Demographic differences in exposure to toxic trace elements in urban South Africa during the 20th century

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Abstract

Exposure to toxic elements is a significant threat to public and individual health worldwide. Toxic elements such as heavy metals are associated with increased mortality and morbidity in both men and women and are a substantial contributor to neurological deficits and developmental delay in children. Analysis of skeletal material yields important information regarding exposure to toxic elements in a given population. This project has investigated toxic element exposure in 215 adults living in urban South Africa who died between 1960 and 1999. Exposure to toxic elements, particularly exposure to lead, has significant impacts on human health, even at very low levels. To date, little research has been conducted on human exposure to toxic elements in adult urban South Africans and a clear gap exists regarding toxic element exposure rates during the latter half of the 20th century. Among the primary aims of this research is to address this gap in knowledge and to quantify human exposure to these elements during the apartheid era. Bone element concentration was analysed by ICP-MS to determine the concentration of six elements that are toxic to humans: lead, cadmium, manganese, arsenic, antimony and vanadium. The results of this research demonstrate clear racial divisions in toxic element exposure in all but one element investigated. In the case of lead and cadmium, white males in the sampled population show significantly higher bone element concentrations than either black males or black females. It is surmised that apartheid-era separation of racial groups in regards to residence, occupation and movement within the urban landscape are partly, if not significantly, responsible for these differences in toxic element exposure. Lead exposure is strongly associated with exposure to traffic in urban Pretoria and Johannesburg, which is evident in both the limited environmental data available and the present study. Designated residential areas for white individuals were situated in and adjacent to the central business districts of both cities and are the areas associated with high traffic. Black residential areas were located on the urban periphery, often near industrial areas and mine dumps. The result is a lead exposure pattern by which white individuals in the sampled population yield double the bone lead concentration of black individuals. The wide divide in socioeconomic strata between the black and white population also factors significantly and is an additional result of apartheid policy. For arsenic and antimony, black individuals, particularly females, show significantly higher bone element concentration than white individuals. These elements are strongly associated with acid mine drainage, a form of pollution which results from mining activity. The close proximity of black residential areas to mining activities and the heavy reliance on

contaminated surface water is likely responsible for higher exposure rates to these elements in the black population. This research has established that rates of exposure to toxic elements in urban Transvaal were moderate considering the level of industrial and mining activity in the region and the notably lax environmental regulations in place during the latter half of the 20th century. Despite this, bone element levels, particularly that of lead, cadmium and manganese are within ranges documented to cause negative impacts on human health. It is highly likely, given the bone element concentrations reported here, that these elements caused significant and negative health effects in the sampled population and were a clear threat to overall public health in urban South Africans.

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Common Abbreviations

AMD	Acid Mine Drainage
As	Arsenic
Be	Beryllium
Bt20	Birth-to-Twenty
Ca	Calcium
Cd	Cadmium
CRM	Certified Reference Material
Cu	Copper
Fe	Iron
HCl	Hydrochloric acid
HNO_3	Nitric acid
ICP-MS	Inductively Coupled Plasma Mass Spectrometer
Mg	Magnesium
Mn	Manganese
MMT	Methylcyclopentadienyl manganese tricarbonyl
NIST	National Institute for Standards and Technology (United States)
Pb	Lead
PM	Particulate Matter
Sb	Antimony
V	Vanadium
Zn	Zinc

Publications and conference papers

Hess CA, Cooper, MJ, Smith, MJ, Trueman CJ, Schutkowski H. *In press*. Lead exposure in adult males in urban Transvaal Province, South Africa during the apartheid era. PLoS One.

Hess CA, Smith MJ, Trueman C, Schutkowski, H. 2012. Human exposure to lead and manganese in South Africa during the 20th century. Presented at the 111th Annual Conference of the American Anthropological Association, San Francisco, USA.

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List of Figures

List of Tables

Table of Contents

1 Introduction

The analysis of heavy metals and environmental toxins in the human skeletal tissue are of great importance not only to archaeologists but to historians, environmental ecologists, epidemiologists and forensic researchers. The presence or absence of heavy metals and toxic elements in the skeletal remains of past populations offers great insight into the daily lives of the population. Understanding the significance of skeletal heavy metal concentrations enables researchers to establish the individual life histories, occupation, migration-patterns, socio-economic status and health status of members of historical and pre-historical populations. Such analysis can also be of great use to modern forensic investigators needing to establish the identity of an individual using only skeletal remains (Aufderheide et al. 1985; Aufderheide et al. 1981; Aufderheide et al. 1988; Corruccini et al. 1987; Handler et al. 1986; Turner et al. 2009; Wittmers et al. 2002). This project will investigate the concentrations of the metals lead, cadmium, vanadium and manganese; the metalloids arsenic and antimony; and the essential trace elements (metals) iron, copper, zinc and magnesium in human skeletal material from urban South Africans.

Toxic trace elements and heavy metals cause significant health issues worldwide. Elements such as lead, which is toxic to humans in even small quantities, and is stored for decades in human bone, are known to contribute substantially to chronic non-communicable disease in any given population. Elements such as cadmium, manganese and arsenic can exacerbate existing health conditions such as hypertension and kidney disease. These elements are known to cause neurological damage with chronic exposure. In a developing country such as South Africa, the impact that even low levels of chronic exposure can have on the population is substantial, as the effects of exposure to these elements are often made worse in individuals with marginal health and nutritional deficiencies. Lastly, many of these elements are either stored in bone long enough to become a secondary source of endogenous exposure, where they cause health issues that may persist for years, even decades after exposure. In this way, it is critical to understand how toxic trace element exposure impacted the population in the immediate past, as many individuals born and living during the past 40 years are still alive and still experiencing the ill effects of exposure.

In the case of the current investigation, trace element analysis of historical bone fills a critical gap in understanding human exposure to toxic elements in urban South Africans in

the latter half of the 20th century. Indeed, it is the only way to investigate these exposure rates as the more traditional epidemiological and medical investigations into trace element exposure rates were simply not conducted on the living population of Pretoria or Johannesburg during this time.

The critical and evidence-based assumption of the present research is that bone tissue is an effective post-mortem biomonitor of toxic trace elements and heavy metals in humans, and that important information regarding exposure rates, toxicity, health and disease can be inferred from the element concentrations in bone. The Pretoria Identified Bone Collection is well suited to this research as demographic variables are well documented and because of South Africa's recent history regarding the separation of whole demographic groups both socially and physically, the analysis of this collection enables the tracking of trace element exposure across the social as well as physical landscape.

1.1 Toxic elements worldwide: the silent epidemic

It is estimated that exposure to toxic elements, particularly lead, causes a substantial burden of disease across populations worldwide. A substantial portion of this burden comes not from outright metal poisoning, but from clinical and sub-clinical toxicity that damages individual health and contributes to overall morbidity and mortality in a population. Often, toxic metal toxicity exacerbates pre-existing medical conditions or causes health complications that may have numerous causes, making it difficult to determine whether metal exposure is the root cause of the illness.

Confounding the matter is the fact that across many parts of the world, the extent to which populations are exposed to toxic metals is unknown. For this reason, metal toxicity has been called the "silent epidemic" by epidemiologists (Nriagu 1988). In Africa in particular, the need for epidemiological research on metal exposure is often overshadowed by more immediate and dire public health epidemics such as HIV/AIDS, tuberculosis, malaria and malnutrition, resulting in undiagnosed metal toxicity that may have a profound effect on the population. Over the last 30 years a growing body of research has demonstrated that low-level metal exposure may be as damaging to human health as Western-style high-fat, high sugar diets – with many of the same physical consequences – and yet without the same level of public awareness and concern. Briggs (2003) estimates that as much as nine percent of the global burden of disease is caused by exposure to pollution, much of which takes the form of toxic metal pollution. Moreover, this percentage is likely greater in developing countries such as those in Africa.

1.1.1 Public health and economic impacts of toxic element exposure (or, lead will make you dumb and violent)

This research will focus on a small sub-population of urban residents in South Africa, and as such it is narrowly focused. However the society-wide implications of toxic metal and element exposure are worth discussing as they place individual-level findings into a greater social context. Spurious subject headings aside, the role of heavy metal exposure in shaping the social, psychological, physical and even economic framework of an entire society is very tangible.

In the weeks leading up the submission of this thesis (Winter 2012-2013), there has been much interest in lead exposure by the popular media, following research that has demonstrated a very clear association between violent crime and lead exposure. This research, which has been conducted over the last decade, is striking and very succinctly captures the societal impact of exposure to toxic metals.

The recent popular interest in the relationship between crime and lead is based largely on work conducted over a decade ago by Nevin (2000). Nevin measured IQ and blood lead in US children between 1976 and 1991. He reports that a decline in lead in petrol beginning in the late 1970s tracked significantly with an increase in children's IQ over the same time period. He found a similar correlation between reduction in lead and a reduction in unwed pregnancies and violent crime. Nevin concludes that approximately 90% of the reduction in violent crime in the US can be attributed to the reduction of lead in the environment. Figure 1-1, below, presents one of Nevin's findings regarding the relationship between leaded petrol in the US and rape. The graph is a dramatic indication of the effect of lead on the population, in which rate of rape within the US population correlates significantly with the reduction of lead in petrol, on a 20-year lag (the approximate time it takes for lead exposed children to reach maturity). Other research has shown strong correlations between bone lead and anti-social behaviour and delinquency, which will be discussed in greater detail in the following chapter.

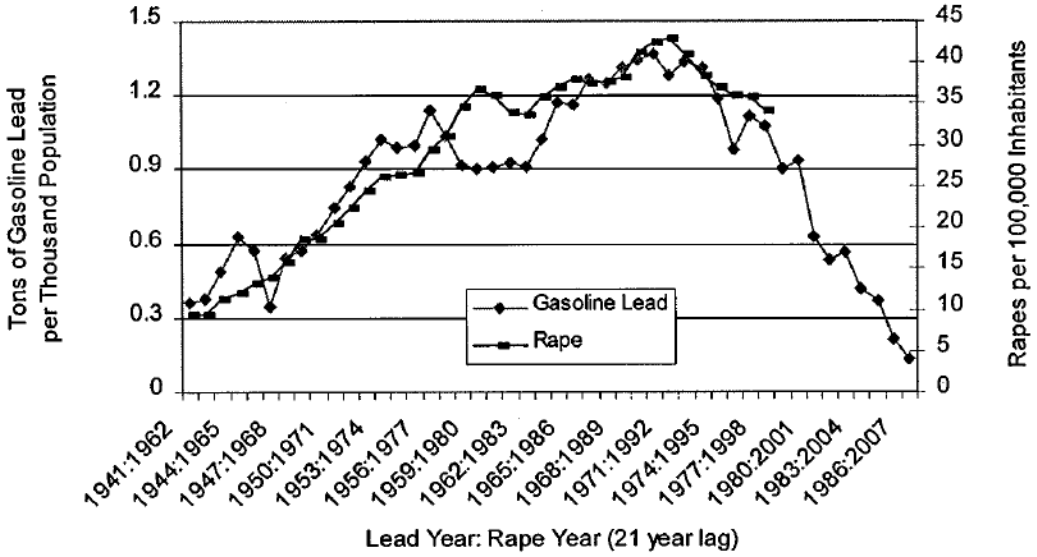


Figure 1-1. Rape and petrol lead in the United States. From Nevin (2000).

The loss of IQ due to lead exposure is a health outcome experienced globally. Fewtrell et al. (2004) have estimated that as much as 13% of all mild mental retardation, worldwide, is caused by lead exposure. This statistic is of value in and of itself, but is compounded by the economic side effects. It is estimated that for each lost IQ point, worker productivity declines by as much as 2.4 %, and that in the US alone, the economic gains associated with the elimination of lead from petrol was as much as $320 billion (Grosse et al. 2002) .

Other toxic metals, such as cadmium and arsenic, cause substantial public health burdens as well. Both elements cause diabetes and renal failure. Chronic exposure to arsenic is linked to cancer, and may affect tens of millions of people worldwide, and is widely considered to be one of the most significant environmental causes of cancer globally (Bhattacharya et al. 2002; Ng et al. 2003). In Asia alone, nearly 50 million people drink water containing arsenic concentration above the minimum concentration recommended by the World Health Organisation (Ravenscroft et al. 2011). Cadmium causes both kidney disease and osteoporosis, much of which occurs at low level exposures (Satarug et al. 2003). Osteoporosis in particular is estimated to represent approximately 1% of non-communicable disease globally, and in some regions cadmium exposure may play a significant role in its onset (Johnell and Kanis 2006).

Despite its role as an essential trace element, manganese is highly neurotoxic at low levels. It is known to cause cognitive impairment and motor impairment in both children and adults. At present, there is a worldwide debate as to whether manganese should replace lead as an octane enhancer in petrol. The use of an organic manganese compounds, MMT

(methylcyclopentadienyl manganese tricarbonyl) as a petrol additive is currently banned in many countries, but its use is common practice in many others worldwide, including South Africa (Okonkwo et al. 2009). Thus in many regions, environmental lead has simply been replaced by another, equally toxic element. Given the significant reductions in the blood lead levels of children worldwide following the removal of lead from petrol, it stands to reason that the use of MMT may result in a similar "silent epidemic" of manganese exposure, with the concomitant social and economic costs (Walsh 2007).

On a global scale exposure to the elements of interest in this research cause significant illness and social and economic disruption. When combined with poverty, deprivation and malnutrition, such as that experienced by most of the population of South Africa during apartheid, the toxic effects of heavy metal and toxic element exposure may be significantly enhanced, causing a greater burden of disease than in developed countries (Briggs 2003). Understanding how this exposure is distributed across a given population is critical to establishing the scale and scope of toxic element-related morbidity. It is hoped that this research will make a contribution to such understanding in South Africa.

1.2 Toxic elements in South Africa

The six toxic elements to be investigated within this project are: lead, manganese, cadmium, arsenic, antimony and vanadium. Among these, lead, cadmium and arsenic comprise three of a group of four elements which Nriagu (1988) refers to as "the big four". These elements are highly toxic to humans, ubiquitous in urban/industrial environments and well-researched in regards to their effects on health. The elements cadmium and arsenic are included in this research for the above reasons and because their presence in the urban environment of Pretoria and Johannesburg is well documented and because they are associated with the mining activities that are prevalent in the region. Manganese is included in this study because manganese has been mined extensively in the southern regions of South Africa and processed in Transvaal/Gauteng. In addition, manganese has recently replaced lead as a petrol additive and environmental manganese levels are expected to increase. By including manganese in this study it is possible to yield data that can be used to investigate change in human exposure to this element over time. Lead is included in this research for several specific reasons. Firstly, the element is extremely toxic to humans in very low environmental concentrations. Secondly, South Africa's late cessation of leaded petrol means lead was present in the environment in significant concentrations in the period of interest in this project (1960-1999).

Though mercury is present in the South African environment and may be of some health concern to the population (Kootbodien et al. 2012; Papu-Zamxaka et al. 2010; Rollin et al.

2009). The analysis of mercury however requires the use of an alternative method to ICP-MS such as Cold-Vapour Atomic Absorption Spectrometry (CV-AAS) (Tyson and Yourd 2004). Moreover, mercury analysis requires the addition of pre-treatment steps with oxidizing agents and a further reduction stage with sodium chloride or potassium borohydride (Morales and Segura 2013). Owing to both time and funding constraints, the additional preparation steps and alternative methods required for mercury analysis resulted in the decision not to include the element in this research.

1.3 The use of racial terms in this research

This research deals extensively with racial differences in toxic element exposure, which necessitates the use of terms which characterise an individual solely on the basis of skin colour. In many fields, particularly in Anthropology and the social sciences, researchers are hesitant to employ the term "race" or to imply that differences in health and health outcomes are purely racial. Within anthropology, specifically, there is a push to debunk racially delineated public health phenomena. Dressler et al. (2005) discuss this at length, in a review of literature regarding racial differences among black and white Americans in regards to low birth rate and high blood pressure. These authors argue against common models that attempt to explain racial differences in health and health outcomes such as genetic differences, health behaviour differences, socioeconomic factors, psychosocial stress and structural constructivism. The authors also purport that the processes and effects of racial differences in outcomes are too complex to be explained by any one model, and that racial differences in health outcomes are more complex than race alone can explain. This is certainly true in integrated societies such as Europe or North America. Marks (1996) argues that classification of individuals into race categories involves cultural knowledge, as opposed to biological knowledge. He further argues that referring to racial issues as such creates a false focus on biology rather than social inequity. These researchers are correct in their assertion that race is largely a social construct, one that is imposed on individuals by society and at the same time one that influences an individual's behaviour because s/he adopts the world-view of the social group, or race, to which s/he is assigned.

Gravlee (2009), concedes that whilst health disparities do exist between individuals of different races, he warns against allowing race to become a biological explanation of these disparities. Human phenotypes are plastic, and can change between generations of individuals of the same "race" or ancestral background, it has been argued, lending credence to the concept that race is a cultural construct. Since the mid-1990s, research into the human genome has emphasised the stunning lack of genetic variation among and between human populations. This has led to the concept of race falling out of favour with anthropologists ,

and could certainly open this particular project to criticism within the field of anthropological theory.

However the terms "black" and "white" are used throughout this project. It is argued here that to do otherwise would be naïve and would overlook very tangible trends in health in apartheid South Africa. Certainly this research does not imply, nor place any significance in theories that imply, that the differences in health and health outcomes between "races" in South Africa are in any way genetic or biological in nature, nor does it imply that the racial classification of the South African population was in any way legitimate. The author agrees wholeheartedly with the concept of race as one that is both imposed on the individual by society and one that shapes the behaviour and cultural construct to which an individual identifies, and it is in this sense and this sense alone, that the concept of race is used in this research. It is for this reason that, unlike apartheid-era literature and some current literature, the terms "black" and "white" are not capitalised. The labels Black and White are politically and emotionally loaded and to continue to capitalise them would lend credence to the view that they are legitimate political labels.

In this and subsequent chapters the terms "black" and "white" will be used to distinguish between groups. These groups are actually cultural and socioeconomic groups but ones that are divided based on skin colour alone. This is because during Apartheid, socioeconomic and racial differences within the population were one and the same. In a sense, when investigating racial differences in health during Apartheid, researchers are examining two distinct populations, as opposed to one diverse national population. Among the lowest socioeconomic groups, there was little mixing between races, and no fluidity between groups. Even the poorest white individuals were legally allowed to live in white residential areas. They did not inhabit the townships or the hostels or the informal settlements surrounding the cities alongside black South Africans, no matter what their economic circumstances. Similarly, black individuals who may have been relatively prosperous could not hope to escape either townships or Bantustans. In this way, race absolutely determined residence, access to healthcare, clean water, diet, and other factors in determining toxic element exposure and health outcome.

1.4 Aims and objectives

Broadly, the overall aim of this project is to establish baseline data regarding human exposure to heavy metals and toxic trace elements in humans in Gauteng, South Africa in the latter half of the 20th century. It is hoped that this data can serve as both historical knowledge in its own right and as comparative data by which to put present-day analyses into context.

In addition, there are several specific objectives:

1. To examine and record demographic differences in element exposure between individuals of African ancestry and those of European ancestry based on analysis of bone element concentration
2. To examine heavy metal and trace element exposure between African men and women
3. To infer the potential health effects on the studied individuals resulting from toxic trace element exposure, given the measured bone element concentrations
4. To analyse the exposure rates of the studied population in the context of published knowledge regarding the state of inorganic pollution in Gauteng, South Africa during the latter half of the 20th century – and to compare these rates with those of other industrialised regions
5. To establish how Apartheid policies regarding the movement and residential arrangements of Africans and the white population contributed to differences in exposure rates to toxic trace elements
6. To compare the bone-element concentration that is gathered in this research to recent studies of element exposure rates in South Africa.

These objectives will be met by the chemical analysis of cortical bone from 215 individual remains from the Pretoria Bone Collection. Analysis was conducted by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Results will be analysed quantitatively to examine relationships between elements of interest and to examine demographic patterns in element exposure. Specifically, each objective will be met in the following way:

1. ICP-MS analysis of cortical bone in black and white individuals to establish bone element concentration of lead, cadmium, arsenic, antimony, and manganese and vanadium. In addition, copper, iron, magnesium, and zinc were measured. Bone element concentration was compared between black and white males and black males and black females across approximately 40 years. Bone element and age was also examined between and within ancestral groups.
2. Bone element concentration for all elements was measured and compared between black males and females.
3. Analysis of bone element concentration in the study population in the context of published data regarding the clinical and subclinical effects of bone element concentration on human health. For each individual element analysed, it is established which members of the study population were most at risk for negative health consequences of element exposure and what those effects were likely to have been. In addition, this objective includes the comparison of essential trace elements

to infer, as best as possible, likely dietary status and how this may have influenced both individual bone element concentration and individual health outcomes. Differences in essential trace elements within and between demographic groups are explored statistically.

4. Using bone element concentration to infer rate of exposure, this is analysed qualitatively and discussed in the context of inorganic pollution in South Africa during the late 20th century. Exposure rates and likely exposure source of the study population is compared against what would be expected given pollution levels in Gauteng.
5. By examining the social and political policies of Apartheid, specifically in the context of the Group Areas Act, and data regarding the distribution of pollution across the landscape during this period, the impact of these policies on toxic trace element exposure is discussed.
6. This objective is met by comparing data on this study population with recent, present-day investigations of toxic element exposure in Gauteng.

1.5 South Africa: Geography, economics and demography

South Africa is located in the southern-most tip of the African continent (Fig. 1-2). It covers an area of 472,281 square miles. It's population is approximately 50 million in 2012, of which 79.4 % are black African, 9.2 % are white, 8.8 % are coloured (mixed race) and 2.6 % are Indian or Asian (Statistics South Africa, 2010).

Figure 1-2. South Africa, present day (google maps).

The skeletal remains used in this study come from individuals who were resident in the Transvaal Province (encompassing the region now known as Gauteng Province) at the time of their death between 1960 and 1998. It is difficult to estimate the population of Transvaal during apartheid. In 1989, the population of South Africa was approximately 30 million, a number which did not include the residents of four of the homelands (see Section 1.2.4, below) which were considered independent nations. The estimated number of black Africans living in South Africa as of 1989 was 21 million. The number of white South Africans was 5.1 million. As black Africans were technically resident in the homelands (even those residing in urban areas) they were largely excluded from the census, and the total black African population of Transvaal is not clear. In Pretoria and Johannesburg, specifically, the total population in 1985 was 4 million in Johannesburg and 2 million in Pretoria. However in greater Pretoria, there were approximately 650,000 informal dwellings housing an estimated 2.5 million people (Smith 1992). In Johannesburg, the number of informal dwellings was similar. The number of black Africans living in Johannesburg by the 1980s

was unclear, but by the 1986 census it was estimated that as much as 25% of the population of the city was black (Parnell and Pirie 1991).

The primary industry in the Transvaal, and in South Africa as a whole is mining. Gold was discovered on the Witwatersrand in Transvaal in the 1860s and the region was once home to the richest gold deposits in the world. In addition to gold, platinum, diamonds and coal are the primary minerals mined in the region, but lead, antimony, uranium, manganese, iron, vanadium and many other elements are mined in South Africa as well (Beck 2000). The country is the wealthiest in Africa, but is classed as a middle income country, primarily due to the level of income inequality that characterises it.

The dependence on mining and associated industries has been a mixed blessing. Whilst South Africa's mineral wealth has brought a certain level of prosperity to some of the population, it has resulted in successive governments that have been reluctant to impose environmental restrictions on industrial polluters (Van Eeden 2008). During apartheid and before, the Anglo American Corporation, founded in 1917 were active in supporting government policy that maintained lax control over the mining industry, most of which was located in or near Transvaal (Thompson 2001). Coupled with South Africa's use of leaded petrol well after most countries had switched to unleaded petrol, the general view of Transvaal is that of a region that is highly polluted. As little environmental monitoring took place in the region during apartheid, the level of human exposure to inorganic pollution during this time is essentially unknown.

1.5.1 **European settlement to apartheid**

The history of South Africa is a typically colonial one, with Dutch settlers arriving in the Cape Colony in 1652 and establishing a trading station for the Dutch East India Company. The following hundred years saw the expansion of Dutch settlement, and the establishment of the cultural dominance of the Afrikaners or "Boers" in the southern half of the region, the Boer expansion included the importation of slaves from the Indian sub-continent and Madagascar, as well as neighbouring Mozambique. In 1795, the British conquered the Cape Colony, which was regained by the Boers in 1805 and lost again to the British in 1806, marking the beginning of nearly 100 years of sparring between the British and the Boers in South Africa, with Afrikaners gaining control of the Transvaal and Free States in 1852 and 1854, respectively, and losing the Transvaal to the British in 1877. The century of tit-for-tat between the British and the Afrikaners culminated in the Boer Wars of 1899 -1902, in which the British were victorious and South Africa formally became a British colony. During this time the native African populations, comprised of distinct tribes and kingdoms, were expelled from ancestral lands prompting uprisings against the colonial powers. These

uprisings ended with the British defeat of the Zulus in 1879 and the Afrikaner defeat of the Venda in 1898 (Thompson 2001).

In 1906, all of the former Afrikaner republics of South Africa were granted parliamentary government by the British, though only white individuals were enfranchised. Four years later, in 1910, the Union of South Africa was formed with the union of the Cape Colony, Natal, Free State and Transvaal (Beck 2000). After the formation of the Union, African reservations were formed, which would later become the basis of the apartheid-era bantustans or homelands. In 1913, the Natives Land Act was passed by the newly formed government which restricted land ownership by Africans to the reserves, at this time the African National Congress (ANC) was formed by black Africans as a response to increasing marginalisation (Thompson 2001).

In the following years, the Union consolidated power within South Africa, removing any powers held by the British. Also during this time, South Africans of British descent or origin held most of the country's wealth and power, relegating many Afrikaners to the status of "poor whites", despite the fact that they comprised over 50 % of the population. Many Afrikaners worked as labourers or engaged in more "working class" employment, in many cases in direct competition with black Africans. Increasingly racist employment practices however, resulted in an improvement of the social and economic conditions of the Afrikaners, and by mid-century, white poverty had largely been eliminated. This period also saw the rise of the so-called "pass-laws", which by 1930, prevented the free movement of Africans within South Africa. In Transvaal in particular, black individuals entering urban areas such as Pretoria or Johannesburg, were required to register with authorities within 24 hours or face jail (Thompson 2001). Despite these attempts to curb African presence in urban areas, the population of black Africans in urban areas increased significantly throughout the 1930s. The increasing marginalisation of the African population of South Africa culminated in the rise to power of the Afrikaner National Party (ANP), which gained control of South Africa during the general election of 1948. Thompson (2001) points out that during the years leading up to the ascendency of the ANP, race and class were nearly one in the same in South Africa, with white individuals relatively well-off compared to the black population. In 1951 the wages of white gold miners were nearly 15 times that of black miners, despite the fact that both black and white miners did essentially the same work.

1.5.2 **Apartheid**

Among the first acts of the new ANP government was the passage of a number of segregation laws including the Population Registration and Group Areas Acts. These acts codified racial segregation in South Africa. The former required the official classification of

all South Africans into one of four racial groups: White, Coloured, Indian and African. The latter enforced strict racial zoning in all parts of South Africa (Beinart 2001). Also at this time the Union was officially decentralised into "nations" which gave the white population complete control over most of the country and relegated the black and coloured populations to one of ten ancestral "homelands" or bantustans, which were essentially reservations. Figure 1-2, below is an apartheid-era map of South Africa showing the white union and the black homelands.

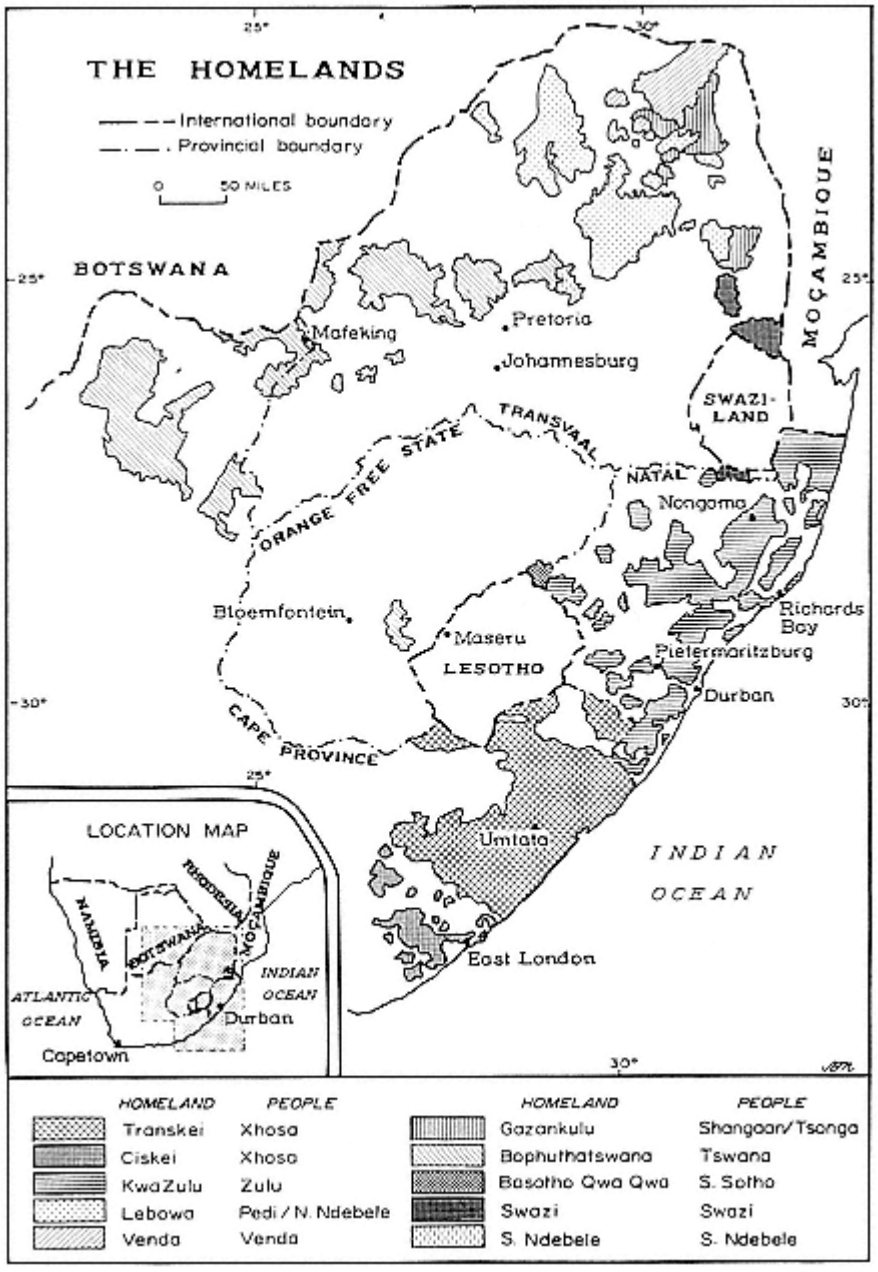


Figure 1-3. Locations of the 10 black African homelands or bantustans. The non-marked areas were white-controlled regions, including Transvaal (upper right) which included Johannesburg and Pretoria. From (Butler et al. 1978).

From the 1960s to the 1980s, many of these homelands were granted "independence" from South Africa, which viewed them as their own distinct countries. Residents of these new "nations" were now denied South African citizenship. As is evident in the figure above, many of these homelands were highly fragmented and were separated in many cases, by privately owned white land. In some cases the different parts of a homeland could be over 100 miles apart. The white population was legally barred from investing in homelands which relied largely on meagre subsidies from the South African government (Thompson 2001).

During this period black individuals were allowed in white-controlled areas and cities, but they were only considered temporary residents and were only allowed insofar as they had legitimate employment. Any black individual who could not provide authorities with the requisite documents proving permission to reside in an urban area was arrested. In one year, 1975-1976, approximately 350,000 such arrests were made. Under the Group Areas Act, black individuals who lived in urban areas were forced into suburban townships. In many cases, black and coloured individuals were forcibly removed to these townships from other parts of the cities. Despite the very permanent nature of these townships, residents were officially classed as temporary, nonetheless, these laws were not able to stem the flow of rural to urban migration (Beck 2000).

The forced residence into townships and homelands saw living conditions deteriorate for black Africans. Compounding this was the intensified exploitation of black labour. By the 1970s mining wages for black Africans were less than what they had been in 1911, in real wages. As will be discussed in greater detail in Chapter 3, conditions in many townships were crowded and extremely poor. A combination of internal rebellion among black Africans, recession and changing attitudes among the Afrikaner ruling class led to the slow disintegration of apartheid throughout the 1970s and 1980s. In 1984, the Union government reformed parliament, creating three racially distinct assemblies – white, coloured and black, which met together in certain circumstances. This marked the first time that black Africans had been given a role in government. Despite this, living conditions for black Africans improved little during this time. By 1986, many of the pass-laws, restricting black migration to and residence in urban areas, were repealed. The Group Areas act remained in place however, and black Africans were restricted in where they could settle in urban areas. Also in 1986 the government repealed many segregation laws, including restrictions on inter-racial marriage, business dealings and sporting activites. However, increasing government resistance, rebellion and violence led to the disintegration of the apartheid government. Despite its continued unravelling, and the government's willingness to engage with the ANC, the period between 1990 and 1994 was characterised by violent suppression of strikes and uprisings and the political oppression of black Africans. In this four year period alone,

there were over 13,500 political killings in South Africa, most of which were perpetrated by the government (Thompson 2001). In spite of its attempts to maintain control, apartheid officially ended in 1994 with the transfer of government power from the Afrikaner nationalists to the ANC.

1.5.3 **Post-apartheid**

The post-apartheid era and the formation of the new South African Republic has brought about many changes, although in many parts of the country little has changed in real terms. At the time of the formation of the new republic, South Africa had one of the most unequal societies on earth, with white individuals living in affluence and Western-style conditions, and black Africans living lives equivalent to most other sub-Saharan Africans – impoverished and poorly educated. In 1997, nearly half of the population of South Africa lived below the poverty line of US $60 per month. The health of black South Africans also remained poor. Though the new government directed some resources towards improving healthcare, many urban hospitals, such as Baragwanath, which served Soweto, were plagued by corruption, crime (including theft and assaults on medical staff). In addition, the epidemic of AIDS was killing nearly 250,000 South Africans per year. The health of urban South Africans was believed to be worsened by exposure to air and water pollution, including the use of leaded petrol, the mining of coal, acid mine drainage and the burning of kerosene in many households (Thompson 2001). Thompson also points out that environmental health was very low on the list of priorities of the new government. In addition violent crime was rampant during the immediate post-apartheid period. By 1998 South Africa had the highest rate of rape in the world and had one of the highest violent crime rates in the world (Thompson 2001).

The post-apartheid period has seen the rise of a black African middle class and greater opportunities for black Africans. The end of the Group Areas Act means that black individuals are legally free to live wherever they can afford to and the demographic backgrounds of many neighbourhoods are changing, though the change is slow.

1.5.4 **Contributions to anthropology and to public health research**

This project provides data regarding exposure to toxic elements in residents of Pretoria and Johannesburg during apartheid. As no such analysis was undertaken or published on this population during this time, this research has generated important quantitative data that was absent from the current body of knowledge regarding pollution and human health in South Africa. This data is valuable both as stand-alone information regarding one facet of human health during apartheid. It also serves to highlight the way in which political and social policies directly affect human exposure to toxic pollution. Lastly, the data presented here

also provides significant background information by which to consider present-day trends in human exposure to toxic elements in South Africa.

Chapter 2 provides a comprehensive review of the elements of interest: lead, cadmium, manganese, arsenic, antimony and vanadium, as well as four essential trace elements: iron, zinc, copper and magnesium. Mean bone element concentrations from 20th century cadaver bone or in vivo measurements are presented for a number of modern populations. This information is used to place the bone element concentrations reported in this project into a wider global context. The process of incorporation of these elements into bone tissue is discussed. More importantly, the known health effects of these elements are discussed in detail, and wherever possible, the specific bone-element concentration at which health effects occur is discussed. This information allows for the formulation of hypotheses regarding the likely effects of toxic element exposure in the study population.

Along with quantitative analysis of toxic elements in bone tissue, this research presents a detailed discussion of the demographic and health consequences of apartheid, which is given in Chapter 3. This information is critical to a contextual understanding of how toxic element exposure was distributed across the population, particularly between black and white individuals. In addition, the health status of black and white individuals differed substantially during apartheid, to the extent that black Africans were living in what was essentially a developing country and white South Africans were living in a Western industrial country. This dichotomy is likely to have played a significant role in the effects of toxic element exposure on each demographic group and is an important factor in understanding the likely health implications of this exposure across the population.

The literature is lacking with regards to environmental studies within South Africa. The substantial mining industry in South Africa, coupled with the country's persistent use of leaded petrol into the current century, have conspired to make South Africa one of the most polluted countries in sub-Saharan Africa. As such, it is important to understand how this pollution affected the population and how official Apartheid policy may have exacerbated the problem of toxic trace element exposure for some or all of the population. Despite the suspicion that the region is highly polluted, little research (relative to other countries) has been conducted on toxic element pollution in South Africa. To make sense of human rates of exposure to inorganic pollution, it is first necessary to discuss the level and distribution of pollution in South Africa, the details of which are presented in Chapter 4. It is also critical to establish the likely sources of and pathways of exposure to inorganic pollutants so that differences in exposure within the population can be better understood. This research includes a discussion of the state of environmental research in South Africa during the latter half of the 20th century. Recent studies of pollution in South Africa, specifically Gauteng are

also discussed and compared with more historical research, in order to provide a complete profile of inorganic pollution in the region.

A review of the literature regarding human exposure to toxic metals in South Africa yields no broad reviews of the state of knowledge. Rather, studies of exposure to inorganic pollutants have been piecemeal. Over the last 20 years, a handful of studies have sought to quantify toxic metal exposure in South Africa. Whilst these have focused on lead and manganese levels in children, some were conducted during apartheid. These studies are explored in detail and are very useful as comparative data by which to compare the results of this research. Chapter 5 includes a detailed synthesis of the current state of knowledge regarding human exposure to inorganic pollution in South Africa, from the earliest monitoring in the 1980s to the present day. This synthesis is critical to placing the results generated within this project into context within South Africa.

Chapter 6 includes a detailed description of the materials and methods used in this research. A demographic profile of the sampled remains is presented, along with a comparison of how the sampled remains compare to the greater bone collection with regards to demography. The sampling method is discussed, including both the method used to determine which remains to sample, as well as the physical sampling method. Analytical method is also presented in this chapter, including early analytical attempts using ICP-AES and the decision to use ICP-MS instead. The theoretical aspects of method development are presented, from choice of digestion acids to the use of internal standards and quality control. Lastly, the statistical methods used to conduct the analysis of results are described, including the justification for the use of each statistic, and the parameters applied (including the treatment of outliers, data distribution and transformation).

Results of the analysis of South African bone material are presented in Chapter 7. Descriptive statistics, including frequencies and distributions are given for each element. Detailed descriptive statistics for each primary demographic group (black males, black females, and white males) are presented as well as descriptive statistics for age, decade of death and city of residence. Multivariate analysis is used to explore potential relationships between individual elements or element clusters are presented, as well as correlation statistics for each element within each demographic group.

Chapter 8 provides a thorough discussion of the results presented in Chapter 7. Each element is discussed individually and the demographic trends uncovered in Chapter 7 are discussed and the likely reasons behind these trends are explored. The potential health effects of each element on the sample population are also discussed. Bone element concentrations reported in Chapter 7 are placed into a wider context and compared with data from other countries. In

addition, the causative factors behind relationships between elements in the study population are explored in detail.

Finally, Chapter 9 concludes by revisiting the overall aim and specific objectives set forth in this chapter and examines how each objective and overall aim was met during the course of this research.

2 Trace elements and health

A review of the literature regarding health and the element of interest to this research yields vast amounts of information. As such, this chapter is certainly not an exhaustive review of the literature as to provide such detail is beyond the scope of this project. Rather, this chapter will review the major health effects of lead, manganese, arsenic, cadmium, antimony and vanadium, and will focus, as much as possible on the most recent research regarding these elements and health. The basic biology of bone tissue and the primary roles and mechanisms of uptake of each element is discussed in brief. The information presented here will serve as the framework for understanding the potential consequences of exposure to these elements on the health of apartheid-era residents of urban South Africa.

Exposure to toxic trace elements such as lead, cadmium, arsenic, antimony and manganese are known to cause a wide array of serious health conditions and can even cause death if ingested in high enough quantities. Nriagu (1988) has written extensively about toxic metal exposure throughout the world and in Africa, has warned of a 'silent epidemic' of clinical and subclinical metal poisoning affecting millions of people worldwide and particularly prevalent in urban regions of developing countries. He writes of the 'big four' toxic elements: lead, mercury, cadmium and arsenic as the four most nefarious metals in regards to their impact on human health, three of which are examined in this project. For elements such as lead and cadmium and arsenic, even low levels of exposure can cause significant health effects and can cause illness affecting multiple organ systems within the human body. Lead has been associated with renal failure, hypertension, neurological damage, psychiatric disorder and psychopathy, as well as osteoporosis, and infectious disease. Cadmium causes severe bone pathology. Arsenic is known to cause hypertension, cancer and Blackfoot Disease, and manganese is a severe neurotoxin. The potential health consequences of each element of interest in this project are discussed in this chapter. In addition, *cortical* bone element concentrations from modern populations worldwide are compared. In all cases, only bone element concentrations from non-buried, non-diagenetically altered bone is included.

Bone concentrations of these elements can vary substantially in modern populations and depend on the demographic profile of the population, nutritional status, exposure rate and sex. Very high ($>200 \mu g \cdot g^{-1}$) levels of lead have been reported in some occupationally or industrially exposed populations, and is very often found in concentrations in parts per million in bone tissue in industrial societies, though in some cases even in industrial societies, bone lead concentration can be lower, in the range of 2 to $10 \mu g \cdot g^{-}1$ (Baranowska et al. 1995; Jurkiewicz et al. 2005). Background lead from local geology can also result in

the uptake of lead and lead is found in very low levels in populations unexposed to lead, generally on the order of parts per billion in bone (generally archaeological populations with little chance of post-depositional diagenesis) (Patterson et al. 1991).

Bone is generally not used for the biomonitoring of arsenic exposure in humans, though exposure rates can be extrapolated using bone tissue. Arsenic is generally found in very low concentrations in the order of parts per billion in cortical bone or can be so low as to be undetected by modern analytical equipment. Concentrations of 0.10 to 0.510$\mu g \cdot g^{-1}$ have been reported in an industrial population (Brodziak-Dopierala et al. 2011; Jurkiewicz et al. 2004; Wiechula et al. 2003).

Cadmium is also found in relatively low quantities in bone tissue, and range in concentration from parts per million to parts per billion. Concentrations of up to 1.5$\mu g \cdot g^{-1}$ have been reported in the bones of individuals living in a highly polluted industrial region (Baranowska et al. 1995). Concentrations as low as 0.035$\mu g \cdot g^{-1}$ have been measured in the skeletal remains of North American Indians (archaeological specimens) who were never exposed to anthropogenic cadmium (Ericson et al. 1991).

Manganese is found in concentrations in parts per billion in human bone tissue. Bone manganese concentrations of between 0.017 and 0.06$\mu g \cdot g^{-1}$ have been measured in industrially exposed populations (Bocio et al. 2005; Garcia et al. 2001).

Antimony is found in concentrations in parts per billion in human bone and is not commonly monitored in bone tissue. Bone antimony concentrations of 0.07 to 0.1 have been reported in a modern industrial population (Lindh et al. 1980).

Table 2-1, below gives comparative cortical bone element concentrations from a number of modern populations worldwide, and whether or not the individuals studied in each were occupationally exposed or living adjacent to an industrial area.

Table 2-1. Mean cortical bone element concentrations in modern populations in $\mu g \cdot g^{-1}$. *Non-occupationally exposed. **Occupationally exposed. [T]Industrially exposed. [TT]Environmentally exposed. [§]No Itai-Itai Disease. [§§]Itai Itai Disease.

Element	Sweden	Europe	Poland	Mexico	Spain	Poland	Poland	Korea	Taiwan	Czech Republic	Japan	UK	Canada	New Zealand	Russia
As	-				0.06	0.18-0.41	0.09-0.16	2.6-3.0	3.6						
Pb	2.85*-15**	19.9*	20-200[†]	11.71[††]	3.71			1.4-1.6	7.1	4.4		9.7 *– 30.9**			2.24
Cd	-		0.4-1.5		0.04			0.13	1.2	.04	0.5[§]-1.9[§§]			0.20*	0.04*
Mn					0.06			0.07	.07	0.110-0.215			0.1* 2.9**	0.36*	0.36*
Sb	0.007 0.015				-					0.005					0.009*
V	-							1.3							
Source	Lindh et al. 1980	Van de Vyver et al. 1988	Baranowska et al. 1995	Hernandez-Avila et al. 1998	Llobet et al. 1998	Wiechula et al. 2003	Brodziak-Dopierala et al. 2011	Yoo et al. 2002	Kuo et al. 2000	Benes et al. 2000	Noda et al. 1990	Sommervaille et al. 1988	Pejović-Milić et al. 2009	Casey et al. 1982	Zaichick et al. 2009

Bone tissue is a particularly useful matrix for the study of trace element exposure in animals. The relatively low turnover of bone compared to other tissues means that years, even decades of exposure to trace elements can be easily measured. In adults, human bone is categorized into two types, cortical bone, of which approximately 80 % of the skeleton is comprised, and trabecular bone which makes up the remaining 20 % (Ott 1996). In most simple terms, cortical bone is characterized by its compactness and density, and trabecular bone is considered "spongy" in that it is highly porous (White & Folkens 2005). Bone tissue itself consists of collagen and hydroxyapatite, a form of calcium phosphate. Calcification takes place by way of cells known as osteoblasts, which deposit hydroxyapatite into tissue called osteoid, essentially, pre-mineralized bone matrix. Once calcified, osteoblasts are known as osteocytes, which are the cells that maintain bone tissue (White and Folkens 2005).

