.19	61.49
	203.53	251.45	15.57	30.72	237.79	255.01	32.23	37.31
	211.49	239.48	39.07	50.97	216.79	244.61	38.01	48.90
Human T 2167 A	223.21	260.53	90.50	127.02	218.23	256.00	90.50	137.66
	209.14	266.31	55.90	65.88	214.09	276.08	46.15	62.75
	189.25	310.59	78.16	92.87	196.90	331.63	77.91	96.39
Human T 2167 B	249.79	515.15	64.85	94.76	255.26	520.15	52.77	93.44
	204.81	216.95	83.34	102.19	220.29	226.95	88.37	104.87

APPENDIX F
(continue)

Sample	External observer 1				External observer 2			
	Min_ost	Max_ost	Min_can	Max_can	Min_ost	Max_ost	Min_can	Max_can
Horse T 1 B	241.80	293.39	45.31	79.48	219.12	263.78	36.38	74.56
	299.24	395.94	51.42	82.17	263.29	386.04	41.79	63.45
	278.40	294.65	44.80	47.51	249.99	263.20	37.14	47.37
	320.37	372.50	80.09	90.95	318.23	372.49	54.78	72.87
	210.39	334.24	31.25	39.95	185.19	284.66	26.09	37.37
Horse T 1 C	282.38	300.00	50.23	52.96	265.15	282.33	52.49	65.78
	382.83	365.18	40.72	55.66	323.42	376.31	48.87	47.06
	174.68	208.24	24.96	29.77	164.14	193.46	23.18	25.60
Horse T 2 A	267.64	271.50	26.67	27.28	247.06	298.42	17.92	28.62
	247.20	258.25	18.11	43.14	230.39	247.99	18.63	34.77
	229.75	230.44	48.57	60.71	223.03	241.26	37.37	45.25
Human T 2167 A	222.50	256.58	113.38	135.26	231.26	236.02	80.15	135.41
	238.72	303.13	69.63	71.78	199.34	276.02	54.45	63.04
	218.09	345.13	80.72	110.54	188.60	313.52	71.24	94.98
Human T 2167 B	246.81	524.20	67.33	100.29	245.20	520.90	53.23	92.87
	220.06	229.75	88.83	91.31	219.61	223.50	81.29	100.60