2.1 Bone growth and development

All bone used in this research is cortical bone, because it is the primary storage location for many heavy metals entering the body and is the primary source of endogenous release of these metals back into the blood stream. Bone consists of an organic phase, collagen, and an inorganic phase of calcium hydroxyapatite. The inorganic phase is approximately 60% of bone, with 30% organic matrix and 10% water and lipids (Clarke 2008). It is thought that toxic metals and metalloids are incorporated into bone by supplanting calcium in hydroxyapatite (Bronner 1996). These elements have a strong affinity for bone and are incorporated by surface exchange, diffuse exchange or ionic substitution with calcium (Dahl et al. 2001).

Cortical bone, particularly in long bones (of which all of the bones in this study derive), is dense and solid and extends from the periosteal surface of the bone to the marrow cavity or endosteal surface within. Bone growth in cortical bone these takes place in the Haversian canals. After new bone formation is complete, the Haversian canal becomes the conduit by which nutrients and blood reach cortical bone, which is not as metabolically active as trabecular bone (White and Folkens 2005), and are the foci of remodelling activity within the bone tissue. After epiphyseal surfaces have fused and longitudinal growth has completed, most bone modelling and remodelling takes place throughout the Haversian system. Blood vessels, osteoclasts, osteoblasts and nerves are found in the periosteum and endosteum. Most bone growth typically takes place along the periosteal surface, which has a higher rate of new bone formation than the endosteal surface. For this reason, bones tend to become thicker with age and the marrow cavity widens (Buckwalter et al. 1995).

There are two forms of new bone formation, bone modelling and bone remodelling. Bone modelling entails the change in shape of new bone as a response to stress placed on the bone by the muscles. An example of this would be the increase in bone density and muscle attachments commonly seen in individuals who engage in heavy physical labour either occupationally or recreationally (through sports) (Buckwalter et al. 1995). The second form of new bone tissue formation is remodelling, which is the resorption of existing bone tissue and the deposition of new tissue. Remodelling takes place throughout life, and is the form of bone growth most likely to result in the uptake and endogenous release of heavy metals. During remodelling, osteoclasts first breakdown and resorb existing bone, before osteoblasts form new bone. The rate at which this process occurs is relatively stable through adulthood, however in older males and especially in peri- and menopausal women the process may quicken substantially (Buckwalter et al. 1995).

2.1.1 **Molecular processes during bone remodelling**

Bone remodelling and modelling involves the mobilisation of two types of cell: osteoclasts, which resorb bone and osteoblasts, which create new bone. During remodelling, osteoclasts secrete hydrogen at a low pH, which breaks down the mineral phase of bone. These cells also secrete a substance (containing acid phosphatase, cathepsin K, matrix metalloproteinase 9 and gelatinase) which digests the inorganic matrix. This process results in the formation of a Haversian canal in cortical bone (Clarke 2008). By the end of the resorption phase, these canals contain monocytes, osteocytes and cells known as pre-osteoblasts which begin the formation of new bone. The process by which bone formation begins and resorption ends is not known, however it is theorised that the release of a chemical signal (most likely a hormone or growth factor) is responsible. Other studies have suggested that the increase in strain within cortical bone (at the microscopic level) as a result of resorption may result in the activation of osteoblasts (Clarke 2008).

Once the formation phase has been activated, osteoblasts synthesize collagen to create the bone matrix. Osteoblasts also secrete small vesicles that concentrate calcium, phosphate and enzymes that destroy mineralisation inhibitors. At the end of this phase, an osteon is formed. The overall function of bone remodelling is to repair damaged bone (damage in this sense means primarily micro-damage, as opposed to fractures etc.) and calcium and phosphate homeostasis (Clarke 2008).

Homeostasis is maintained by the uptake of calcium, magnesium and phosphorus into the inorganic matrix of bone tissue. In the absence of other competing elements, calcium homeostasis is essentially an isoionic exchange of calcium ions within the matrix (Heaney 2003). This exchange takes place between calcium ions in extra cellular fluid (ECF) and

bone. In heteroionic exchange, the calcium ion in bone is replaced with an ion of another element that mimics calcium in the bone matrix (O'flaherty 1998a). Bone remodelling is a constant process. The kinetics of mineral release and uptake in bone has been modelled by many and varies according to factors which will be discussed in greater detail in the following sections. To give an idea of the *volumes* of elements in question, Heany (2003) estimates that the calcium into and out of bone (formation and resorption) is approximately 6mg/kg (body weight) per day. This is largely based on an estimate of total cortical bone turnover rate of approximately 3% per year (Clarke 2008). Though trace elements will necessarily be cycled in and out of bone in greatly lower volumes, the overall turnover of bone is substantial. This has significant implications for the ability of bone to act as an endogenous source of toxic trace elements, a phenomenon which will be discussed in the following section.

2.1.2 **Uptake of heavy metals into bone- mechanisms**

As mentioned above, heteroionic exchange is thought to be the primary means by which elements such as lead and other elements are incorporated into bone tissue. Research involving lead and uranium tracer isotopes suggest several potential pathways by which these bone-volume-seeking elements enter the bone: surface exchange, deposition with forming bone and slow migration throughout the bone matrix (Leggett 1993). Lead in particular appears to follow the same pattern as calcium in regards to uptake into bone, and may actually compete with calcium for deposition into bone (O'flaherty 1998b). In addition, studies of bone lead in autopsy subjects have shown a significant correlation between bone lead/calcium ratio. The lead/calcium ratio was constant between bone types (tibia, iliac crest and iliac bone) (Van De Vyver et al. 1988).

For elements that are not thought to be bone-seeking or easily exchangeable with calcium in bone matrix, the information regarding the mechanism of incorporation into bone is less clear. For arsenic in particular, there is some data regarding the effect of the element on bone cells, but very little is known about its kinetics in bone tissue. The same is true of antimony, cadmium and manganese, though each of these metals does interact with bone tissue. The protein metallothionein plays a significant role in uptake and metabolism of these metals (Bremner and Beattie 1990; Hogstrand and Haux 1991). Thought the physiological processes which involve metallothionein are still being studied, it is a known intracellular storage location for both essential and toxic elements (George Cherian and Goyer 1978). Studies of the protein in mice have demonstrated that in animals genetically altered to produce high concentrations, and whilst the uptake and distribution of elements such as cadmium remained the same as in normal mice, the excretion rate was increased (Probst et al. 1977). Glutathione, a non-essential peptide, may function as a chelating agent for

elements such as arsenic and cadmium. Glutathione can be synthesized within the human body, but its production is depressed in when nutrition is inadequate (Bray and Taylor 1993; Taylor et al. 1996). Subsequently, poor nutritional status affects the human body's ability to metabolise and excrete certain heavy metals (O'flaherty 1998b).

Many metals and metalloids in the body are protein bound and tend to be chemically labile. Whether or not they are incorporated into the bone matrix depends on chemical factors such as charge and ionic radius. In addition, the uptake of a given element often depends on the presence and concentration of other elements, as some metals inhibit or encourage cellular uptake or the body absorbs a toxic element in the absence of an essential element. These elements do remain in bone tissue in the same way as lead, however a much lower concentrations, as a large percentage of the ingested amount is excreted through urine. For this reason, in living populations, exposure to these elements is often monitored by urine concentration as opposed to blood or bone, with cadmium as one such element. Interestingly, there is very little research that could be located by the author which investigates the potential of any of these elements, save lead, to become an endogenous source of exposure due to bone turnover. It may be that these elements are found in too low concentrations for release from bone to matter, or it may be that this is simply and understudied facet of each element's biokinetics.

As lead is so toxic at even low concentrations, and because it interacts so readily with bone tissue, there is much discussion within the literature about the relationship between bone and lead. For this reason, the biokinetics and effects of lead in bone will be discussed in greater detail than for other elements, about which there is far less information regarding biokinetics.

2.1.3 **Uptake pathways**

For the majority of metals of interest in this study, the primary uptake pathways are inhalation and ingestion via which they are incorporated into the bloodstream and subsequently, into organ tissues (including bone). Worldwide, contaminated food and/or water is responsible for a substantial portion of non-occupational exposure to elements such as arsenic and cadmium (Hutton 1987). Lead and manganese are often inhaled, as they were common additives to petrol during most of the 20th century and are still present in soil and dusts in high-traffic areas worldwide (Frumkin and Solomon 1997; Miguel et al. 1997; Shy 1990; Walsh 2007). Exposure sources and uptake pathways specific to urban Gauteng are discussed at length in Chapter 4. For some elements, manganese in particular, inhalation may be more dangerous to human health due to its more direct introduction to the bloodstream (as opposed to being processed in the digestive tract first). Whatever the uptake

pathway, once entered into the bloodstream, the mechanisms of element incorporation into bone tissue are the same.

2.1.3.1 Manganese

Unlike lead, manganese is an essential nutrient, and is both necessary for health and detrimental to it in high concentrations (Santamaria and Sulsky 2010). Manganese is thought to follow the same kinetic pathway as iron, and whilst manganese is often included in trace element studies of bone tissue, as it is in this project, little is written about manganese-bone interaction and how bone manganese concentration can be interpreted. Moreover, manganese is thought to be essential in bone remodelling, with lower bone remodelling rates evident in manganese-deficient animals, and lower serum manganese concentrations evident in osteoporotic, postmenopausal humans (Odabasi et al. 2008; Strause and Saltman 1987; Strause et al. 1987). It is believed that manganese regulates the formation of the osteoid matrix, in that this process is dependent on glycosyltranspherase, a manganese-dependent enzyme (Gonzalez-Perez et al. 2012).

Studies of bone manganese concentration are few in number, but the use of bone tissue as a biomonitor of manganese exposure is being studied. (Arnold et al. 2002; Aslam et al. 2008; Pejović-Milić et al. 2009; Zheng et al. 2011). Manganese is a bone-seeking element, with approximately 50 percent of manganese stored in bone, making bone useful as a matrix for studying past exposure (Weiss, 2001).

2.1.3.2 Cadmium

Bone cadmium is not a commonly used measurement of cadmium exposure. Bone cadmium has been measured in archaeological populations and in a handful of studies in living populations (Apostoli et al. 2009; Arnay-De-La-Rosa et al. 2003; Bronner 1996; Garcia et al. 2001; Gonzalez-Reimers et al. 2003). Few studies however, have investigated the biokinetics of cadmium uptake into bone, despite the long-standing body of evidence that demonstrates that cadmium exposure causes significant reduction in bone mass, primarily by interfering with osteoblast and osteoclast activity (Akesson 2012; Engstrom et al. 2012a; Lindh et al. 1980). Recent research has suggested that different exposure levels of cadmium affect osteoblasts and osteoclasts differently, with low cadmium exposure could enhance bone resorption by enhancing osteoclastic activity, and inhibit bone formation at high levels through interference with osteoblastic activity (Chen et al. 2009a; Iwami and Moriyama 1993). Cadmium is thought to supress the metabolism of vitamin D (Chalkley et al. 1998).

Some limited studies of animal analogues, primarily rats, have shown that cadmium ions can be found in osteoblasts a short time after injection into the subject, but few studies have

investigated time-dependent kinetics and the possibility of bone turnover releasing low levels of cadmium back into the bloodstream (Bawden and Hammarstrom 1975). Levesque et al. (2008) examined this in greater detail, and concluded that cadmium may follow the same uptake mechanism as calcium and magnesium (Thevenod 2010). This has led to the suggestion that cadmium uptake into osteoblasts may increase when calcium and magnesium levels are reduced. Cadmium interaction with other elements such as zinc and magnesium, in particular, may also affect bone development (Matovic et al. 2010; Noel et al. 2004). It may also inhibit uptake of calcium into bone (Kippler et al. 2009). In a study of women living near a smelting facility in China, bone density was seen to decrease linearly with cadmium excretion, suggesting that cadmium affects bone density in a dose-dependent manner (Kazantzis 2004).

In this research, bone cadmium concentration is considered a means by which to assess exposure to and possible health effects of cadmium. Cadmium concentration will also be investigated in relation to calcium, manganese, lead, magnesium and zinc concentrations to explore potential interactions in bone tissue.

2.1.3.3 Arsenic

As with cadmium, arsenic exposure is not generally measured with bone arsenic concentration, however the presence of the element in bone can provide valuable data regarding exposure, particularly in unburied reference collections such as the Pretoria Collection. In archaeological collections arsenic concentration in bone is often disregarded due to its affinity for uptake into bone post-mortem due to diagenetic processes (Pike and Richards 2002; Rasmussen et al. 2009; Özdemir et al. 2010). Unlike lead, arsenic is not commonly measured in vivo and bone is not a common tissue for biomonitoring. Nevertheless, arsenic does affect bone tissue, and is incorporated into bone tissue as a result of chronic exposure. Arsenic is known to have an affinity for calcium hydroxyapatite, and in fact, hydroxyapatite is a commonly used substance for removing arsenic from water sources (Boisson et al. 1999; Nakahira et al. 2006).

Most studies of arsenic in bone concern arsenic trioxide, as this is a commonly used treatment for cancer as it causes cell apoptosis. Arsenic is thought to cause premature apoptosis of osteoblasts, which inhibits new bone formation (Hu et al. 2012; Tang et al. 2009). Adeyemi et al. (2010) have investigated arsenic biokinetics and have suggested that arsenic, whatever the compound, follows the uptake and absorption pathway of phosphate (PO_4^{-3}) from the digestive tract. Up to 30% of arsenic ingested will incorporate into bone tissue (Adeyemi et al. 2010). Once ingested, arsenic tends to be metabolised into one of several forms of methylated metabolites of arsenic. Essentially, this is a detoxification

response, however methylated arsenic may still be toxic (Tseng 2009). Methylation of arsenic is also known to be nutritionally dependant and requires glutathione (O'flaherty 1998b).

Measurements of bone arsenic concentration in modern populations have involved bones removed from autopsy or biopsy and can be considered analogous to the bone collection used in this project. Like lead, bone arsenic concentration appears to increase with age and correlates to iron, cadmium and manganese concentration (Brodziak-Dopierala et al. 2011; Kuo et al. 2000). Even these studies however, have little comparative material with which to juxtapose results, and data regarding modern bone arsenic concentration is often compared with archaeological data, which is unwise due to reasons discussed above. In this project, bone arsenic concentration will only be analysed against other non-archaeological studies.

2.1.3.4 Antimony

There is little data regarding human absorption of antimony. Yet the element is highly toxic, and in recent years, has been suspected as a factor in Sudden Infant Death Syndrome (SIDS) (Boex et al. 1998). Biomonitoring of chronic antimony exposure is generally conducted through analysis of human hair and nail tissue and the biokinetics is largely unknown. Despite this, it is known that antimony is incorporated into human bone tissue, and its subsequent release, especially in pregnant women, has been associated with neonatal antimony exposure. Uptake through the digestive system is thought to be low, due in part, to the fact that antimony is an emetic agent and generally causes vomiting and diarrhoea when ingested orally, which decreases the amount that is able to be absorbed (Filella et al. 2012). Most antimony is therefore generally inhaled in the form of particulate matter associated with air pollution. How much antimony is absorbed into the body from inhalation is unquantified and unclear. Studies involving the analysis of trace elements in bone do occasionally include antimony and as it is toxic, it is included in this research.

2.1.3.5 Vanadium

Though vanadium is not widely studied in bone tissue, it is considered a bone-seeking element and replaces phosphate in bone apatite (Etcheverry et al. 1984; Facchini et al. 2004). Vanadium seems to be preferentially stored in bone as compared to other tissues (Hansen et al. 1982). Some studies have reported that up to 85% of vanadium stored in the body is found in bone tissue, due in large part to the substitution of VO_4^{3-} for PO_4^{3-}, which appears to occur rapidly (Barrio and Etcheverry 2006).

2.1.3.6 Copper, Iron, Zinc and Magnesium

Heavy metals and trace elements do not act on bone tissue as individual elements. The importance of the interaction between metabolic processes, essential elements and toxic elements cannot be understated. Deficiencies in essential trace elements may significantly increase the absorption and negative effects of heavy metals. In addition, nutritional deficiencies can cause negative effects on bone tissue even in the absence of toxic elements. The four elements copper, iron, zinc and magnesium are all essential trace elements, and are generally not toxic to adult humans, even at higher concentrations. They are included in the present research for four reasons: 1) they are indicative of nutritional deficiencies when found in very low bone concentrations, 2) they are known to interact with toxic elements in bone, 3) some toxic elements follow similar uptake pathways as these elements and bone concentrations may be correlated, and 4) they are critical to bone remodelling processes. The first reason will not be discussed in great detail in the present research as the focus of this project is on toxic elements. However bone concentrations of these elements were measured and are reported here, and will be discussed in relation to their impact on bone tissue and their interaction with toxic trace elements.

Iron

Iron is an essential nutrient that regulates red blood cell production and oxygen transport throughout the body. It is present in in the bone matrix and is likely incorporated there as a result of heteroionic exchange with calcium ions (Bauminger et al. 1985). Iron is also essential to proper bone formation. In animal models, chronic iron deficiency has been demonstrated to inhibit bone formation by interfering with osteocalcin levels and inhibits osteoclast activity (Katsumata et al. 2009; Roodman 2009). When combine with a low-calcium diet, a lack of iron results in greater cortical bone density than lack of calcium alone (Medeiros et al. 2002; Medeiros et al. 2004). The same appears to be true in humans as well. Yanovich et al. (2011) studied female soldiers serving in the Israeli Defence Force, and measured both blood iron levels and stress fractures. The authors found that the prevalence of long bone stress fractures was higher in women who were also suffering from iron deficiency anaemia. Studies of bone mineral concentration and bone mineral density in postmenopausal women also demonstrate a relationship between bone health and iron. Two distinct studies have found a positive correlation between iron intake and bone mineral density in this population (Harris et al. 2003; Maurer et al. 2005). The results of these studies suggest that iron is essential for the uptake of calcium into the bone matrix. It may also be the case that iron is necessary for the proper metabolism of Vitamin D, as iron deficiency is often associated with vitamin D deficiency (Lawson et al. 1999; Mcgillivray et al. 2007). It is known to interact with cadmium, and low blood iron concentration is correlated with

increased cadmium absorption, and Vitamin D may be the link. Studies of dietary Vitamin D and cadmium, found that cadmium uptake and the negative effect of cadmium on bone were increased in Vitamin D deficient animals (Uchida et al. 2010). However the relationship between cadmium and Vitamin D is subject to debate (Engstrom et al. 2009).

Zinc

Zinc is essential to the human body and is found in bone matrix. Bone is the largest tissue repository of zinc in the body (Murray and Messer 1981). Its uptake into bone tissue is likely via ion exchange with calcium within bone apatite (Matsunaga et al. 2010). Like lead, it is believed that the zinc found in bone tissue can act as an endogenous source, supplying other organs and tissues with zinc in times of dietary deficiency (Calhoun et al. 1978; Ohyama et al. 2002). Also similar to lead, bone zinc concentration is considered an adequate measure of zinc status (Bobilya et al. 1994).

Several studies have demonstrated that zinc is essential to bone health. The element plays a vital role in the activity of osteoblasts, though the mechanism is not yet clear, and zinc deficiency is linked to poor bone remodelling and reduction in bone density (Cerovic et al. 2007; Eberle et al. 1999). In menopausal women, and interestingly, in older men, zinc deficiency is linked to a significantly higher rate of bone fracture than in individuals with adequate zinc intake (Elmstahl et al. 1998; Gur et al. 2002; Mir et al. 2007).

Zinc may also interact with copper to decrease bone density. A study of men with a high copper/zinc ratio (high serum copper and low serum zinc) had substantially lower bone density and mortality rate than men with a lower copper/zinc ratio (Gaier et al. 2012).

Magnesium

Magnesium is a serum electrolyte and is critical for human health, and specifically, bone health. Magnesium regulates both mineral and bone metabolism, and comprises and is necessary for the proper function of osteoblasts and osteoclasts (Sojka and Weaver 1995). Within the bone matrix itself, magnesium appears to regulate and inhibit hydroxyapatite crystal formation and 60% of the total body store of magnesium is found in bone tissue (Palacios 2006). Like other trace elements, magnesium ions are substituted for calcium in the bone matrix, though in the case of bone, this occurs preferentially (Laurencin et al. 2011). In the absence of magnesium, the calcium crystals formed are larger and more brittle that otherwise, meaning magnesium regulates the brittleness of bone tissue (Sojka and Weaver 1995). Magnesium is essential for the normal metabolism of other essential trace elements including calcium, zinc, copper and iron, as well as toxic elements such as lead and

cadmium. Deficiency can result in reduced calcium uptake and subsequently, osteoporosis, iron accumulation within the body (to toxic levels) (Johnson 2001).

2.1.3.7 Lead

Because bone acts as a repository for trace elements to which humans are exposed, it is an excellent marker of chronic as opposed to acute exposure (Hu 1998; Hu et al. 1998). As the subsequent sections in this chapter will demonstrate, lead in bone is a biomarker by which to measure long-term lead exposure, but, more importantly, lead in bone is a significant source of lead in itself. This means that lead stored in bone has the potential to be released into the blood slowly as natural remodelling processes take place. Bone lead then, can cause clinical symptoms of lead toxicity years, even decades after the initial environmental exposure (Morrow et al. 2007).

2.1.3.8 Bone lead uptake and concentration

It is estimated that 90% of the lead in the human body at any one point in time is stored in bone tissue (Barry 1975). Lead appears to be preferentially incorporated into bone matrix heteroionically, and at the expense of calcium ions (O'flaherty 1998a). The amount of lead in bone varies in relation to the amount of individual environmental exposure, the rate of bone turnover, age and gender. In humans with no environmental lead exposure, such as the pre-Columbian Southwest American Indians from the Pacific coast of North America (Patterson et al. 1991). These individuals had no known exposure to lead other than that in the natural environment. A mean concentration of $0.013 \mu g \cdot g^{-1}$ was reported in this population. When compared to the bone lead concentrations measured in individuals in Silesia, a highly industrial region of Poland, with an average of $3 \mu g \cdot g^{-1}$ in cortical bone, it is evident that anthropogenic sources of lead have had a substantial effect on human bone lead levels (Jurkiewicz et al. 2005). Lead levels have been recorded in the same region at over $200 \mu g \cdot g^{-1}$ in bone (Baranowska et al. 1995).

These values mean little however, unless placed into the context of human health. It is important to understand the threshold at which bone lead concentration is considered potentially toxic. Studies of in vivo bone lead concentration have established the link between bone lead concentration and a host of associated morbidities. Bone lead levels as low as $5 \mu g \cdot g^{-1}$ have been associated with cognitive impairment in children and adults (Nevin 2000; Van Wijngaarden et al. 2009; Weisskopf et al. 2004b). Additional research has found strong evidence of correlation between bone lead and conditions such as osteoporosis, renal disease and hypertension (Nash et al. 2004; Navas-Acien et al. 2008; Raafat et al. 2012).

2.1.3.9 Past exposure as a source of future exposure: endogenous lead.

Among the most insidious aspects of bone lead is its release into the bloodstream years, even decades after the initial uptake into bone. Before bone lead biokinetics were fully understood, researchers identified a deficit between the amount of lead an individual was exposed to and the amount the same individual excreted. Among the first researchers to investigate the difference between lead intake and excretion was Kehoe et al. (1935) who assumed, in essence, that lead in roughly equalled lead out. Kehoe's study used a "chemical correction" to correct for chemical loss, which was ascribed to analytical factors, but not lead uptake into any tissues. With the increasing use of isotopic tracers to track lead kinetics, this discrepancy became more apparent. Rather than finding any equilibrium between lead intake and excretion, isotope tracer studies demonstrated the existence of lead pools or repositories within the body. By measuring the excretion rate of a specific lead isotope (generally radioactive isotopes such of lead), it was clear that lead did not clear the body quickly and that some lead appeared to not leave the body at all (Rabinowitz et al. 1976). These early tracer studies allowed the development of biokinetic models that could determine specific exchange rates and elimination rates of lead in human tissues, including bone.

2.1.3.10 Lead as endogenous lead source and effects on blood lead

In cortical bone, lead is stored within the inorganic matrix. Unlike lead in blood and other tissues, it is not possible to introduce chelating agents into the bone matrix to rid the body of bone lead. Lead in bone is released only as a result of natural osteoclastic remodelling, which occurs at different rates across an age/sex/health spectrum (Leggett 1993). As bone is remodelled, the lead bound in the matrix is released into the bloodstream. This occurs via the same mechanism that maintains calcium homeostasis between blood and bone.

Rabinowitz et al. (1976) developed a three-pool model of lead intake and excretion after conducting a lead tracer study which identified a very slow disappearance of tracer lead from the bloodstream following cessation of tracer ingestion. The rate of tracer lead disappearance recorded by the authors was far slower than would be expected. The authors concluded that the slow, continuous release of lead into the blood stream from a bone lead pool must be responsible. Other researchers have found similar patterns, along with a "sluggish" response of blood to changes in lead source. In other words, a delay was identified between the ingestion or inhalation of a lead tracer and the dominance of that tracer in the blood stream (Rabinowitz 1991). Essentially, no matter what the acute lead exposure source, endogenous lead will generally account for some proportion of blood lead. After exposure to a given source, it will persist in the blood stream for approximately a month due to endogenous lead pools within the body (Rabinowitz 1998). To account for this when investigating excretion

rates, Rabinowitz et al.'s (1976) three-pool model to explain blood lead concentration vis. a vis. external exposure. This model included inhaled lead, ingested lead and bone lead as three potential sources of blood lead. The model has been expanded to include differences in cortical and trabecular bone, and soft tissues.

2.1.3.11 Half-life of lead in the body

In a study of individuals suffering from chronic occupational exposure to lead, Hryhorczuk et al. (1985) investigated approximately 60 individuals with chronic occupational lead exposure and who were also suffering from lead toxicity. Patients were removed from occupational exposure and administered chelating agents (calcium disodium edentate) to remove lead from the blood. The mean blood lead half-life was 619 days. In addition, workers with longer exposure periods had longer blood lead-half lives. In patients with renal impairment (due to lead poisoning), mean blood lead half- life was 1,907 days. Lastly, the authors note two key findings: 1) a positive association between blood lead half -life and age of the patient and 2) cessation of chelation therapy – even after acceptable reduction in blood lead levels – resulted in a substantial rebound increase in blood lead levels even with no return to occupational exposure. In some cases a return of clinical symptoms of lead exposure returned. Additional research has reported that endogenous lead is found in higher concentrations in blood plasma than in whole blood. This is a critical factor, as it is believed that lead in plasma is more diffusible within body tissues and is more dangerous than lead bound to red blood cells (Cake et al. 1996). This further suggests that endogenous lead is potentially more toxic than exogenous exposure.

2.1.3.12 Quantifying the contribution of bone lead to blood lead concentration

Chronic lead exposure creates an internal supply of lead that continues to release lead into the body long after environmental exposure has been removed or improved. In this way, lead exposure, even acute lead exposure has long term consequences on the health of exposed individuals. In an attempt to quantify the exposure risk associated with bone lead, Rabinowitz et al. (1991) developed a theoretical model that may allow for the approximation of blood lead concentration from bone lead concentration. The equation:

$$C = S(R2 - R1)$$

where *C* is a change in daily bone lead output (µg/day) from a change in bone turnover rate (1 unit/day) and where *R1* is the initial rate and *R2* is the new rate and *S* is the total skeletal lead mass (µg). Using this value of C to quantify the corresponding change in blood lead concentration:

$$B = \frac{C}{(M(RB))}$$

where *B* is the change in blood lead (µg/dL) associated with change in *C*, *RB* is the blood pool turnover rate (1 unit/day), and *M* is the volume of the blood pool.

It is clear that the contribution of bone lead to blood lead concentration is dependent on knowing the rate at which bone remodels, and is dependent therefore on bone type, an individual's age and sex and other factors. The above equations are at best, hypothetical. However, they do illustrate quite succinctly, the potential of bone to serve as an endogenous source of lead within the body, especially in populations with higher rates of bone turnover such as children and osteoporotic adults, and older individuals (as evident in Hryhorczuk et al). This hypothesis is borne out in the data – women, older individuals, those with higher rates of bone turnover do indeed appear to have higher blood lead levels as well, regardless of exposure.

The O'Flaherty Model was devised by Fleming et al. (1999), to test and quantify lead kinetics in smelters with occupational exposure to lead. Similarly, to Rabinowitz and others, the O'Flaherty Model includes bone as an endogenous source of lead, and the authors separate bone into trabecular and cortical bone. But the model also further distinguishes between two types of cortical bone: metabolically active and quiescent. In the adult, remodelling processes affect lead kinetics in metabolically active bone. In quiescent, the authors posit that a slow exchange of calcium and lead ions occurs between bone and blood. In trabecular bone, only metabolic processes occur, as there is no quiescent bone. Taking into account both processes, (metabolic and exchange), The O'Flaherty model suggests that an individual with a tibia lead concentration of 100µg·g^{-1} will experience a continuous endogenous lead release of 16µg/dL.

2.1.3.13 Evidence for bone-turnover related increases in blood lead

Data gathered from the US Third National Health and Nutrition Examination Survey (US NHANES III), conducted between 1988 and 1994 provides the quantitative evidence that would support Rabinowitz' hypothesis. Using NHANES data, Nash et al. examined blood lead levels in 2,575 pre- and postmenopausal women and controlled for factors such as the use of Hormone Replacement Therapy (HRT) and demographic variables (Nash et al. 2004). They concluded that bone density was a significant predictor of blood lead concentration. Pre-menopausal women had a mean blood lead concentration of 1.9µg/dL versus 2.7 and 2.9µg/dL in surgically menopausal and naturally menopausal women. Moreover, women undergoing HRT, which is known to prevent an increase in bone turnover rates, had

significantly lower blood lead concentration than women not on HRT. Femoral bone density was inversely associated with blood lead concentration.

Silbergeld et al. (1988) conducted a similar study on 2,981 women using NHANES II and reported similar results. In this study, the authors made no correlation between bone density and blood lead. Only menopausal status was examined. Notably, both studies using NHANES data found that blood lead levels declined with the number of years since menopause. The authors do not proffer any explanations for this, but it is not unreasonable to suspect that the cause is the decrease in bone lead stores due to bone remodelling. Silbergeld et al. also note that increased bone lead may play a causal role in osteoporosis, due to the calcium interfering characteristics of lead.

These findings have been confirmed in more recent studies in Korea. Using the Korean NHANES (2008-1009), Lee and Kim (2012a) also found that bone mineral density was significantly and inversely associated with blood lead concentration in menopausal women.

2.2 Exposure pathways: the role of particulate matter in health and toxic metal intake

Exposure pathways often have a role in an element's toxicity. Inhaled metals can be particularly detrimental to human health as they are absorbed into the blood stream faster than ingested metals. In adults, who rarely suffer from conditions such as ingested lead from paint, inhalation of metals is often the most prominent exposure pathway. There is a substantial body of evidence that inhaled air pollution is highly damaging to health, though the mechanisms involved, and the determination of which combinations of elements is the most dangerous has yet to be determined (Davidson et al. 2005; Harrison and Yin 2000; Schlesinger and Cassee 2003). Overall exposure to air pollution is one of the major causes of morbidity and mortality worldwide (Lippmann et al. 2000; Ostro and Chestnut 1998; Son et al. 2012). Pope et al. (2002) conducted one of the largest studies of the health consequences of exposure to particulate matter (PM). The authors studied 50,000 individuals and their exposure to PM. They found that for every 10μg/m^3 increase in PM exposure, there was a corresponding 4, 6 and 8% increase in all-cause mortality, cardiopulmonary mortality and lung cancer respectively. These results have been reported by many other authors worldwide (Laden et al. 2000; Patel et al. 2009; Son et al. 2012). Bell et al. (2009) studied the association between hospitalisation and PM in 106 counties in America over a six year period. They report that counties with higher concentrations of PM had higher rates of hospitalisations than other counties, even if the particulate matter concentrations were elevated in the short term.

The effect of PM on health highlights the fact that toxic metals do not act alone in harming human health. Where one element is present, a whole array of toxic and non-toxic elements are generally present as well, some mitigating and some magnifying the effects of other elements. Generally, toxic elements such as lead and manganese occur simultaneously in atmospheric pollution, resulting in a much greater threat to human health than one element acting alone.

2.3 Toxic trace elements and health

2.3.1 **Lead**

Of all the possible environmental poisons humans face, lead is among the most destructive to the human body and also among the easiest to identify in skeletal remains. Humans have been processing lead for several millennia and the metal has been used in tableware, food storage, jewellery, decorations, industrial processes and childrens' toys to name but a few. Since it was first discovered lead has been used substantially in most societies and its use continues today (Knudson and Stojanowski 2008). Whilst there is a minimum blood concentration considered acceptable in humans, there is no concentration that is considered "safe" for humans (Gavaghan 2002). Large-scale studies tracking the health of individuals over time has found that lead exposed individuals have higher mortality rates than non-lead exposed individuals (Lustberg and Silbergeld 2002; Menke et al. 2006).

Bone lead concentration is of particular interest to public health researchers as isotopic studies have shown that, especially in older individuals, up to 60% of blood lead comes from lead that has been sequestered in bone following previous (up to decades) exposure (Barry 1975; Gulson et al. 1995). This phenomenon may be particularly prevalent in women, for whom bone turnover and release of stored lead is higher than in males. Lead may also disrupt calcium and vitamin D-3 metabolism, causing differences in lead deposition and uptake between men and women (Berglund et al. 1999; Vahter et al. 2007). In almost all studies, bone, blood and soft tissue lead concentrations are higher in males than in females. Barry (1975) reports that in all soft tissues in males in which mean tissue lead concentration is $0.2\mu g \cdot g^{-1}$ or greater, mean lead concentration was 30% lower in equivalent tissues in women.

With regards to bone lead and total body lead burden, Barry has calculated that in an adult non-occupationally exposed male, aged 20-29, with a tibia lead concentration of approximately $7\mu g \cdot g^{-1}$ will have a total body lead burden of approximately 63mg. The same study indicated that 90% of total body burden of lead is stored in bone tissue, with 70% of that in cortical bone. Schroeder and Tipton (1968) estimated that in an adult male, aged 70,

with a tibia bone lead concentration of $5\mu g \cdot g^{-1}$ the total body burden of lead is as high as 200mg.

Among the primary disorders caused by lead exposure is neurological disease. Clinical, acute lead poisoning results in peripheral nerve pathology. In severe cases, symptoms include psychosis, confusion and loss of consciousness. Long term chronic exposure to lead may lead to confusion and memory deterioration and recent research has suggested that lead exposure may be a risk factor for the development of Alzheimer's Disease (Jarup 2003; Loef et al. 2011; Prince 1998). Beyond this, long term lead exposure has been linked to Parkinson's disease (Gorell et al. 1999b). Coon et al. (2006) in a case-controlled study found an increased incidence of Parkinson's disease in individuals with high lead concentrations in the calcaneus and tibia.

The relationship between bone lead concentration and bone mineral mobilization has been further established in studies investigating bone disease and lead. Adachi et al. (1998) found an association between bone lead concentration and osteopoenia and Paget's disease. In addition, lead was found to increase osteoclastic resorption in animal models. Adachi et al. (1998) further found that individuals with metabolic bone disease had higher lead concentrations in cortical bone than individuals without bone disease. Release of bone lead into the blood stream may result in lead being absorbed by the body's organs, which could have serious health implications for ostoeporotic women with past lead exposure (Theppeang et al. 2008a).

Whilst lead is known to affect the health of adults, the effects of lead on children and infants is substantial, and it is important to include juvenile and infant bones in any archaeological study of the impact of lead on a population. Lead is able to pass through the placental barrier, and is transferred from mother to child before birth and after through breast milk (Vahter et al. 2007). Lead exposure in children is known to cause diminished intellectual capacity, even at the sub-clinical level (Ronchetti et al. 2006). Much of the damage may come from pre-natal lead exposure. Recent studies indicate that up to 75% of maternal blood lead during pregnancy comes from lead stored in the mother's bone (Ronchetti et al. 2006). In essence this means that the effects of long-term lead exposure are multi-generational; a mother's lifetime lead exposure has a direct impact on the health of her children.

2.3.1.1 Neurological effects of lead exposure

Children

Exposure to lead during childhood has been demonstrated to have permanent negative effects on IQ, behaviour, mood and cognition. The potential link between lead exposure and

children's intelligence was suggested in the 1970s though the link was considered somewhat controversial at the time (Lansdown et al. 1974). Since that time, the link between lead exposure in childhood and diminished intelligence has been established and is considered one of the most nefarious effects of lead on children's health. Most early studies of lead exposure in children focused almost exclusively on acute lead intoxication and the subsequent symptoms, however, the link between lead and brain development was established by the 1940s. Byers (1943) studied 20 children with acute lead poisoning and reported that 19 of these children suffered permanent developmental delays and learning disabilities as a result.

Among the methodological issues confronted by these early studies was the means by which lead exposure was measured, which was primarily blood lead concentration (Brycesmi and Waldron 1974). By the late 1980s, the dangers of long term low level exposure in children were being studied through the use of bone and teeth, as biomarkers of chronic exposure (Needleman and Bellinger 1987, 1991). Needleman and Bellinger (1991) investigated tooth lead concentration and children's performance on an extensive array of cognitive tests. The authors report that the children with the highest tooth lead concentration scored the lowest on measurements of IQ, speech and language, and attention. Moreover, these children displayed the highest levels of non-adaptive (disruptive behaviour and lack of attention) behaviour in the classroom and the degree of non-adaptive behaviour appeared to be dose-dependent based on tooth lead concentration.

Bellinger et al. (1991) studied lead and academic performance in children and controlled for socioeconomic factors by including only children of high socioeconomic status. They found that children whose blood lead concentration was high (greater than 10µg/dL) at age 24 months scored worse on cognitive tests at 57 months than children with lower blood lead concentration. Tests of children at ages 6 and 10 years found that lead exposure at 24 months had significant long-term effects on development and intellectual achievement (Bellinger et al. 1992). Additional research has confirmed these results and suggested that blood lead concentration at 24 month has a more significant effect on IQ than blood lead concentration at subsequent ages (Chen et al. 2005; Nie et al. 2011). These results have led to researchers calling for a the establishment of a threshold limit of 10µg/dL lead in blood as an intervention point (as opposed to a toxicity threshold) (Bellinger 2004). More recently, it has been established that lead affects children's development at even lower levels than described above, indicating that there is no toxicologically "safe" concentration of lead in blood (Bellinger 2011; Jusko et al. 2008; Koller 2004; Needleman 2004; Schwartz 1994a).

In addition, pre-natal lead exposure has also proven to be significant in relation to children's neurological development (Bellinger et al. 1987; Cory-Slechta et al. 2008; Gomaa et al. 2002; Neal and Guilarte 2010; Needleman et al. 1984; Ronchetti et al. 2006; Wasserman et al. 2000). This would indicate that the mother's environment and her bone lead concentration will impact the IQ and neurological development of her children. As bone lead is known to be released from bone into the bloodstream during pregnancy, a woman's past exposure (up to 10 years) prior to pregnancy could be a significant source of pre-natal lead exposure (Gulson et al. 1997; Tellez-Rojo et al. 2004).

Delinquency and lead exposure

Recent research has shown a positive correlation between lead exposure in childhood and antisocial and delinquent behaviour in adolescence and adulthood (Nevin 2000). This relationship manifests itself in higher levels of aggression and impulsiveness among lead-exposed children. Nevin (2000) tracked environmental and blood lead levels in American children and found a correlation between lead and crime statistics, noting that the cessation of leaded gasoline in the US was inversely related to violent crime. Haynes et al. (2011) found a similar relationship between environmental exposure and criminal convictions among teens in Ohio. Whilst there are many confounding variables that would affect this statistic, there has been much research in the past decade that has established a link between delinquency and lead.

Needleman et al. (2002) conducted a case-controlled study of bone lead levels in adjudicated delinquents between the ages of 12 and 16, and compared them with bone lead levels of non-delinquent adolescents of the same age group. The authors found that when controlling for covariates such as race and socioeconomic status, delinquents had significantly and substantially higher bone lead concentrations than non-delinquents at 15µg·g-1 and 1.5µg·g-1 respectively. Other researchers have found similar trends (Abrahams et al. 2011; Liu 2011; Marcus et al. 2010; Nicolescu et al. 2010; Olympio et al. 2009; Olympio et al. 2010). Wright et al. (2008) have found that individuals with higher post-natal blood lead levels and whose mothers had higher pre-natal blood lead levels were more likely to be arrested for criminal activity later in life. Moreover, arrest rates for violent crimes were greater for each 5µg/dL increase in blood lead. Wright has subsequently found evidence that lead exposure in early childhood has permanent effects on adult behaviour, and that when controlling for other confounding variables, lead exposure in childhood can be positively correlated with psychopathy in adulthood (Wright et al. 2009). Lastly, in the months leading up to the completion of this thesis, Guilarte et al. (2012) have reported a positive association between lead exposure and schizophrenia.

These trends have broad implications not just for the field of criminology but for society as a whole (Bellinger 2011; Narag et al. 2009). From a purely social standpoint, even low levels of lead are positively associated with lowered IQ and criminal behaviour, both of which affect economic and social well-being (Landrigan and Goldman 2011; Schwartz 1994b). Research has shown that the economic gains that result from reduced lead exposure are very real (Grosse et al. 2002; Muennig 2009; Pichery et al. 2011; Tsai and Hatfield 2011).

Adults

Lead exposure in adulthood is thought to be equally detrimental to cognitive function. Whilst childhood lead exposure has been associated with decreased grey matter and brain volume, long term, chronic lead exposure has also been associated with decreased cognition and even Parkinson's Disease in adults (Brubaker et al. 2010; Brubaker et al. 2009; Cecil et al. 2008; Coon et al. 2006; Weisskopf et al. 2010). Bandeen-Roche et al. (2009) found a positive correlation between bone lead concentration and hand-eye coordination in older (ages 50 to 70) adults. Additional research has demonstrated a correlation between bone lead correlation, drug abuse and cognitive function (Fishbein et al. 2008).

Schwartz et al. (2007) has suggested that what is currently believed to be normal cognitive decline in adults may actually be the result of cumulative exposure to lead. Investigations into older adults with age-related Mild Cognitive Impairment and Alzheimer's Disease reported positive correlations between both conditions and cortical bone lead concentration (Van Wijngaarden et al. 2009). Anecdotally, Weisskopf et al. (2004a) describe the association between cumulative lead exposure and cognitive impairment in two identical adult male twins, only one of whom had significant chronic lead exposure. That twin showed greater cognitive impairment with age than the non-exposed twin. In a study of over 450 adult men, this relationship between chronic lead exposure (as measured by bone lead concentration) and cognitive impairment was found to be statistically significant (Weisskopf et al. 2004b).

In the oldest males, endogenous release of lead into the bloodstream can cause cognitive decline years after exposure (Weisskopf et al. 2004b). Bandeen-Roche et al. (2009) found a clear association between persistent cognitive decline in terms of hand-eye coordination and verbal learning and memory and tibia lead concentration. The scale of this decline is presented in Fig. 2-1, below.

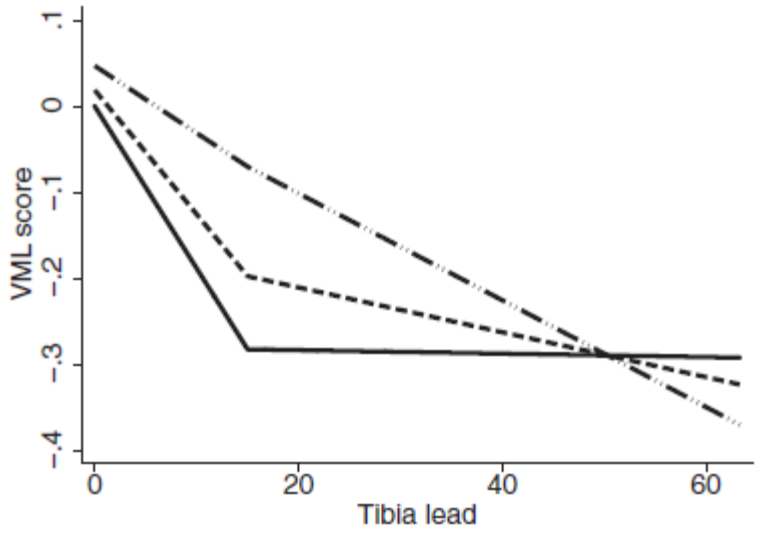


Figure 2-1. Decline in cognitive function with increasing tibia lead concentration. Solid line is baseline decline in function, dashed and dot-dashed lines are first and second follow up visits with the same individuals, demonstrating a persistent decline in cognitive function over time. From Bandeen-Roche et al. (2009).

2.3.1.2 Cardiovascular health and lead

Adults

Lead is known to have detrimental effects on the human cardiovascular system, and is associated with hypertension, in both adults and children, as well as cardiovascular disease (Simoes et al. 2011). Among the strongest evidence of impairment of cardiovascular function is that linking lead exposure with hypertension (Lackland et al. 1992). Interestingly, there does not appear to be a correlation between blood lead and blood pressure, indicating that it may be chronic, as opposed to acute exposure that has the greatest effect on blood pressure (Cheng et al. 2001; Nawrot et al. 2002).

Studies have investigated blood pressure and blood lead concentration, with mixed results (Cheng et al. 2001; Glenn et al. 2006; Nash et al. 2003). Bone lead concentration however, does appear to be associated with an increased prevalence of hypertension. Recent meta-analysis has identified a correlation between bone lead concentration and hypertension (Navas-Acien et al. 2008). Among the ramifications for this trend on the present project, is the possibility of predicting whether or not an individual from the Pretoria Collection suffered from hypertension based on cortical bone lead concentration. Cheng et al. (2001) reported a significant hazard ratio of 1.7 (controlling for age, body mass index and family history of hypertension) between tibia bone lead and hypertension, indicating a causative effect. The authors report that lead levels from the lowest quintiles to the highest quintiles was positively associated with increased incidence of hypertension, suggesting that even low

levels of chronic lead exposure will affect blood pressure. This same correlation has been reported by other researchers (Korrick et al. 1999) . Wedeen (1988) has reported that bone lead concentration as low as 5µg·g^{-1} is associated with an increased risk of hypertension. This same correlation has been reported by other researchers (Korrick et al. 1999).

Other hypertension-related disorders are greater in lead exposed individuals than in non-lead exposed individuals. Hu et al. (1994) have reported an inverse relationship between haematocrit (the percentage of red blood cells in total blood volume) and haemoglobin (the iron-containing metalloprotein in blood which transports oxygen throughout the body) and bone lead, even when blood lead concentration was very low. Any bone lead concentration (from lowest to highest quintiles of bone lead), was associated with decreased haematocrit and haemoglobin. Again, this demonstrates a strong association with chronic lead exposure as measured by bone lead and cardiovascular health. It also underlines the growing consensus that blood lead, whilst an indicator if acute and very recent lead exposure, is not necessarily as strong and indicator of the health effects that occur as a result of lead exposure.

Poreba et al. (2011) have recently reported that in individuals with hypertension, those who were also occupationally exposed to lead had a higher prevalence of left ventricular diastolic dysfunction and an increase in local arterial stiffness, than non-exposed individuals. These two pathologies both affect the heart's ability to efficiently circulate blood.

Children

This relationship also appears to manifest during pregnancy, a particularly dangerous condition. Research has shown that bone lead concentration is a predictor of third-trimester hypertension. Rothenberg et al. (2002) report that in pregnant women, for every 10µg·g^{-1} increase in bone lead, the odds ratio of third trimester hypertension was 1.86. Hypertension in pregnancy can lead to both foetal and maternal mortality. As will been discussed in Chapter 3, black individuals in South Africa already appear to be predisposed to hypertension and maternal mortality is higher than in the white population (Rayner 2010; Stewart et al. 2011b). Chronic lead exposure among women may exacerbate these phenomena. In addition, pre-natal exposure to lead may contribute to high blood pressure in children (Zhang et al. 2012). It has been found that maternal tibia lead concentration is a predisposing factor to hypertension in girls, but not boys. Other research has found a negative association between pre- and post-natal blood lead concentrations and hypertension in children. Most notably, this relationship was significant for blood lead concentrations below 10µg/dL, indicating that even low levels of lead can affect blood pressure in children

aged 9.5 years (Gump et al. 2005). The correlation between blood lead concentration and blood pressure has been found in children as young as 5.5 years (Factorlitvak et al. 1996). As with adults, research examining the blood lead/hypertension relationship yields mixed results. Chen et al. (2006) found no association between blood lead concentration and blood pressure.

Confounding the issue is the relationship between maternal lead burden and birth weight and birth weight and hypertension. There is an inverse relationship between maternal bone lead concentration and birth weight (Gonzalezcossio et al. 1997). In addition, for girls in particular, birth weight appears to be associated with hypertension, though there were differences in the mechanism of the association between girls and boys – in boys, lead appears to affect vascular resistance, and in girls, cardiac sympathetic activation (constriction of blood vessels) (Jones et al. 2008; Loos et al. 2001; Taylor et al. 1997; Te Velde et al. 2004).

2.3.1.3 Other effects of lead

Renal function

Recent research has found a correlation between renal function and lead exposure, though this is currently subject to debate (Evans and Elinder 2011). Several studies have found evidence of renal dysfunction in lead exposed adults. In South Africa, Ehrlich et al. (1998) found a correlation between blood lead concentration and renal function, with an exposure-response correlation between blood lead concentration and renal function. Sun et al. (2008) report similar findings in China.

Lin et al. (2003) have quantified the relationship between renal function and lead in non-diabetic adults. The authors report that for every increase of 100µg total body lead burden, there is a significant associated decrease in the glomerular filtration rate in the kidneys. Van de Vyver et al. (1988) also found increased bone lead concentrations in patients undergoing kidney dialysis.

Oral health

Dye et al. (2002) and Saraiva et al. (2007) using the NHANES III data found a positive correlation between periodontal bone loss and blood lead levels in over 10,000 individuals. The relationship was stronger in men than women. Arora et al. (2009) also found a positive correlation between tooth loss and bone lead in men. Lead has been associated with elevated incidence of periodontal disease, which has recently been discovered to be associated with heart disease (Saraiva et al. 2007).

Bone health

Lead exposure is significantly correlated with bone density in both children and adults (Raafat et al. 2012). In children, lead exposure is associated with significantly reduced bone mineral density and increased skeletal maturity – both conditions which are prevalent in black South African children (Campbell et al. 2004; Hawley et al. 2012). In adults, both osteoporosis and osteopenia are positively correlated with lead exposure in both men and women (Raafat et al. 2012; Theppeang et al. 2008a; Vahter et al. 2002).

2.3.2 **Arsenic**

Arsenic is a highly toxic element often produced as a by-product of copper, silver and lead mining and until recent times, was widely used as a wood preservative and insecticide (Doyle 2009). It has been used medicinally for centuries and is known to have been used by Hippocrates. Arsenic sulphide was used in the 10th century to treat skin lesions, syphilis and haemorrhoids (Lev 2010). Interestingly, arsenic is currently used in medicine in the form of arsenic trioxide in the treatment of leukaemia (George et al. 2004). In most parts of the world arsenic is ingested via contaminated water (Meharg and Raab 2010; Meliker et al. 2006; Rahman et al. 2009; Ratnaike 2003; Tsai et al. 1999). Whilst the effects of ingesting high levels of exposure (approximately 300µg/L in drinking water) on the human body are well known, recent research has focused on the potential health effects of moderate to low rates of exposure (10 to 300µg/L) (Chen et al. 2009b). This research reveals that low to moderate arsenic exposure can cause skin lesions (pre-malignant), hypertension, neurological dysfunctions, and all-cause and chronic disease mortality. The study, of over 20,000 individuals in Bangladesh, also noted that the skin effects of arsenic toxicity can be mediated by adequate intake of selenium and folate.

Chronic arsenic toxicity manifests in almost all of the systems within the human body (Ratnaike 2003). Exposure to arsenic is known to be linked to increased incidence of cardiovascular disease, cancers of the lungs, urinary tract, and gastrointestinal tract (Tollestrup et al. 2003; Tsai et al. 1999). Long term chronic exposure is associated with liver disease and most notably, Blackfoot Disease, a type of peripheral vascular disease in which decreased circulation to the extremities, particularly the feet can lead to gangrene (Hall 2002).

2.3.2.1 Children

Among the potential risks of exposure to arsenic in children is cancer. Investigations into a leukemia cluster among children in the US, as well as an arsenic contaminated copper smelting region, found high levels of arsenic in local water supplies, prompting researchers to suggest a link between the two (Chervona et al. 2012; Moore et al. 2002; Tollestrup et al.

2003; Tsai et al. 1999). Wasserman et al. (2011) found a correlation between cognitive development in children and arsenic exposure, though only at high levels of exposure. In this study the authors report reduced working memory and perceptual reasoning were impaired in children with exposure to high levels of arsenic. Pre-natal exposure to arsenic has been shown to induce liver pathologies and potentially chronic liver disease later in life in animal models, though this link has yet to be examined in humans (States et al. 2012).

2.3.2.2 Adults

Cardiovascular disease and arsenic

Like lead, there is a growing body of evidence to demonstrate that arsenic affects blood pressure and is associated with hypertension (Abhyankar et al. 2012). More importantly, even low level arsenic exposure has been positively correlated with increase prevalence of cardiovascular disease in exposed communities. Gong and O'Bryant (2012) studied a population in rural Texas that is chronically exposed to low levels of arsenic. Controlling for any confounding factors, the authors report that coronary artery disease and hypertension were associated with low-level arsenic exposure (approximately 2 to 15µg/L in drinking water) and the prevalence of these diseases increased with increased exposure to arsenic. Additional research has also found a dose-dependent relationship between hypertension and arsenic exposure (Hossain et al. 2012). This dose-dependent relationship has been reported by Mazumder et al. (2012) in a study of exposed and non-exposed populations in India. Hypertension was significantly associated with exposure to arsenic in drinking water. The exact mechanism of the effect of arsenic on hypertension prevalence (and other diseases) is not clear (States et al. 2011). However recent research using animal analogues suggests that arsenic may increase inflammation in blood vessels and vascular hypertrophy, contributing to heart disease and hypertension (Ma et al. 2012; Sanchez-Soria et al. 2012). The role of arsenic in promoting vascular inflammation has been reported in humans as well (Wu et al. 2012). In addition, arsenic may play a contributing role in prevalence of myocardial infarction (heart attack), though the mechanism is unclear (Afridi et al. 2011b).

Most studies of arsenic and health measure arsenic exposure by investigating the concentration of arsenic in drinking water and associating this value with disease prevalence. Few studies have investigated the association between the concentration of arsenic in human tissues and disease prevalence. Mordukhovich et al. (2012) measured arsenic concentration in human tissue – toenails – and investigated the association between nail arsenic concentration and hypertension. These authors found a significant association between arsenic concentration and increased prevalence of hypertension, with some interaction by manganese.

It is difficult to relate tissue arsenic concentration and pathology, however several studies have measured bone arsenic concentration in exposed and non-exposed populations, and the rate of exposure of a given population can be extracted from this data. Wang et al. (1997) investigated bone arsenic and bone zinc and selenium concentration in patients with Blackfoot Disease and those without. The difference in bone arsenic concentration between the two groups was not substantial, however the difference in bone zinc and selenium were, with lower bone concentrations of these two elements associated with Blackfoot Disease. Like Chen et al. (2009) the authors surmise that zinc and selenium may inhibit the disease. Subsequent research has confirmed the mitigating effect of selenium on acute arsenicosis (Yang et al. 2002). This relationship has also been confirmed in animal models, in which mice supplemented with selenium had lower rates of arsenic induced carcinoma than non-supplemented animals (Stepnik et al. 2009).

2.3.3 **Cadmium**

Cadmium is a highly toxic element that causes a number of pathologies and morbidity in humans (Dokmeci et al. 2009). Most notable among these is the effect of cadmium on human bone tissue and the bone disease *Itai Itai* Disease (literal translation in Japanese: Ouch Ouch Disease), so called because it was first identified in Japan and causes sufferers severe bone pain. Cadmium is also known to cause renal dysfunction and osteoporosis as well as pathologies relating to the prostate gland in men. Unlike lead, for which exposure level is generally monitored by bone or blood concentration, and arsenic, monitored by drinking water concentration, cadmium exposure is measured by urine cadmium output, however some researchers have pointed out that urinary cadmium may be increased as a function of kidney damage and could be misleading (Alfven et al. 2000; Alfvén et al. 2002). It is difficult then to compare studies of bone cadmium to urine cadmium and make meaningful comparisons, as just how urinary and blood cadmium concentration relates to bone concentration is unclear, however it is possible to glean exposure rate from bone based on previous comparative studies. In addition, the very presence of cadmium in bone is an indicator that an individual was exposed to cadmium during life.

There is limited research regarding bone cadmium concentration and pathology. Noda and Masanoku (1990) however measured cadmium concentration in the iliac crest of patients diagnosed with Itai Itai Disease and report a mean bone cadmium of $1.9\mu g \cdot g^{-1}$ compared to $0.5\mu g \cdot g^{-1}$ in controls.

2.3.3.1 Cadmium and Itai Itai Disease

Itai Itai disease is actually a combination of osteomalacia – a chronic softening of the bones due to improper mineralisation – and renal disease. The disease causes intense pain and

increased risk of fractures and was first identified in Japan after World War II (Nordberg 2004). Whilst the individuals in Japanese studies were exposed to fairly high concentrations of cadmium, recent research has demonstrated that the effects of cadmium on bone mineralisation can occur at much lower rates of exposure than previously thought (Alfven et al. 2000; Jarup and Alfven 2004). Prior to the beginning of the 21st century, it was widely held that urinary creatine (an indicator of cadmium excretion) output of 10nmol/nmol (cadmium/creatine) was the minimum or critical threshold for cadmium exposure below which renal disease would not occur. Recent research however has demonstrated that both kidney damage and risk of fracture occurred at much lower concentrations (Brzoska 2012; Brzoska and Moniuszko-Jakoniuk 2004; Jarup and Alfven 2004; Noel et al. 2004). Renal dysfunction occurs in conjunction with low bone density in cadmium exposed individuals. The half-life of cadmium is 10 to 30 years, and most of the bodily burden of cadmium is stored in the kidneys (Dokmeci et al. 2009). Kidney damage manifests in increased urinary protein, a symptom of kidney disease (Jakubowski et al. 1987).

Kazantzis (2004) has reported decreased bone density and increased risk of fracture in women and loss of height in men with exposure as low as 1μg·g-1 cadmium concentration in urine. As discussed previously, the mechanism behind this is the interference between cadmium and calcium and phosphorus in bone mineralisation, as well as the interference between cadmium and the metabolism of vitamin D (Alfven et al. 2000; Chalkley et al. 1998; Christoffersen et al. 1988). Zhu et al. (2004) measured cadmium exposure and bone density among men and women in a cadmium-polluted region of China. The prevalence of osteoporosis in non-cadmium exposed women was 34 % compared to 52% in those exposed to cadmium. Subsequent research has reported similar findings, and cadmium exposure is considered a risk factor in osteopenia, osteoporosis and fracture (Afonso et al. 2009; Brzoska 2012; Brzoska and Moniuszko-Jakoniuk 2004, 2005; Engstrom et al. 2012a, 2012b; Wu et al. 2010). The risk of low bone density and fracture is also more prevalent in men exposed to cadmium (Thomas et al. 2011).

2.3.3.2 Cadmium and cancer

Several studies have reported increases in mortality among cadmium exposed populations. In Japan, China and the United States and Europe, research has demonstrated that cadmium exposed individuals have higher mortality rates in general than the non-cadmium exposed. (Järup et al. 1998a; Kazantzis 1990, 1991; Menke et al. 2009; Nishijo et al. 2004). Whilst some of this increase in mortality is related to kidney disease, the role of cadmium as a carcinogen is also to blame (Järup et al. 1998a). Much of the cancer caused by cadmium is respiratory-tract-related. Jarup et al. (1998) noted that individuals occupationally exposed to cadmium had significantly increased rates of sinus and nasal cancers than the general

population. This research also found an increased risk of lung cancer among the cadmium exposed population, though this was not an exposure- or dose-dependent relationship. Data from the 1970s in the United States estimated that the adult mean daily intake of cadmium was 50µg per day (Fox 1979) and the WHO during the same decade estimated a provisional tolerable intake in humans at 57-81µg/day, above which clinical symptoms of toxicity would occur.

Data from the NHANES III study indicate increase risk of mortality from cancers of the lung and pancreas as well as non-Hodgkins lymphoma in association with urinary cadmium (Adams et al. 2012b). Similar increase in cancers of the lung, ovaries and uterus were found in women in relation to urine cadmium, though the association was weaker. Further research examined a potential effect of cadmium exposure on breast cancer, though no association was found (Adams et al. 2012a; Asara et al. 2012). The association between cadmium and lung cancer is a significant one (Kazantzis 1987, 1989, 1990; Kazantzis and Lam 1986; Park et al. 2012). Kazantzis et al. (1988) conducted a five year study of UK workers occupationally exposed to cadmium and found a substantial and significant increase in incidence of both chronic bronchitis and lung cancer.

2.3.3.3 Cadmium and cardiovascular health

Recent research has demonstrated a clear link between cardiovascular disease and cadmium exposure. Data from the Korean NHANES found that blood cadmium concentration was associated with an increased risk of cardiovascular disease with an odds ratio of 2.1 (Lee et al. 2011). Data from the US NHANES III found an association between urinary cadmium and heart attack. Individuals with urinary creatinine greater than 0.88µg·g-1 had an odds ratio of 1.86 for myocardial infarction compared to individuals with urinary creatinine of less than 0.43µg·g-1. Cadmium is also significantly associated with hypertension. Eum et al. (2008) using the Korean NHANES 2005 data found that hypertensive individuals had significantly higher blood cadmium concentrations that those without hypertension and the relationship was clearly dose-dependent. Similarly, Al-Saleh et al. (2006) report that in Saudi women, individuals with blood cadmium concentrations above 0.627µg/L were nearly four times more likely to be hypertensive than women with cadmium concentrations below this threshold. A 2010 meta-analysis of cadmium and blood pressure confirmed this relationship (Gallagher and Meliker 2010).

2.3.3.4 Cadmium and prostate pathology

Recent research has suggested a link between pathologies of the prostate and cadmium exposure. Zeng et al. (2004) report an increased prevalence of prostate pathology in cadmium-exposed men in China. The authors suggest that an increase in follicle stimulating

hormone in men with prostate pathology (as diagnosed by digital rectal exam) may be caused by hormonal interference by cadmium, which may go on to cause prostate cancer in these individuals. Aimola et al. (2012) have found evidence that cadmium causes apoptosis (cell death) in the epithelial cells of the prostate, suggesting that this may be a causative factor in cadmium-induced prostate cancer. Still, the mechanism behind the relationship between prostate cancer and cadmium remains unclear.

2.3.4 **Manganese**

Manganese is both an essential and a toxic element. It is necessary, in particular for proper development and maintenance of the skeletal and nervous systems. It is also an important antioxidant (Santamaria and Sulsky 2010). The recommended daily adequate intake of manganese in adults and adolescents is between 2.0 and 5.0 mg/day which is obtained entirely through diet (Iom 2001). Generally, manganese absorption is about one to 3% of total dietary intake, the rest is excreted via urine (Boyes 2010; Santamaria and Sulsky 2010). Manganese in excess quantities is a potent neurotoxin, particularly in children. This is particularly true of inhaled manganese. To date, there is little evidence to suggest that toxic levels of manganese can accumulate in the body through ingestion. Numerous studies have been conducted involving the dietary supplementation of adults with manganese, up to 15mg/day, with no adverse effects (Finley et al. 1994). Clinical manganese neurotoxicity, however is evident at airborne manganese concentrations greater than 1.0 mg/m^3, indicating that inhaled manganese affects the body differently, though the mechanism is still unclear (Santamaria and Sulsky 2010). It is also unclear what effect or interaction ingested or dietary manganese has with inhaled manganese. What is known is that inhaled manganese adversely affects neurological, pulmonary and reproductive function in exposed adults and children (Boyes 2010).

Manganese is generally biomonitored in blood, however hair, nail and bone manganese are indicators of chronic exposure (Smith et al. 2007; Sriram et al. 2012; Zheng et al. 2011). Some studies have purported that it is unclear as to the relationship between blood and urine manganese and airborne manganese, however blood manganese concentrations in individuals working in areas with high levels of airborne manganese do tend to be significantly higher than those in low-airborne manganese areas (Lucchini et al. 1999). Though the exposure/tissue uptake may not be clear, it is still possible to biomonitor manganese exposure through human tissue concentration.

Recent research has brought to light the suitability of bone tissue with regards to biomonitoring. As with lead, manganese is stored in bone (though not to the same degree) and the use of bone for assessing long term exposure. Smith et al. (2007) have stressed that

there may be a complex relationship between blood manganese and exposure that may be confounded by the mechanism of exposure or the latency of exposure, rendering blood manganese concentrations difficult to interpret. It is possible that bone manganese is a more accurate measure of chronic exposure than blood, plasma or urine (Aslam et al. 2009; Aslam et al. 2008; Pejović-Milić et al. 2009). Unlike lead, there is no established bone manganese concentration at which concern or intervention is warranted.

Manganese is thought to affect neurological development and the central nervous system by interfering with the function of the neurotransmitter dopamine (Aschner and Aschner 1991; Butterworth et al. 1995; Normandin and Hazell 2002; Verity 1999). Manganese may accumulate in the central nervous system resulting in increasing damage with long-term exposure (Rivera-Mancia et al. 2011). Recent research has demonstrated that both inhaled and ingested manganese has a significant effect on intellectual function in children (Riojas-Rodríguez et al. 2010; Sharma 2006). There is still debate as to the level at which manganese exposure becomes dangerous to children's neurological development and caused impairment. Recent research suggests that manganese levels commonly found in tap water, approximately 34µg/L, is enough to cause a reduction in IQ (Bouchard et al. 2011). Manganese is also known to affect sight and can cause night blindness in exposed children (Afridi et al. 2011c). Manganese is associated with neurological and liver pathologies in adults (Butterworth et al. 1995; Laohaudomchok et al. 2011).

Manganese affects human health at the acute level (manganism), and the chronic, sub-clinical level. Acute exposure is generally characterised by severe neurological disturbance and with Parkinson's like pathology (tremors, loss of coordination, motor skill deficits) whereas chronic exposure can result in milder motor skills deficits and mood disturbances.

2.3.4.1 Manganese neurotoxicity in children

There is substantial evidence regarding the toxic effect of excess manganese on the neurological development of children. Interestingly, much of this research is very recent, and comes on the heels of the WHO's (World Health Organisation) discontinuation of water manganese concentration guidelines. Until 2011, the WHO maintained guidelines recommending an upper limit of manganese at 400µg/L in drinking water (Frisbie et al. 2012). Researchers have suggested that this value was too high, and that damage to individual health occurs at concentrations far below this, as was demonstrated by Bouchard et al. (2011). Rates of exposure as high as 400µg/L have been positively associated with a host of neurological and psychosocial deficits (Bouchard et al. 2007). Kahn et al. (2011) found that schoolchildren with higher rates of exposure were associated with increased incidence of anti-social behaviour including impulsivity, aggression and irritability. Chung

et al. (2011a; 2011b) also report an increased prevalence of Attention Deficit Disorder and behavioural problems among manganese exposed children. In addition, manganese has been shown to affect the motor skills of exposed children (Hernandez-Bonilla et al. 2011; Takser et al. 2003). In infants exposed to manganese in utero, the effects of the element may have long term consequences. The concentration of manganese in the cord blood of newborns was negatively associated with psychomotor function at three and six years of age (Takser et al. 2003). In addition, foetal exposure to manganese may adversely affect birth weight (Zota et al. 2009).

Subsequent studies have found that children consuming water with a manganese concentration in excess of 400µg/L demonstrated a substantial decrease in mathematical aptitude (Khan et al. 2012). Menzes et al. (2011) found that blood manganese concentration and hair manganese concentration are negatively associated with IQ, particularly verbal skills, in children living near a metal smelting plant in Brazil. The neurological effects of manganese have been shown to occur along a continuum of exposure and severity. Lower exposure rates are associated with neurological and cognitive pathology which are less severe at low manganese exposure and increase in severity as exposure increases (Collipp et al. 1983; Mergler et al. 1999).

2.3.4.2 Manganese neurotoxicity in adults

As with lead, manganese significantly affects the neurology of adults. Gorrell (1999a) has reported an increased incidence of Idiopathic Parkinson's Disease (IDP) among individuals exposed to manganese for more than 20 years. After this time, the odds ratio of developing Parkinson's for manganese exposed individuals was 10.61. Subsequent research has suggested that manganese plays a role in the development and onset of IDP (Martin 2006). In a recent study of manganese exposed welders, 42% suffered tremors, 60% suffered numbness, 65% excessive fatigue, 79% sleep disturbances, and 53% suffered from depression. A further 18% suffered from toxic hallucinations associated with long-term manganese exposure. The mean blood manganese concentration in this study group was 10µg/dL (Bowler et al. 2007). Moreover, manganese exposure in adults is associated with Parkinson's-like motor skills deficits and possibly with mood disorders, even at relatively low exposures (Laohaudomchok et al. 2011). These deficits, particularly the IDP tremors are irreversible (Levy and Nassetta 2003). Bowler et al. (2006) reported mood disturbances, including anxiety, depression and confusion and neurological effects including deficits in motor skills, visual tracking, verbal proficiency and memory among welders occupationally exposed to manganese. These effects are particularly pronounced in older men and with increasing manganese exposure (Bowler et al. 1999).

2.3.5 **Vanadium**

Vanadium has recently been established as an essential ultra-trace element in humans, however like manganese, it is toxic in higher concentrations. Vanadium is also essential for bone maintenance and has very recently been examined as a potential treatment for diabetes due to its insulin-like effects on glucose (Badmaev et al. 1999; Poucheret et al. 1998). Little is known about the specific mechanisms of vanadium toxicity. Vanadium is a common element found particulate matter resulting from industrial activities and transportation emissions (French and Jones 1993). Vanadium may be of special concern in South Africa, as the country is a major producer of vanadium.

The level and degree of vanadium essentiality and toxicity has not been established in humans, and no threshold dose by which to measure toxicity is available. In the past decade it has been established that vanadium may be a potent carcinogen, particularly in its pentavalent form (most commonly found in nature and used industrially) (Assem and Levy 2009; Chen et al. 2001; Montiel-Davalos et al. 2012). In its inorganic form, vanadium in high doses can cause gastrointestinal discomfort and liver and kidney toxicity (Srivastava 2000). Among the unknown issues with use of vanadium as a therapeutic agent is its established affinity for bone. Much like lead, vanadium is a bone-seeking element and is sequestered in bone tissue in much the same way. In addition, vanadium is stored in and toxic to kidney tissue (Parker and Sharma 1978). Studies in rats have found that in otherwise normal and healthy rats, the ability to learn new tasks is diminished with vanadium administration (Sanchez et al. 1998). Still other studies have found that in animal analogues, vanadium increases bone density in diabetic and non-diabetic subjects and its accumulation appears to have no toxic effects (Chiu et al. 2006a; Chiu et al. 2006b; Facchini et al. 2004; Facchini et al. 2006).

Environmental vanadium exposure may be toxic to humans when inhaled in particulate matter (PM) (Woodin et al. 2000). The primary effect of inhaled vanadium may be pulmonary disease and inflammation, potentially in the presence of nickel (Campen et al. 2001; Dominici et al. 2007; Lippmann et al. 2007; Rice et al. 2001). In 2009, Bell et al. (2009) examined hospital admissions on one day (per year) in 106 U.S. counties between 1999 and 2005. This data was examined against total atmospheric PM, both fine and course ($PM_{2.5}$ and PM_{10}), in each county on the same day. The authors found an association between hospitalisation and vanadium and nickel in ambient air particles. Those counties with higher vanadium and nickel concentrations in PM had greater hospital admissions for respiratory complaints than counties with lower atmospheric vanadium and nickel concentrations.

Patel et al. (2009) found a similar trend among children from birth to age 24 months in New York City seen at a local hospital for cough and wheeze. The authors report that total $PM_{2.5}$ concentration in ambient air was not associated with either symptom but that both nickel and vanadium concentration of PM was.

2.3.6 **Antimony**

The toxicity of antimony is not well understood. Antimony is frequently associated with arsenic, environmentally, and it is difficult to determine the toxic effects of antimony as a result (De Boeck et al. 2003). The element is suspected to be both carcinogenic and genotoxic, though this remains unclear (Gebel 1998; Léonard and Gerber 1996; Winship 1987). Most interestingly, despite its likely carcinogenic properties, antimony may potentially reducing the genotoxic effects of arsenic (Gebel 1998).

2.4 Sex differences in the toxicity of metals

Confounding the investigation of the health effects of toxic metals in humans is the role that biological sex plays in the uptake, metabolism and consequences of these elements. Toxic elements can affect men and women quite differently. The differences in health effects can be the result of dietary differences and cultural practices (such as occupation, smoking prevalence, alcohol consumption). In addition, the differences in the physiology and life history between men and women play a role in the effect of metals on health. For example, the reduction in hormones caused by menopause causes increased bone turnover in women, which in turn, mobilises bone lead back into the bloodstream. As women age the release of endogenous lead in causing clinical and subclinical symptoms of lead exposure increases. Men, who experience lower rates of bone turnover as they age, are less at risk of endogenous lead exposure than women.

Recently, the role of sex and gender has begun to be explored in the investigation of toxic element exposure (Arbuckle 2006; Clougherty 2010). As Clougherty (2010) demonstrates, a literature search of key words relating to air pollution and respiratory and health effects shows that in a majority of published studies, the reported health effects of environmental toxins affect women and girls more than men and boys. It has become clear over the past decade that toxic metals affect men and women differently, yet much remains unknown about sex and metal metabolism and exposure. What is abundantly clear from these differences, is that any analysis of toxic element exposure and the probably effects on health must be examined within a sex-specific framework, if the data is to be interpreted in a meaningful way.

2.4.1 **Lead**

Lead toxicity is evident in bone tissues and may affect women and children more acutely than men (Vahter et al. 2007). Paradoxically, lead exposure tends to be higher in men than in women, even in occupational settings (Popovic et al. 2005). As was discussed in previously in this chapter, lead is thought to displace calcium cations in the bone matrix, leading to weakening of bones, delayed healing and osteoporosis (Carmouche et al. 2005; Pounds et al. 1991; Vahter et al. 2007). There is also sufficient research to suggest that women may be more susceptible to the effects of lead in the body due to hormone-related changes in bone density and bone mineral mobilization during pregnancy and menopause. This research will, in part, investigate bone lead concentrations in women in order to contrast with recent in vivo studies of bone lead in modern women and its effect on health at the clinical and sub-clinical level (Adachi et al. 1998; Theppeang et al. 2008a; Vahter et al. 2007).

In utero, there is currently evidence that lead exposure affects boys more significantly than girls. Jedrychowski (2009) has demonstrated that by 36 months of age, pre-natal lead exposure is significantly and inversely related to cognitive development, more so than for girls. This relationship was evident at low exposure levels (maternal blood lead concentration) of less than 5µg/dL.

2.4.2 **Cadmium**

Itai Itai disease was originally considered a women-specific disease, and women appear to be affected by the disorder in greater numbers than men (Vahter et al. 2007). It is believed that this is due to greater gastro-intenstinal absorption of cadmium in women when bodily iron stores are low, a well-documented phenomenon (Kippler et al. 2009; Lee and Kim 2012b; Vahter et al. 2007). Vahter et al. further point out that despite *Itai Itai* disease garnering the label as a "women's" illness, little sex-specific research has been conducted since the identification of the disease.

The cardiovascular effects of cadmium also appear to manifest differently in men and women. Despite links between cadmium and myocardial infarction (MI), or heart-attack, and cadmium and cardiovascular diseases, each condition only manifested in one sex. The urinary cadmium MI association brought to light by the NHAHES III data is only significant for women (Everett and Frithsen 2008). Conversely, the relationship between blood cadmium and prevalence of cardiovascular disease found in the Korean NHANES was only significant in men (Lee et al. 2011). In some cases, particularly in regards to cadmium exposure, the data is somewhat contradictory (Akesson et al. 2005; Jin et al. 2004). Ferraro et al. (2012) report higher rates of cardiovascular mortality in cadmium exposed women using data from NHANES. In addition, whilst research suggests that blood cadmium

concentration tends to be higher in men, cadmium is more significantly associated with cardiovascular risk in women than for men. This may indicate that women are more physiologically more susceptible to cadmium toxicity than men even at lower doses (Olsen et al. 2012).

2.4.3 **Manganese**

Sex may also play a role in manganese uptake and toxicity however this is thought to be primarily a function of iron status. Iron deficiency is generally associated with women, which has led researchers to conclude that iron status and manganese uptake may be higher in women (Finley et al. 1994). Other research has suggested that men are more susceptible to the neurotoxic effects of manganese (Mergler et al. 1999).

2.4.4 **Arsenic and antimony**

Some studies have suggested the presence of sex-related differences in arsenic and antimony uptake between men and women (Buchet et al. 1996; Gebel et al. 1998). Both Buchet et al. and Gebel et al. report higher urinary arsenic excretion in men than women in Belgian and German populations respectively, which is ascribed to higher uptake due to greater exposure. It is also possible that there are differences in arsenic metabolism and toxicokinetics of arsenic between men and women. Likewise, Gebel et al (1998) also report greater antimony burden in men.

Conversely, Berglund et al. (2011) report higher urinary arsenic concentrations in the tissues of women (along with cadmium and manganese) than in men in Bangladesh. These authors also report that women in this population had lower urinary concentrations of essential elements including calcium, magnesium and zinc, indicating that nutritional status among women may be worse than men. The poorer nutritional status of women may account for the increased uptake of toxic elements in this population, including arsenic.

2.5 Toxic element interactions

There is a significant amount of interaction between elements in the human body. Interactions between essential elements and toxic elements have been discussed in regards to element uptake and metabolism. However these elements also interact to exacerbate or mitigate the health effects of one another. In some cases, it is unclear how these interactions occur and which element may be acting upon the other, however most of the elements of interest in this project do interact in ways that have clear consequences for health.

2.5.1 **Manganese, lead and cadmium**

The interaction between lead and manganese may contribute to human morbidity more than either element. Manganese may increase the effects of lead toxicity. Studies of children

exposed to lead found that those who had high blood manganese levels showed greater levels of neurotoxicity than those with lower manganese exposures (Henn et al. 2012). Kim et al. (2009) investigated the association between manganese and lead in children's intelligence. The authors report that in children with blood manganese levels above 14µg/dL^{-1}, IQ and verbal IQ were significantly associated with blood lead concentration, whereas children whose blood manganese was below 14µg/dL^{-1} did not show a lead/IQ relationship. This indicates that manganese may significantly alter the neurotoxic effects of lead in exposed children.

Studies of individuals who have suffered multiple myocardial infarctions (MI), or heart attacks, have shown that prevalence of MI is associated with increased blood levels of lead, arsenic and cadmium and the levels of these elements were significantly higher in MI sufferers than in a control group. Moreover, the levels of these elements were highest in the group suffering three MI incidents (Afridi et al. 2011b). These same three elements have also been shown to be significantly higher in the scalp hair of individuals with diabetes mellitus than in those without (Afridi et al. 2008).

2.6 Essential elements and toxic elements

2.6.1 **Magnesium**

Magnesium is a bone-seeking, essential trace element and 50% of the body total body volume of magnesium is stored in bone (Elin 1988). Worldwide, magnesium deficiency is not uncommon (Elin 1988; Johnson 2001; Reinhart 1992). It appears to be particularly sensitive to alcohol and it is well established that even moderate consumption of alcohol can disturb magnesium metabolism, resulting in decreased absorption and overall deficiency (Afridi et al. 2011a; Kärkkälnen et al. 1988; Lieber 1988; Romani 2008; Rylander 2001). Excess body fat and excess salt intake, Vitamin D deficiency are also known to disrupt magnesium metabolism and result in deficiency of the element in humans (Johnson 2001).

The three elements, magnesium, calcium and lead appear to have an antagonistic relationship with regards to uptake and deposition on the uptake of each element into bone tissue (Todorovic et al. 2008). Research has demonstrated that lead toxicity may be more acute in the presence of magnesium deficiency (Anetor et al. 2007). The primary mechanism by which magnesium may result in increased lead deposition in bone tissue may be related to the relationship between magnesium and calcium. Magnesium deficiency is associated with decreased calcium uptake into bone tissue and increased risk of osteoporosis (Gur et al. 2002; Mutlu et al. 2007; Odabasi et al. 2008; Sojka and Weaver 1995). It is believed that magnesium deficiency may cause increased bone turnover, which releases endogenous lead

into the blood stream. In addition, because lead is a bone seeking element and is readily absorbed into bone tissue in place of calcium, the effect of magnesium in the metabolism and uptake of calcium is a critical factor in lead uptake.

In the absence of magnesium, calcium uptake may be decreased and lead uptake increased. Magnesium is also required for proper elimination of lead via the kidneys and deficiency results in less lead excretion from the body following exposure (Johnson 2000). In both animal and human studies, reduced magnesium intake was significantly associated with higher tissue lead uptake and toxicity (Ahamed and Siddiqui 2007; Herman et al. 2009; Jamieson et al. 2005; Lech 2002; Todorovic et al. 2008; Tonelli et al. 2009). In studies of children with neurological disorders thought to be caused by lead intoxication, an inverse relationship between lead and magnesium was identified. Low magnesium/lead ratios may indicate that magnesium deficiency may increase uptake of lead into human tissues (Lech 2002). Other research has confirmed this relationship in animal models and it has been hypothesised that lead may supress the uptake of calcium, zinc and magnesium (Todorovic et al. 2008).

Magnesium also interacts with cadmium. There is evidence that cadmium prevents magnesium absorption in the gastro-intestinal tract and that cadmium excretion in urine is associated with increased magnesium excretion (Järup 2002). Conversely, magnesium supplementation may reduce cadmium absorption. Magnesium has a two-faceted relationship to cadmium in that it plays a role in cadmium elimination and toxicity, but magnesium metabolism appears to be disturbed by the presence of cadmium (Matovic et al. 2010).

2.6.2 **Zinc**

Zinc interacts with several toxic trace elements, including lead however the interaction between cadmium and zinc is the most clearly understood. Like magnesium, zinc deficiency is prevalent throughout the world and due to the numerous functions the element plays in the body, deficiency can result in severe impairment to many metabolic processes in humans (Brown et al. 2001; Hambidge 2000). Zinc plays an important role in the formation of bone and deficiency can cause a reduction in bone density and osteoporosis (Brown et al. 2001; Eberle et al. 1999; Elmstahl et al. 1998; Mir et al. 2007; Mutlu et al. 2007; Yamaguchi 1998).

Recent research has demonstrated that lead uptake into bone tissue is increased during marginal zinc deficiency in rats. The same study, however found that zinc deficiency did not reduce lead toxicity (Jamieson et al. 2005). Other research has contradicted this showing no

increased cytotoxicity in lead administered rats in the absence of zinc, indicating that the relationship between zinc and lead toxicity may be limited to specific organ systems (Piao et al. 2007).

Zinc is also a potent antioxidant and may play a significant role in the metabolic elimination of arsenic and cadmium, with zinc-deficient individuals less able to methylate arsenic, in particular (Patrick 2003). Rats fed a diet deficient in both zinc and iron demonstrate a substantial increase in cadmium uptake than those fed standard diets, further indicating that zinc (and iron) may be critical to the body's elimination of cadmium (Reeves and Chaney 2002). Zinc homeostasis is believed to be disrupted by the presence of cadmium (Jarup 2002; Noel et al. 2004). The mechanism behind this is believed to be the role of zinc in the production of metallothionein, which is responsible for the elimination of cadmium (Patrick 2003). Inadequate synthesis of metallothionein due in part, to zinc deficiency, may cause the release of cadmium into body tissues where it can cause damage, not just to bone, but to other organ systems as well. Moreover, in the absence of zinc, cadmium may replace it in metallothionein, which may increase the rate of uptake into the body (Goyer 1997).

2.6.3 **Iron**

Iron is known to interact with cadmium, and low blood iron concentration is correlated with increased cadmium absorption, and vitamin D may be the link. Studies of dietary vitamin D and cadmium, found that cadmium uptake and the negative effect of cadmium on bone (severity of *Itai-Itai*- Disease) were increased in Vitamin D deficient animals (Uchida et al. 2010). However the relationship between cadmium and Vitamin D is subject to debate (Engstrom et al. 2009). Other studies seem to confirm a potential indirect relationship between iron and cadmium, and have demonstrated that increased dietary intake of iron reduces the uptake of the cadmium metallothionein (Groten et al. 1992). The relationship between low serum iron concentration and blood cadmium concentration has been shown to be an inverse one, though the implications of this in regards to cadmium uptake in to other tissues is not yet clear (Lee and Kim 2012b).

Iron deficiency has also been associated with increased lead absorption and uptake from the intestinal tract (Elsenhans et al. 2011; Goyer 1995; Goyer 1997; O'flaherty 1998b). Iron deficiency and lead exposure are often linked socioeconomically, with the poorest individuals, usually children, exposed to higher rates of lead and suffering from poor diets and inadequate intake of iron (Yip 1989; Yip et al. 1981a, 1981b). It is thought that the uptake of lead is not linked to blood iron concentration but the production of red blood cells. Choi et al. (2003, 2005) studied blood lead and body iron stores in children and adolescents

and found that blood lead increased not as a function of the reduction of blood iron but as a function of erythropoiesis during iron deficiency.

Whilst most of the studies of lead and iron have concerned the relationship in children, the relationship between the two elements is also evident in adults. Kim et al. (2003) examined iron and lead status in lead workers and non-lead workers in Korea. They report that lead workers had significantly lower hemoglobin, hematocrit, serum-iron levels than non-lead workers. These findings have been both confirmed and rejected elsewhere (Alabdullah et al. 2005; Karita et al. 2005; Keramati et al. 2010). However it has been pointed out that studies that have rejected the association between lead and iron have focused on different iron measurements such as total blood iron, which may be leading to false-negative results (Kwong et al. 2004). With regards to bone iron and bone lead, few studies have sought to establish a relationship. Hu et al. (1994) report a significant negative relationship between trabecular bone lead and haemoglobin, with no concurrent relationship between blood lead and haemoglobin. The authors conclude that release of lead from bone may be responsible for decreased iron uptake.

Iron also interacts with manganese (Aschner and Aschner 1990; Mena et al. 1969; Rossander-Hultén et al. 1991; Smith et al. 2012). Studies in rats and mice have demonstrated that animals deficient in iron have higher uptake of manganese, and the mechanism is believed to be iron's ability to reduce manganese absorption into mucosal cells of the gastrointestinal tract (Davis et al. 1992). A study of humans from the Korean National Health and Nutrition Survey Examination (NHANES) also found that individuals with decreased iron status had higher blood manganese concentrations than individuals who were not deficient in iron (Kim and Lee 2011). However, this relationship may not be detrimental to health. In small quantities, the increased uptake of manganese during iron deficiency may actually protect the brain. Kim et al. (2012) found that in iron-deficient individuals, manganese may improve motor function deficits brought about by lack of iron.

2.6.4 **Copper**

Copper is an anti-oxidant that is essential to human health. It is generally ingested through food intake and is absorbed via the gastro-intestinal track by binding to metallothionein (Burch et al. 1975). Copper d is rare and generally occurs only in infants and those receiving long-term parenteral nutrition (Williams, 1983). Copper interacts primarily with zinc, which has been demonstrated to have an inhibitory effect on copper uptake in humans (Valberg et al. 1984; Yadrick et al. 1989). In addition, copper may play an important role in facilitating iron absorption (Tapiero et al. 2003). With regards to toxic elements, several studies have shown that copper, in conjunction with zinc and iron may play a protective role against

uptake of lead in animals (Klauder and Petering 1975, 1977; Petering 1978). Copper may also interact with both lead and iron. In animal models, rats given high doses of lead developed anaemia. This is believed to be secondary to depressed copper intake, which in turn, interferes with iron uptake (Klauder and Petering 1977). Copper uptake may also be depressed in the presence of cadmium (Petering 1978).

3 Health and demography and race in apartheid South Africa

Understanding the demographic, social and health conditions of urban society is critical to the understanding of trace element exposure in South Africa, perhaps more so than would be necessary in another population. The phenomenon of apartheid has caused a societal division that is unlike that of any other 20th century population. This divide permeated every aspect of life, from health, nutrition and disease, to place of residence (both within urban areas and on a national scale). A discussion of urban apartheid and its effects on human health in both black and white individuals is presented in this chapter. More importantly, the information presented here highlights the substantial lack of data regarding the health of black South Africans during the apartheid era and underlines the need for studies such as this project. This chapter examines, briefly, the political, social and demographic policies that resulted in geographic and resource division in South Africa during apartheid. Disparities in living conditions, health and nutrition between the black and white populations is also discussed in order to provide context to differences in toxic element exposure and the potential social and health ramifications arising from this exposure.

South Africa is a unique country in which to study correlation between demography toxic trace element exposure. This is largely due to the nearly 50 year period in which demographic groups, split along purely racial lines, were separated by law. Whilst trace element exposure does generally differ among socioeconomic groups in other countries, the clear division between racial groups in Apartheid South Africa, and the restriction of movements and residential choice among black South Africans has led to differences in health and health outcomes that are not merely socioeconomically, but racially different.

In 1950, the Population Registration Act classified every individual living in South Africa into one of four racial groups: White, black, Asian and Coloured. White individuals included people of European origin, excluding individuals who were of mixed ancestry. Black individuals were those who were indigenous to Africa, except the Khoisan. Asians were primarily from India, and immigrants from China (until 1984). 'Coloureds' was generally anyone who did not fit into the previous three categories. These policies further entrenched the differences in health and living conditions that existed previously between the black and white populations. The forced removal of black individuals to arid bantustans and overcrowded and unsanitary townships had a direct impact on the already marginal to poor health of black South Africans. The direct result of apartheid policy was a substantial gap in health, nutrition, living conditions and life expectancy between the two populations. By the 1990s and into the present day, South Africa has had one of the highest income inequality

rates in the world, with black South Africans bearing the brunt of the poverty gap (Fig. 3-1) (Hirschowitz and Orkin 1997; Klasen 1997).

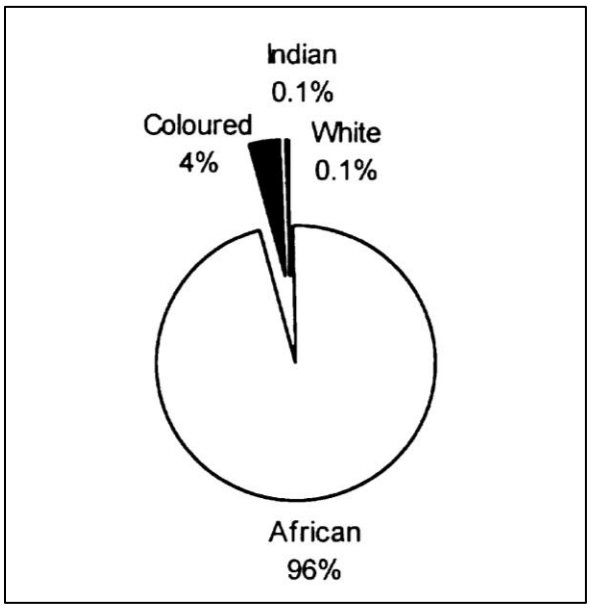


Figure 3-1. Percentage of South Africans living below the poverty line by racial group. From Klasen (1997).

3.1 South Africa and apartheid

South Africa has been referred to as one of the most unequal countries on earth (Mathee et al. 2009a). The legacy of complete racial segregation is one in which differential access to resources is a persistent problem. An additional legacy of Apartheid is a lack of consistent data regarding health discrepancies between black and white populations. Among the current public health literature, there is often little mention of the lasting impact of Apartheid on public health inequity. Whilst much has been written regarding the lack of adequate healthcare among large segments of the population, there appears to be hesitation among researchers to identify racial discrepancies regarding access, despite race continuing to be a significant barrier to public health care. Social scientists and demographers have been less reluctant to refrain from examining inequality among racial lines, and often highlight racial differences in cases in which public health researchers may not. Current public health literature often uses phrases such as "urban dwellers" living in informal squatter settlements and tenements, with little or no mention of the fact that these urban dwellers are almost wholly black and still disadvantaged due to the legacy of apartheid (Mathee et al. 2009a). Studies of national or provincial public health issues in the present day often do not make any demographic distinctions other than age and sex, despite persistent evidence of racial disparities in health outcomes. Moreover, during Apartheid, there was little public health research into just how different health outcomes were and still are due to Apartheid policy,

for reasons that are quite obvious. Even basic information such as infant mortality rates and census data were not collected for the majority of the black population. As with data regarding inorganic pollution in South Africa, much of the difference in Apartheid-era health and health outcomes between the black and white populations must be gleaned from post-Apartheid studies of South African health.

3.2 Health and health outcomes - definitions

Within this research, the terms health and health outcomes will be used. These terms define two distinct phenomena. Health refers to the physical state of an individual or group based on factors such as genetics, diet, lifestyle, and, most importantly for this project, exposure to pollution and toxic elements. Diabetes and hypertension are two different health *conditions* that are both individual and group-level health problems. Exposure to lead and subsequent lead-related illness is a health condition. Health *outcome* refers to the change or lack of change in a given health condition due to intervention or lack thereof. Diabetes that remains uncontrolled in a given individual due to lack of adequate health care is a poor health outcome. A reduction in blood lead levels among a group of school children after leaded paint is removed from their school is a positive health outcome. Within all populations, both health and health outcomes vary based on socioeconomic and, in South Africa's case, racial differences.

Both terms are important in understanding the consequences of toxic metal exposure because both factors are in effect. Different segments of a population may be exposed to the same or different levels of lead, and may face different health outcomes due to differences in access to health care or treatment.

3.3 Health care Apartheid in South Africa

Present-day proxy data can be used to infer past health conditions in South African primarily because change has occurred slowly (Christopher 1997, 2005). Prinsloo and Cloete (2002) noted that the process of racial integration in post-Apartheid cities was slow in the years following the abolition of the Group Areas Act. Mathee et al. (2009a) conducted an extensive health survey of five study sites in Johannesburg in 2006. This study found that Apartheid-era racial residential patterns persisted at each of the study sites, despite the research taking place a dozen years after the end of Apartheid. Mathee et al. (2009a) reported stark living conditions among the almost entirely black study sites. Poor access to hot, running water, household degradation, including peeling paint, and damp were reported in each study site. In addition, poor health was a persistent problem across all five sites, including both chronic and acute illness, but these issues were highest in the two study areas

that were formal Apartheid-era settlements for black and coloured (largely Asian) populations.

Peltzer (2002) studied health behaviour of 250 black individuals and 250 white individuals in formerly Black and White residential areas of the Northern Province. Among the information Pelzter gathered was socioeconomic data, which illuminates the stark contrast between the black and white populations. Among respondents, 66% of whites were categorised as financially well-off or wealthy, whilst 76% of black individuals were not well-off or poor. No black individuals were considered wealthy and no white individuals were considered poor. Access to automobiles, another indicator of socioeconomic status was also reported. Among black households, 55% had no car. Not one white household was without a car and 59% of white households had more than two.

With regards to access to medical care, the disparities between black and white populations were perhaps the most striking. Kon and Lackan (2008) have examined the persistent inequality of health care in South Africa as a legacy of Apartheid. The authors note that in 1981 there was one physician for every 330 white individuals in South Africa, and one physician for every 91,000 black individuals. Much of this disparity has to do with the administration of bantustans, each of which was responsible for its own health care system, despite almost total lack of funding (AAAS 1998). Within urban areas, like Pretoria and Johannesburg, access to healthcare for black individuals during Apartheid would have been limited. They would not have been able to be treated in most urban hospitals as these were reserved for the white population, though some urban hospitals had black wards (AAAS 1998). Furthermore, black individuals were technically only allowed treatment at hospitals in their homelands, meaning economic migrants living in squatter communities near urban areas could be refused health care at a black hospital if it was not in their homeland (AAAS 1988). This means many black individuals resident outside of their designated homelands – as many working in Pretoria and Johannesburg were – would have had to travel long distances to obtain medical care, an expense that would have likely been beyond the reach of most black labourers (AAAS 1988). Wages of black individuals were predominantly set by government regulation and worsened over the apartheid period. In 1930, black gold miners were paid 11 times less than white mine employees and by 1970 black wages were 20 times less than white wages (Coovadia et al. 2009).

During apartheid, health care across South Africa was decentralised and at the end of the apartheid government there were 14 distinct health departments in the country, most of them chronically underfunded (Coovadia et al. 2009). Nightingale et al., (1990) provide a telling description of the considerable disparity in health care by comparing annual per capital

health expenditure between population groups in 1985. The South African government spent $201 per white individual and $51 per black individual. At that time 14% of the population was white and 74.7% of the population was black. The Nightingale et al. study offers a rare though limited glimpse into the delivery of health services between populations during apartheid from an outside perspective. Published as the findings of an international delegation sponsored, in part by the US National Academy of Sciences and the American Public Health Association, it is one of the few non-South African investigations of the health consequences of apartheid to take place during apartheid. The authors describe the vast magnitude between white and black medical care between Baragwanath Hospital in Soweto, versus Johannesburg Hospital, a white only facility. In Baragwanath, the authors find a male ward with 40 beds and two toilets serving over 80 patients. Many were sleeping on the floor. In contrast, Johannesburg Hospital a few miles away, a new facility with 1800 beds, had 1000 empty beds. In 2011, Baragwanath Hospital served over one million individuals in Soweto (Stewart et al. 2011a). With lack of adequate healthcare, both health and health outcomes were substantially compromised for township residents. Many of South Africa's poorest individuals have not sought adequate health care when ill, citing to poor access to transportation, and prohibitive treatment costs as the two most prevalent reasons (Klasen 1997).

Whilst many view these health disparities as a result of or symptom of, apartheid, others have argued that the health care system in South Africa was actually an instrument of apartheid. Proponents of this concept argue that the health care services provided for the black population served the white agenda, as opposed to the medical needs of the black population. One example if this was the conversion of Hillbrow Hospital in Johannesburg from a white to a black hospital in the early 1980s. The hospital was converted without a paediatrics ward, despite the need for one, because there were ostensibly no black children officially resident in Johannesburg (Price 1986). Because children were not labourers, they were expected to live in the bantustans, not urban areas. Price argues that the increase in urban health services in the 1980s was primarily due to an increase in the need for skilled labour in the mines and industries. Skilled labourers are more expensive to train (and replace when lost to illness) and so white employers and the government had a financial interest in improving urban health care. Further, he argues, that the insistence on black individuals receiving primary health care in the bantustans was aimed at preventing greater black migration to urban areas, thus keeping these areas white (Price 1986).

The focus of medical care was on hospitals, and to a great extent, primary health care was overlooked and essentially the health system was one of acute as opposed to chronic or preventative care (Mayosi et al. 2009). This has strong implications for the health

consequences and outcomes associated with all types of disease, but in particular, exposure to toxic elements. With primary health care services lacking or beyond the reach of most black individuals, both clinical and subclinical toxicity is likely missed. In addition, the nutritional deficiencies that can exacerbate heavy metal toxicity would likely have been undiagnosed as well. As Kon and Lackan (2008) have pointed out, this lack of adequate health care plays out in the substantial difference in life expectancy between black and white individuals. In 1980, the median year for the samples included in this project, the life expectancy for black males was 55 years. For white males, life expectancy was 70 years.

Disparities in living conditions also contribute to different health and health outcomes for black and white individuals living under apartheid. In a 1989 study of living conditions and environmental health services in urban and peri-urban South Africa the South African Medical Research Council estimated that the number of black individuals living in "informal" (not purpose built or planned) dwellings was nearly 50%. Only 4% of these dwellings had either a flush toilet or clean water tap and only 5% had electricity. A majority of peri-urban households used coal, wood or paraffin for cooking and heating, which carries its own health consequences as will be discussed in subsequent chapters.

3.4 Urban demography and residential geography

During apartheid, both Johannesburg and Pretoria, like all other cities, was reserved entirely for the white population. Residential patterns followed strict racial lines. The Group Areas Act of 1950 ensured no mixing of races in neighbourhoods and complete separation of races in regards to residence, shopping, markets, services, and public amenities such as parks. Black individuals in each city were expected to live in townships outside of the city, hostels, if they were men migrating in from a Bantustan. Many black individuals also lived in informal squatter camps that appeared around the cities towards the end of apartheid, as more individuals migrated from rural areas in search of work. The aim of the Act was to segregate the white population as much as possible from the other three racial groups. This was nearly achieved by the late 1980s, which saw most white individuals living in areas almost exclusively white. Other areas were more mixed, with the black, coloured and Asian groups living in less segregated communities (Christopher 1990).

3.4.1 **Johannesburg**

Prior to apartheid, and particularly during the interwar period between the two World Wars, both cities, particularly Johannesburg, were not completely segregated. Inner city slums, near the central business district of Johannesburg housed nearly 8,000 poor white individuals (Parnell 1988). Many of these white individuals were relocated to council houses during the clearance of the slums in the pre-apartheid years. Most of these homes were located in high-

density neighbourhoods near the central business district. According to Parnell (1988), the shortage of housing for white families in Johannesburg was effectively relieved during this period. In addition, a shortage of skilled labour contributed to a backlog of construction (Crankshaw and White 1995). The situation for the black individuals uprooted during slum clearance programs was less positive. Despite needing approximately 42,000 new housing units to shelter these individuals, less than 2% of building materials were allocated for this purpose (Parnell 1988).

The program of urban slum clearing was effectively codified into law with the 1950 Group Areas Act. In the 1950s, forced removal of black individuals from the city was carried out, and formerly black neighbourhoods were razed. Many of these individuals were no longer considered urban residents, but temporary migrants, whose real residence was on one of the Bantustans. In Soweto, the permanent black township in the southwest of the city, black individuals were segregated *within* the township according to tribal or ethnic background. This was to ensure cultural identity with a tribe (which likely had a corresponding Bantustan), rather than identify as urban residents of Johannesburg(Mashile and Pirie 1977). To further reinforce the temporary nature of their presence in the city, the Group Areas Act also ensured that ownership of land within the townships could was only tenable for a maximum of 30 years.

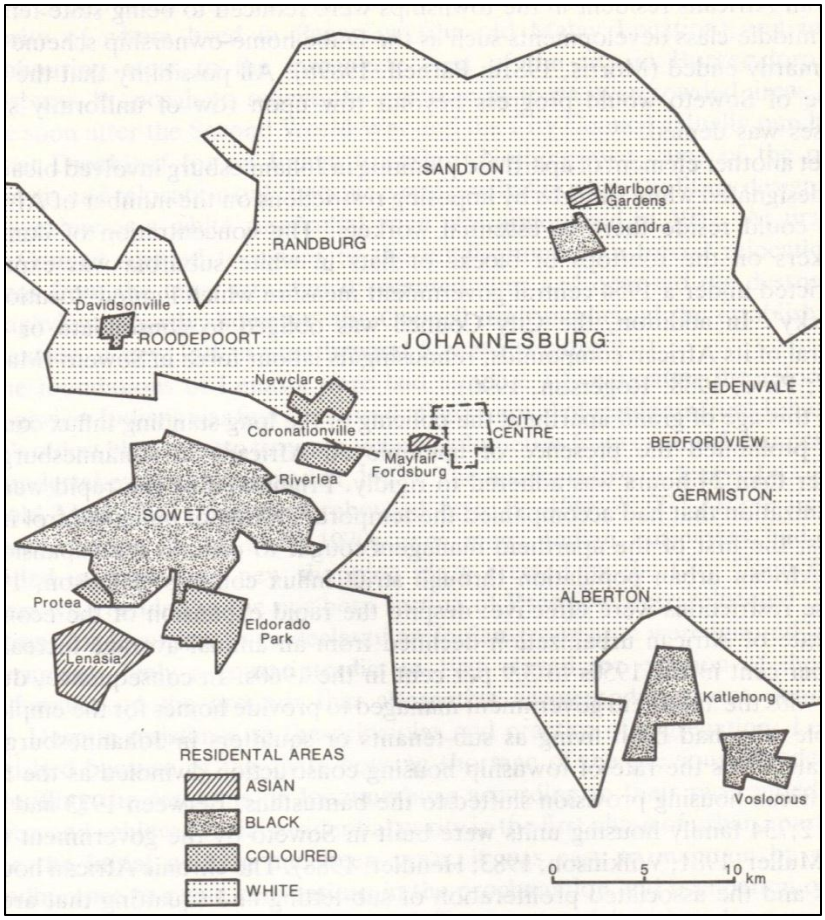


Figure 3-2. Designated racial group areas in Johannesburg circa 1970. From Parnell and Pirie (1991).

As is evident in Figure 3-2, the overall effect of the Group Areas Act was the creation of a white-centred urban core, insulated by white neighbourhoods and services, with black townships and hostels on the periphery. Even black individuals who worked in white neighbourhoods as domestic labourers were restricted. The number of black individuals who could live in white neighbourhoods, was reduced and closely monitored by the apartheid government. Within the white core, unemployed black individuals could not be present for more than 24 hours before being subject to arrest or removal. There were officially no black residents of Johannesburg. All black individuals living in Johannesburg were technically migrant labourers resident in one of the homelands and living temporarily in Johannesburg. The townships however, proved insufficient to house the black population. By 1990, it was estimated that nearly two million black individuals lived in informal housing or squatter camps on the urban periphery.

3.4.2 **Pretoria**

The urban demography of Pretoria is similar to that of Johannesburg, in that it follows closely the white urban core/black periphery model envisioned by the Group Areas Act. Even before the rise of the apartheid government, Pretoria generally followed this model, with a white urban core and a number of black satellite settlements (both official and unofficial) around the outskirts. By 1970, almost all black individuals were living in one of the peripheral townships: Atteridgeville and Mamelodi. In Pretoria, the white residential areas surround the urban core, and comprised most of the city. The areas between the white and black areas were industrial zones. As Hattingh and Horn (1991) point out, the location of the industrial areas adjacent to black townships appears to be deliberate, which should and did have health consequences for the township residents, but also, as this project demonstrates, had consequences on white health as well.

The Group Areas Act enforcement in Pretoria, also served to create distinct cities within the greater Pretoria urban area. The multi-city model was aimed at removing the non-white population from Pretoria, making it a white only city. Non-white individuals were forcibly removed to one of the new black towns. As part of "grand apartheid" – the creation of independent bantustans, the government tried to establish urban areas within bantustans and sited these areas adjacent to industrial activities, in addition a policy of industrial decentralisation was adopted in an attempt to move industry to the urban periphery or to the bantustans to help stem rural to urban migration (Bell 1973; Wellings and Black 1986). In the Pretoria region, this included the establishment of Ga-Rankuwa, an urban township within the boundaries of Bophuthatswana and adjacent to Pretoria. Another black township, Mabopane, was also incorporated in Bophuthatswana. The Bantustan KwaNdebele also borders Pretoria, and thought it lacked an "urban centre" like Ga-Rankuwa, it was home to a number of settlements on the urban periphery (Hattingh and Horn 1991).

3.4.3 **Urban demography of Johannesburg and Pretoria – implications for health**

The organisation of urban areas in apartheid South Africa, particularly in Pretoria has three primary implications for health as it relates to the current project:

1. Crowding and poor health conditions in townships and squatter camps.
2. The need for black individuals living on the periphery to commute daily to work in the urban core
3. The impact of mass commuting and daily transit on air quality and pollution in the urban core

Each of these factors is important in understanding how heavy metal exposure affects different segments of the population, and how the health and health outcomes of this exposure may also be different among black and white individuals. The first two factors, crowding and conditions in townships are discussed in this chapter, and the third factor, air quality and pollution is discussed at length in Chapter 4.

The Group Areas act and the forced relocation of hundreds of thousands of black urban residents caused a severe housing shortage in both cities. The construction of homes in the townships could not keep up with the population growth in these areas, resulting in vast squatter settlements or "informal' housing on the periphery of urban areas in South Africa. In Pretoria, this phenomenon saw the rise of the Winterveld squatter camps. In the late 1960s, housing allocation and building ceased in the townships of Atteridgeville and Mamelodi. Whilst accommodation existed in Ga-Rankuwa and KwaNdebele, the Bophuthatswana Bantustan was intended as a homeland for the Tswana people, and housing in these townships was earmarked primarily for Tswana. Non-Tswana individuals migrating to the Pretoria urban region were left to settle on the Winterveld north of Pretoria. The result was that this area became densely populated with squatter camps, as the poor farming and economic conditions in the bantustans pushed their populations to urban areas in search of better opportunities.

Conditions in the Winterveld were quantitatively assessed in the late 1980s by Vermaak (1992) . In the southern end of the settlements, closest to Pretoria, only 8% of homes had foundations. Nearly 50% of the population were under 20 years of age at this time, and approximately 75% were unskilled or semi-skilled. Sewage systems were non-existent and water was obtained by boreholes and wells, which were often contaminated with human waste. There was little refuse removal in the area. Conditions in townships were (and still remain) little better.

3.4.4 **Commuting and movement within urban centres**

Among the key characteristics of the removal of black urban residents to the urban periphery was an increase in transport into and out of the central business district and industrial areas each day (Pirie 1986). During apartheid, urban transportation from black areas into Johannesburg and Pretoria was subsidised by the South African government to ensure a reliable labour force (Pirie 1986). It has been estimated that by the 1980s nearly 1.5 million intra-urban commuters moved between townships and urban centres daily (Khosa 1998). In Pretoria, an estimated 400,000 labourers travelled into the city core each day, with an average commuting distance of 52km (Khosa 1998). Black workers could spend up to seven hours per day commuting between work and home, using public transportation,

predominantly, a feature of apartheid policy meant to further control the movements of the black population (Khosa 1995). The "kombi taxi" (minibuses) transporting labourers were introduced in the 1970s which increased efficiency, however this has impacted traffic in and around the urban core on a daily basis in vehicles, many of which burned leaded petrol. This phenomenon of massive movement of labour from the urban periphery to the urban core each day has had a dramatic effect on urban pollution. This is discussed in greater detail in Chapter 4.

3.4.5 **Post-apartheid demographic change**

Since the abolition of the apartheid government and the establishment of the new Republic of South Africa in 1994, the restrictions imposed by the Group Areas Act became obsolete. All South Africans were legally allowed to live where they chose. The transfer of power from white South Africans to black South Africans however, was political rather than economic since the end of apartheid, financial constraints have prevented many black South Africans from moving out of townships and ghettos (Christopher 2005). In short, the physical dismantling of apartheid residential constraints has been slow. The majority of black South Africans remain in townships or informal settlements on the urban periphery, whilst white (and coloured) South Africans remain in the urban core.

There is evidence that this pattern is slowly changing. As Prinsloo and Cloete (2002) note, many black South Africans are moving out of the previously black residential areas. In both cities black residents are following a similar pattern of moving to areas that are between the previously black suburbs or townships and the central business district (Fig. 3-2). Relatively speaking, there is a trend towards greater urbanisation of the black population, even as circular migration persists and township populations grow (Clark et al. 2007; Ginsburg et al. 2009; Kok and Collinson 2006). In Johannesburg, this is evident in one formerly white residential area known as Hillbrow, which is adjacent to the central business district. Racial desegregation of this area began in the 1980s when the South African government began to relax influx control laws and Group Areas Act restrictions that controlled the movement of black Africans from one region to another (Morris 1994, 1999). The shift in demography in areas such as Hillbrow has resulted in a corresponding physical decline of the neighbourhood not unlike that if the typical "inner city" neighbourhoods in the United States where inadequate maintenance of buildings and exploitative tenant-landlord relationships result in slum-like conditions. In the early post-apartheid period, the slow pace of movement into formerly white areas was due largely to the unaffordability of these areas for most black individuals, however this is changing (Crankshaw and White 1995; Gilbert and Crankshaw 1999; Morris 1999).

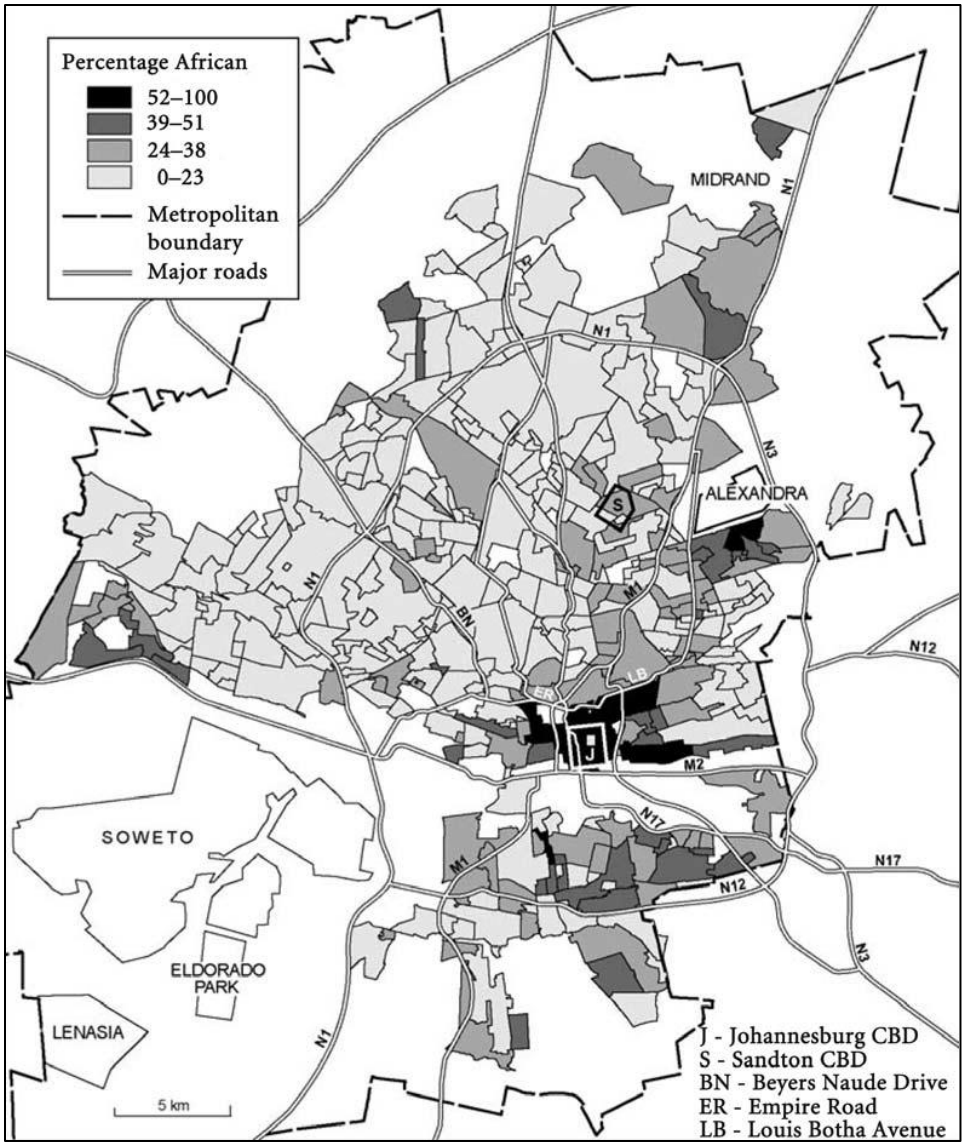


Figure 3-3. Percent of black residents in formerly white-only residential areas in Johannesburg in 2001. From Crankshaw (2008).

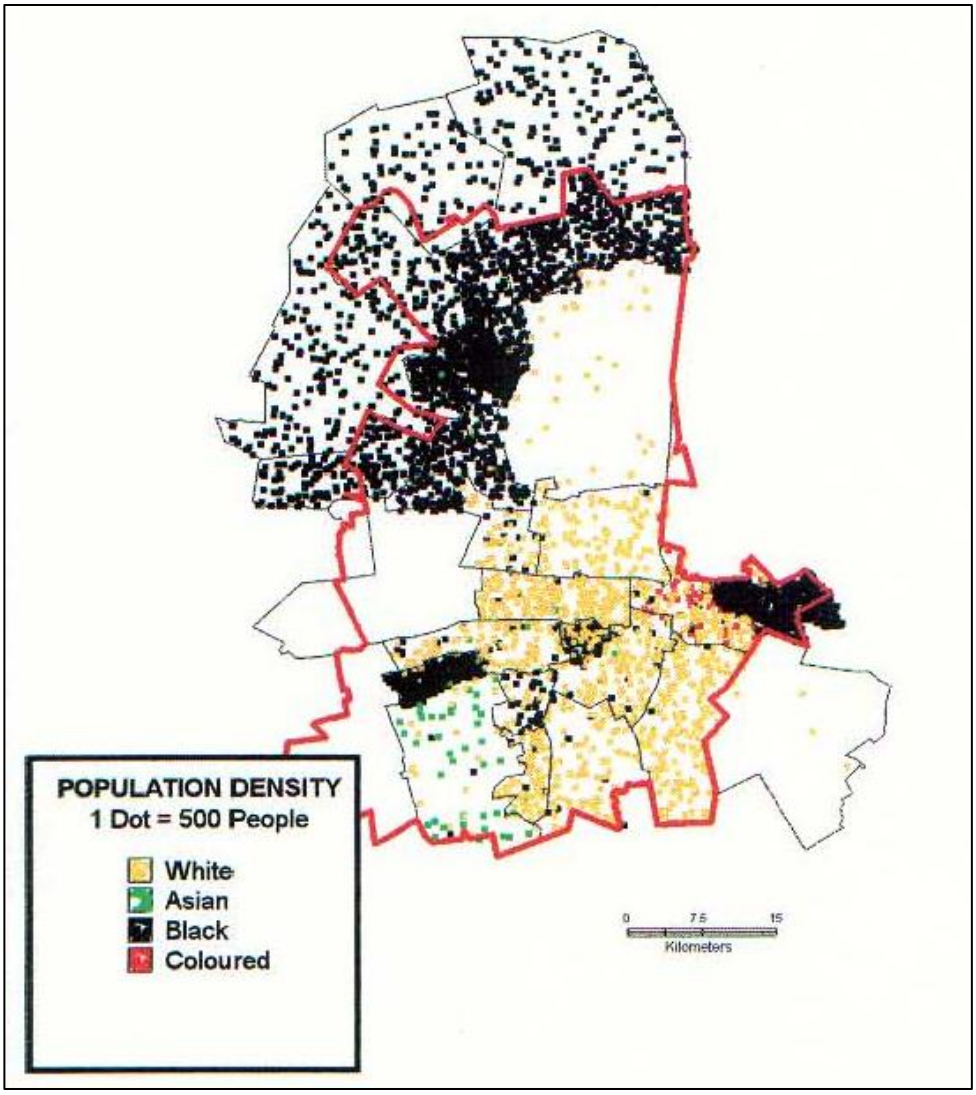


Figure 3-4. Population density by race in Pretoria in 2000 (Lombard and Olivier 2000).

Much like cities in North America, South African cities – Johannesburg in particular- have undergone deindustrialisation and a decentralisation of the industries that remain. In Johannesburg, many industries and businesses that once occupied the central business district have moved into the northern and persistently white suburbs (Crankshaw 2008). By 2001, the demographic make-up of the formerly white-only neighbourhoods of inner city of Johannesburg was 88% black, indicating a massive shift (Crankshaw 2008). In Pretoria, the change has occurred more slowly (Fig. 3-3). With the exception of a few pockets, most of central Pretoria has remained relatively white.

Much of the influx of black individuals into cities comes as a result of rural to urban migration. In the period between 1996 and 2001, the urban population of South Africa increased by over 17% , with Gauteng as the greatest recipient of rural to urban migrants (Christopher 2005). Much of this urban expansion has taken the form of the expansion of the

townships and black residential zones, and white residential areas have remained particularly segregated. But whilst the movement from rural to urban areas is often seen as a positive one, with greater access to education, and health and welfare services, Ginsburg et al. (2009) have pointed out that in South Africa in particular, urban migration can have serious negative health and social consequences for migrants, particularly children. Ginsburg et al.'s study of the residential mobility of children included in the Birth to Twenty Cohort study is one of the most extensive post-apartheid studies of intra-urban migration in Gauteng. This study showed that the households in the lowest and highest socioeconomic strata were most likely to move.

Whilst many black households have moved into established, formerly white areas, more still have moved into the burgeoning informal settlements in the Pretoria/Johannesburg region. In 2005, 1.376 million black South Africans lived in informal/squatter settlements (Richards et al. 2007).

3.5 Health among black South Africans

Apartheid policy affected health in many distinct ways. In addition to lack of access to adequate medical care, as well as poor living conditions, lack of education and within group ethnic divisions all contributed to the poor health and health outcomes of the black population.

3.5.1 Living conditions in townships and squatter camps

In 1955, five years after the introduction of the Group Areas Act, the township of Alexandra, Johannesburg had roughly 80,000 people living in just 1.5 square miles of land. The township lacked a sewer system and refuse collection system and the Alexandra Health Committee had a budget of less than £1 per head (Susser et al. 1955). Whist Alexandra was just one smaller township, the conditions within its boundaries were characteristic of most townships, and squatter camps were notoriously worse. By the 1980s townships in Pretoria and Johannesburg were so overcrowded that sub-tenancy (shack homes on larger plots within the townships) and squatting were rife (Fig. 3-4). In 1990, it was believed that over two million people lived in squatter camps in the Johannesburg region. Because of their informal, unsanctioned nature, these squatter camps are characterised by some of the worst urban living conditions (Parnell and Pirie 1991). These conditions are directly responsible for the poor health of black South Africans during apartheid.

A 1991 demographic and health survey of Alexandra paints a bleak picture of the township, which the South African Medical Journal describes as "distressing" (Ferrinho et al. 1991). By 1991, the population of Alexandra was 200,000 people living in under five square

kilometres. Despite an ambitious 1979 plan to upgrade Alexandra away from a hostel city and into a model urban township, Ferrinho et al. point out that the upgraded homes and services were out of reach financially for the majority of residents, many of whom were displaced into the informal sector as a result. The survey highlights high infant and child morbidity, communicable and sexually transmitted disease and violence. From the 1950s to the 1970s the township had only three general practitioners, which increased to 17 by 1991. The referral hospital for Alexandra, Tembisa was over 25 kilometres away. With respect to facilities and quality, a 1987 study of patients receiving sutures at the Alexandra Health Centre found that over 22% of patients returned to the clinic with septic suture sites attributed to the lack of aseptic conditions during suturing (Reitenberg et al. 1991).

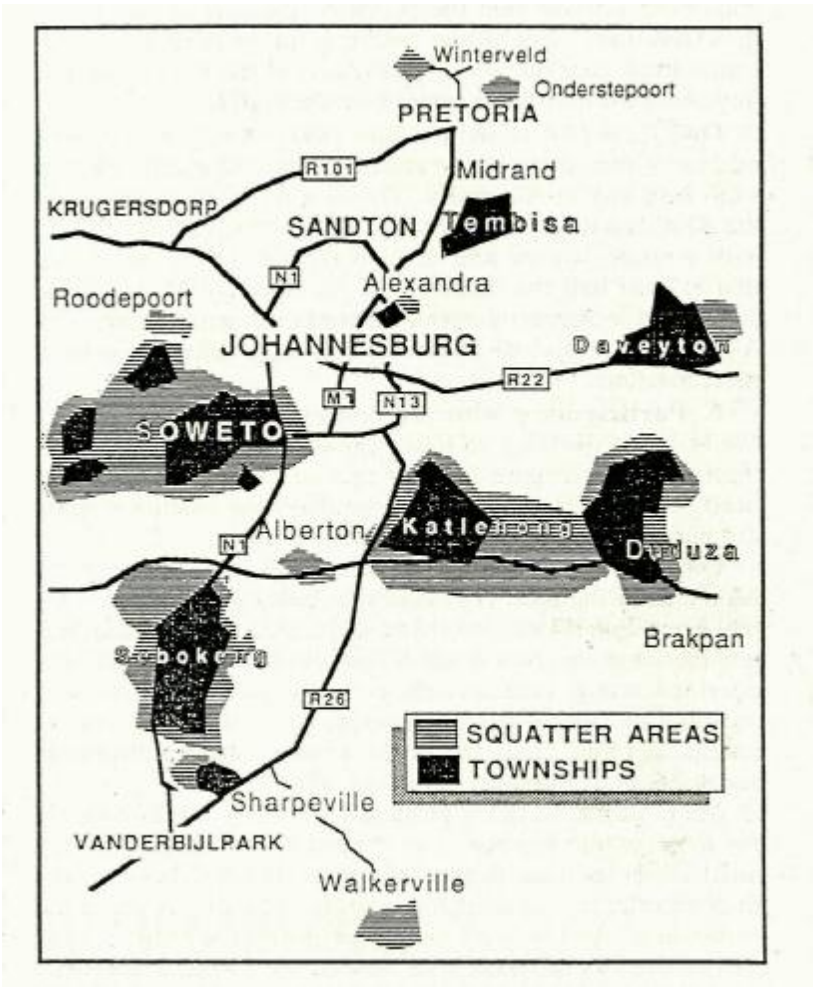


Figure 3-5. The borders of both townships and informal/squatter camps in Johannesburg in 1991 (Ferrinho et al. 1991). The map shows the extensive nature of squatter settlements surrounding Johannesburg, and to a lesser extent, Pretoria, most of which were characterised by poor living conditions.

3.5.2 **Infant mortality as an index of black health status**

Andersson and Marks (1988), following on the WHO report Apartheid and Health (1983) examined the impact of apartheid on health. They note that disease patterns in South Africa closely followed income, which is a function of racial classification. Damningly, they note that as late as 1986, data on health indices such as infant mortality were lacking for large regions of the country. The South African government stopped officially publishing data on black infant and child mortality in 1963 (Wisner 1989). Even official census data did not include mortality data for many Bantustans, as they were considered independent countries by this time, and not subject to basic data collection (Thompson 2001). As late as the 1970's the three poorest homelands were not included in the census, and urban black residential areas were under-sampled (Klasen 1997). Moreover, much of the data that was gathered was piecemeal and methodologically different, making comparison difficult. What was clear was that perinatal and infant mortality was substantially higher among the black population than the white (Andersson and Marks 1988). In 1950 infant mortality rates among black infants at Baragwanath Hospital was 233 per 1000 live births. The infant mortality rate for white infants in Johannesburg in 1950 was 32 (Stein and Rosen 1980).

In the bantustans, mortality by age five was thought to be nearly 50% (Wisner 1989). Perinatal death due to causes such as gastroenteritis, chest infection and "peri-natal problems" was 11 times higher in black children than white children in the city of Kimberley, a pattern repeated in other urban areas. Maternal deaths were also higher in black populations, with illegal and unsafe abortions causing most maternal deaths among black women, a factor Andersson and Marks ascribe to differential access to safe medical care between black and white women. In a pilot study of peri-natal deaths in a Lebowa hospital (a black hospital), 30% of all peri-natal deaths were attributed directly to medical mistakes or omissions in care (Wilkinson 1991).

Nannan et al.(2007) reported similar trends using the Demographic and Health surveys. In the 1980s, infant mortality rates were 13 per 1000 live births for white children and 68 per 1000 live births for black children. The 1996 census was the first to collect vital information for South Africa as a whole, incorporating the former homelands. In 1995 16% of infant deaths, and 20% of child deaths (under five years) were attributed to diarrhoea (Choi 2003). Among the factors that are correlated to childhood diarrhoea are sanitation, hygiene and maternal education (Gyimah 2003). All of these factors were directly influenced by apartheid policy – the forced residence in townships with no access to sanitation or clean water, and limited educational opportunities for black individuals (Choi 2003). Again, this data was collected post-apartheid, but it is believed that these trends in infant mortality are

persistent ones, and given the slow rate of change in health care access in post-apartheid South Africa, they are accurate proxies for apartheid era trends (Burgard and Treiman 2006).

The 1998 Demographic and Health Survey has been used to identify another significant cause of apartheid (and present-day) infant and child mortality: indoor air pollution. The trends associated with indoor pollution in relation to heating and cooking fuel are discussed in greater detail in Chapter 4. Use of coal and wood fuel for heating and cooking in impoverished areas of South Africa was significantly correlated to infant and child mortality (1-59 months), although the authors of this study do not speculate as to the mechanism responsible for increased mortality associated with indoor pollution (Wichmann and Voyi 2006).

3.5.2.1 Chronic disease

There is little information regarding chronic disease among black South Africans during apartheid. Again, this is largely due to lack of inclusion in demographic and health surveys prior to 1995 and lack of access to medical care for diagnosis. Since the late 1990s, several studies have examined differences in chronic disease between the black and white population. These studies focus primarily on non-communicable disease such as heart disease, hypertension and diabetes. It is difficult to compare pre-and post-apartheid non-communicable diseases such as these for a number of reasons. First, is the aforementioned lack of baseline data. Secondly, is the concept of an epidemiological transition, which South Africa is currently undergoing (Stewart et al. 2011). This phenomenon marks the transition from communicable disease associated with developing countries to non-communicable diseases prevalent in the developed world.

Whilst historically, the black population has had lower rates of death due to heart disease and Type II diabetes, there are chronic diseases that were more prevalent in the black population than the white. In a 1984 study of all cardiovascular deaths between black and white individuals in Cape Town and Durban, the black population had a higher rate of death from cerebrovascular events, i.e. stroke than white individuals with 36% and 20% of all cardiovascular deaths, respectively (Andersson and Marks 1988). The black population also had a higher rate of death from hypertension, at 3% and 0.5% respectively, though overall, a substantially lower rate of cardiovascular deaths (values given are for Cape Town, Durban values followed the same trend). In a 1982 study of urban Zulus versus urban whites, 25% of Zulus were hypertensive, compared to 22.8 of whites (Seedat and Hackland 1984). Both Andersson and Marks (1988) and Seedat and Hackland (1984) attribute the higher rates of hypertension and stroke to higher levels of stress among the black population.

Diseases such as tuberculosis (TB) were also more prevalent in the black population during apartheid. Prevalence rates for TB in the black population were between 18 and 47 times higher than in the white population, depending on location. Cholera was believed to be endemic in several of the bantustans, due predominantly to poor sanitation, however the exclusion of bantustans from national health surveys means there was little data on the prevalence of cholera during apartheid (Andersson and Marks 1988). Neither the cholera, nor similar typhoid outbreaks in black areas seemed to reach the white population, as its primary cause is due to lack of clean drinking water (Coovadia et al. 2009). Tuberculosis in particular, is nutrition-related. Individuals who have iron-deficiency anaemia are more likely to contract and die from tuberculosis than individuals who are not (Schaible and Kaufmann 2004).

3.5.2.2 Nutrition

Poor nutrition is a major factor not only to overall health, but in the uptake of heavy metals within the human body. As discussed previously, in Chapter 2, deficiencies in some essential trace elements can result in increased uptake of toxic trace elements into the body, which can cause illness or exacerbate existing illness. Again, wide-scale nutritional surveys of the black population during apartheid were not undertaken. However the information that does exist demonstrates that a large percentage of black individuals were chronically malnourished to some degree.

Among the issues pertaining to poor nutrition among black individuals, was the presence of Bantustan and townships. By the mid-1980s the bantustans were theoretically home to 24 million black individuals (74% of the population). The area encompassed by all of the bantustans was only 13% of the total area of South Africa. Additionally this land was among the most arid in the region, and yet it was expected that the bantustans would be self-sufficient food producers (Wisner 1989). This meant that township and squatter camp residents and bantustans had to purchase food at market prices, despite poverty. The result was wide-spread hunger and malnutrition. In 1983, the WHO estimated that vitamin A deficiency, scurvy (in an orange-growing country), rickets, Kwashiorkor (a protein deficiency) and pellagra, were all extremely common, based on information gleaned from small-scale studies (WHO 1983). In addition, the WHO estimated that incidence of marasmus (protein deficiency plus inadequate caloric intake), an acute form of malnutrition was approximately 15-20% in rural areas. Overall, the WHO estimated that the number of black children in South Africa suffering from some form of malnutrition was nearly one million. In 1978 alone, over 1100 children were seen at Baragwanath Hospital, Johannesburg suffering from acute malnutrition and an estimated 25% of child deaths were caused by Kwashiorkor, marasmus or a combination of both (Stein and Rosen 1980).

In a rare investigation of the state of affairs in a peri-urban township near Johannesburg, a 1955 report published in the *Lancet* highlighted conditions in Alexandra Township. The authors note that the primary food source among township households was maize and potatoes with little meat. Little to no vegetables were consumed and despite the availability of vegetables in shops, the high prices meant vegetables were out of reach of most households (Susser et al. 1955). This appears to be the case still in many townships, and the present South African government is promoting the use of urban vegetable gardens, a practice that may be problematic in regards to toxic elements (see Chapter 4) (Crush et al. 2011; Keatinge et al. 2012; Wills et al. 2010).

As Faber et al (1999) points out and the few studies mentioned above reiterate, national food security does not equal household food security, and despite the fact that South Africa produces adequate food for its population, large gaps in nutrition between the white and black populations exist into the present day . Food insecurity in some townships is as high as 85% in Johannesburg (Naicker et al. 2009). Faber at al. (1999) conducted a study of nutrition among primary school children in a rural area outside Durban. They found persistent deficiencies in several micronutrients, and an overall low intake of calcium and vitamin A. In addition, chronic malnutrition was prevalent as was the associated growth stunting.

Among black adults, nutrition is also lacking in key nutrients. Whilst there is no real data regarding adult nutrition in this population during apartheid, a handful of post-apartheid studies make it clear that nutritional gaps existed. Women in particular may be lacking in vitamins D and A, as well as calcium, iron and zinc (Hattingh et al. 2008; Kruger et al. 2011; Oldewage-Theron et al. 2008; Steyn et al. 2000). Most nutritional studies focus on children and infants as they are more susceptible to health problems from a poor diet. However as was discussed in Chapter 2, deficiencies in essential trace elements such as zinc, calcium, iron and vitamin D may have a direct effect on uptake of toxic trace elements, putting nutritionally deficient adults at risk for higher rates of uptake of these elements. Iron, magnesium and zinc deficiencies are prevalent in black men, the former two are due to inadequate nutritional intake and the latter due both to nutritional deficiency and the prevalence of alcohol consumption (Elin 1988; Kärkkälnen et al. 1988; Lieber 1988; Nojilana et al. 2007; Parry et al. 2005; Peltzer 2002; R. Rylander 2001; Romani 2008; Schneider et al. 2007).

Among the most substantial research into health in South Africa is the Birth to Twenty Cohort (BT20) research. The study began in 1990 and followed 3273 children who were born in Soweto in 1990 (Naicker et al. 2010a; Richter et al. 2007). The BT20 research

examined all facets of the health of the children included in the study and data from the project is still being published in the present day. Most critically for this project, the research included data regarding lead exposure among Soweto children despite its post-Apartheid focus, the children included in the study were born into Apartheid-era conditions and this data can be used as a proxy to infer conditions for black South Africans prior to the 1990s (Richter et al. 2007). Bearing in mind, the black population in South Africa is currently undergoing a nutritional and epidemiological transition towards a Western, higher fat diet and towards obesity and related health conditions, the status of children in townships such as Soweto is still far from healthy (Abrahams et al. 2011; Bourne et al. 2002; Bourne and Steyn 2000).

Studies of the BT20 cohort have yielded much information about the health of individuals growing up in an urban township. MacKeown et al. (2007) report the mean intake of macro and micro nutrient intake of 143 children from the cohort at ages 10 and 13. They found moderate deficiencies in iron, zinc, copper and vitamin A, pantothenic acid and biotin at both ages. More serious deficiencies in mean calcium intake (less than 70% recommended daily intake) were reported at both ages. The children consumed more protein than recommended, but less over all calories than recommended at each age. In 2000 (age 10), 73% of children consumed below the recommended daily energy (caloric) intake. Eighty-five and 90% of children were deficient in zinc and calcium respectively and in almost half of children calcium intake was less than 50% recommended intake.

3.6 Health of white South Africans during Apartheid

The focus in this chapter has been largely on the health of the black population, but the white population of South Africa is not without its health concerns, despite western standards of living and access to world-class medical care. The health of white individuals in South Africa is on par with European and North American health. The epidemiological and nutrition transitions took place there at the same pace as western countries so the diet is high in fat and carbohydrate, and diabetes, heart disease and obesity are similar to European countries (Faber and Wenhold 2007; Steyn et al. 1992). As was discussed above, white life expectancy is approximately 70 for males and 77 for females, compared with 52 and 55 in black males and females, respectively (as of 1997) (Kinsella and Ferreira 1997). Communicable diseases such as cholera, HIV/AIDS, tuberculosis and typhoid are nearly non-existent in the white population. Sanitation, population density, and food security are similar to western countries.

Several dietary deficiencies are prevalent in the white population, namely calcium, iron, zinc and magnesium. As with the black population, this is primarily due to consumption of a

Western diet, high in fat, carbohydrate, and sugar. Smoking and alcohol use, which is most prevalent in white males also contributes to deficiency in some essential elements such as magnesium and zinc (Steyn et al. 2006).

There are differences in health however, in which the white population is at a disadvantage to the black population. White individuals are more likely to suffer from diabetes, and non-hypertensive heart disease than the black population. Primarily, this is due to lifestyle differences and the influence of the western diet. However there are other differences that are more difficult to explain. Post-menopausal white women have demonstrably lower bone mineral density than black women, despite better nutrition (Chantler et al. 2012). Post-menopausal black women have higher femoral neck and total hip bone mineral density but lower lumbar spine density than white women. The authors attribute this to socioeconomic and lifestyle differences, but point to a paucity of explanatory data (Chantler et al. 2012). Interestingly, this difference in bone mineral density between those of African and European ancestry is seen in other countries such as the United States (Luckey et al. 1996). Other authors have found similar trends among post-menopausal women in South Africa, attributing this to higher body mass index in post-menopausal black women (Daniels et al. 1995).

Mickelsfield et al. (2011) conducted a thorough study of bone differences between black and white women in South Africa and, controlling for socioeconomic factors, found a significantly lower rate of bone fracture among black women as well as higher femoral bone density. In this study, black women had a significantly lower mean intake of calcium than white women, at approximately 400 to 500 mg per day. Other researchers have reported differences in calcium intake between black and white women at approximately 100 and 1000 mg per day respectively (Kruger et al. 2007). Nutritionally, post-apartheid urban black women consume more fat than white women and have less lean mass and less visceral fat and greater peripheral fat than white women, which significantly affects bone mineral density in these women (Micklesfield et al. 2011). The difference in bone density is manifested in higher femoral neck and total hip density in black females, and is highly correlated to socioeconomic status and degree of physical activity (Chantler et al. 2012).

The phenomenon of lower calcium intake yet higher fat intake among urban black South Africans is part of a wider paradox of both under- and over-nutrition occurring in the same community. In a study commissioned by the Mines Safety Commission, South Africa, Dias et al. (2003) found that among mine workers across all mine types (gold, coal and diamond), black mine workers were, on average deficient in vitamins C and A despite normal body mass indicies. Mine workers living in hostels had significantly poorer nutrition and lower

energy intakes than men living in non-hostel accommodation. Moreover, all mine workers consumed very little fruit and vegetables (Dias et al. 2003). Puoane et al. (2002) examined under-over nutrition in South African adults and found that in urban black and white South Africans and found malnutrition despite obesity. With regards to weight, Puoane et al. found the highest rates of abdominal obesity (most often associated with heart disease and diabetes) in white men and urban black women. In men, an estimated 12% of black individuals were classified as underweight, compared to just 5% of white individuals (Vorster 2010). As Vorster (2010) points out, South Africa is prone to both infections disease that results from under-nutrition as well as non-communicable disease that results from over-nutrition. Other researchers have suggested that early childhood malnutrition and growth stunting may account for the prevalence of obesity in black South Africans later in life (Kruger, Margetts & Vorster 2004; Mukuddem-Petersen & Kruger 2004). Even in overweight individuals however, there appears to be a prevalence of micro-nutrient deficiency that is likely to affect health and more importantly, for this project, trace element uptake (Vorster 2010).

4 Environmental pollution in South Africa

Of paramount importance to this research is an understanding of the type and prevalence of inorganic pollution in South Africa during the later-half of the 20th century. Without this information, the context of human exposure rates within the greater environment cannot be established. This chapter provides a review of environmental studies conducted in South Africa, specifically those dealing with inorganic pollutants, and will include discussion of the state of the environment in South Africa, particularly Gauteng Province, incorporating the few environmental studies conducted in the region prior to 1990 with present-day data. In doing so will present as thorough analysis of inorganic pollution as is possible, given the paucity of data. This chapter also includes a discussion of the likely sources of exposure to toxic metals and trace elements for individuals living in urban Gauteng. As with data on human health, however, this information is quite piece-meal, and often lacking altogether. In addition, much of what is known about the state of inorganic pollution in South Africa comes from studies conducted since the end of apartheid, meaning any robust review of the environment during this period will entail a certain amount of educated conjecture.

Information regarding heavy metals and trace elements in the South African environment is sparse. The need for a system of environmental monitoring for trace elements has only recently begun to be recognized in South Africa. Despite the country's long history of mining and industrial activity, environmental policy has been largely lax compared to other developed nations (Van Eeden 2008). This is due, in part to the historical domination of industrial and mining companies in the political realm in South Africa. As a public health concern, human exposure to heavy metals has been relegated to the background and public health discourse tends to be centred on HIV/AIDS, as this is among the more pressing of health crises in southern Africa. Nonetheless, as this review demonstrates, heavy metal pollution is worryingly high across Africa as a continent and in South Africa in particular (Nriagu 1988; Nriagu 1992; Yabe et al. 2010).

There are certain environmental trends that are clear regarding the distribution and primary sources of pollution and toxic metal exposure in South Africa. The majority of pollution comes from motor vehicle emissions, followed by industrial activities such as the burning of fossil fuels (coal and oil), and domestic wood and coal burning. In urban Gauteng (Transvaal), mining and smelting are also pollution sources. As Norman (2007a; 2007b)

points out, South Africa is at once an industrialised and undeveloped country, with many of the environmental issues that plague each.

However data regarding atmospheric concentrations of heavy metals and their behaviour from other South Africa countries can shed light on the likely distribution of these elements in the past. In South Africa, as elsewhere, the majority of exposure to inorganic pollution is in the form of airborne particulate matter which is suspended in the air in aerosol form. Aerosol pollution contains both organic (including volatile organic compounds) and inorganic components, most of which are harmful to humans. Much of the inorganic matter in particulate matter (PM) has been shown to be bioavailable to humans, meaning it can be taken up into human organs (Lum et al. 1982). Inorganic constituents of aerosol fall into one of two types of PM: fine and coarse. Fine PM is any which contains particle sizes up to 1µm in diameter. Fine PM (PM_{FINE}) is almost always anthropogenic in origin. Coarse PM, which includes two types: $PM_{2.5}$ and PM_{10}, contains much larger particles and can be anthropogenic and natural (e.g.. volcanic) in origin. The numeric subscript indicates the size, in microns, of the particulate matter with $PM_{2.5}$ consisting of particulate matter of 2.5µm and PM_{10} consisting of particulate matter of 2.5 to 10µm. PM_{FINE} and coarse PM can be readily inhaled by humans and absorbed by the human body, and as such are dangerous to human health (Davidson et al. 2005; Harrison and Yin 2000; Lippmann et al. 2000; Oberdorster et al. 2004).

The size of the particulate matter plays a role in the extent of dispersion of the pollutants, with PM_{FINE} and $PM_{2.5}$ generally dispersing more widely than PM_{10}, and have a longer "lifetime" (time spent airborne) as the smaller particle size allows for greater atmospheric mobility through wind and air current patterns, though the practical modelling of PM dispersion is quite complex (Claiborn et al. 2000). Adding further complexity is the fact that the same element can be present in each type of PM, often this is a function of its source. Of the recent PM aerosol monitoring conducted in South Africa, several trends emerge. Present day studies of manganese point to its high concentration in PM_{10}, with vehicle emissions as a source. During the period from 1960 to 2002, manganese was not added to South African petrol. During this period atmospheric manganese in Gauteng likely came from ferromanganese smelting activities, which produces manganese particles which fall into $PM_{2.5}$ (Who 2001) which tends to have a wider dispersal pattern than PM_{10}. Vanadium is a by-product of steel production and smelting and is found in PM_{10} (Moja et al. 2013). Cadmium is likely present in both $PM_{2.5}$ and PM_{10}, however the proportion of the element in each has not been established in South Africa. $PM_{2.5}$ is not well-studied in South Africa.

This lack of widespread PM monitoring is just beginning to be addressed. In the past four years, several studies of PM and its impact on human health were conducted in South Africa. Kaonga and Kgabi (2009) measured PM_{FINE}, $PM_{2.5}$ and PM_{10} in a platinum mining area in Rustenburg, in northwest South Africa (Fig 4-1). They found that in the morning hours, when traffic is at its peak, PM was dominated by $PM_{2.5}$ (particularly in winter, when cold and dry weather conditions cause concentration of PM) but that levels of all PM were highest during this time. They also note that this is a peak time for burning of domestic coal. PM valued dropped midday when mining activity peaked. The authors conclude that domestic coal burning and traffic accounted for the bulk of atmospheric PM in the region.

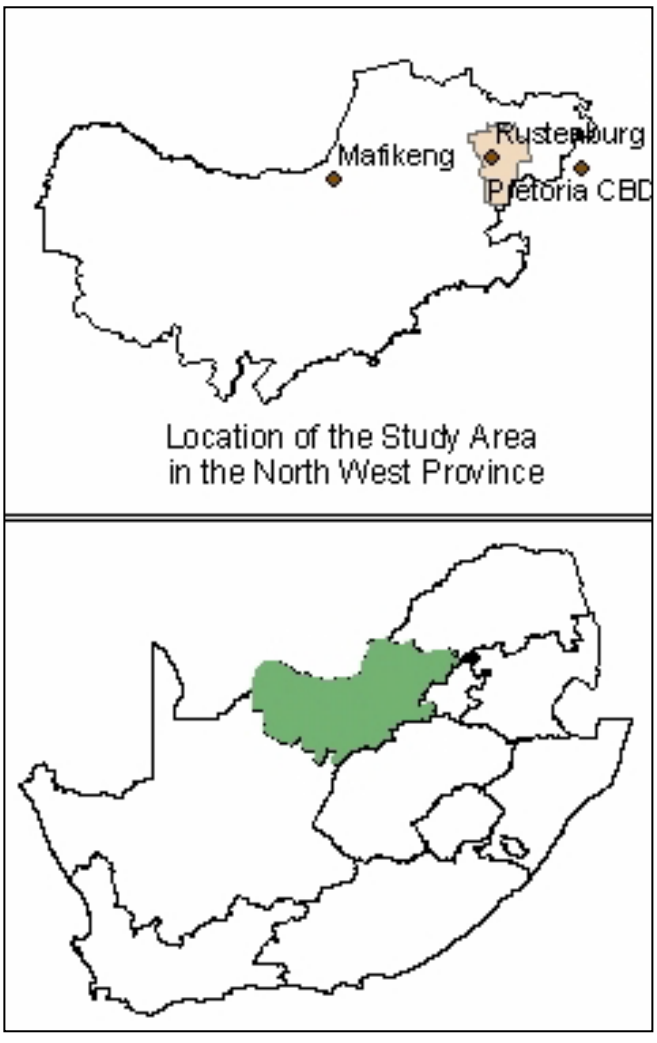


Figure 4-1. From Kaonga and Kgabi (2009). PM monitoring in a mining area in Rustenburg, South Africa. The platinum mining area is east of Pretoria and adjacent to present-day Gauteng.

In 2012, Kgabi (2012) reported levels of toxic elements associated with PM_{10} in near Rustenburg. Of the elements of interest to this project were lead and vanadium. Source apportionment of PM_{10} determined that both lead and vanadium come from vehicle traffic, as opposed to domestic heating or industrial activities. Kgabi also reported that as levels of total PM_{10} increased, so did levels of inhalable lead and vanadium. Other authors report the presence of lead in both PM_{FINE} and $PM_{2.5}$ and vanadium in the PM resulting from oil-fired industrial activities (Dominici et al. 2007; Kgabi et al. 2011; Woodin et al. 2000). Kgabi et al. (2012) monitored PM concentrations in the mining area of Rustenburg as well as the Rustenburg central business district. They report higher levels of PM_{10} in the mining area regardless of season or time of day. Notably the air quality in the mining area, classified by the degree of PM into categories ranging from good to hazardous,

Since the 1990s, following the formation of the new South African Republic, few researchers have undertaken the task of monitoring urban and suburban air and soil quality in South Africa. To date, eight studies have been conducted on atmospheric inorganic pollution in Pretoria and Johannesburg. Several studies have investigated air quality and health, including the Vaal Triangle Air Pollution studies, which have sought to quantify the health effects of the highly industrialised and polluted Vaal Triangle in southern Gauteng (Moja et al. 2013; Terblanche et al. 1993; Terblanche et al. 1992). Few of these studies have investigated inorganic pollution, with Moja et al. (2013) and Maenhaut et al. (1996) the exceptions. These two studies sought to identify the constituent metals in the coarse and fine fractions of PM. Maenhaut et al. measured trace metals in aerosols in eastern Transvaal. They report that lead and antimony were found in the coarse fraction, and manganese in the fine fraction. Zinc was found in both and copper in the course fraction. As this region is not particularly industrialised, they attribute the PM to burning biomass. Moja et al. (2013) found lead in both coarse and fine PM, and attributed PM in the industrialised Vaal Triangle to industrial, motor vehicle and domestic coal burning. One other study, conducted by Batterman et al. (2011) found lead and manganese in both $PM_{2.5}$ and PM_{10} in Durban, however manganese measured in this study included MMT emissions, which would not have been present during the period from 1960 to 2000.

PM studies are critical to the study of trace element exposure patterns, particularly for elements such as lead, manganese and vanadium which are predominantly airborne. The presence of lead in PM_{10}, the heavier component of PM, explains why lead is concentrated around roadways. PM_{10} does not disperse as far and remains concentrated near its source. It also explains the presence of lead, albeit in lower concentrations, in areas far from traffic, as lead is also found in $PM_{2.5}$, which can disperse quite widely across great distances. Moreover, the finer particles of $PM_{2.5}$ may be more dangerous to humans as they are more

easily absorbed into the lungs. These particles stay airborne for longer periods, where they are more readily inhaled. Again, the kinetics and movement of PM in the atmosphere add a level of complexity to studies of trace element exposure and are not as well documented in South Africa as in other parts of the world.

Fewer studies still have examined water and soil pollution. In Pretoria/Tshwane, much of the published data regarding urban heavy metal pollution derives from one study of lichen and jacaranda trees that was conducted during a three-month period. Moreover, studies of urban pollution in South Africa are largely non-existent prior to 1985. This means that any analysis of pre-1990 human exposure to heavy metals is largely framed in the context of present-day environmental data. Despite a lack of temporal environmental data, and with the exception of the (very) recent cessation of leaded-petrol use, very little is believed to have changed in the urban environment in Gauteng in the past several decades and apartheid era environmental conditions can be adequately extrapolated.

It is critical to note that data on inorganic environmental pollution in South Africa are heavily biased towards the study of lead. Whilst other element concentrations are reported along with lead, these are rarely given more than cursory discussion. Moreover, within the handful of studies conducted on trace elements in the Gauteng environment, a given element may be included in one study and omitted in another. As a result, data is often piecemeal and the overall state of urban pollution must first be pieced together from sometimes difficult to compare studies. Subsequently, the state of the Gauteng urban environment *in the past* must be extrapolated from these present –day studies.

Despite a lack of data regarding the state of the environment in South Africa prior to the 1990s, researchers have characterised the period as one in which industrial pollution and "general air quality issues" were the norm (Naiker et al. 2012). During this period, the Vaal Triangle, encompassing Gauteng Province, was a known "hotspot" of air pollution. In 1965 the government of South Africa enacted the Atmospheric Pollution Prevention Act, with the general aim of addressing industrial pollution. Notably, however, the Act exercised little regulatory control over vehicle emissions, which have since been determined to be among the primary sources of lead pollution in urban South Africa (Diab 1999; Monna et al. 2006; Naiker et al. 2012). What can be gleaned from the sparse data regarding atmospheric pollution in urban South Africa prior to 1990 is that the environment was largely characterised by industrial, vehicle and smoke-related pollution, with lax government control. Studies primarily investigated trace element and heavy metals present in the atmosphere in Cape Town, Durban, Port Elizabeth, Pretoria and Johannesburg. More

recently, multi-site studies have monitored air quality in Pretoria and Johannesburg and the greater Tshwane metropolis in Gauteng Province.

In the past decade, South African researchers have conducted limited studies of trace element concentrations in lichen transplants throughout Pretoria/Tshwane and Johannesburg. These mark the first studies of this kind in South Africa. Separately, these studies offer a snap-shot view of urban inorganic pollution levels in Pretoria/Tshwane and Johannesburg. Taken together, the data presented show some consistent patterns in urban pollution, particularly with regards to lead. When these patterns are combined with data gathered from the Pretoria Bone collection, and with blood lead data from the region, a more detailed picture emerges, in which urban pollution and human exposure rates are characterised both by urban geography and urban demography.

Lastly, research into the source of heavy metal exposure in humans is sorely needed in South Africa. In the case of lead, in particular, no analysis of lead isotope ratios have been conducted on blood or bone lead, meaning that any conclusions regarding both exposure source and pathway are, on balance, conjectural. Whilst it is still possible to make meaningful and significant connections between a given element source and human exposure to it, these relationships and correlations must be taken with a proverbial grain of salt until more quantitative data is available.

4.1 Environmental monitoring in Gauteng prior to 1994

A review of the literature yields almost no information regarding the state of the environment in urban South Africa prior to 1990. Studies of urban atmospheric pollution and heavy metals from this time were simply not conducted. Two studies however, were conducted on atmospheric pollution in Johannesburg in 1978 and 1982-1984 by PIXE (Particle Induced X-ray Emission) analysis (Annegarn et al. 1981; Formenti et al. 1998). These analyses, though limited, shed substantial light on the state of the environment during this time, and for a variety of reasons, are of great value to the present research. Annegarn et al. (1981) conducted atmospheric monitoring of three sites in Johannesburg: the urban core (central business district), Soweto – the Southwestern Township 15km south of the city, and Lanseria Airport, approximately 30km outside of Johannesburg. Monitoring was conducted over a two-month period from September to October (Spring). The study included the monitoring of atmospheric trace element concentrations including lead.

Two patterns emerge from the Annegarn et al. (1981) study. Firstly, atmospheric lead concentrations were substantially lower in Soweto than in central Johannesburg, with concentrations of 0.33µg/m^3 and 0.90µg/m^3, respectively. Unlike the lichen studies that will

be discussed in Section 4.2.2, this analysis allowed for the continuous monitoring of pollution levels, including diurnal, nocturnal and hourly fluctuations in element concentrations. This allows for the uncovering of a second pattern: significant differences in temporal variation in atmospheric lead concentration between study sites. The authors report consistently high lead levels in the central core of Johannesburg throughout the day, with atmospheric lead concentration dropping only at night on most nights, and remaining high on others. Conversely, in Soweto, daily peaks occurred which corresponded to peak traffic times, which the authors ascribe to the transport of black workers into and out of the city each morning and evening (Annegarn et al. 1981).

Formenti et al. (1998) examined these patterns in further detail. The time-resolved atmospheric monitoring conducted in this study found that the two peaks in atmospheric lead concentration peaked strongly between 7:00 and 10:00am, and again from 17:00 and 21:00pm. Furthermore, during the mid-day period, lead levels dropped in the order of ng/m^3. Formenti et al. also posit that the burning of domestic coal accounted for between 40% and 50% of atmospheric pollution (including sulphur, copper, zinc, and manganese), with the rest stemming from street dust (on unpaved Soweto roads) and traffic. No isotopic studies were included in this analysis, so it is unclear as to the source of individual elements, however the authors employ a source apportionment method involving Principle Component Analysis (PCA) and tracer elements for coal-burning (crust elements) and traffic.

These two studies are interesting, particularly in light of present-day isotopic analysis of lead pollution in Johannesburg (this chapter, section 4.2.3), which conclude that coal contributes little to atmospheric lead in the region.

Lastly, in 1992 a study was conducted by Yousefi and Rama (1992) on trace element composition of the atmosphere in Johannesburg. The monitoring sites included two hospitals, a residential area, an industrial area, a metal-works, a chemical plant and a ceramic factory. Unfortunately, the authors do not identify the specific areas studied nor do they include a map with the data, so it is impossible to identify where the monitoring sites were located within the city, whether they are near to residential areas, townships, the central business district or important road networks. The authors do report that lead concentration at the industrial site was ten times that of the next highest lead concentration, located at a metal works at approximately $6000ng/m^3$ and $600ng/m^3$ respectively. The undisclosed residential area had an atmospheric lead concentration of approximately $300ng/m^3$, higher than one of the hospitals, the chemical plant or the ceramic plant.

Manganese was highest in the ceramic plant and second highest in the residential area at approximately 1800 and $1200ng/m^3$. Manganese in the residential area was double or triple

that of manganese in other areas including one hospital and was three times that of the metal works. Iron followed roughly the same pattern as manganese, with a high concentration in the residential area. Given the fact that manganese is associated with iron ore deposits, this finding is not surprising. Other elements such as cadmium, zinc and antimony were extremely high, by an order of magnitude, at the industrial site. Between 1979-1982, the highest airborne manganese concentration recorded by the U.S. Environmental Protection Agency was 200ng/m^3, and the mean concentration was 20ng/m^3 (WHO 2001). The WHO (2001) also reports that worldwide, regions with ferromanganese foundries or industry, manganese concentration can be as high as 500ng/m^3, still far lower than the values measured by Yousefi and Rama (1992).

Recent data indicates that with manganese in particular, particule size is more important that concentration of manganese in the atmosphere with regards to uptake and toxicity in humans. There is no published data regarding the distribution of manganese in fine and coarse PM in Gauteng. It is assumed that manganese in this region would be mainly found in PM_{FINE} and $PM_{2.5}$ particulate matter, but this is not confirmed. There is growing evidence that manganese found in fine PM is more toxic to humans as it is easily transported from the lungs to the brain (Weiss 2006). The lack of data regarding toxic element particle size makes health predictions based on environmental data substantially more challenging.

4.2 Present-day environmental monitoring Pretoria

The basis of urban atmospheric pollution studies in South Africa is lichen (*Parmelia sulcata)*. The use of lichen for biological monitoring is well established in the field of environmental toxicology, having been first mentioned in the literature as effective biomonitors in 1866. Lichen species are effective accumulative bioindicators because they are both cosmopolite, easily transplanted from one environment to another, and are efficient accumulators of environmental contaminants such as heavy metals. In practice, lichen can be grown in conditions that are free of any anthropogenic contaminants. Individual lichens can then be subsequently transplanted to an area of interest for monitoring, where they will absorb and accumulate any environmental substances in the atmosphere. This allows for quantitative assessment of contaminant or pollutant levels in a given region. Lichen have been used over the past two decades to monitor environmental pollution in a wide range of environments from urban industrial areas to indoor pollution (Canha et al. 2012; Conti et al. 2012; Flegal et al. 2010; Garty et al. 2009; Geagea et al. 2008; Klos et al. 2011; Olmez et al. 1985; Sert et al. 2011; Tretiach et al. 2011).

4.2.1 **Heavy metals**

Lichen are effective bioindicators of both heavy metals and trace elements. Though the exact mechanisms for accumulation are not fully understood, it has been hypothesized that thre are three potential mechanisms for the absorption of heavy metals in lichen organisms (Richardson 1995):

1) intracellular absorption through exchange processes
2) intracellular accumulation
3) entrapment of particles containing heavy metals

These elements and other atmospheric contaminants deposit on lichen through precipitation, including rain, mist, dew, dry sedimentation and gaseous absorption (Conti and Cecchetti 2001). The levels of elements accumulated do vary according to phases of accumulation and release – this is thought to be influenced, in part, by acid rain. In addition, altitude is known to play a role in absorption of lead and cadmium in particular. Lead accumulation has been shown to increase linearly with altitude, whilst Cd follows an inverse trend.

4.2.2 **Lichen studies and heavy metal pollution in urban South Africa**

4.2.2.1 Johannesburg

Lichen were first used as biomonitors in South Africa in 2001. To date, only one study has been conducted. Monna et al. (2006) used *Parmelia sulcata* to monitor atmospheric lead in and around Johannesburg. Between 2001 and 2003, naturally growing lichen (non-transplanted) were collected from throughout the city and investigated using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Thermal Ionisation Mass Spectrometry (TIMS). Samples were taken from several sites, including parks, mine dumps, and industrial areas and roadways. Notably however, is the absence of sampling sites in or near the historically black townships such as Soweto, which the authors ascribe to an absence of lichens in the area.

Table 4-1. Mean trace element concentrations in lichen in Johannesburg by area type. In µg·g^{-1}. From Monna et al (2006).

Area Type	Pb Avg.	Pb Range	Cu	Cu Range	Zn	Zn Range	Cd	Cd Range	As	As Range	Sb	Sb Range
Open Space Urban	81.41	49-179	35.25	15-45	74.25	53-121	0.253	0.05-0.55	4.866	2.5-9.5	1.96	1.1-4.8
Low Traffic Urban	159.16	41-302	51.83	26-81	143.75	70-227	0.213	0.14-0.25	7.82	3.4-13	3.76	1.6-4.7
Med. Traffic Urban	157	157	41	41	124	124	0.18	0.18	6.1	6.1	6.9	6.9
High Traffic Urban	273.5	159-388	53.5	41-66	171	169-173	0.36	0.25-0.47	11.15	7.3-15	6.95	4.2-9.7
Open space Suburb	114.25	47-217	26.5	21-36	139	82-274	0.59	0.23-1.39	6.9	6.1-9.6	3.23	1.8-6.8
Open Space Dump	108.66	53-141	57	21-93	127.33	101-145	0.343	0.15-0.59	11.9	9.7-14	2	1.8-2.3
Open Space Rural	53.5	51-56	29	24-34	61	68-88	0.095	0.08-0.11	5.65	5.3-6	1.1	1-1.2
Pilansburg	6.9	6.9	15	15	37		0.08	0.08	2.6	2.6	<0.1	0

As is evident from both Table 4-1 and Fig. 4-2, central urban areas in Johannesburg yield the highest levels of lead. Monna et al. posit that this is likely due to leaded petrol emissions, a pattern also visible in Pretoria. Interestingly, however is antimony, which is highest in concentration in urban open space. The authors make no mention of this phenomenon.

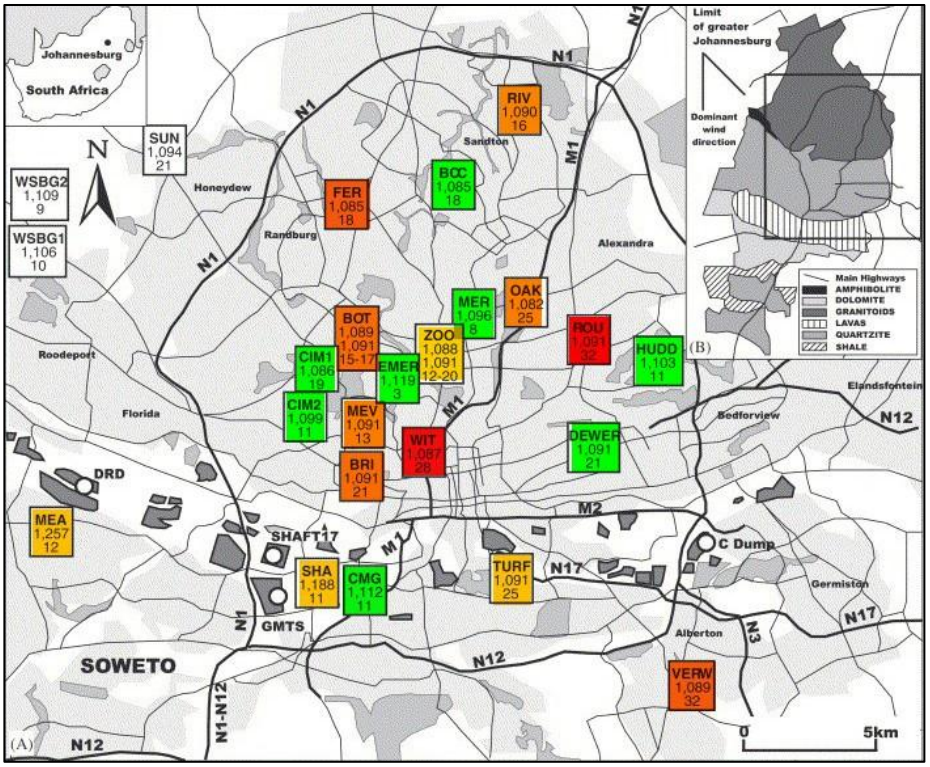


Figure 4-2. From Monna et al. (2006) Atmospheric lead concentration in and around Johannesburg showing an urban to suburban/ high to low (red > orange > yellow > green) lead gradient (colour added).

Unfortunately, information regarding heavy metals, specifically lead, in Soweto and historically black townships is not included. Despite this oversight, the authors provide strong evidence for a distinct, urban to rural gradient for petrol-based lead in the Johannesburg environment. In addition, the areas characterised by mine dumps yield lower lead pollution than even the low traffic urban areas, indicating that mining activities may not contribute to airborne heavy metal pollution. Mining may, however, contribute substantially to water pollution. This is a critical pattern, as urban residential patterns in South Africa followed strict laws which saw the urban core as a white area and the surrounding suburban areas as black areas.

The urban pollution data is moderately better for Pretoria/Tshwane (Table 4-2). To date, one lichen study and three street dust studies and one study of jacaranda (*Jacaranda mimosifolia*) leaves and bark have been conducted, though most are overlapping studies of the same areas during the same time period (Okonkwo et al. 2006; Okonkwo et al. 2009; Olowoyo et al. 2011). Thought the authors do not identify the same pollution distribution as Monna et al. (2006), closer examination of the data results in the identification of a similar urban to rural gradient. Specifically, the lichen transplants and dust samples located in the

city centre yield the highest concentrations of heavy metals – higher than the mining area and industrial dump.

Table 4-2. Mean trace element concentrations in lichen in Pretoria by area type. In µg·g^{-1} from Olowoyo et al. (2010).

Area Type	Pb	Cu	Zn	Cd	Sb	Mn	Ni	Sn	Fe
University	25.4	13.1	35.3	0.2	0.36	232	6.64	1.91	1.47
Residential	14.1	5.46	25.2	0.08	0.18	243	4.12	1.24	1.22
Mining area	36.2	16.5	92.84	0.22	0.32	256	10.71	2.61	1.32
Industrial	40.8	15.2	65.3	0.2	0.28	234	9.18	3.96	1.32
Industrial/Traffic	45.6	19.9	95.1	0.46	0.56	256	11.6	2.32	1.56
Commercial/Traffic	54.4	20.6	130	0.58	0.72	345	12.2	3.32	2.34
Smelting	33.4	17.9	89	0.22	0.32	312	6.78	3.96	1.35
Union	28.0	14.2	48.6	0.22	0.4	208	7.36	1.62	1.43
Airport	13.8	14.3	85.2	0.26	0.26	286	7.16	1.46	1.45
Taxi Rank	46.8	15.3	184	0.51	1.38	368	9.28	4.26	3.42

From the data sets presented in Olowoyo et al. and Monna et al. it is possible to conduct bivariate correlation to investigate how elements are distributed across the urban landscape. Spearman's rank correlations between elements are given below. Each area was coded to represent the amount of traffic likely present. The data set used to conduct these tests is included in Appendix G.

Table 4-3. Spearman's Rank correlation matrix for trace elements in lichen in Pretoria and Johannesburg. In a) in mining and industrial areas and b) in high traffic areas. Significant correlations are in boldface. Bonferroni's correction reduced the p-value associated with significance to 0.005.

a.	Pb	Cu	Zn	Cd	Sb
Pb	1.000				
	.				
Cu	.771	1.000			
	.072	.			
Zn	.829	.486	1.000		
	.042	.329	.		
Cd	.812	.522	**.986**	1.000	
	.050	.288	**.000**	.	
Sb	**.986**	.812	.812	.824	1.000
	.000	.050	.050	.044	.

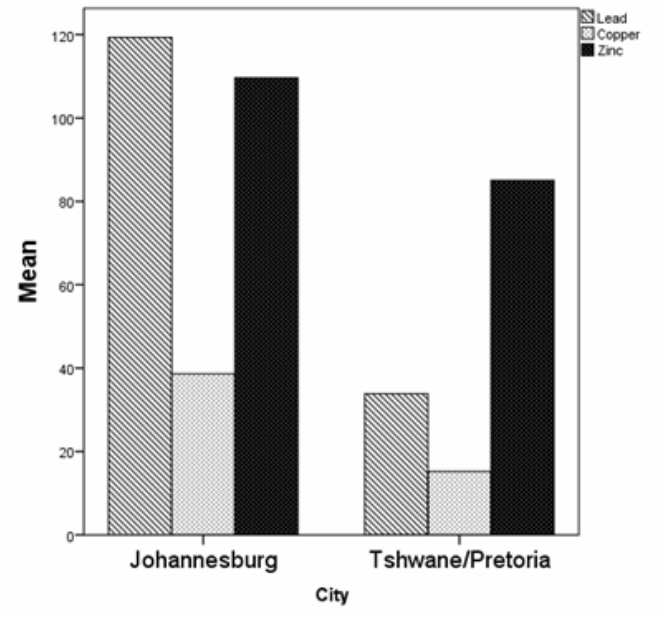
b.	Pb	Cu	Zn	Cd	Sb
Pb	1.000				
	.				
Cu	**.951**	1.000			
	.000	.			
Zn	.727	.776	1.000		
	.007	.003	.		
Cd	.238	.326	.637	1.000	
	.456	.301	.026	.	
Sb	**.958**	**.916**	**.748**	.277	1.000
	.000	**.000**	**.005**	.384	.

There are trends that become apparent when high traffic and mining and industrial areas are compared. In both areas, antimony is highly and significantly correlated with lead. Given the use of antimony in brake pads, particularly on heavy goods vehicles, this is not surprising. As most of the lead in the atmosphere in Gauteng has been attributed to motor vehicle traffic, it is not surprising that antimony correlates highly to lead. What is striking is that lead and cadmium are only correlated in high traffic areas, and not in mining and industrial areas. Antimony correlates with cadmium only in low traffic areas. These relationships may indicate slightly different element sources in these areas. Or, they may indicate that elements such as lead and cadmium are present in different fractions of PM and are distributed differently across the landscape. Until further research on atmospheric PM is conducted in Gauteng, there is no way to be sure.

4.2.3 **Differences between Johannesburg and Pretoria**

Examining the mean heavy metal concentration in lichen between the two cities, Johannesburg and Pretoria/Tshwane uncovers interesting differences. There is no

statistically significant difference between the mean cadmium concentration in lichen between the two cities (ANOVA, $p > 0.05$). Significant differences do exist between lead $t = 2.97$ (two-tailed) ($p < 0.05$), antimony $t = 3.07$ (two-tailed) ($p < 0.05$), and copper $t = 4.31$ (two-tailed) ($p < 0.05$). Mean concentrations in lichen for these three elements are higher in Johannesburg than in Pretoria/Tshwane (Figure 4-3). Unfortunately, arsenic, iron, manganese and nickel concentrations could not be compared. These differences have yet to be analysed or reported in the literature, and it is unclear whether the differences in mean element concentration are methodological or due to differences in size, traffic congestion and industrial activity between the two cities.



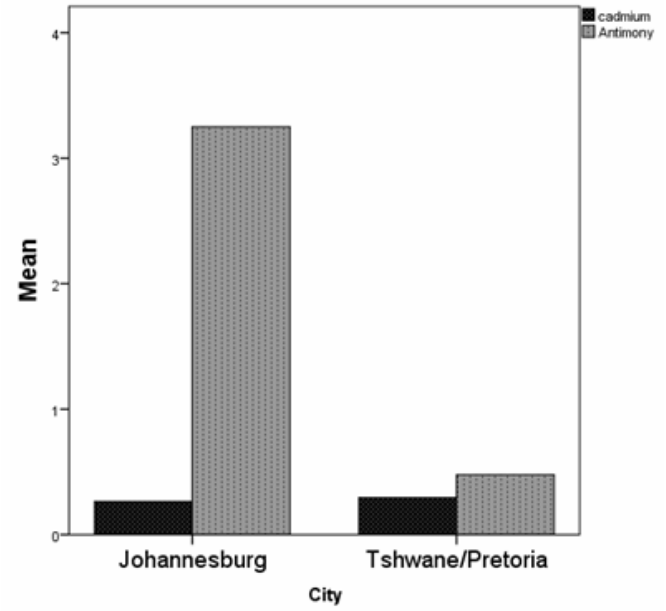


Figure 4-3. Mean concentrations ($\mu g \cdot g^{-1}$) of trace elements in Johannesburg and Tshwane/Pretoria. In a) copper, lead and zinc; and b) cadmium and antimony. From Monna et al., (2006) and Olowoyo et al. (2010).

4.3 Urban Pollution – Sources

4.3.1 **Lead**

The bulk of atmospheric lead, like that from automobile emissions is found in PM_{10}. Information regarding the source of inorganic pollution in Gauteng is also lacking. Anecdotal evidence suggests that industrial and mining activity would account for much of

the non-lead heavy metal pollution, though this has not been quantified. Conversely, lead is known to stem largely from automobile emissions – as a result of South Africa's persistent use of leaded petrol (Bollhofer and Rosman 2000; De Villiers et al. 2010; Monna et al. 2006; Okonkwo et al. 2009). Coal is believed to be the second most prevalent source of environmental lead pollution. Lastly, industrial and mining activities make up the remainder of potential lead sources in Gauteng.

During the 20th century, most environmental lead worldwide came from leaded petrol. However the environmental and public health consequences of this led to the reduction or cessation of leaded petrol in most countries beginning in the 1970s. South Africa however, continued to use leaded petrol until 2006. The late cessation of the use of lead in petrol has been ascribed to the country's robust lead mining industry and the fact that lead is a cheaper way to increase fuel octane than the alternatives.

4.3.1.1 Determination of lead source – Isotopic studies

Identifying the source of lead entails the analysis of the distinct isotopic signature of a given source. This ratio can be used to establish the source or provenance of a given lead product, such as leaded gasoline, paint, mine or industrial product. As with environmental pollution studies, few studies have attempted to quantify lead source in South Africa. Monna et al. (2006) measured lead isotope signatures in atmospheric lead in Johannesburg and compared these isotope ratios against the known isotopic signatures of leaded petrol for sale in the city, coal used for domestic heating and cooking and industrial/mining fly ash. Monna et al. (2006) determined that the bulk of atmospheric lead surrounding Johannesburg is from leaded petrol. The authors conclude that sources such as domestic coal contribute very little to the overall atmospheric lead concentration. Whilst this clearly evident from the results, there have yet to be any studies to examine the contribution of each lead source to human tissue-lead concentration (blood or bone). Nor are there adequate studies of indoor air pollution which may differ substantially from the outdoors.

Recent studies of lead concentration and lead isotopes in soil have confirmed that lead levels in the Johannesburg area are substantially elevated compared to background levels. De Villiers et al. (2010) report that areas of active lead mining exhibit high surface soil lead levels, which is to be expected. Areas of coal mining, whether active or inactive, exhibited low levels of soil lead, which the authors attribute to the relatively low concentration of lead in South African coal (Diaz-Somoano et al. 2009; Wagner and Hlatshwayo 2005). Other researchers however, have reported lead concentrations in coal fly ash from coal-powered electric plants at nearly 25$\mu g \cdot g^{-1}$ (Ayanda et al. 2012). When compared to recent studies of

coal fly ash in China (3077 $\mu g \cdot g^{-1}$) and India ($35 \mu g \cdot g^{-1}$), the South African value is low (Bhangare et al. 2011; Liang et al. 2010).

Overall, the patterns found correlate to previous studies, showing that the areas with the highest lead levels correspond to areas of high traffic and industrial activity. Studies of peat layers on the Witwatersrand also attribute high peat lead levels not to mining runoff, but to automobile traffic (Mccarthy and Venter 2006). Furthermore, a 2009 analysis of isotope ratios in lead aerosols worldwide found that despite a now global phasing out of leaded petrol, lead from petrol products, as opposed to coal combustion, still accounts for the bulk of atmospheric lead in South Africa (Diaz-Somoano et al. 2009).

Whilst lichen studies from Pretoria/Tshwane do not include isotopic analyses, they do show the same pattern as Johannesburg, of higher lead levels in the urban/industrial areas associated with high traffic. This would further indicate that total atmospheric lead is largely a result of the persistent use of leaded petrol. This has been confirmed by the reduction in total atmospheric lead that has followed South Africa's reduction in the use of leaded petrol, and follows the worldwide trend. Prior to its use being curbed, petrol in South Africa contained the highest levels of lead in the world, primarily because it is a relatively inexpensive and efficient means to increase the octane rating of petrol. From a high of 0.5 – $1.0 g/L^{-1}$ in the 1980s, the concentration of lead in petrol was reduced in the early 1990s, and unleaded petrol was introduced in 1996. Leaded petrol was not removed from the South African market until 2007. During the 1990s, Gauteng province consumed 35% of all petrol sold in South Africa. During a two-year monitoring period from 1996-1997, lead levels in Pretoria exceeded the WHO minimum mean monthly guideline value of $1.0 \mu g/m^{-3}$, a total of eight times. This is twice as many instances as neighbouring Johannesburg and four times as many as Durban (Diab 1999). Post-1999, these peaks have dropped below WHO minimum guidelines, however they remain higher than those of Europe or North America (Diab 1999).

Mining is a major industry in Gauteng (Transvaal) and in South Africa as a nation. Between 1980 and 1984, total lead mined in South Africa exceeded 85,000 tonnes per year, compared to just hundreds of tonnes in the early 20th century (Snodgrass 1986). Pretoria in particular is home to the Edenvale mining sites, just northeast of the city near Mamelodi. This was an active lead mine from 1898 to 1932 and activities included onsite lead smelting. Recent environmental studies of the abandoned mines, has reported hazardous levels of lead in the soils near the mines. The ranges of lead concentrations at slag and dump sites are above EU intervention levels of 580 parts per million, indicating that the area remains highly contaminated over 80 years after the cessation of mining activity (Glass 2006). Lead smelting activities at Edenvale would have been a source of atmospheric and water pollution

of lead, iron and zinc oxides, arsenic and antimony which may have affected the nearby townships (Glass 2006).

As Monna et al. (2006) point out, the location of historically disadvantaged populations near mine dump sites, and the concomitant exposure to airborne mine pollution continues to be a source of debate into the present day. However as is evident in Fig. 4-2, these authors noted that lead levels at mine dumps in Johannesburg are relatively low in lead. This may not have been the case at Edenvale, however, as these mines were active lead, as opposed to primarily gold mines.

4.3.2 **Cadmium**

As with other heavy metals, cadmium in the South African environment is a by-product of mining and smelting operations as well as petroleum (De Villiers et al. 2010; Van Aardt and Erdmann 2004). It is generally not mined on its own, but is a by-product in the mining and refining and smelting of other elements such as zinc (Nordberg et al. 2004). In drinking water the WHO maximum recommended concentration is 5µg/kg (5 ppb).

As is evident in Monna et al. (2006) and Olowoyo et al. (2010), in Tables 4-1 and 4-2, cadmium is present in the atmosphere in urban Gauteng, and at high levels in some areas. Despite being included in studies of atmospheric pollution, there is little to no published information regarding cadmium pollution in South Africa, other than its inclusion in studies focused on other elements such as lead. It is known that in polluted areas, cadmium is often found in water resources, and it is unlikely that South Africa is any different. Naicker et al. (2003) reported cadmium levels as high as 10mg/L (10 ppb) in acid mine drainage in Johannesburg, which is double the WHO maximum concentration for water.

De Villiers et al. (2010) identified substantial cadmium concentrations in soil samples in Gauteng, and attribute this to motor vehicle exhaust. These authors also identify high soil cadmium levels along the Namibian border, a region home to the only remaining active lead mine in South Africa (Figure 4-4a). Importantly, cadmium concentration is related to lead concentration in soil and other sources both at the local and national level as is evident in Fig. 4-4b.

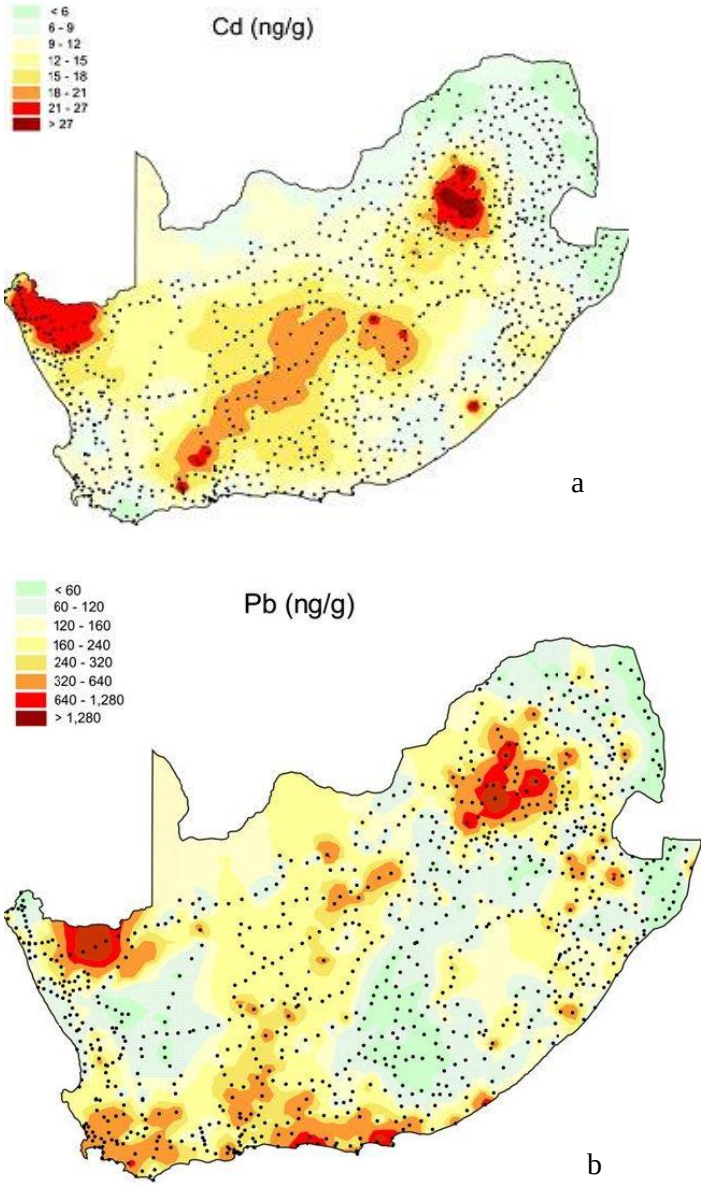


Figure 4-4. Soil lead and cadmium concentrations in South Africa. a) soil cadmium distribution in South Africa. b) soil lead distribution in South Africa. From de Villiers et al. (2010).

4.3.3 **Manganese**

It is difficult to compare present-day levels of manganese in the urban South African environment with bone levels from the last century. This is primarily because the use of manganese has increased following the phasing out of leaded petrol. Since the 1990s, methylclycopentadienyl manganese tricarbonyl (MMT) has been added to petrol as an anti-knock and octane enhancer as a replacement for lead (Batterman et al. 2011; Forbes et al. 2009; Röllin et al. 2005). Manganese levels in the environment, especially the urban environment are likely to be higher in the present day than would have been prior to 1990.

Though the data on environmental manganese in South Africa prior to the mid-1990s is almost non-existent, Formenti et al. (1998) conducted PIXE analysis on urban aerosols in

Soweto from 1982-1984. This study concluded that atmospheric manganese in Soweto was likely due to manganese smelting activities near Johannesburg. There is a significant ferromanganese processing economy in Gauteng. The authors also note that the presence of manganese in the atmosphere was intermittent and dependent on southerly wind, which would not have been often. With regards to the substantial mining activities in the region, aerosol monitoring of a Transvaal goldmine conducted in the late 1980s found negligible manganese concentrations in dust resulting from any mining activities (Annegarn et al. 1987). Prior to the use of MMT, the most likely source of manganese in urban Gauteng would have been smelting activities as it is used extensively in the production of steel, and ferromanganese alloys.

4.3.4 **Arsenic**

Like manganese, arsenic is largely a by-product of mining, specifically gold mining (Armah et al. 2012; Davies 2010; Dzoma et al. 2010; Ogola et al. 2011). Almost no research has been undertaken regarding the measurement of arsenic in the environment in either Gauteng or the country at large. However, studies of water resources do report high levels of arsenic in surface and groundwater in Gauteng. This is largely due to the region's substantial gold-mining industry (Durand 2012).

Historical and present-day gold mining in Gauteng has left the region with substantially elevated arsenic levels in water resources. In addition, South Africa has no formal arsenic monitoring programme to track arsenic concentration in water resources, and the presence of arsenic in the South African environment is under-studied (Kempster et al. 2007). However there is evidence that the region's gold mining heritage has resulted in extensive arsenic pollution (Figure 4-5.).

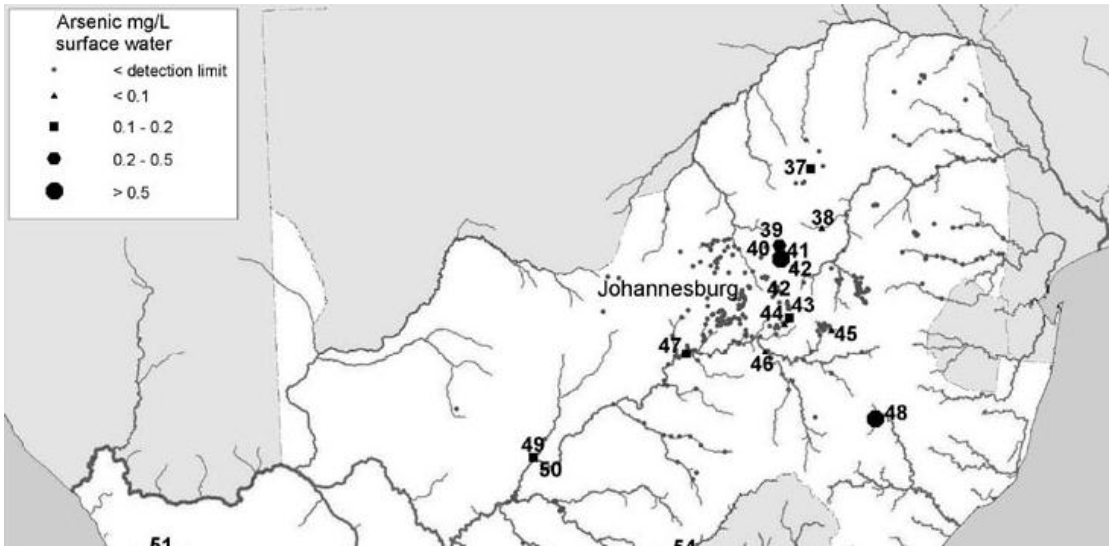


Figure 4-5. Aresnic concentration in groundwater in Gauteng. From Kempster et al. (2007)

Rosner et al. (2000) identified over 200 mine damn tailings (the materials that remain after a valuable metal has been extracted from the surrounding ore) in South Africa, many of which are located in or near urban areas. Most of this material is not treated in any way, allowing seepage of heavy metals into ground and surface water sources. International target and "intervention" thresholds for arsenic in the environment are 29 and 55mg/kg respectively – in this case the intervention value signifies significant risk of toxicity. Among seven sites in and around Johannesburg, one had a soil concentration of 53mg/kg, and the remaining six sites were close to or exceeding the target value for arsenic and are considered moderately to highly contaminated (Rösner and Van Schalkwyk 2000).

Outside of urban areas, the prevalence of arsenic in the environment can be equally substantial. Dzoma et al. (2010) cite nearby mining activity for elevated arsenic levels in water, grass, soil and the blood of grazing cattle in the Northwest Province, South Africa. In this study, arsenic levels in water in the cattle farming area of Koekemoerspruit were 12 times higher than the WHO/U.S. EPA recommended maximum of 10µg/L.

4.3.5 **Vanadium**

South Africa is home to the world's largest reserves of vanadium, some 85 percent of which is used in steel production (Edel et al. 2009; Feichtinger 1993; Jochens 1985; Rohrmann 1985; Young 1985). Nearly 50% of the vanadium produced in South Africa is vanadium pentoxide, widely considered the most dangerous form of vanadium in regards to human health (Moskalyk and Alfantazi 2003). Despite the scale of vanadium production in the country, little research has been conducted on environmental vanadium in South Africa. Two studies have found vanadium toxicity in sentinel cattle adjacent to vanadium mines

(Gummow et al. 2006; Mccrindle et al. 2001). The authors found no toxicity from mere background vanadium, even at elevated background concentrations. Investigation of PM10 in the Vaal Triangle found vanadium concentrations in PM as high as 0.36µg/L (Moja et al. 2013).

4.3.6 **Antimony**

There is little to no research into the source of antimony in the South African environment. Highveld coal products are low in antimony but there is extensive antimony mining in Limpopo Province north of Gauteng in former Transvaal (Davis et al. 1989; Feichtinger 1993; Jaguin et al.; Wagner and Hlatshwayo 2005). Antimony is not routinely monitored in the urban environment.

4.4 Urban pollution and heavy metal exposure pathways

4.4.1 **Sewer sludge**

As in many countries, the use of disinfected sewer sludge as agricultural fertiliser is not uncommon in South Africa. The use of sludge provides crop soil with needed nitrogen and phosphorus, which increased agricultural output. Sewer sludge is thoroughly disinfected prior to agricultural application and any organic contaminants are removed. However, disinfection does not remove inorganic contaminants and toxic substances such as heavy metals and trace elements of interest to this research. These substances can be incorporated into growing plants and work their way into the food chain either by direct consumption of plant products by humans or by grazing animals. In 1991, mean lead concentrations in sewer sludge in South Africa was 452mg/kg^{-1}. The mean lead concentration for UK sewer sludge from the same time period was 400mg/kg^{-1} (Hutton and Symon 1986).

4.4.2 **Water**

Water pollution is a persistent problem in South Africa. Despite this, extensive studies of heavy metal concentration in water were not adequately conducted prior to the mid-1990s (Roux et al. 1994). Studies specific to metals in water in Gauteng are equally scant. Approximately 70% of all water in South Africa is surface water (Ochieng et al. 2010). Moreover, Gauteng derives all of its water from surface sources, particularly the Vaal River (Mckenzie and Wegelin 2009). This is a critical issue with regards to heavy metal exposure, as the Vaal Triangle and the surface water sources that supply Gauteng are highly polluted with mining effluent (Potgieter-Vermaak et al. 2006). The country's substantial mining industry produces a form of pollution called acid mine drainage (AMD), which can and does contaminate South Africa's largely groundwater water resources, and has been referred to as the a significant threat to South Africa's environment (Mccarthy 2011). AMD is formed

when mine runoff comes into contact with rock formations containing sulphur. The result is acidic, metal-rich water, specifically in lead and manganese, which can be highly toxic. The low pH of the water allows for a high uptake of metals into the water and when the drainage meets with a groundwater source, can contaminate the source with heavy metals. AMD in Gauteng is particularly high in metals such as manganese, lead, iron, nickel and zinc (Durand 2012).

The level of heavy metal contamination can be severe enough to cause health effects in individuals who drink contaminated water or eat fish or plants that have been exposed. Naicker et al. (2003) reported high levels of heavy metal contamination in soil and water near a mining area of Johannesburg. In addition, the authors report that the water contamination continued up to 10km from the AMD source. AMD in Gauteng (and in mining areas worldwide), is known to persist for long periods after the closure of a mine or the removal of mine dump materials (Rösner and Van Schalkwyk 2000).

Studies of water metal concentration in three rivers in Limpopo have reported lead and cadmium levels that exceed WHO maximum recommended concentrations for drinking water (Okonkwo and Mothiba 2005; Okonkwo et al. 2005). Interestingly, this study reported higher metal concentrations in the wet season than in the dry, which the authors ascribe to run-off from road (petrol) pollution further contaminating rivers, a well-documented phenomenon (Dunlap et al. 2008; Kayhanian 2012).

4.4.3 **Soil/Ingestion**

Though soil is generally not the proximal source of toxic metals in the environment, it often acts as the immediate source in that lead and other trace elements are readily absorbed into soil and then released into the food chain and water supply. Environmental cadmium in particular is often found in soil. Cadmium has a long half-life and can remain in soil for decades, even centuries. The primary pathway from soil to humans is the ingestion of food grown in metal rich soils (Oliver 1997).

Elevated soil lead levels resulting from acid mine drainage can also be a significant source of lead, especially in children. Soil lead levels have been positively correlated with childrens' blood lead levels (Zahran et al. 2010). The US Centers for Disease control have estimated that for every 1000 ppm increase in soil lead concentration, a corresponding increase of 3-7µg/dL occurs in the blood lead concentration of exposed individuals (Mielke and Reagan 1998). Moreover, highly acidic soil, similar to that caused by AMD, results in greater lead mobility and greater uptake of lead into plants (Davies 2010). This is potentially an issue in areas such as the historically black townships in Gauteng. In these areas where

food security is and has traditionally been poor, individuals turn to small urban farming to meet household food needs.

Though there is little quantitative data regarding the prevalence of urban farming, it is estimated that the practice is common. In a survey of five settlements within the Atteridgeville township in Pretoria/Tshwane, 54% of households were actively involved in urban farming. In addition, it has been reported that as much as 25% of total annual food requirements in these areas (Van Averbeke 2007). A similar study of the informal settlement of Orange Farm near Johannesburg found that 88% of households participated in some form of urban farming (Crush et al. 2011). Provincially, urban farming is on the increase in Gauteng. Between 2002 and 2007 the total percentage of households participating in urban cultivation increased from 5% to 15% (Burger et al. 2009). This increase in the practice of urban farming over time may be one contributing factor to the high levels of blood metal concentration reported in present day studies. Kootbodien at al. (2012) have recently measured heavy metal contamination in soil in a school vegetable garden in Johannesburg which was located near a mine tailing. Levels of lead and arsenic were elevated in both soil and vegetables, though each element concentration was within acceptable limits in South Africa.

There is a dearth of data on soil-trace element concentrations in the townships, but it is not unreasonable to assume, given the high concentration of metals in urban street dusts in the area, that urban farming in heavy metal contaminated soils may be one pathway of exposure to these elements (Okonkwo et al. 2006). Atteridgeville in particular is situated parallel to Church Street, one of the busiest thoroughfares in the city. Olowoyo et al. (2010) study of trace element concentration in jacaranda also included data for soil metal concentration. The authors report lead levels as high as 99.7$\mu g \cdot g^{-1}$ in high traffic areas of the city, which is just at the 100$\mu g \cdot g^{-}1$ threshold considered dangerous to children by the US EPA (1991). Cadmium concentration was not reported in this study. From this it may be extrapolated that soil plots historically used for urban cultivation may be contaminated with heavy metals.

Olowoyo at al. (2012) also measured element concentration in soil at this site. Lead concentration ranged from 11.62$\mu g/g^{-1}$ (0-15cm) and 9.92$\mu g/g^{-1}$ (15-30cm). Manganese levels were reported at 78.94$\mu g/g^{-1}$ (0-15cm) and 73.39$\mu g/g^{-1}$ (15-30cm). In this instance, jimson weed and amaranth demonstrated a low uptake rate of trace elements.

With regards to cadmium, it seems that soil contamination may be a primary exposure pathway. An estimated 65% of total cadmium in soil is considered bioavailable to living organisms (Scheyer 2004; Wilson et al. 2008). Again, data on soil cadmium concentration is sparse Gauteng. However it is possible to extrapolate cadmium concentration from

measurements of cadmium in street dusts in Pretoria/Tshwane. Okonkwo et al. (2009) reported a cadmium concentration of 0.17µg/g^{-1} in Pretoria. This is relatively low compared to Hong Kong and London with reported cadmium concentrations of 3.77µg/g^{-1} and 4.2µg/g^{-1} respectively (Li et al. 2001; Thornton et al. 1985). Cadmium in any level is toxic to humans however, and even low concentrations can have subclinical and clinical effects on human health.

4.4.3.1 Plant element concentration

One proxy measure that may be a good indicator of trace elements in urban soil is the measurement of trace elements and heavy metals in medicinal plants that are grown in these areas. Use of plants as traditional medicine is still widely practiced among urban black populations and country-wide it has been estimated that as many as 27 million South Africans, primarily black, make use of locally grown plants for medicinal purposes (Mander 1998). Across plant species, metal concentration varies widely and is largely a function of soil characteristics such as metal concentration, pH and the bioaccumulative properties of each individual plant species. Still, South Africa lacks an official monitoring program to regulate metal concentration in medicinal plants (Street et al. 2008b). What is clear is that these plants contribute both a direct exposure pathway as well as a proxy (albeit a less than ideal one) for data regarding soil contamination.

Most medicinal plants are sold without packaging at open-air markets near busy traffic corridors where they can be contaminated with street dust containing heavy metals (Street et al. 2008b). Ingestion of these plants has been associated with renal pathology leading to speculation that heavy metal poisoning may be the cause (Steenkamp et al. 2000). Whilst medicinal plants tend to be low in lead, manganese levels are high (Olowoyo et al. 2012; Steenkamp et al. 2000; Street et al.2008). Urine manganese concentrations after ingestion of medicinal plants were shown to be higher than in control individuals (Steenkamp et al. 2000). Street et al. (2008b) measured manganese concentrations as high as 2089µg/g^{-1} in the leaves and stems of some species.

In a 2012 study of trace elements in medicinal plants collected from a waste dump site in Pretoria near the town of Ga-Rankuwa, Olowoyo et al. (2012) reported a mean lead concentration of 1.98µg/g^{-1} in roots and 1.31µg/g^{-1} in leaves. This is substantially lower than the 10µg/g^{-1} limit recommended by the WHO (Olowoyo et al. 2012). Mean manganese was also reported at 21.94µg/g^{-1} in leaves and 44.41µg/g^{-1} in roots. Cadmium concentration was not analysed.

4.4.4 **Ambient air**

It is difficult to quantify the difference in exposure between inhaled and ingested trace elements. For some elements, such as cadmium, the most likely pathway is through ingestion of contaminated water and food. Elements such as lead and manganese are also ingested through contaminated substances, however the high levels of atmospheric lead in countries such as South Africa, make it equally likely that some lead exposure stems from lead that is suspended in the atmosphere in particulate matter. Environmental lead suspended in the air maintains the same isotopic signature once it has settled into soil or water, meaning isotopic studies of blood and bone lead provide little information as to exposure pathways.

Ranft et al. (2008) established a quantitative relationship between atmospheric lead and blood lead concentration in children. These authors report 48 % of the variation in children's blood lead could be explained by variation in lead in ambient air. Similarly, 17 % of variation in blood lead could be explained by changes in soil lead concentration. For individuals living adjacent to roadways in Gauteng, inhaled lead may be a significant contributor to overall lead burden.

Only one similar study has been conducted in South Africa. Batterman et al. (2011) studied blood lead levels in children following the cessation of leaded petrol. Children attending schools in more industrial areas of Durban showed higher blood lead levels than other children. However, no corresponding differences were visible in atmospheric lead in these areas, leading the authors to conclude that non-airborne lead plays a more significant role in blood lead concentration than airborne lead. The authors further speculate that lead from sources other than petrol may be a substantial contributor to children's lead exposure in Durban. It is unclear if this is the case in Gauteng, as no similar studies have been undertaken. As with other aspects of environmental pollution in urban South Africa, the dearth of data renders comparisons both within South Africa and between South Africa and other countries, quite difficult.

4.4.5 **Indoor exposure**

Exposure to indoor air pollution, primarily from smoke and its associated particulate matter is a major public health concern for developing countries worldwide (Ezzati et al. 2002). In Africa particularly, the burden of diseases such as respiratory disease, chronic obstructive pulmonary disease, lung cancer and others are attributed directly to exposure to often toxic coal and wood smoke (Norman et al. 2007a). This can be particularly dangerous for impoverished populations in which individuals are already nutritionally and physically stressed (Goyer 1995; Mahaffey 1983).

4.4.5.1 The role of domestic coal

During Apartheid and continuing into the present, the primary fuel for cooking and heating used in townships and historically black neighbourhoods in South Africa has been coal or wood (Kimemia and Annegarn 2011; Monna et al. 2006; Wesley and Loening 1996). This is true even of households with electricity (Mdluli and Vogel 2010). Of the 50% of households that use coal or wood for heating and cooking, 95% are black African households (Norman et al. 2007a). Whilst the few studies investigating this phenomenon are primarily concerned with respiratory and cardiovascular disease, it is critical to consider that indoor smoke from coal contains lead and may well be a contributor to the overall lead burden within a given household, despite the relatively low lead concentration of South African coal (De Villiers et al. 2010; Wagner and Hlatshwayo 2005).

So, whilst researchers conclude that the lead in coal provides very little contribution to *overall atmospheric* lead in urban South Africa, it would be naïve to discount coal as a substantial contributor to individual lead exposure in black households, especially in regards to lower level chronic exposure. Domestic coal use in general is attributed to higher rates of lead exposure in lower-income households worldwide (Barnes et al. 2009; Ezzati et al. 2002). Recent studies of the blood lead concentration in children in Cape Town may help to confirm this (Mathee et al. 2004). What is needed however, are isotopic studies of blood or bone lead concentration of a cross section of South Africans. This would provide researchers with much critical information regarding both sources and pathways of lead exposure.

4.4.5.2 Lead in paint

Lead concentration in residential paint has been of great concern worldwide (Clark et al. 1985). Lead in paint is particularly dangerous for young children, who are likely to exhibit pica during infancy/toddlerhood and who may ingest lead-based paint chips. And whilst leaded paint is considered a problem unique to older homes in the West, this may not be the case in South Africa. Lead-containing paint as an exposure pathway did not garner attention in South Africa until the first decade of the 21st century. The first study of its kind, Montgomery and Mathee (2005) investigated the lead content of 60 homes throughout Johannesburg. The authors found that 20% of all homes surveyed contained lead-based paint, in both new and old homes and across socioeconomic backgrounds.

More importantly, despite an agreement to stop the practice of adding lead to paint in the mid-1970s, Mathee et al. (2004) report that 60% of paint being sold to the public in Cape Town and Johannesburg were lead-based. In addition, some lead concentrations in these paints were as high as 189,000$\mu g \cdot g^{-1}$. The U.S. reference level for lead concentration in paint

is 5,000µg·g^{-1}. In addition, lead concentration in the paint on widely available children's toys were measured at levels as high as 145,000µg·g^{-1} (Mathee et al. 2006).

The paint used in children's playground equipment is also a source of lead exposure. A reported 48% of lead used in play equipment in Johannesburg, Tshwane and Ekurhuleni, contained lead in concentrations higher than international reference values, signifying a substantial risk to the children that use them (Mathee et al. 2009b). Currently, and certainly during the latter half of the 20th century, no government legislation has existed in South Africa regulating lead in paint, and given the high concentrations reported by Montgomery and Mathee (2005) and Mathee et al. (2006), it appears that this may be a substantial source of exposure for South Africans, particularly children. Unfortunately, no isotopic studies of lead isotope ratios in paint have been conducted, making it difficult to ascertain just how much of the total lead burden in the population comes from residential paint.

4.4.6 **Tobacco**

Tobacco smoke is an often overlooked yet potentially significant source of toxic element exposure in both smokers and those exposed to second-hand smoke (Chiba and Masironi 1992; Mishra et al. 1986). Tobacco is known to contain arsenic, cadmium, lead, manganese and vanadium (Bernhard et al. 2005). These elements are then inhaled into the lungs where they are quickly absorbed into the blood stream. Cadmium is particularly prevalent in tobacco, and contributes to elevated blood cadmium levels in populations worldwide, though this varies based on several factors including the region in which the tobacco is grown (Järup et al. 1998b; Stephens et al. 2005; Tsadilas 2000).

4.4.7 **Secondary exposure**

Lastly, very little research into secondary exposure pathways has been investigated in South Africa. In this sense, para-occupational exposure – the exposure to heavy metals of individuals living with occupationally exposed household members is also under-reported. Anecdotal evidence exists regarding children who are exposed to lead, cadmium or arsenic from backyard motor repairs, car battery recycling, jewellery making, and mining dust, but no studies have been conducted into the prevalence of these activities nor their effect on heavy metal exposure rates (Rees and Schneider 1993; Von Schirnding et al. 2003).

5 Toxic elements and health in South Africa

The discussion of the current state of knowledge regarding toxic elements in the South African environment is critical to understanding the risks to humans. Studies of inorganic pollution in South Africa have primarily concerned children, as opposed to adults and the majority of were conducted in Cape Town or Cape Province. The environment of urban Gauteng differs from the Cape in many ways, not the least of which is the predominance of mining and industry activities that are located so close to urban areas. Nonetheless the information presented in this chapter, particularly the studies of metal exposure conducted in Gauteng after apartheid and more recently, after the reduction of lead in petrol, provide a means by which to directly compare the data generated by this project.

As with environmental studies, research regarding human exposure to toxic metals and trace elements is in its infancy in South Africa. The majority of studies that have been undertaken in the past two decades have focused on exposure rates in children as opposed to adults. In addition these studies have primarily involved lead and manganese exposure, and little information is available regarding rates of exposure to other toxic elements. Nevertheless, the studies that have been conducted provide two critical sources of information by which to compare data from this project. Firstly, historical exposure can be compared against modern to identify any changes in exposure rates or patterns. Secondly, and subsequently, any identification of changes over time can be used to infer and predict potential future changes in toxic element exposure within the South African population.

The distribution of human lead exposure in South Africa has only been monitored since 1982, and until the 2000s no multi-city study was undertaken. Most published studies have focused on Cape Town, and since 2000 only a few have focused on Johannesburg. No investigations of human lead exposure have been undertaken in Pretoria/Tshwane. Some overall trends are apparent however, with regards to lead exposure across the population. In most urban centres in South Africa, lead exposure follows a negative urban to rural gradient, with the highest lead concentrations found in city centres. Within that trend however there are many factors that appear to influence lead exposure, socioeconomic status most prominent among them. The major source of human lead exposure has likely been leaded petrol, however as some more recent studies have shown, lead exposure persists even after the phasing out of leaded petrol and other sources are likely to play a role as well.

The role of lead in the total disease burden of South Africa is substantial. Norman et al. (2007c) estimate that in the year 2000, approximately 23 million South Africans had a blood

lead concentration between 5 and 10µg/dL-1. A further four million individuals were estimated to have blood lead concentrations above 10µg/dL-1. Given these lead exposure level, Norman et al. estimate that in children, 530 in every 1000 suffer a reduction in IQ at blood lead levels of between 5 and 10µg/dL-1. At high levels of exposure - > 20µg/dL-1, an estimated three children in every 1000 suffer a loss in IQ of 3.5 points.

In adults with blood lead levels between 5 and 10µg/dL-1, an estimated 0.4 to 0.6 increase in mmHg (systolic blood pressure) occurs in 527 out of every 1000 individuals. In adults with blood lead concentrations above 20µg/dL-1, a corresponding increase of 3.75 mmHg occurs in 2.3 per 1000 adults.

The authors report that 75% of the total Disability Adjusted Life Years (DALYs) in the population as a whole can be attributed to mild cognitive disability in children caused by lead exposure. Cardiovascular diseases such as stroke, hypertension and heart disease make up the remaining 25% of DALYs, all of which can be brought about and exacerbated by exposure to lead. Thus the role of lead exposure in the total burden of disease in South Africa is quite substantial.

With regards to other elements such as manganese, the research is less well defined and under-reported. To date, only a handful of studies have investigated manganese exposure in the population, and as with lead, these tend to focus primarily on children. There does appear to be a link between lead and manganese in some parts of the population however this has been explored in only one published report. Only one study to date has investigated cadmium exposure in the South African population.

It is critical to note that studies of human exposure to several elements included in this research, namely, vanadium, antimony and arsenic have yet to be conducted in South Africa and there is no way to compare the data gathered in this project to present-day trends.

5.1 Lead

5.1.1 **Early lead monitoring – 1982 to 1999**

The earliest investigation into urban lead exposure in South Africa is that of White et al. (1982), in which the authors measured the blood lead concentration of 226 children residing in Cape Town. Among the findings of the study is that children residing in the Cape Town urban area had blood lead concentrations approaching those of children in other major urban centres worldwide. The ethnic/socioeconomic background of the children studied was not reported by the authors, however 23 of the children were inpatients at a Ciskei homeland hospital and were thought to have been residents of a township near East London, which

would indicate that they were black children. These children, and those white children residing in the Cape Peninsula but not necessarily in Cape Town, yielded lower blood lead concentrations (mean 7.8 and 8.2µg/dL blood lead concentration vs. 12.7µg/dL) than children residing in Cape Town, though the range in blood lead levels was the same. The authors recognize the urban/rural dichotomy in lead exposure. In this study however, blood lead levels in Cape Town children were still lower than those of children living in a lead-exposed area of Philidelphia, United States.

Though White et al. do not report the race of the children with the highest blood lead levels among the Cape Town dwellers, based on previously-reported sociological trends, it may be that they were black or of low socioeconomic status. The authors investigated each child with a blood lead concentration above 24µg/dL. Among these nine children, the father of one worked in a factory using lead, the father of another child collected and burnt batteries to sell for scrap metal, and three of the homes were in disrepair with visible paint. The collection and burning of batteries in particular is a predominantly black activity, and homes with peeling paint indicate lower socioeconomic status. Lastly, six of the children lived adjacent to major roadways.

These trends are mirrored in subsequent research by Deveaux et al. (1986). This study investigated blood lead levels of 293 children between the ages of 4 and 6 and attending pre-school in Cape Town. All of the children resided in central Cape Town and all were classified as "coloured". Devereaux et al. also applied a socioeconomic score to each child based on family income, mother's education level and parents' occupation. The average blood lead concentration in this study was approximately 16µg/dL and there was no correlation between socioeconomic status and blood lead level.

In children with the highest blood lead levels (> 29µg/dL) lead-based paint was found in the home of the majority, indicating that among children, lead paint was and likely still is a substantial source of lead exposure. Measurements of house dust in the homes of a cross-section of children were taken and there was no correlation between the lead concentration in house dust and those children with the highest or lowest blood lead concentrations. Notably however, the children with the highest and toxic levels of blood lead all came from the most socioeconomically deprived areas of the city. Lastly, the authors report that they did not find an association between petrol-derived aerosols and blood lead levels.

Von Schirnding (1991) and von Schirnding et al. (1991) subsequently explored the potential causes behind the elevated blood lead levels in coloured children residing in urban Cape Town. Von Schirnding analysed the blood lead levels of 1,234 school children residing in a working-class neighborhood near central Cape Town. Potential sources of lead were

explored as well as socioeconomic status and potential health effects of high levels of lead exposure. The author found that dusty homes and homes in disrepair were significantly correlated with increased blood lead concentration. Among the other factors found to be correlated with higher blood lead concentration were overcrowded living conditions, high number of siblings, low parental education, single parents and lack of home ownership. Thus *within* the urban core, von Schirnding found a socioeconomic gradient with regards to lead exposure. Most strikingly, there appears in this study to be a negative correlation between attendance at a crèche (only available to higher income households) and blood lead concentration. The author further posits that the difference in blood lead levels may be ultimately related to the quality of care-giving received by lower income children, as overcrowding, large families and single parenthood may greatly reduce the time spent monitoring children by caregivers.

von Schirnding et al. (1991) expanded the study to include comparison between mixed-race and white children, as the neighborhood studied was among the few mixed areas in South Africa. The authors reported that extremes of wealth were present within the study area. The study area was approximately 4km from the central business district and almost all traffic into and out of the central business district traveled through the area.

The authors found significant differences in mean blood lead between mixed-race and white children with mixed-race children yielding higher blood lead levels. All of the children with elevated blood lead were mixed-race. The primary differences between the two groups were socioeconomic. Nutritional and health status between the white and mixed-race children were negligible. The white children tended to live in larger homes and in smaller families, even those at lower incomes.

Another significant factor influencing blood lead concentration was residence and school. Children of either racial group attending one of three schools closest to the main roads yielded significantly higher blood lead levels than children in other schools, even when residence and socioeconomic factors were taken into account. The increase in blood lead level associated with attendance at these schools was between 5 to 7µg/dL. Atmospheric lead levels obtained at these schools confirmed greater air-lead concentration than at schools further from roadways. This study was the first in South Africa to associate traffic with lead exposure, although it is clear that other variables contribute strongly to lead exposure in the region. In addition, this particular study highlights the potential for substantial intra-urban variability in exposure to lead based on multiple risk-factors.

In 1996, von Schirnding and Fuggle (1996) studied the distribution of lead exposure in urban Cape Town. They found high levels of variation in urban environmental lead levels across

the study sites. Sites situated near roads yielded atmospheric lead levels nearly double that of those sites located further from major roads.

Examining the rural/urban differences in blood lead, Grobler et al. (1986) measured the blood lead concentration of adult long-distance runners in both urban and rural settings. The authors analysed the blood of participants in one of two ultra-marathons in either Cape Town or the area between Durban and Pietermaritzburg. Mean blood lead among rural runners was 20.1µg/dL and for urban runners was 51.9µg/dL indicating significant difference in lead exposure between urban and rural areas of South Africa.

In 1993, Maresky and Grobler (1993) investigated the effect of the reduction of lead in petrol (though not its complete removal on blood lead between 1984 and 1990. During this time, lead additives in petrol were reduced from 0.84g/L to 0.4g/L. The authors report that between 1984 and 1990, the average blood lead concentration of urban Cape Town residents with no unusual or occupational exposure to lead fell from 9.7 to 7.2µg/dL, bringing urban blood lead concentration in line with urban areas in Europe and North America. This trend was confirmed in 1999 by Diab (1999).

5.1.2 **Kwazulu-Natal and Durban: Post-Apartheid**

Also among the first researchers to investigate blood lead in urban South Africa were physicians working in an obstetrics ward in Durban (Chetty et al. 1993). In this small study, the maternal and cord blood levels of 21 women were investigated. Among women, 95% were above the 10µg/dL threshold and 25% had blood lead levels at or above 25µg/dL. Among newborns (as measured by umbilical cord blood) 95% had blood lead levels at or above 10µg/dL, indicating high infant blood lead levels. Of contributing socioeconomic and sociological factors, only the amount of physical labour engaged in by the mother was a significant predictor of elevated blood and cord blood lead concentration.

In 1997, Nrigau et al. (1997) studied 1200 children in and around Durban and rural Kwazulu-Natal. This study was among the first to investigate blood lead in children resident in black townships. This is also one of the first large scale studies to study a city or region other than Cape Town or Cape Province. The "Besters" township area included in the study is semi-urban, like many townships, and consists largely of informal shanty towns and represents the most socioeconomically disadvantaged segment of the Durban population.

Of children resident in the townships, 50% had blood lead levels above 10µg/dL, the level at which the US CDC recommends intervention. Children resident in rural areas of KwaZulu-Natal had significantly lower blood lead levels than township children. Among the significant risk factors for children living in Besters was proximity of residence to a

tarmacked road, number of rooms in the house, cooking fuel used and the presence of smokers in the house. Contrary to von Schirnding, Nriagu et al did not find any association between the education level of parents and blood lead, however Besters is considered socioeconomically uniform and this was not expected.

Although Nriagu et al. do not compare black children to white or coloured children, one important comparison is found between blood lead levels measured in a formal Durban township in 1985 and the Besters study. A blood lead level difference of just 2µg/dL^{-1} was apparent despite a 10 year difference in the date of the studies. Nriagu et al. state that this may be an indicator that despite reduction in lead in petrol, the corresponding reduction in human lead exposure may be slower than in developed countries.

Bazzi et al. (2008) studied a number of trace elements in children from rural Kwazulu-Natal aged 8-10 years. The children studied lived approximately 140km from the nearest city, Durban. Among the sampled children the mean blood lead was 5.64µg/dL-1. No information was given regarding the race/sex of the studied children.

Batterman et al. (2011) studied 408 children in urban Durban 14 years later and reports a dramatic reduction in mean blood lead, and 3.4% had blood lead concentrations above 10µg/dL-1. Blood lead concentration was highly correlated with school and with sex. Blood lead concentration was lower in white children than in black or Indian children. Most critically, Batterman et al. suggest that lead exposure in present-day Durban may not be linked to petrol emissions. The authors measured airborne lead in addition to blood lead across the city and did not see a gradient from high traffic to low traffic areas, despite the trend of higher blood lead concentration in children attending schools near transport networks.

5.1.3 **Gauteng (Transvaal)**

Despite being the largest and most industrialised urban areas, Johannesburg (and Pretoria) were not subject to human lead exposure monitoring until 2001. As with studies of other cities, lead exposure studies in these two cities has focused primarily on exposure rates in children.

Among the earliest mentions of lead exposure is a case report of a young girl from Soweto undergoing treatment for lead poisoning at Baragwanath Hospital, Soweto. The authors report that her blood lead concentration was 100µg/dL-1. Subsequent investigation revealed that the girl's grandfather with whom she resided regularly burned and dismantled batteries near the home to sell the scrap metal. Most strikingly, the authors recommend that a blood lead level at or above 30µg/dL-1 in children under the age of 14 should be referred for

clinical intervention, a level substantially higher than the US CDC recommends as a level for intervention. To lower the actionable standard, the authors argue would be counter-productive, as lower levels are likely to be the result of common environmental exposure, indicating belief among the medical establishment that high levels of lead in the environment were likely causing elevated lead levels in the region's children (Rees and Schneider 1993).

The first major study of lead exposure was reported by Mathee et al. (2002). As with other studies, the study focuses on exposure in children. The study included 433 children from the inner urban core, the Alexandra township and the Westbury suburb. The authors describe each area as relatively low on the socioeconomic scale. The study did not include statistics regarding lead exposure and race or lead exposure across socioeconomic groups.

Mathee et al. (2002) found that the highest blood lead levels were in children living in the inner city and in the Alexandra township. Overall, the authors report that 78% of children had blood lead concentrations at or above 10μg/dL. Significant differences in mean blood lead concentration were found between male and female children, with males having higher blood lead levels than females. Like von Schirnding et al. (1991), the risk factors for elevated blood lead concentration in Johannesburg children included parental education level, living in an informal dwelling, the presence of smokers in the household and the regular consumption of canned foods.

Notably, Mathee et al. (2002) are the first researchers in South Africa to examine behaviour and lead exposure within the study population. Parents of the children studied were asked about the perceived quality of the children's schoolwork and whether or not they perceived the children as being hyperactive. Children whose parents described them as doing poorly in school or as hyperactive had higher blood lead levels than other children, indicating that some neurological deficits may be present among lead exposed children.

It is critical to note that this study was conducted after the reduction in lead in petrol in South Africa, and yet the authors found persistently high blood lead levels in the study population, indicating that sources other than petrol are responsible for toxic levels of lead in the environment. Mathee et al. hypothesize that lead in paint and the rise in "cottage industries" in the townships (i.e. battery recycling, welding and metalworking, motor vehicle repair) are contributing factors to lead exposure in urban children.

Mathee et al. (2004) explored the matter further and examined the potential sources of household lead across South Africa. Though to date, no isotopic studies have been conducted to conclusively identify the primary sources of lead exposure in Johannesburg,

particularly in children, it is likely that household sources to contribute to lead exposure in the region, in addition to lead from petrol. Mathee et al. identify several potential sources, including lead paint, cottage industries, and the grinding of batteries to use the lead in mud coated walls in traditional dwellings.

In 2009 Rollin et al. (2009) investigated maternal and umbilical cord blood in women at several sites across South Africa, including urban Gauteng (city not specified). Urban Gauteng women had the highest levels of blood lead with a median concentration of 32.9µg/dL. Women in coastal, inland, industrial and rural areas all had lower blood lead concentrations than urban dwellers. Median cord blood concentration was also highest in urban Gauteng, at 24µg/dL.

Most recently, Wilson et al. (2011) investigated trace element concentrations in non-occupationally exposed residents of Gauteng. The study included 107 men and women across racial groups who were between the ages of 18 and 73, and most closely aligns with the research being undertaken in the present project. Participants were considered environmentally exposed to trace elements if they lived within 2km of a motorway, mine dump, incinerator or power station. This included 68% of the population, although the authors do not report the results according to environmental exposure, nor are the results reported according to socioeconomic status (though they are all workers in a local electric utility office) or racial background. The authors do report that the blood lead levels measured in the study are similar to those reported in Germany and the UK.

5.1.3.1 Lead and the Birth-to-Twenty Cohort Study

The Birth-to-Twenty Cohort Study (Bt20) is a longitudinal study of the health and wellbeing of children born in Soweto between the months of April and June 1990. The study has included 16 follow up studies of approximately 2300 children and their families. Among these, blood lead data was gathered for 1546 children aged 13, making this the largest study of lead exposure and its associated health effects in Africa (Naicker et al. 2010a; Naicker et al. 2010b; Naicker et al. 2012).

At age 13, the mean blood lead for all sampled children was 5.7µg/dL-1. Three percent of children had blood lead concentrations at or above 10µg/dL-1. (Naicker et al. 2012). Among the critical findings of these studies is the association between blood lead and a number of pathologies and negative health outcomes. Among the black females included in the study (n=725), the mean blood lead concentration was 4.9µg/dL-1 (Naicker et al. 2010a). Among females with higher levels of lead, the onset of puberty was significantly delayed. Based on previous research, the mean age of the onset of puberty for black females in South Africa is

10.42 to 11.69 years of age. By 13, most of the females in the Bt20 study are expected to have begun puberty. Naicker et al. report that in girls with blood lead concentrations above 5µg/dL-1, the onset of puberty (including breast development, pubic hair growth and onset of menarche) was delayed, even when controlling for socioeconomic and anthropometric factors. This follows the same trend found in other countries (Wu et al. 2003).

In 2012, Naicker et al., using the same data investigated the potential neurological effects of lead exposure among the sampled children. Specifically, the authors examined the relationship between blood lead and socio-behavioural adjustment and aggressive and delinquent behaviours. In all the children, two anti-social behaviours were significantly associated with higher blood lead concentrations: "threatening others" and "destroys own things". In boys, in which the mean blood lead concentration was 6µg/dL-1, four anti-social behaviours were associated with elevated blood lead levels: "argues", "destroys own things", "attacks people", and "loud". No significant relationships were found between anti-social behaviours in females. White children were not included in the study, and the majority of children included in the study (coloured, mixed race and black) were from a low socioeconomic group.

Among the Bt20 cohort, 618 maternal and cord blood samples were taken at birth and of these, 312 repeat blood samples from this subset of children were sampled again at age 13 (Naicker et al. 2010b). At birth the mean cord blood concentration was 5.9µg/dL-1. By age 13, blood lead decreases in females but not in boys. Of the children sampled at birth and at 13 years, blood lead concentration increased in 42.3% of the population, with the rest of the population either decreasing or staying the same. The authors also found that children born to teenaged mothers (<20 years) had higher blood lead levels at birth than those born to mothers aged 20 years or older. Similarly, there was a positive association with low educational status of the mother and the blood lead concentration of the child at birth.

By age 13, children born to adult mothers and with lower cord blood concentrations at birth had higher blood lead concentrations than those born to teenaged mothers, indicating that different environmental or household factors may be affecting lead exposure (Naicker et al. 2010b). Among these factors, low education level of mothers, lack of home ownership and lack of household telephone were significantly associated with elevated blood lead concentration, indicating that those children living at the lowest end of the socioeconomic spectrum were more significantly exposed to lead at aged 13. In addition, children with higher blood lead concentrations at birth were 1.9 times more likely to have elevated blood lead concentrations at age 13.

5.1.3.2 Lead exposure in a mining region

Von Schirnding et al (2003) measured blood lead levels of children living in a lead mining town in the Northern Cape Province and compared this data to that gathered from a non-mining community 40km away. The authors report several interesting findings. Mean blood lead level between the two rural communities was 15.9 and 13.2µg/dL in the mining and non-mining towns respectively, which is, overall quite similar. In the mining community, 98% of the study population had blood lead levels above 10µg/dL-1. In the non-mining community, 85% had a blood lead level at or above 10µg/dL-1. Among the key differences between the communities is the level of parental education and level of poverty, with higher educated parents in the mining community and a higher level of poverty in the non-mining community. Children in the non-mining community were also more likely to live in households with more than 3 children under the age of five years.

Within the more affluent mining community, children from higher socioeconomic backgrounds had lower blood lead concentrations than children from lower socioeconomic households, even when the exposure rate of the former group was higher than in the latter. These findings are similar to those of von Schirnding, (1991) and Mathee et al. (2002) which found a clear link between blood lead concentration and socioeconomic status even when exposure rates are roughly similar, or, in the case of the mining community, higher.

5.2 Manganese

The post-2000 studies of manganese exposure in South Africa have largely assumed that the bulk of manganese exposure stems from the use of MMT as a petrol additive. Whilst this is likely so, manganese is also mined and processed in South Africa, and some exposure may be due to other sources of manganese in the environment. One study in particular, Rollin et al. (2009), reports data in which rural residents and urban residents have roughly similar blood manganese concentrations, which calls into question the traffic/automotive explanation for human manganese exposure.

There are no published investigations into manganese exposure in South Africa before 2005. At that time, blood manganese concentrations in 430 children were measured in Cape Town and 384 children in Johannesburg (Rollin et al. 2005). This study compared children living in the inner city of each city, those living in former townships, and those in white or coloured suburbs. Children attending school in the inner city of Johannesburg had the highest blood manganese concentrations. Inner city children had a mean blood manganese concentration of approximately 11µg/L-1 whilst children from schools in Soweto had a blood manganese concentration of approximately 8µg/L-1. In Cape Town, children from the formerly coloured suburb of Mitchell's Plain had the highest blood manangese

concentrations at approximately 5µg/L-1 compared to > 5.5 µg/L-1 in the inner city. In both cities, black children had the highest blood concentration of any demographic group, although the authors did not report results according to race, so the exact blood manganese concentrations per racial group is unknown. The authors report that in Johannesburg, 12.5% of children had blood manganese concentrations above 14µg/L-1, the Agency for Toxic Studies and Disease Registry (ATSDR) threshold for intervention. In Cape Town, this percentage is much lower at 4.2% of children.

In 2007, Rollin et al. included the city of Kimberley and three rural sites in the Northern Cape to the 2005 study. Children living in the mining town of Aggeneys had the highest blood manganese concentration with a mean of 9.86µg/L-1. Children in Johannesburg and Kimberley had blood manganese concentrations of 9.76 and 9.72µg/L-1 respectively. Rural children from Pella and Onseepkans had blood manganese concentrations of 8.30 and 7.75µg/L-1 respectively and children in Cape Town had the lowest blood manganese concentration at 6.75µg/L-1.

Paradoxically, Rollin et al. (2009) found that blood manganese and cord blood manganese levels in women and infants from urban Gauteng were substantially lower than those in women and infants from any other region, including mining and industrial areas. Median cord blood manganese concentration in Gauteng was 19.7µg/L-1 as opposed to 34.5 and 36.6µg/L-1 in mining and industrial areas. Maternal blood manganese concentration was substantially lower in each region except Gauteng, with median blood manganese of approximately 16µg/L-1 in mining, industrial and rural areas, and 17.7µg/L-1 in urban Gauteng. Notably, this does not follow the same trend as lead which shows a clear urban/rural, high/low dichotomy in regards to blood concentration.

Rollin et al. (2007) also examined the relationship between blood lead concentration and blood manganese concentration. When blood lead was fitted as the response variable, and manganese as the explanatory factor, there was no consistent relationship between blood lead and manganese concentration in any of the study areas. The authors did find that blood manganese concentrations were higher in females than in males and lower in black individuals than in those of other races, a trend found by Batterman et al. (2011) in Durban. In addition, the authors report an overall positive linear trend in which manganese concentration increased with increasing blood lead, but this trend was not found to be significant across each study location. In Johannesburg, no linear effect between blood manganese and blood lead was apparent (Röllin et al. 2007).

Wilson et al. (2010) report blood manganese concentration of 9.7µg/L-1 in non-occupationally exposed Gauteng residents. This value is higher than those reported in Italy in 1990 and the Germany in 2002 at 8.8 and 0.6µg/L-1, respectively.

Bazzi et al. (2008) report a mean blood manganese concentration of 8.48µg/L-1 in children from rural Kwazulu-Natal.

5.3 Cadmium

There is scant information regarding cadmium exposure in South Africa. Rollin et al. (2009) and Wilson et al. (2010) are the only two studies to date that have examined cadmium concentration in human tissues in South Africa. These studies do indicate that cadmium exposure is higher in South Africa than in many other under-developed countries, and is on par with Western countries.

Wilson et al. (2010) report median adult cadmium concentration at 1.1µg/L-1 with a range of 0.3-2.3µg/L-1. This is compared with Italy (1990) and Germany (2002) with median reported blood concentrations of 0.6µg/L-1. The range in Italy is lower than that of South Africa at 0.1 to 1.7µg/L-1 and the range in the sampled German population is 0.1-3.3µg/L-1.

The values reported by Rollin et al. in maternal blood is substantially lower than those reported by Wilson et al. In rural samples median blood cadmium concentration is 0.10µg/L-1 and 0.15µg/L-1 in women from urban Gauteng, despite the fact that these women come from roughly the same region and time period as those potentially sampled by Wilson et al. The highest cadmium concentrations reported by Rollin et al. are from women living on either South Africa coast, with women on the Atlantic ocean having the highest blood cadmium concentrations in the study, with 0.25µg/L-1, and women living near the Indian Ocean having the second highest at 0.16µg/L-1.

5.4 Arsenic

To date, only two published studies have investigated blood arsenic concentration in the South African population, Bazzi et al. (2008) and Rollin et al. (2009). Bazzi et al. report a mean blood arsenic concentration of 1.5µg/L-1 in children from rural Kwazulu-Natal. Conversely, Rollin et al. report much lower blood arsenic concentrations in the blood of women across South Africa, with maternal blood arsenic concentration from women in urban Gauteng at 0.43µg/L-1. In Rollin et al., women living in a mining community and women living in a rural inland malaria region had the highest blood arsenic concentrations at 0.73 and 0.74µg/L-1 respectively. The lowest concentration came from women living in the industrial area at 0.33µg/L-1. Cord blood taken from infants at the same time as maternal blood was sampled also showed lower blood arsenic concentrations than that measured by

Bazzi et al., with the highest concentration of blood arsenic found in infants from the inland malaria zone at 0.79µg/L-1. The lowest blood arsenic concentration in cord blood came from infants born in urban Gauteng at 0.37µg/L-1.

5.5 Antimony and Vanadium

No monitoring of antimony or vanadium has been conducted in humans in South Africa.

6 Materials and methods

6.1 Materials

6.1.1 Skeletal materials

Skeletal material was sampled from incomplete postcranial remains from Pretoria Identified Bone Collection at the University of Pretoria, South Africa and the Dart Student Bone Collection at Witswatersrand University, Johannesburg. The Pretoria Bone Collection is an identified reference collection held at the University of Pretoria, School of Medicine. The skeletal remains are those of individuals who died in the Pretoria area between 1943 and 2012 and whose bodies were either unclaimed or donated. In the former case, unclaimed bodies become the property of the University of Pretoria to be used for teaching and research, subject to the South Africa Human Tissues Act of 1983 (L'abbe et al. 2005). The collection consists of individuals who range in age from neonates to 95 years of age. The predominant demographic within the collection is black males, followed by white males, white females, and black females. This is largely to do with both overall demographic patterns within South Africa and to economic conditions during Apartheid, in which circulating migration brought black males to urban areas from Bantustans for work (Byerlee 1974; L'abbe et al. 2005; Smit 2001). The Raymond Dart Collection is housed at the University of Witswatersrand, School of Medicine and is similar in demographic composition to the Pretoria Collection. Skeletal remains in the Dart collection date to 1928 (Dayal et al. 2009). Only 12 of the femora included in this study are from the Dart collection.

6.1.1.1 Collection background

The Pretoria collection was founded in 1942 with skeletal material derived from research and teaching cadavers from the University of Pretoria School of Medicine (L'abbe et al. 2005). The collection does include forensic cases, however, none are included in this research as the remains are too valuable for destructive sampling. The remains in this collection were never buried, and so there is little possibility of post-mortem exposure to any of the elements of interest in this research. This factor, above all others, makes this collection ideal for trace element research.

All remains that arrive in the School of Medicine are given a cadaver number, and their identity is expunged from the database used by students, staff and researchers. The identity of each individual is known to select staff, as the school maintains the policy of returning remains to family upon request. Therefore, these samples are technically linked, in that it would be possible to identify them individually. However, identity information was not

made available to the author, and the bone samples were re-coded with completely unlinked numbers upon arrival at the University of Southampton.

6.1.1.2 Sample selection

Whilst the collection itself is vast and the documentation is excellent, the physical organisation of the collection made sampling difficult. Years of neglect (L'abbe, 2011) meant that there is little organisational logic with the exception of forensic cases, which are stored in numbered boxes. The materials sampled for this research came largely from the Student Collection, and from materials slated for destruction. A majority of the bones were stored in large plastic or cloth bags which contained bones of one type and side. There was no organisation according to cadaver number, sex, age or any other demographic factor. These bones are regularly given out to students for study purposes.

Sample selection was difficult due to the haphazard nature of selecting one bone, checking its cadaver number against the University's database and deciding whether to include or reject it. Moreover, because the collection is biased heavily towards black males, it was difficult to find white males and females from among the bags of bones. It was decided that all white males and black females that were located would be sampled, and that black males would be sampled in such a way as to attempt to match the white and female samples for age at death and date of death. Despite being statistically more prevalent, no white females were found among the incomplete postcrania. Time and organisational constraints led to the decision not to include white females. Younger black males were also included to establish a range of ages, despite the inclusion of very few young white males. The nature of this sampling method invariable introduced a significant sampling bias into the process. Because the nature of the collection's organisation was unknown prior to arrival in Pretoria, there was little time to prepare and attempt to correct for any sampling bias that occurred. Age, sex and race statistics for the sampled remains are given in Table 6-1.

Table 6-1 Demographic breakdown of sampled remains. Includes age and hospital (residence) by race and sex.

	N	Percent
Males	165	76
Females	49	23
Black	179	82.5
White	35	16.1

	Age	Mean	Median	SD	Min	Max.
All		53.01	55	16.06	20	95
White Males		66	67	13.1	42	95
Black Females		48.2	46	14.67	25	80
Black Males		51.2	50.5	15.28	20	80

	Hospital	Pretoria	Johannesburg	Rural	Unk.	Total
All		153	40	6	16	217
White Males		16	6	2	11	35
Black Females		38	8	1	2	49
Black Males		98	26	3	3	127

Given the sampling method, the remains in this study conform rather closely with the demographic profile of the collection as a whole[1]. Black females comprise 23% of the sampled remains and make up 16% of the total collection. The mean age for black females in the collection is 47.4, and is 48.2 in the sampled remains. White males comprise 16% of both the total collection and the sampled remains. The mean age of white males in the whole collection is 66.5 and mean age in the sampled remains is 66. Black males comprise 60% of the complete collection and comprise 60% of the sampled remains. In terms of age, the mean age of black males in the complete collection is 53.9, and is 51.2 in the sampled remains. Thus, excepting the omission of white females, the sampled remains are highly representative of the Pretoria Collection.

In addition, the nature of the collection itself, in that it is comprised predominantly of unclaimed bodies, lends to a bias as well. Komar and Grivas (2008) refer to these as "manufactured populations" as they tend to be heavily biased towards males, and older individuals, and certainly this is the case with the Pretoria Collection. In much the same way that researchers question whether contemporary populations in reference questions can be used as analogues to historical populations, the same can question can be asked of the

[1] The 12 samples from the Dart Collection are considered with the Pretoria Collection. These remains are of 12 black males and as such, are not considered representative of the large Dart Collection. Moreover, there is considerable overlap between the two collections with regards to admitting hospital. The remains are treated as coming from one population, as opposed to two distinct populations.

Pretoria collection. With regards to the research at hand, it can be argued that the use of a reference collection is quite appropriate.

6.1.1.3 Diagenesis and the advantage of cadaver remains

Diagenesis is the most formidable obstacle facing any chemical analysis of archaeological material. Diagenetic processes must be accounted for and controlled for in order for any examination of bone chemistry to be worthwhile and before results can be properly interpreted (Elliott and Grime 1993). The effects of diagenetic processes are controversial (Martinez-Garcia et al. 2005; Millard 2006). An example of this controversy is the long held assumption that long-term lead exposure and widespread lead poisoning led to the decline of the Roman Empire. This assumption was based on chemical analyses of bone lead concentrations in Roman skeletal remains. Research has questioned whether the high levels of lead in Roman remains is actually representative of in vivo lead concentrations or rather the effects of diagenesis (Patterson et al. 1987)

Bone tissue in burial environments is subject to ground water, soil conditions, exposure to burial artefacts etc. The porous nature of bone makes it predisposed to the absorption of organic and inorganic material in the immediate environment. Archaeologists must determine whether the concentrations of metals and trace elements present in any previously buried bone are representative of in vivo exposure, or diagenetic, resulting from post-mortem exposure in the burial environment. Complicating matters is the fact that bone porosity varies for a variety of reasons, and there are countless processes by which elemental uptake occurs in bone and most are site-specific (Hedges 2002). Exposure to groundwater is the most common diagenetic process (Hedges and Millard 1995). Reference collections like the Pretoria Collection are not subject to diagenetic processes and as such are ideal for biochemical analysis.

The primary aim of this research is to understand present-day exposure to trace elements and heavy metals through a framework of exposure rates in the recent past. With regards to the Pretoria collection's bias towards African males, this is true of many skeletal reference collections, and should not be an argument against using them for epidemiological studies – especially in previously unstudied populations. Many present-day epidemiological studies of heavy metals in humans are biased towards one demographic group or another – often these studies are biased towards women and children and this is certainly the case in South Africa. Lastly, it is an unfortunate reality that investigations of heavy metal exposure were not conducted in South Africa prior to 1980. Analysis of skeletal reference material is the only means by which to establish any understanding of past exposure rates and by which to frame

present-day studies. In this respect, the value of the Pretoria Collection in this research cannot be understated.

In total, 346 individual bones were sampled from 214 individuals, including femora, fibulae, radii, ulnae, humerii, and tibiae. In addition, multiple bones were removed from 48 individuals (excluding individuals from whom the same bone was sampled only on different sides).

6.1.2 **Sampling Method**

6.1.2.1 Sampling procedure

Sampling began on July 12, 2011. In the first instance, fibulae, radii and ulnae were taken from materials that were slated for destruction. These remains had been stored in the basement morgue of the Department of Anatomy for some time, and it was agreed that they would be sampled first. These remains were fully traceable, as they were all labelled with cadaver numbers. Fibulae, radii and ulnae were sampled using a Dremel saw with corundum cutting blade. Samples were taken from the distal end of the fibula approximately 2-3cm from the distal end. Radial samples were taken from the proximal end on the anterior surface. Ulnar samples were taken from the proximal/anterior surface as well. The bone samples were not weighed, but a 1.5cm by 1.5cm square of bone was taken and the samples consisted of the full thickness of the cortical bone.

Femoral bone samples were removed by drill. A 10mm diamond-tipped core drill was attached to a conventional drill press. Femora were held securely by placing the bone between two blocks of wood to prevent the bone from rotating counter to the drill during sampling. The same drill bit was used for multiple bones and was not rinsed between bones, primarily because to do so would have required a substantial amount of time, slowing the process as there was no water source available in near the drilling aparatus. Any bone dust that may have been transferred between samples was removed in the lab. A full-thickness core of cortical bone was removed from each femur from the distal/posterior surface approximately 3-4 cm above the intercondylar fossa. This location was chosen because of the ease of sampling, and because it provided a substantial amount of cortical bone without interfering with any femoral landmarks or osteometric measurements (Gibbon et al. 2009).

Previous research has demonstrated that bone element concentration can differ substantially within the same bone, depending on sampling site. Todd et al. (2001) found that bone lead concentration differed along the diaphysis of the tibia. To correct for this, every effort was made to sample each bone in precisely the same location give or take one centimetre.

After removal from the bone, all samples were placed into zip-closure plastic bags and labelled with cadaver number, bone, and side. A database was also maintained with the same information. Sampling concluded on August 13, 2011. Sample bags were stored at the University of Pretoria until transport to the UK in March, 2012. A complete list of the bones sampled, including the bone, sex, age and ancestry of each sample, is provided in Appendix G.

6.2 Analytical method

6.2.1 **Sample pre-treatment**

6.2.1.1 Method choice constraints

In this analysis, several constraints were placed upon the project that had to be taken into consideration when choosing a sample preparation method. The most critical constraint was time. Only five working days were allowed from the time the samples were signed for by Dr. Trueman, to render the bone tissue completely acellular. This timeline was imposed by the University of Southampton, in accordance with the University's Human Tissue Licence as legislated by the UK Human Tissues Act of 1994. Lab access was restricted on weekends, mornings before 9:00 and evenings after 19:00, so all analysis was carried out during normal working hours between Friday, March 2, 2012 and Thursday, March 8, 2012.

The second primary constraint was lab equipment/space. Whilst the NOC clean geochemistry laboratory is considered cutting edge, it is not set up to process large batches of biological samples. The time and equipment needed to dry ash over 350 samples is substantial and would have resulted in the analysis falling afoul of the five-day processing restriction. Space within the clean laboratory is also at a premium. The clean fume cupboards lack the space necessary to process 350 samples on the lab's hotplates without displacing other researchers and research projects. Thus processing the samples at temperature would have to have been accomplished in small batches, which would have likely resulted in failure to meet the University's deadline.

The decision was made to process the samples in the most simple, timely method possible so as not to violate the five-day deadline.

6.2.2 **Digest Method**

Sample digestion was conducted at the University of Southampton Geochemisty Class 100 Clean laboratory at the National Oceanography Centre Southampton. All reagents used were Fisher Trace Element grade and further sub-boiled in Teflon® stills to ensure ultra-purity. Water used was MilliQ® Millipore ultra-pure water (18.2 MΩ). All tubes and bottles,

including caps used in this analysis were washed with 10% HNO_3 for a minimum of 48 hours and rinsed clean three times with MilliQ. Complete washing protocol can be found in Appendix B. Labware was then placed in a clean dryer with HEPA filtration system to dry. All materials were stored in acid-washed plastic bags until use. Solution bottles were left capped until use. Pipettes and pipette tips used to transfer acid to sample tubes were washed three times with 6M HCl and rinsed three times (including internally) with MilliQ. Nitrile gloves, Tyvek clean suits and clean lab shoes were worn at all times during sample preparation.

After arrival, samples were removed from the storage bags within the clean lab and each sample of whole bone was weighed to 0.0001g accuracy. The samples were then placed in 15ml tubes and each was washed to remove surface dust and dirt in ultrapure MilliQ™ water. Washing was accomplished by filling the tube with approximately 8-10ml of water, capping the tube and shaking the sample, the water was then decanted and the process repeated three times, or more if the decanted water did not run clear after the third wash. After washing, 1mL of 69% sub-boiled, ultrapure nitric acid was added to each sample. Samples were left at room temperature, approximately 20°C, loosely capped for 72 hours. After 72 hours, the samples still retained some organic material. They were then diluted to 10mL with MilliQ water. Samples were then left for four days to allow solid phase to settle.

After acid digestion, individual dilution factors were calculated for each sample in order to reach a dilution of 100µg/mL^{-1}. Dilution was carried out for two reasons: the reduction of total dissolved solids in the sample, and to reduce Ca concentration to a level that would minimise matrix effects. This was accomplished by first calculating the percentage of $Ca_3(PO_4)_2$ bone apatite that is comprised of calcium: 38.7% . Because whole bone as opposed to ashed bone was used, the result was multiplied by 0.666, which is the widely held approximation of the percentage of bone tissue that constitutes the inorganic phase. The following equation was used to calculate the dilution needed to achieve 100µg·g^{-1} Ca, where W is the total weight in grams of the bone sample and DF is the dilution factor:

$$((W*0.387)0.666)1000 = DF$$

Aliquots of varying volume (dependent on the individual DF of each sample) for each sample were pipetted into 20mL vials and then 10mL ±0.1 of 3% HNO_3 was added to each bottle by placing the bottle on a scale and adding acid until total solution weight (sample + acid) was as close as possible to 10g.

For the addition of internal standards, rhinium, indium and beryllium were added to each diluted sample at a concentration of 5µg/mL (Re and In) and 20µg/mL (Be). All three are

rare earth or post-transition metals and are used in this instance to control for signal drift. None of these elements is expected to be present in human bone tissue. Samples were stored in capped vials until analysis. Dilution factors, aliquot volumes and total analytical volumes are listed in Appendix G. All results are reported in concentration ($\mu g \cdot g^{-1}$) dry weight.

ICP-MS Equipment was calibrated by the inclusion of nine calibration standards plus five calcium standards. Individual elements of interest were taken from stock element solution (Inorganic Ventures) of either $1000\mu g \cdot g^{-1}$ or $10{,}000\mu g \cdot g^{-1}$ and diluted to below the lowest expected concentration for each element. Element concentrations were then increased across the nine standards until the top standard contained element concentrations above what was expected in analytical samples. The five calcium standards ranged in concentration from $70\mu g \cdot g^{-1}$ to $110\mu g \cdot g^{-1}$. Calibration standards volumes and method are given in Appendix D.

Nine samples of CRM, NIST-SRM 1486 Bone Meal were digested and analysed alongside analytical samples. In addition, 10 reagent blanks of 3% HNO_3 were analysed to detect and control for contamination.

6.2.3 **Instrument operating parameters**

The equipment used was a ThermoScientific XSeries 2 Quadropole ICP-MS. The elements of interest were analyzed in one of two instrument modes depending on signal size and susceptibility to interferences. These were standard mode and continuous count (CCT) mode with 2mL/min. of a mixed He/H_2 gas added to reduce interferences.

6.2.3.1 Limits of Detection and Limits of Quantification

Limits of Detection (LoD) and Limits of Quantification (LoQ) for ICP-MS were calculated for each element and are given in Table 6-2. The procedure used to quantify LoD and LoQ are given in Appendix B.

Element	As	Cd	Mn	Pb	Sb	V	Mg	Zn	Fe	Cu
LoD	0.00003	0.000002	0.00015	0.00012	0.00016	0.000006	0.00077	0.00018	0.00165	0.00000004
LoQ	0.00007	0.000006	0.00036	0.00027	0.00048	0.000018	0.00177	0.00040	0.00372	0.00000009

Table 6-2. LoD and LoQ for each element in $\mu g \cdot g^{-1}$.

6.2.4 **Element recovery, precision and robustness**

CRM recovery rates were consistent and for elements other than Cd, within acceptable ranges. Mean and target recovery rates are given in table 6-3. Recovery rates for certified elements are good. Non-certified elements are provided by NIST for reference but element

concentrations of these elements are not considered accurate or reliable enough for certification. The four certified elements included in this analysis are Fe, Zn, Pb and Sr. Sr is included in this table as an additional measure of recovery rate. Some CRM samples were run more than once, with different sample batches on different days. These are noted by date and time in Table 6-3.

Table 6-3. Recovery rates and target recovery rates for CRM (NIST 1486 Bone Meal). * Indicates certified element concentration. Other elements are present in the target concentration but not certified.

Sample	Mn	Fe*	Cu	Zn*	As	Cd	Sr*	Pb*
	$\mu g \cdot g^{-1}$	$\mu g \cdot g^{-1}$	$\mu g \cdot g^{-1}$	$\mu g \cdot g^{-1}$	$\mu g \cdot g^{-1}$	$\mu g \cdot g^{-1}$	$\mu g \cdot g^{-1}$	$\mu g \cdot g^{-1}$
CRM 1 11/05/2012 13:22:24	1.095	88.78	1.182	119.3	0.1034	0.00137	243.6	1.188
CRM 9 11/05/2012 17:14:24	1.111	83.07	1.066	121.9	0.006704	0.001814	246.9	1.256
CRM 1 11/05/2012 21:39:30	1.054	83.19	1.12	116.2	0.09403	0.001626	236.6	1.207
CRM 1 09/05/2012 17:52:06	1.118	94.86	1.274	126.1	0.04463	0.00203	275.5	1.166
CRM 2 09/05/2012 20:16:27	1.127	103.8	0.861	120.5	0.000192	0.001533	271.9	1.034
CRM 3 09/05/2012 22:28:28	1.268	104.1	1.023	133.8	0.03278	0.002353	299.7	1.167
CRM 4 10/05/2012 00:53:39	1.283	103.1	1.022	140.1	0.03619	0.001321	311	1.217
CRM 1 10/05/2012 14:47:46	1.046	83.18	1.12	115.4	0.01645	0.003027	239.8	1.18
CRM 5 10/05/2012 17:49:37	1.101	79.32	0.872	121.3	0.01177	0.002166	248.2	1.344
CRM 7 10/05/2012 20:07:55	1.038	76.24	0.773	117.1	0.07577	0.002072	237.8	1.229
CRM 8 11/05/2012 00:32:24	0.95	71.86	0.731	107.3	0.03274	0.002001	219.8	1.248
CRM 1 12/05/2012 13:50:43	1.112	89.87	1.151	120	-0.02474	0.002565	245.7	1.18
CRM 10 12/05/2012 15:30:55	1.112	82.85	0.93	125.6	-0.04708	0.002602	248.4	1.259
CRM 9 12/05/2012 21:54:52	1.101	81.41	1.057	122.3	-0.07249	0.001868	247.8	1.257
Mean	1.108286	87.545	1.013	121.9214	0.022168	0.002025	255.1929	1.209429
Target	1	99	0.8	147	0.006	0.003	264	1.33
±		8		16			7	
%Recovery	110	88	127	83	369	67	97	91

Precision for each element as measured by %RSD for each sample, calibration standard and CRM are between 0.5 and 5.0 for certified elements and the internal standards (Be, In and Re). For elements found in very low concentrations, < 1.0$\mu g \cdot g^{-1}$, including As, Cd, and Sb, precision is lower. Precision data for each analytical sample (including calibration standards and CRM are given in Appendix G.

Given the good CRM element recovery rates and precision, the data presented in Chapter 7 are considered robust and fit-for-purpose. No major analytical or pre-analytical issues were experienced with the digestion method. Analytical instruments performed as expected, with no calibration or measurement errors.

6.3 Statistical methods and data analysis

6.3.1 Data preparation

All data was analysed statistically with IBM SPSS 19 (IBM 2010). Analysis was divided into three sections: toxic elements and their relation to one another, essential elements and their relation to toxic elements, and intra-individual variability in element concentration and bone. In the first instance, tests of normality were conducted on all element concentrations. No element was distributed normally across samples, as a result, all element concentrations were transformed by calculating $\log_{10}$ of each concentration value and using only log transformed data for all statistical tests except for descriptive statistics, in which the back-transformed data is reported. Kolmogorov-Smirnov values for non-transformed element means for each population as a whole is given in Table 6-4. Within demographic groups, normality was first determined by application of the Kolmogorov-Smirnov test of normality. In all instances in with the K-S test is significant ($p < 0.05$), non-parametric tests are employed to compare means between groups and noted in the text. To correct for the possibility of Type I error on repeated tests, Bonferroni's Correction ($0.05/k$) where k is the number of tests and 0.05 is the critical value of p. In this case, k=20 so the corrected value for p is 0.0025.

Table 6-4 Normal and non-normally distributed elements. Elements are considered normally distributed if the K-S statistic is not significant.

Element	Statistic	Df	Sig.	Element	Statistic	Df	Sig.
24Mg	0.137	152	.000	LogMg	0.101	152	0.001
51V	0.149	152	.000	LogV	0.052	152	.200
55Mn	0.166	152	.000	LogMn	0.074	152	0.042
56Fe	0.269	152	.000	LogFe	0.169	152	.000
65Cu	0.194	152	.000	LogCu	0.054	152	.200
66Zn	0.201	152	.000	LogZn	0.135	152	.000
75As	0.473	152	.000	LogAs	0.114	152	.000
111Cd	0.198	152	.000	LogCd	0.063	152	.200
121Sb	0.375	152	.000	LogSb	0.146	152	.000
208Pb	0.211	152	.000	LogPb	0.063	152	.200

Data is investigated statistically by comparing individual bone element concentration between and within demographic. In Chapter 7, for each toxic element, mean bone element

concentration is compared among all groups, black and white individuals (males and females), black and white males, black males and black females, age at death, and decade of death. For each race and sex, descriptive statistics are provided (mean, median, standard deviation, range, minimum and maximum). In addition, concentrations of toxic elements are compared against one another to investigate the patterns and interactions between elements as discussed in Chapter 8.

Toxic element concentrations are explored in relation to essential trace element concentrations. In this chapter, the relationships between these element types are subject to statistical analysis to uncover the presence of any statistically significant relationships and interactions between essential and toxic elements as discussed in Chapter 8.

6.3.1.1 Using multiple bones in statistical tests

Duplicate bones were sampled in order to establish whether statistically significant differences in bone element concentration exist between bones. To assess this, bone element concentration in different bone types were first compared by repeated measures ANOVA, however samples sizes were too small for this statistic. Instead, paired t-tests were conducted between six long bones: femora, fibulae, tibiae, radii ulnae and humeri. The results show that bone element concentration does not vary significantly between long bones of the same individual for any element. The results of these analyses are presented in Appendix A. In all cases, effect size is included in analytical results. In all analyses a mix of long bones are used.

6.3.1.2 Variable Coding

Variables were coded into binary dummy variables for race (1, 2), and sex (1, 2). Hospital was used as an approximation of the city of residence. The admitting hospitals were: Pretoria General, HF Verwoerd, Johannesburg, Baragwanath, Ga-Rankuwa, Kalafong, Edenvale, Benoni, Tembisa, Middlesburg, Natalspruit, and Potgietersrus. These were grouped into one of three codes based on which city the hospital was located in. In some cases, admitting hospital information was missing, or the name of the hospital did not match any known hospitals in the region. These cases were omitted.

Age and decade of death were coded into 10 year intervals. Ages were grouped into eight age categories starting with ages 20-29, and continuing to 99. Decade of death similarly coded into four categories starting with 1960-1969 and ending with 1999.

6.3.2 **Comparison of means**

Comparison of mean element concentrations between demographic groups was conducted by Student's T-test and Analysis of Variance, in the case of normally distributed data, and

by Mann Whitney U and Kruskal-Wallis H tests in the case of non-normally distributed data. Bonferroni's procedure (described below) was applied to all independent t-tests or Mann-Whitney U tests. T-tests and Mann-Whitney U tests were used to compare differences in mean or median bone element concentration between three demographic groups: black females, white males and black males. A standard Bonferroni's Correction of 0.05/3 (0.016) was used on all such comparisons and H_0 was rejected if $p > 0.016$. Post-hoc tests for comparison of multiple means was accomplished with Bonferroni's or Games-Howell procedures with ANOVA (the latter used when variances were unequal) and the Mann Whitney U test with Bonferroni's Correction with Kruskal-Wallis test. The critical value for significance of the Bonferroni's correction was determined by dividing 0.5 by the number of comparisons, N and using the critical value obtained to accept or reject the results.

6.3.3 **Correlation and regression**

When comparing element concentrations for multiple elements both bivariate correlation as well as linear regression tests were employed. Both methods were used because it was suspected that a linear relationship between elements was present in some cases.

All bivariate correlations are two-tailed. Correlation coefficient matrices represent Spearman's Rho coefficients only as so many variables are not normally distributed. In addition Pearson's correlation is less robust and more susceptible to influence by outliers. Linear (Pearson's) correlation coefficients are given as the 'B' value in linear regression tables. To correct for Type 1 error in multiple comparisons, Bonferroni's correction was applied to Spearman's Correlation matrices. The adjusted p-value for significant correlation between two elements is 0.0009 (0.5/55, where 55 is *n* comparisons).

In each instance of linear regression, the Unstandardised, Standardised and Deleted residuals were tested for normality using the K-S test. Residuals were considered normally distributed if K-S was not significant at $p > 0.05$. Only those regression models for which residuals are normally distributed and in which no assumptions are violated were considered valid. Any instance in which residuals were not normally distributed is reported within the text.

When reporting regression statistics, both significant and non-significant results are given. Linear regression results are considered significant if they meet all of the following criteria:

1. β is significant with $p < 0.05$
2. Residuals are normally distributed with a K-S $p > 0.05$ (see above)
3. F statistic is significant with $p < 0.05$

6.3.3.1 Treatment of outliers

During the course of statistical analysis it became apparent that in several instances there were outliers that skewed the normal distribution of the data, causing the violation of assumptions necessary for regression and other statistics. In the case of linear regression and Univariate ANOVA, outliers were found to violate normality and homoscedasicity. In these cases the outliers were removed.

Removal of outliers is somewhat controversial, but it was felt that in the case of the present data, removal was the most prudent course, as it became clear that the outliers were influencing the data significantly and preventing the identification of potentially meaningful population trends. However, removal of outliers was conducted with extreme caution and according to statistically sound methods. Outliers were removed from the data prior to multiple regression after first exploring the data. Once it was determined that any given data set was heteroscedastic, both Mahalanobis and Cook's distances were calculated for all data. For an outlier to be excluded it had to clearly and significantly influence the regression model according to all three criteria. A Mahalanobis distance greater than 11, Cook's distance greater than 1 were the ttwo critical values set to determine whether a data point was an influential outlier. In every case in which data was removed the data reported is the data generated *after* outlier removal. In any case in which the removal of an outlier or outliers changed the significance of a test or the strength of an effect of a variable, this is noted in the text.

6.3.4 **Multivariate statistics: Principal Component Analysis**

Following a recent trend in trace element analyses of biological samples, the use of multivariate statistics was explored as a potential means by which to determine, approximately, the environmental sources of toxic elements. To accomplish this, Principal Component Analysis (PCA) with Varimax rotation was used to explore and visually identify similarities within the data set.

PCA was conducted according to procedures outlined in Field (2009). Initially, all elements of interest were included in PCA analysis. After examining the Kaiser-Meyer Olkin measure of sampling adequacy, one element, magnesium, was found to have a KMO value below the critical value of 0.5 (Field, 2009) and was subsequently removed. All other elements: V, Mn, Cd, Cu, As, Pb, Fe and Ni remained. Two critical assumptions were explored prior to accepting PCA results. Firstly, it was determined that the overall KMO measure of sampling adequacy was above the critical threshold of .05. Secondly, Bartlett's Test of Sphericity, as measured by the X^2 statistic was positive and above the critical value for the reported

degrees of freedom. Reliability of results was tested with Cronbach's α and each component was determined to be reliable if overall Cronbach's α was between .7 and .8.

Both the scree plot generated by PCA analysis and the eigenvalues were considered when determining how many factors to extract. Factors were extracted if they met Kaiser's criterion and had an eigenvalue greater than 1 (Field, 2009).

6.3.4.1 Interpretation and use of principal components

The use of PCA analysis in trace element studies of biological tissues is not new, however it can be confounded by several factors, primary among which is differences in metabolic and toxicokinetic characteristics of each element. Several toxic and trace elements have both symbiotic and antagonistic relationships with one another within the human body, which can promote or supress uptake of these elements into bone tissue. In this study, PCA is not used to determine exactly or definitively, the environmental source of these elements, but is used instead to enable the generation of hypotheses regarding likely sources based on known combinations of elements that are often found to be highly correlated in specific environmental contexts. In the case of South Africa, specifically Gauteng, these combinations are based on data reported by Monna et al. (2006), Olowoyo et al. (2011; 2010), Naicker et al. (2003) and de Villiers et al. (2010) and Rohrmann (1985).

After initial PCA analysis of the total sample population, subsequent analyses were conducted on black males, white males and black females separately to determine if differences existed between principal components. In black females, PCA was not found to be useful, as no elements were strongly or significantly correlated, rendering PCA useless.

6.3.4.2 Scatter plots and Box plots

When producing graphs for the purpose of comparing relationships between bone concentrations of toxic and essential elements the log-transformed data is used in some cases. This was done purely for reasons of aesthetics. For some elements graphical representation of the data, particularly scatter plots with regression lines were considered very difficult to interpret due to differences in element concentrations and scale. Particularly in instances in which an element measured in very small concentrations was compared against an element with much higher concentrations, the use of log-transformed data enabled each element to be compared on the same scale (generally between 0 and 5) resulting more manageable plots. When log transformed data is used it is noted on the graph.

All box plots are representative of median values (central line of each box), 2nd and 3rd quartiles (boxes) and range (whiskers).

6.3.5 **Essential trace elements**

The three essential trace elements, zinc, iron and magnesium are investigated. Box plots for these elements are not given, but basic descriptive statics are, as are comparative statistics between demographic groups. Temporal and geographic trends in trace elements are not explored. These elements are only investigated in relation to toxic elements and are not the focus of this research.

7 Results

This chapter investigates the bone element concentration for each element of interest in the collected bone samples. In addition to descriptive statistics, the differences in mean element concentrations are explored in relation to age, sex, race and time, and, in some cases, cause of death. In addition, statistical relationships between toxic metals is explored, as there are previously reported relationships between elements such as lead, manganese, cadmium, arsenic, antimony and vanadium (Chapter 2).

7.1 Descriptive statistics

Descriptive statistics for bone element concentration for all toxic elements of interest are given. The mean, median, standard deviation, range, and minimum and maximum are given for black males, white males, black females in Table 7-1. Frequency distributions for each toxic element, including deciles are given in Figures 7-1 through 7-6 and Tables 7-2 through 7-7. For the elements As and Sb, several sample concentrations are below LoD. Minimum values for these elements are given as 0.5*Sample LoD, where Sample LoD is LoD/sample Dilution Factor.

Table 7-1. Toxic element concentrations in $\mu g \cdot g^{-1}$ by race and sex. Minimum values for As and Sb are reported as 0.5*Sample LoD due to several samples with concentrations below detection limits.

Group	Element	N	Mean	SE of Mean	Median	SD	Minimum	Maximum
Black Males		129	5.71	0.46	4.34	5.23	0.560	32.23
White Males	Pb	35	16.11	2.14	13.26	12.65	1.55	64.09
Black Females		44	3.92	0.39	3.35	2.73	0.57	14.48
Black Males		129	0.030	2.41	.024	0.027	0.002	0.187
White Males	Cd	35	0.037	6.33	.027	0.037	0.007	0.224
Black Females		44	0.028	2.88	.021	0.020	0.007	0.099
Black Males		129	0.481	49.09	.318	0.558	0.046	4.384
White Males	Mn	35	0.444	67.94	.250	0.068	0.094	1.680
Black Females		44	0.410	44.05	.305	0.308	0.105	1.487
Black Males		129	1.03	0.605	.128	6.841	0.004	77.210
White Males	As	35	0.450	0.156	.071	0.924	0.004	3.634
Black Females		44	14.13	9.65	.128	67.565	0.004	453.600
Black Males		129	0.281	0.060	.015	0.680	0.006	4.302
White Males	Sb	35	0.055	0.032	.010	0.189	0.006	1.128
Black Females		44	0.303	0.096	.036	0.671	0.006	3.710
Black Males		129	0.026	0.001	.021	0.016	0.006	0.112
White Males	V	35	0.020	0.004	.012	0.021	0.004	0.111
Black Females		44	0.031	0.003	.024	0.023	0.008	0.124

7.1.1 **Frequency distributions for each group/element**

For each element, the distribution of bone element concentrations is highly skewed to the left tail of the distribution curve. This indicates that the majority of the sample population is characterised by low rates of exposure to each element, relative to the rate of exposure as a whole. This trend is consistent across race and sex. In some cases, such as As and to some extent Mn and Pb, there is a substantial range in concentrations in all groups, In the case of As this occurs to significant degree in black females. Table 7-1 shows that the highest As concentration in black females is 453.6$\mu g \cdot g^{-1}$, with a median of 0.128$\mu g \cdot g^{-1}$. The distribution curve for As, given in Figure 7-8b, shows clearly that there is only a small number of black females with bone As concentrations on the higher end. This is true for white males and black males as well (Fig. 7-8a and 7-8c). In fact it is only at the 8th decile that bone As concentration is above 1000$\mu g \cdot g^{-1}$ in black females.

Lead

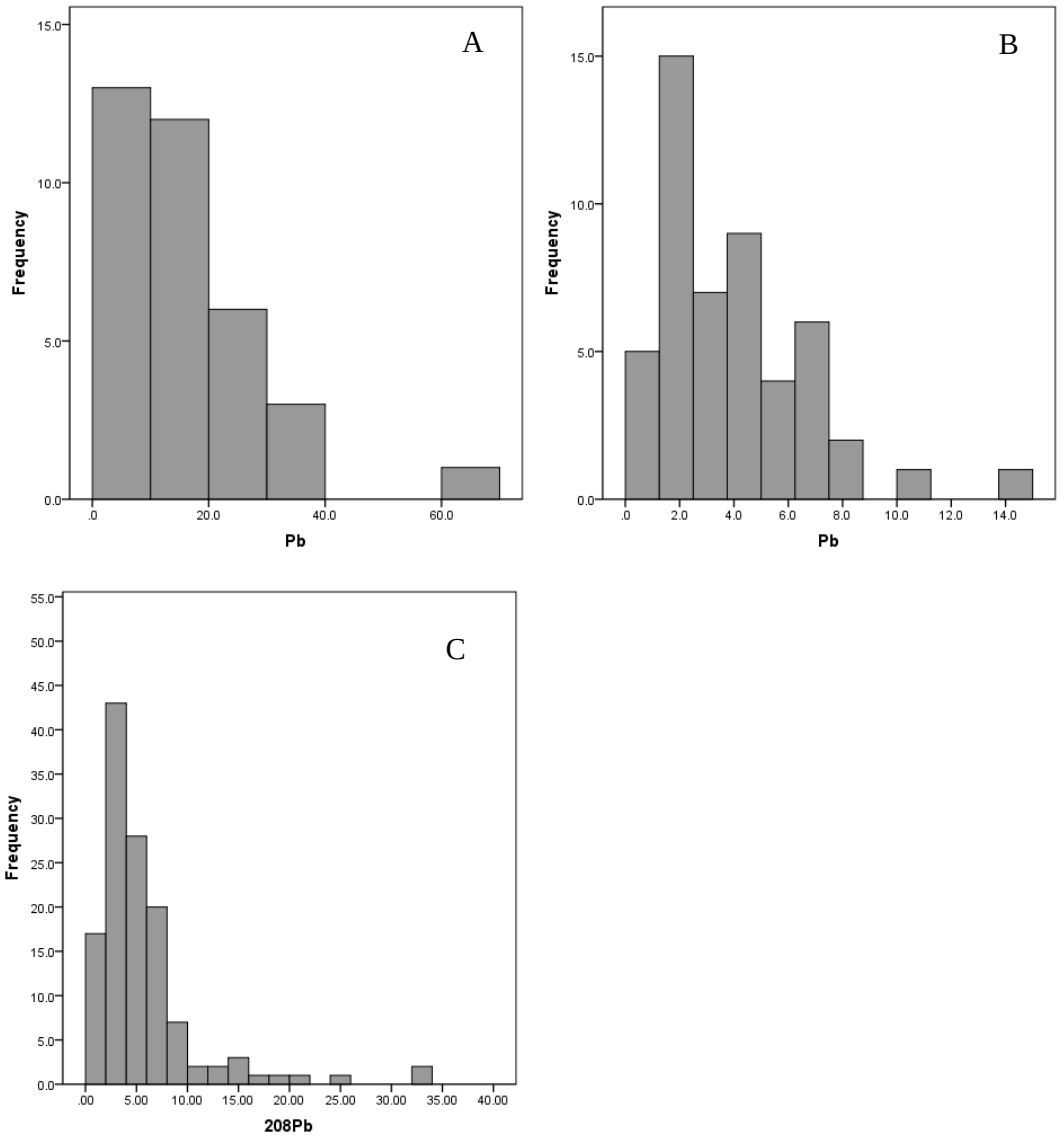


Figure 7-1. Frequency distribution of bone lead. In a) white males, b) black females, and c) black males

Table 7-2. Bone Pb concentration in $\mu g \cdot g^{-1}$ at each decile across the sample population.

Decile Pb	10	20	30	40	50	60	70	80	90
White Males	3.23	7.48	8.71	10.36	13.26	14.51	17.58	26.06	34.68
Black Females	1.23	1.48	2.08	2.61	3.35	3.98	4.75	6.27	7.49
Black Males	1.90	2.22	2.77	3.52	4.30	5.35	6.04	7.38	11.58

Cadmium

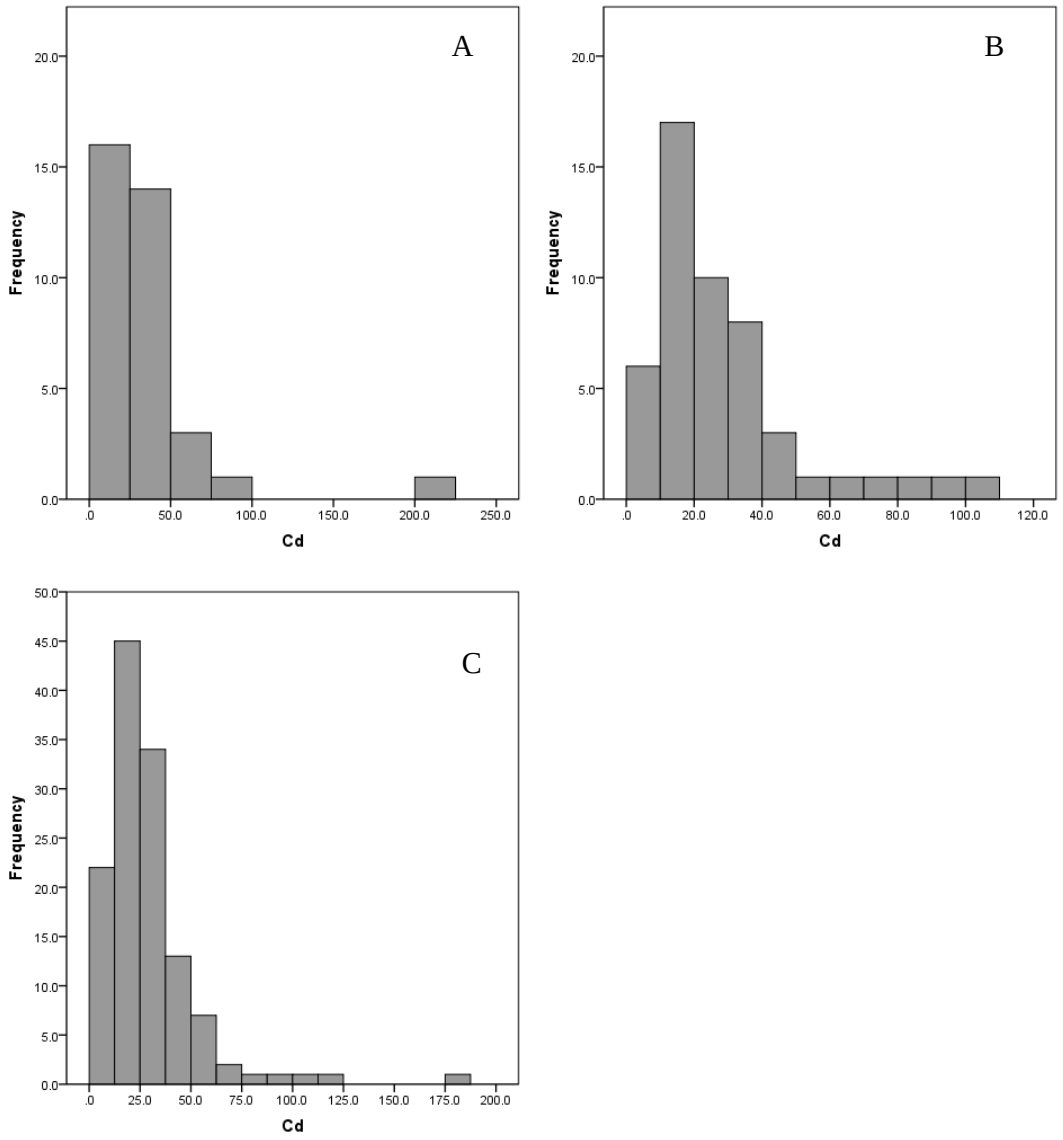


Figure 7-2. Frequency distribution of bone cadmium. In a) white males, b) black females, and c) black males

Table 7-3. Bone Cd concentration in $\mu g \cdot g^{-1}$ at each decile across the sample population.

Decile Cd	10	20	30	40	50	60	70	80	90
White Males	0.011	0.018	0.021	0.024	0.027	0.031	0.036	0.048	0.062
Black Females	0.010	0.013	0.015	0.017	0.021	0.025	0.031	0.039	0.057
Black Males	0.010	0.014	0.016	0.020	0.024	0.028	0.034	0.043	0.052

Manganese

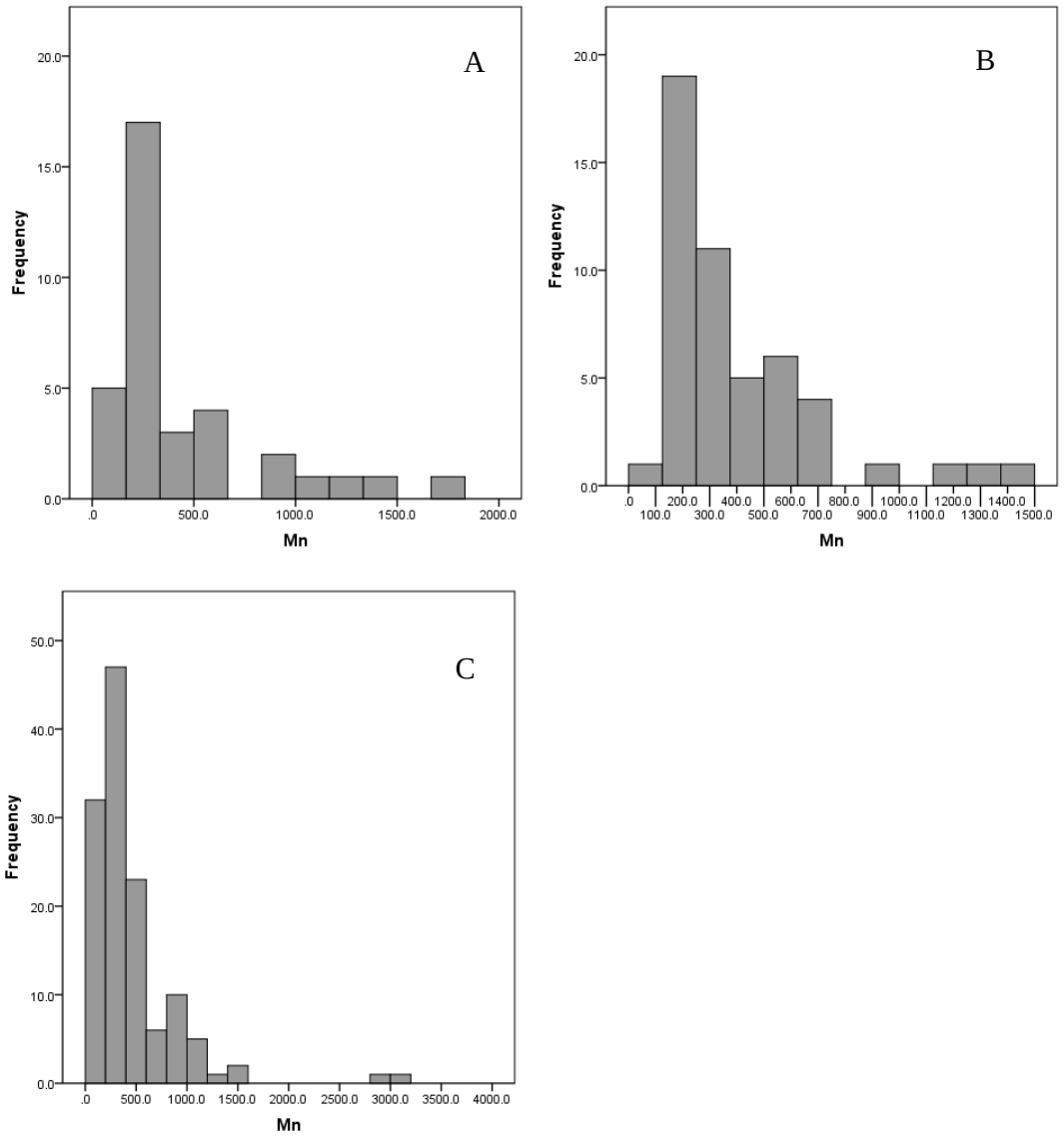


Figure 7-3. Frequency distribution of bone manganese. In a) white males, b) black females, and c) black males

Table 7-4. Bone Mn concentration in $\mu g \cdot g^{-1}$ at each decile in the sample population.

Decile Mn	10	20	30	40	50	60	70	80	90
White Males	0.134	0.175	0.210	0.239	0.250	0.304	0.470	0.646	1.139
Black Females	0.157	0.189	0.205	0.225	0.305	0.370	0.486	0.600	0.738
Black Males	0.139	0.178	0.205	0.258	0.316	0.383	0.474	0.645	0.892

Arsenic

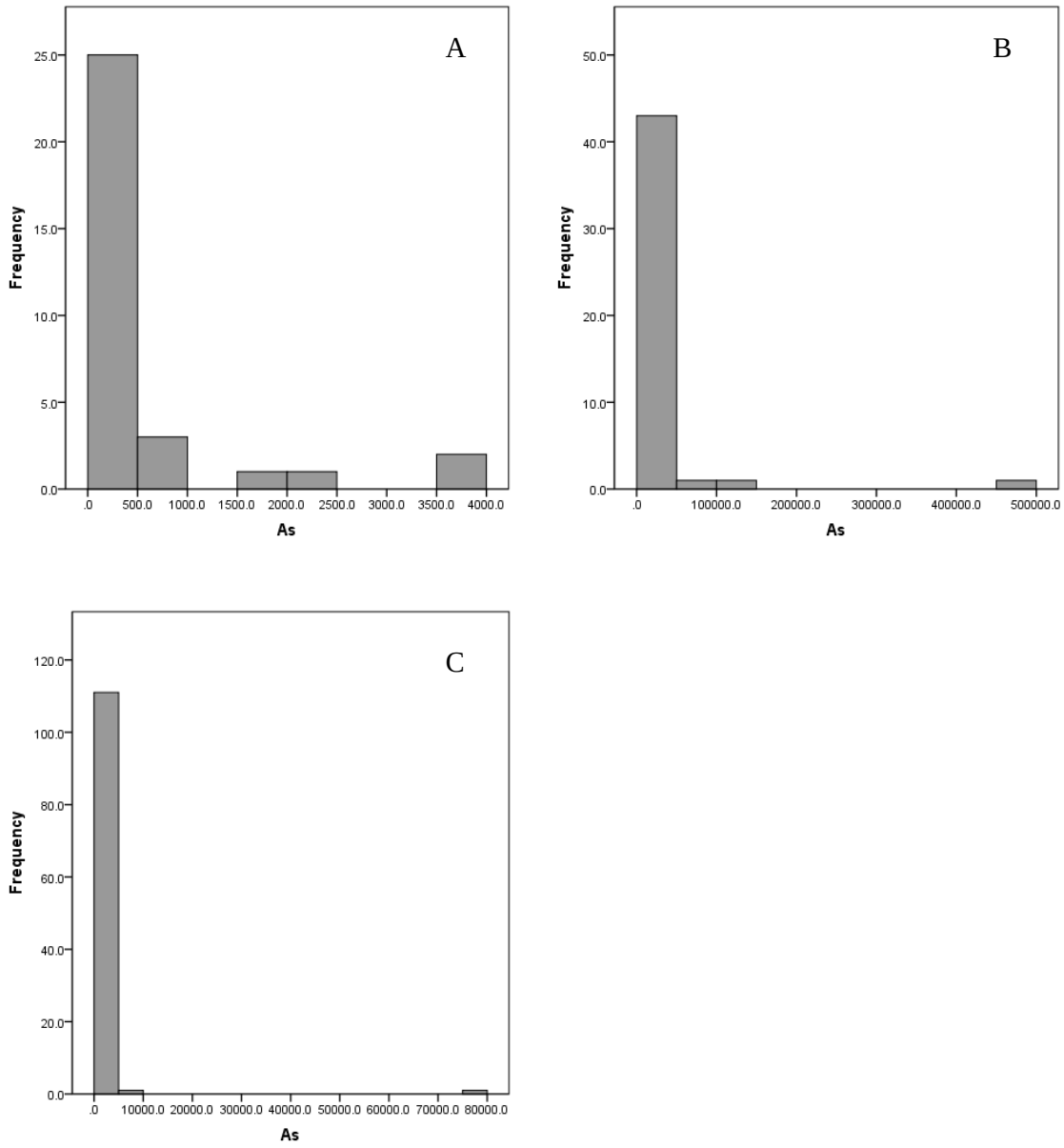


Figure 7-4. Frequency distribution of bone arsenic. In a) white males, b) black females, and c) black males

Table 7-5. Bone As concentration in µg·g^{-1} at each decile in the sample population.

Decile As	10	20	30	40	50	60	70	80	90
White Males	0.013	0.033	0.048	0.057	0.091	0.225	0.277	0.629	2.100
Black Females	0.056	0.074	0.084	0.114	0.136	0.267	0.495	1.674	453.600
Black Males	0.037	0.059	0.080	0.094	0.140	0.192	0.321	0.557	1.818

Vanadium

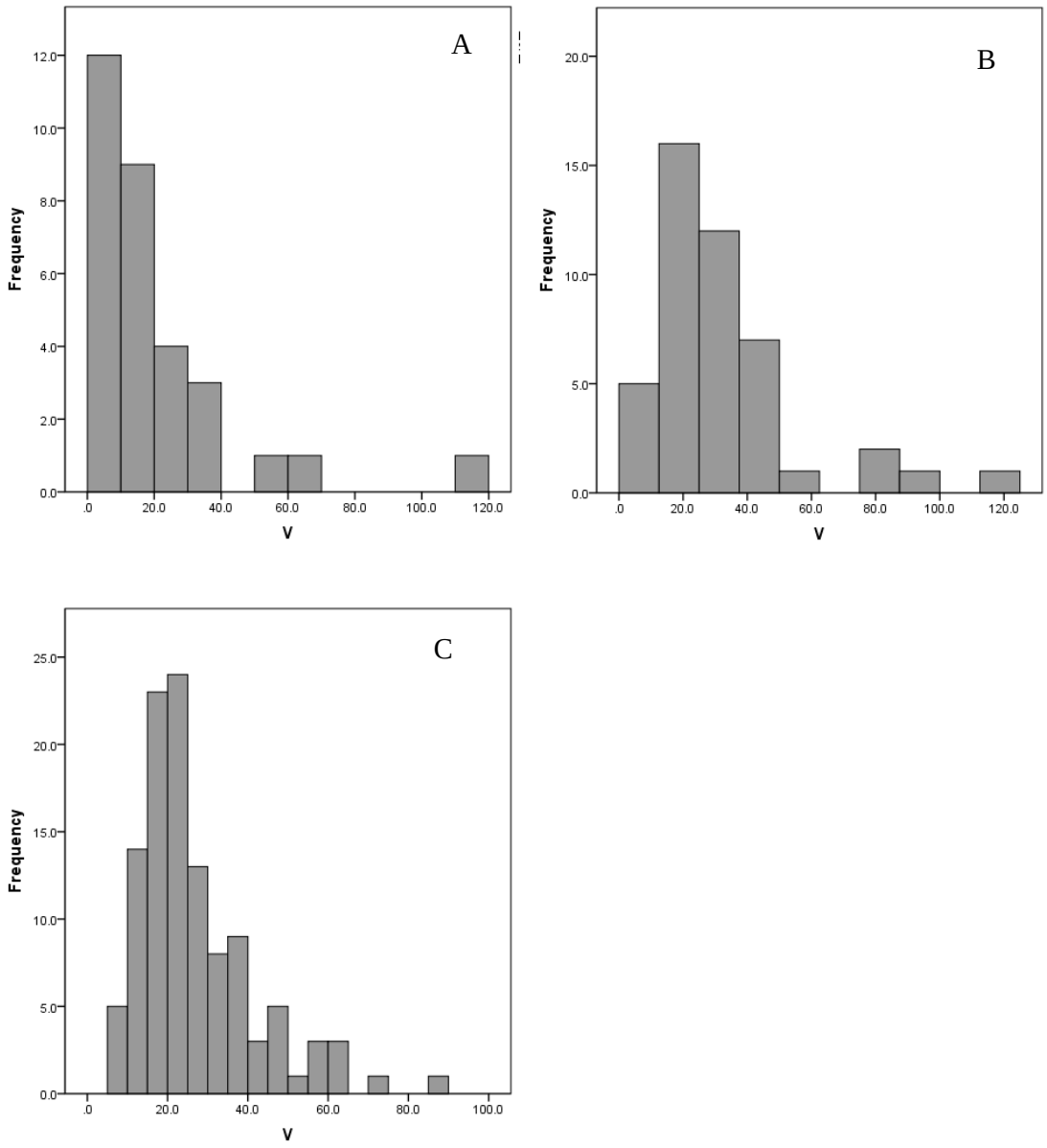


Figure 7-5. Frequency distribution of bone arsenic. In a) white males, b) black females, and c) black males

Table 7-6. Bone V concentration in $\mu g \cdot g^{-1}$ at each decile in the sample population.

Decile V	10	20	30	40	50	60	70	80	90
White Males	0.005	0.008	0.009	0.011	0.013	0.016	0.022	0.029	0.045
Black Females	0.011	0.014	0.020	0.022	0.024	0.029	0.036	0.039	0.060
Black Males	0.011	0.015	0.016	0.020	0.021	0.025	0.028	0.036	0.045

Antimony

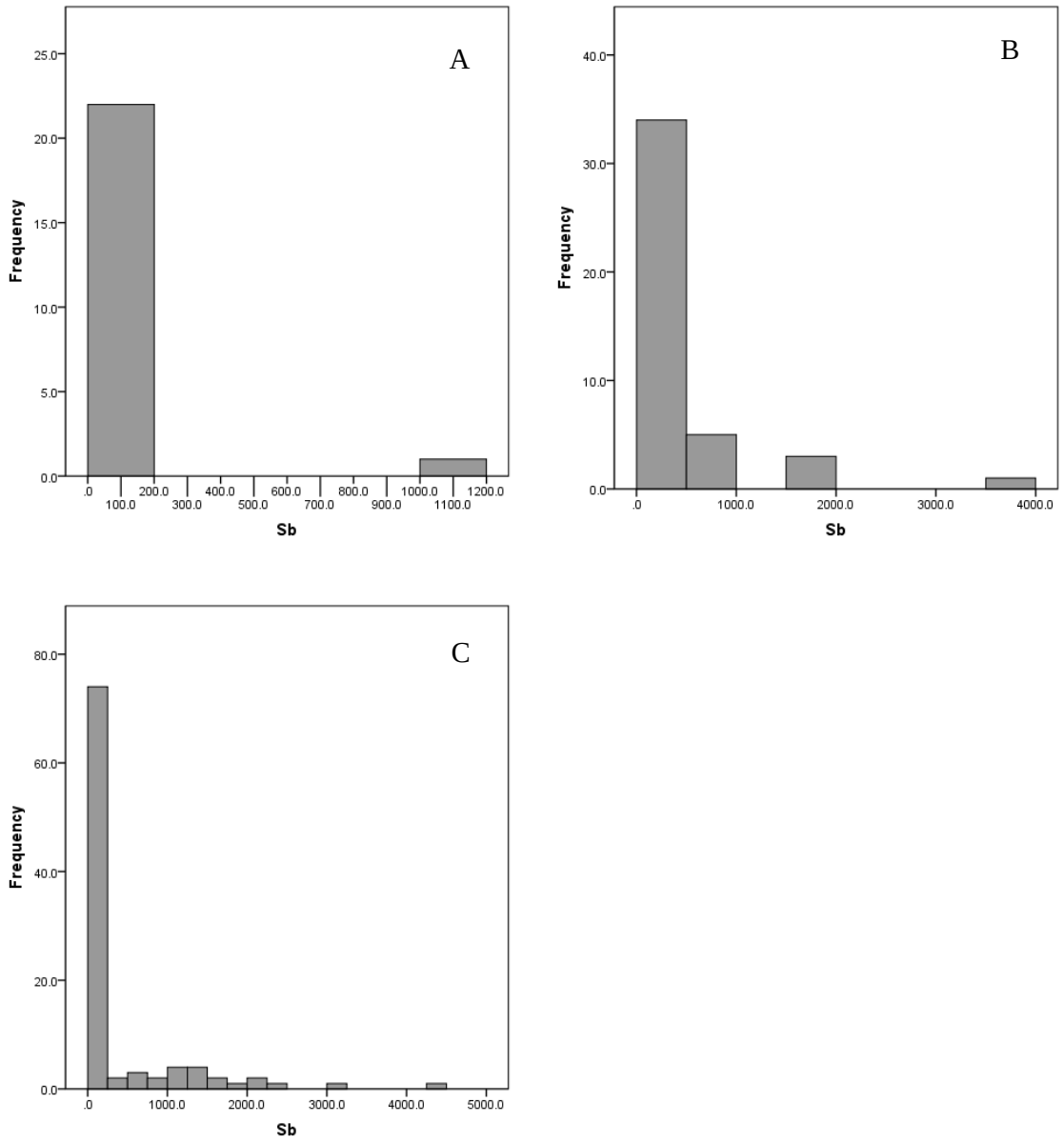


Figure 7-6. Frequency distribution of bone arsenic. In a) white males, b) black females, and c) black males.

Table 7-7. Bone Sb concentration in $\mu g \cdot g^{-1}$ at each decile in the sample population.

Decile Sb	10	20	30	40	50	60	70	80	90
White Males	0.004	0.010	0.012	0.016	0.019	0.030	0.049	0.081	0.118
Black Females	0.006	0.011	0.016	0.028	0.037	0.071	0.332	0.533	1.467
Black Males	0.004	0.007	0.012	0.017	0.026	0.034	0.077	0.624	1.435

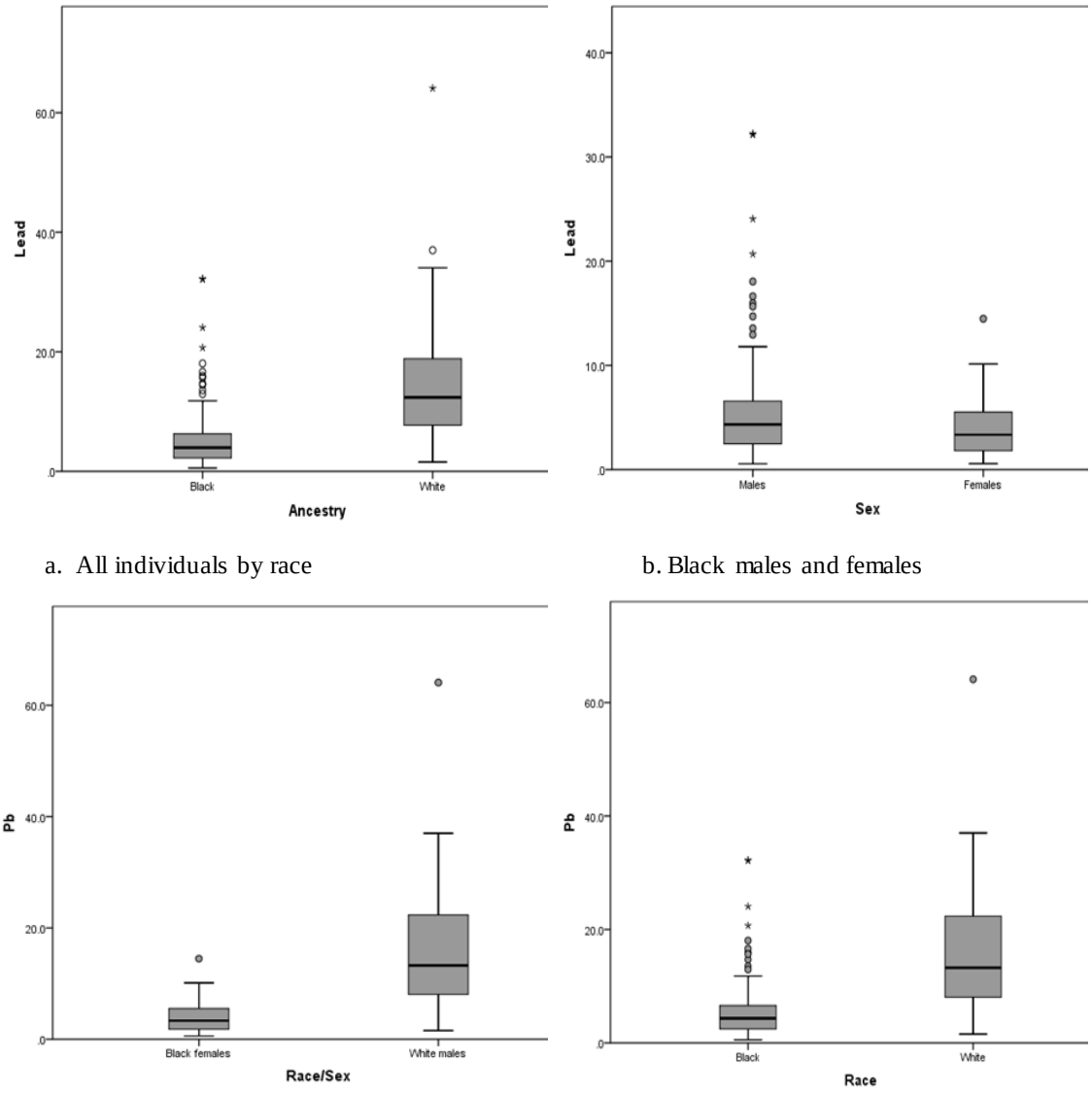
7.2 Differences between demographic groups

7.2.1 **Lead**

Bone lead concentration is lower in black individuals than in white. This is true for both males and females and across ages. Mean bone lead concentration for black individuals is 5.14µg·g^{-1} and for white individuals 16.11µg·g^{-1}. An independent t-test confirmed that the difference in means is significant: $t(47.65) = 7.74$, $p < 0.001$. The effect size is significant, $r = 0.751$.

Though the values for the minimum bone lead concentrations between black and white individuals are not substantially different, 1.55µg·g-1 and 0.56µg·g-1 for black and white individuals respectively, the maximum bone lead concentrations are significantly different at 32.23µg·$^{g-1}$ and 64.09µg·g-1. respectively.

Bone lead concentration between black and white males follows the same trend. When black females are removed, there is little change to the mean bone lead concentrations between black and white males. The differences in means between black and white males is still significant, $t(49.71) = 6.81$, $p < 0.001$, with an effect size, $r = 0.701$.



a. All individuals by race

b. Black males and females

c. Black females and white males

d. Black and white males

Figure 7-7 Comparison of median bone lead by race and sex. Boxes include 2nd and 3rd quartiles, horizontal line represents median and whiskers represent range. In a) all individuals by race; b) black males and white males; c) black males and black females; and d) white males and black females.

Mean bone lead concentration between black females and black males is significantly different. Mean bone lead concentration in black females is 4.14μg·g^{-1}, with a lower range and the maximum concentration is 15.78μg·g^{-1}. Independent t-test confirms the significance between means in black males and females, $t(88.379) = 2.712$, $p < 0.01$, with an effect size, $r = .272$. The difference between black females and white males is also significant $t(68.28) = 7.82$, $p < 0.001$.White males have substantially higher bone lead than black females.

7.2.1.1 Bone lead concentration and age

Differences in bone lead concentration between individuals of different ages was assessed. Black males, black females, and white males were analyses separately. Individual age was coded into age categories, which are given in Table 7-8. As the data in this table and in Fig. 7-8 show, bone Pb increases as age increases within the population as a whole. The highest lead concentrations are found in the oldest individuals. In males, bone Pb increases with age, with some small fluctuations. In white males, there is a drop in bone Pb between the ages of 80-89, when Pb concentration is substantially lower than in other ages. The reasons for this are unclear.

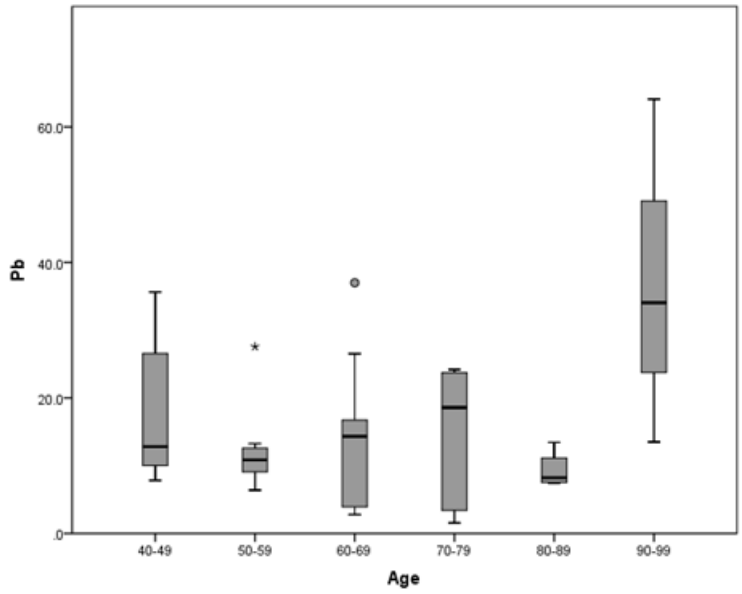
Table 7-8 Bone lead concentration in $\mu g \cdot g^{-1}$ by age, sex and race.

Group	Age Group	N	Mean	SE Mean	Median	SD	Minimum	Maximum
Black Males	20-29	14	3.98	0.64	3.03	2.39	1.55	9.25
	30-39	13	5.26	0.96	4.26	3.47	1.82	13.56
	40-49	26	3.94	0.45	3.29	2.31	1.22	11.56
	50-59	24	6.21	1.14	3.69	5.58	1.61	24.06
	60-69	33	5.34	0.54	4.95	3.12	0.60	16.63
	70-79	16	9.57	2.57	5.61	10.27	0.56	32.23
	80-89	2	14.46	1.51	14.46	2.16	12.95	15.97
White Males	20-29	0	-	-	-	-	-	-
	30-39	0	-	-	-	-	-	-
	40-49	5	18.57	5.37	12.82	12.00	7.53	35.63
	50-59	7	12.59	2.64	10.85	6.99	6.38	27.58
	60-69	10	14.40	3.44	14.34	10.88	2.78	37.00
	70-79	6	15.00	4.13	18.58	10.12	1.55	24.18
	80-89	4	9.38	1.41	8.20	2.82	7.45	13.45
	90-99	3	37.21	14.69	34.04	25.44	13.50	64.09
Black Females	20-29	2	3.90	1.62	3.90	2.28	2.29	5.52
	30-39	14	3.74	0.68	3.04	2.54	0.95	7.49
	40-49	9	5.47	1.46	3.87	4.38	1.81	14.48
	50-59	7	3.17	0.89	2.23	2.36	1.00	7.81
	60-69	10	3.34	0.62	3.90	1.95	0.57	6.27
	70-79	2	3.19	0.15	3.19	0.21	3.04	3.34
	80-89	2	5.10	0.35	5.10	0.50	4.76	5.46

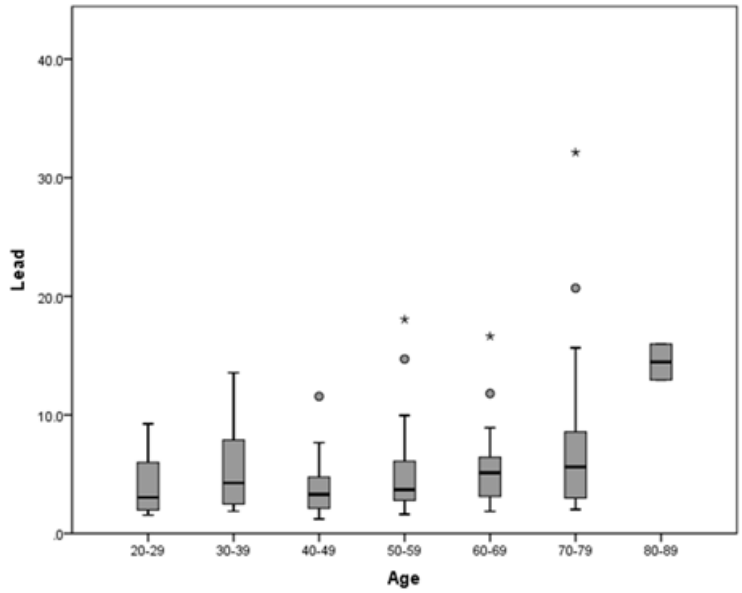
In all individuals the difference in bone lead concentration between age groups is significant, Welch's adjusted $F(7, 27.05) = 4.05$, $p < 0.01$. The effect size is medium, $\omega^2 = .12$.

When each demographic is examined independently, by ANOVA, the trend is the different. In white males bone lead concentration does not differ across age groups, $F(5, 10.34) = 1.405$, $p > 0.05$, even when three outliers are removed due to high Cook's distances (greater than 4/N).

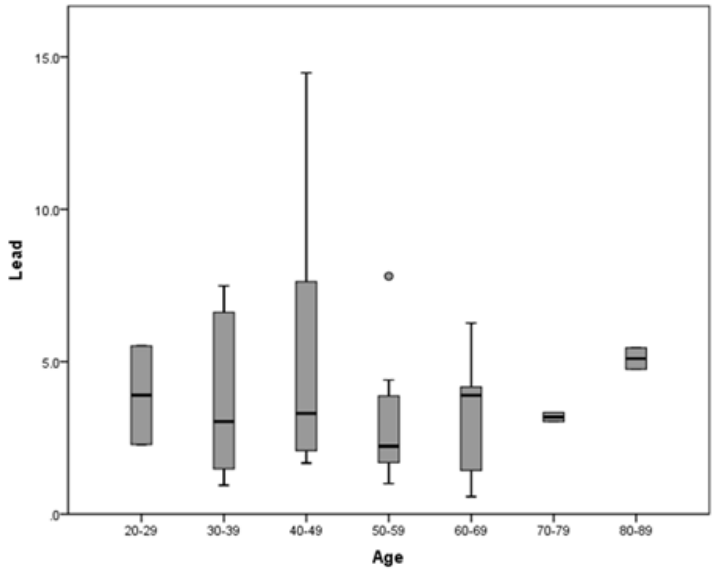
Among black males the difference in bone lead concentration between age groups is significant, $F(6, 117) = 3.03$, $p < 0.01$, with a medium effect size, $\omega^2 = .09$. Post hoc procedures (Hochberg , due to unequal sample sizes between age groups) confirm that the difference in mean bone Pb in individuals aged 80-89 is significantly different ($p < 0.05$) from all other ages 20-29 and ages 40-49. Residuals from Univariate ANOVA identified four samples which yielded a Cook's distance above 4/N. These outliers were removed from the model.



a. White males



b. Black males



c. Black females

Figure 7-8. Bone lead and age in a) white males; b) black males; and c) black females

Among age groups in black females, the greatest differences between bone lead concentration occurs at older ages. Bone lead concentration increases significantly after the age of 70. The lowest mean bone lead concentration is found in 50-59 year olds, and the highest in women ages 40-49. Bone lead concentration then increases beyond age 60. Statistically, however bone lead is not significantly different among age groups in black females, $F(6,39) = 0.489$, $p > 0.05$. One outlier was removed from black females. Removal of outliers did not affect results.

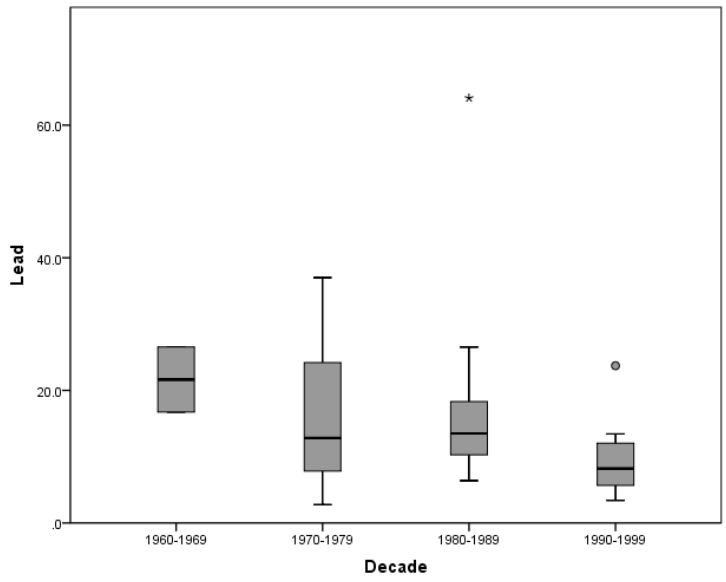
7.2.1.2 Temporal trends in bone lead concentration

Bone lead concentration was investigated across time to determine the existence of temporal patterns in lead exposure. Dates of death for each individual were coded into decade, and bone lead was analysed in ten year intervals. Decade intervals and median bone Pb for each decade for the population as a whole is presented in Table 7-9.

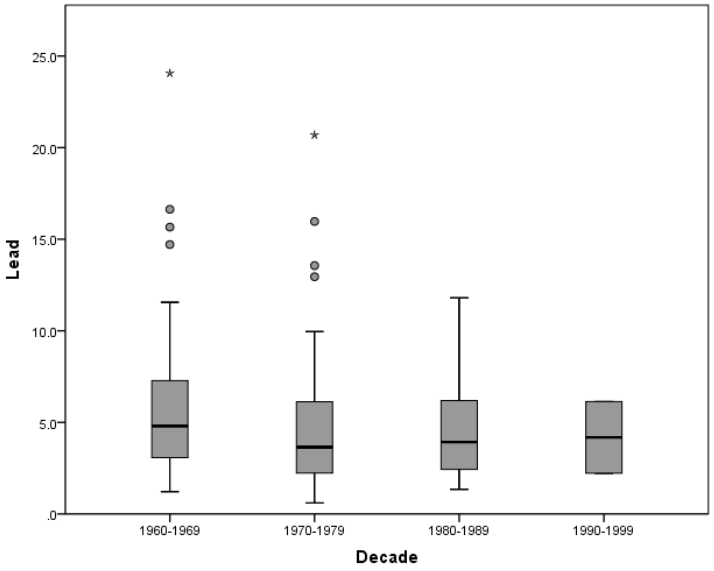
Table 7-9. Bone lead concentration in $\mu g \cdot g^{-1}$ by decade of death, race and sex.

Demographic	Decade	N	Mean	Median	SD	Min.	Max
	1960-1969	41	6.03	4.75	4.63	1.22	24.06
	1970-1979	48	4.94	3.62	4.10	0.56	20.69
	1980-1989	36	6.47	4.35	7.11	1.35	32.23
Black males	1990-1999	3	3.36	2.23	2.42	1.71	6.14
	1960-1969	2	21.64	21.64	6.93	16.74	26.54
	1970-1979	14	16.84	13.04	12.57	1.55	37.00
	1980-1989	12	18.05	13.50	15.51	6.38	64.09
White males	1990-1999	7	9.76	7.59	7.00	3.41	23.72
	1960-1969	23	4.54	3.04	3.31	1.00	14.48
	1970-1979	20	3.34	3.03	2.25	0.57	7.81
	1980-1989	5	3.36	3.87	1.04	1.57	4.01
Black females	1990-1999	1					

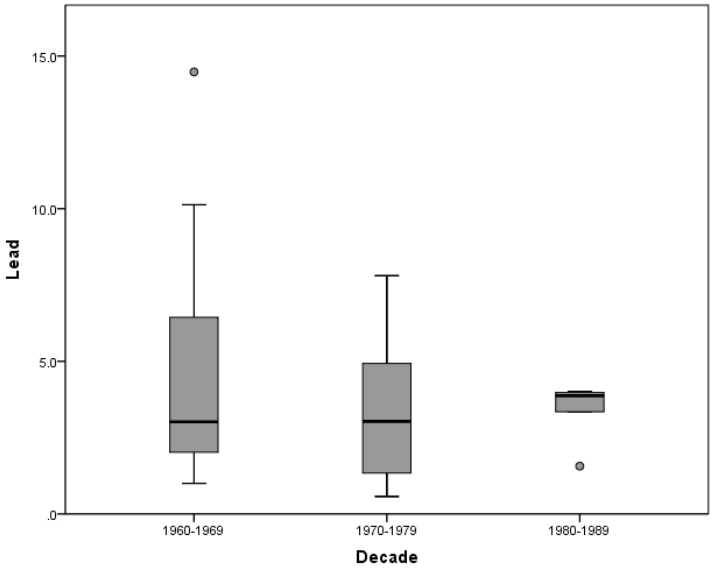
Though it would appear, based on the data presented in Table 7-9, that bone Pb concentration decreases during the 1990s, the trend is not significant. In white males there is no significant change in mean bone Pb over time, $F(3,31) = 1.17$, $p > 0.05$. In black females, there is no difference in Pb concentration across time, $F(2,46) = .922$, $p > 0.05$. One outlier was removed from this. For black males there is also no significant trend, $F(3,122) = 1.31$, $p > 0.05$. Four outliers were removed, according to methods described in Chapter 6. Removal of outliers did not affect the results of any analysis. Median bone Pb for each demographic group for each decade is given in Fig. 7-9.



a. White males



b. Black males



c. Black females

Figure 7-9. Bone Pb concentration per decade in a) white males, b) black males and c) black females.

To determine whether age of death and decade of death co-vary, ANCOVA was carried out for all groups with age group and decade of death as covariables. In white males, the inclusion of age as a covariate does not affect the model, $F(3,31) = 1.62$, $p > 0.05$. Four outliers were removed from white males, in the initial ANCOVA which did not affect the result. In black females, there was no relationship between bone Pb and decade of death, $F(3,42) = 0.670$, $p > 0.05$ when age was included as a covariate. No outliers were removed among black females. The inclusion of age as a covariate in black males yields the same

result, $F(2,116) = 2.945$, $p > 0.05$. Eight outliers were removed from the model with no effect on the results.

7.2.1.3 Geographic trends in bone lead

Bone lead concentration between Johannesburg and Pretoria and rural areas was examined with admitting hospital as a proxy for residence.

Among all individuals there is no difference in bone Pb concentration and location, $F(2,201) = 2.403$, $p. > 0.05$ (Fig. 7-10).

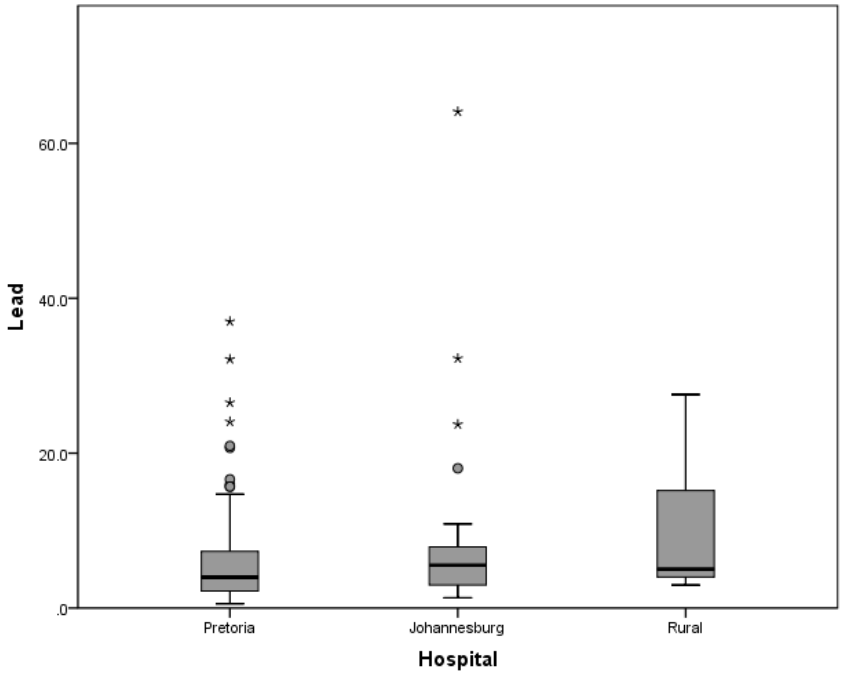
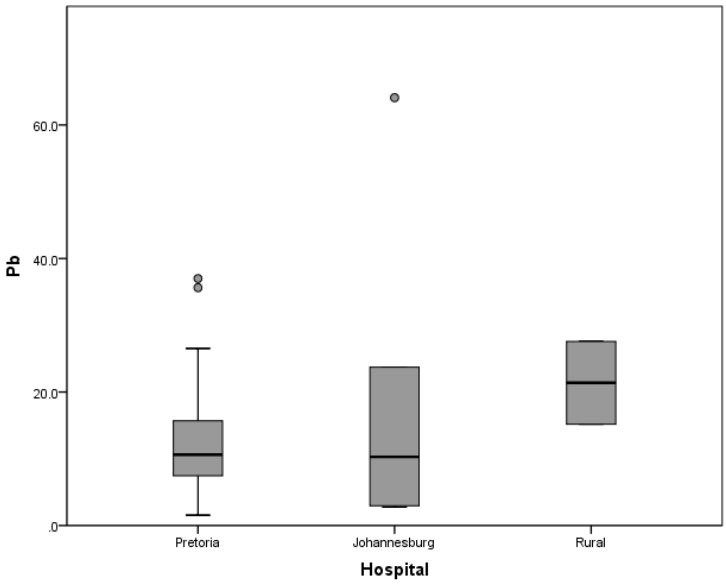
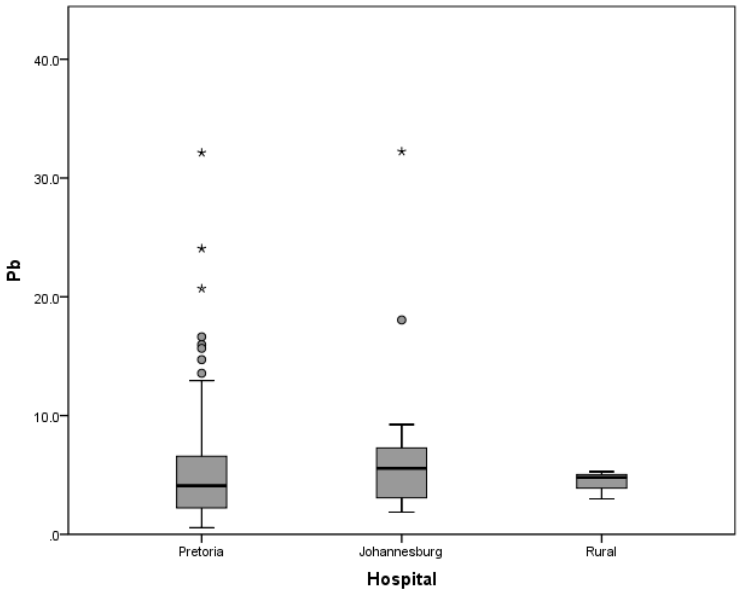


Figure 7-10. Median bone Pb concentration and city in all individuals. Rural residents, N=6.

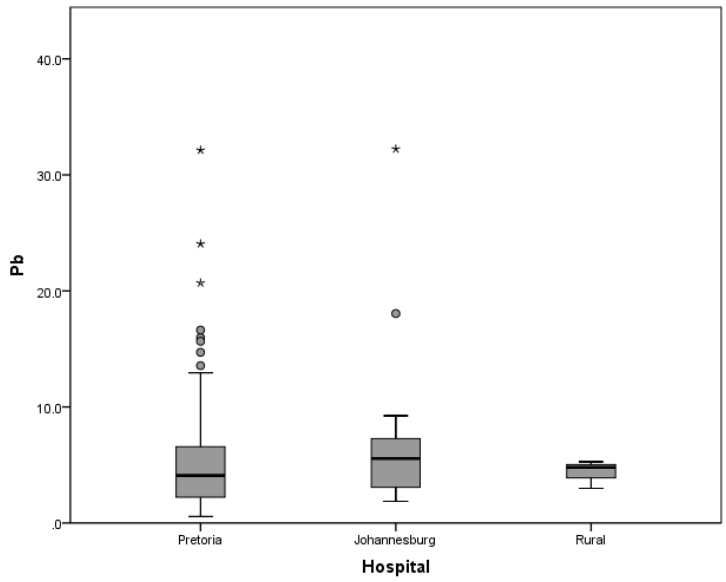
The possibility that bone Pb varies between Pretoria and Johannesburg among individual groups was explored by ANOVA with city of residence. In black females, ANOVA shows that city is not a significant predictor of bone Pb, $F(2, 45) = 0.369$, $p > 0.05$. In white males, there is no variation in bone lead across residence, $F(2,23) = 0.501$, $p > .05$. The lack of relationship between bone lead and city is also evident in black males, $F(2,123) = 1.00$, $p < 0.05$. Box plots for each group and city are given in Figure 7-11, below.



a. White males



b. Black males

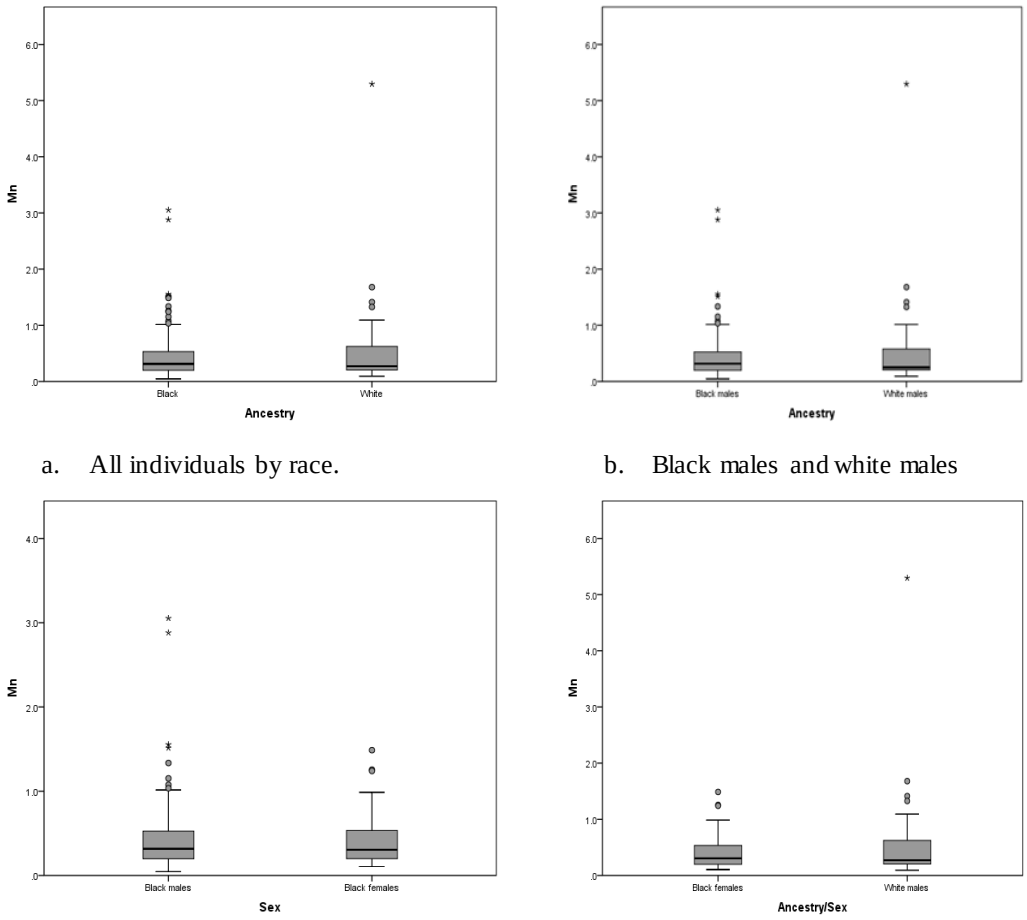


c. Black females

Figure 7-11. City of residence and median bone Pb concentration in a) white males, b) black males and c) black females.

7.2.2 **Manganese**

Manganese data are not normally distributed, even when log transformed. Non-parametric statistics are used. Although median bone Mn appears to be slightly higher in black males than in white males or black females (Table 7-1), when explored statistically, there is no difference in bone Mn concentration between black and white individuals, Mann Whitney $U = 3263$, $p > 0.05$. Among males only, there is no difference in bone Mn between black and white males, $U = 2156$, $p > 0.05$. Between black males and females, there is also no difference in bone Mn, $U = 3114.5$, $p > 0.05$. Between black females and white males, there is no significant difference, $U = 1317$, $p > 0.05$. Box plots for each comparison are given in Fig. 7-12.



a. All individuals by race.

b. Black males and white males

c. Black males and females

d. Black females and white males

Figure 7-12. Median bone Mn concentration between a) all individuals by race, b) all males by race, c) black males and females and d) black females and white males.

7.2.2.1 Bone Mn concentration and age at death

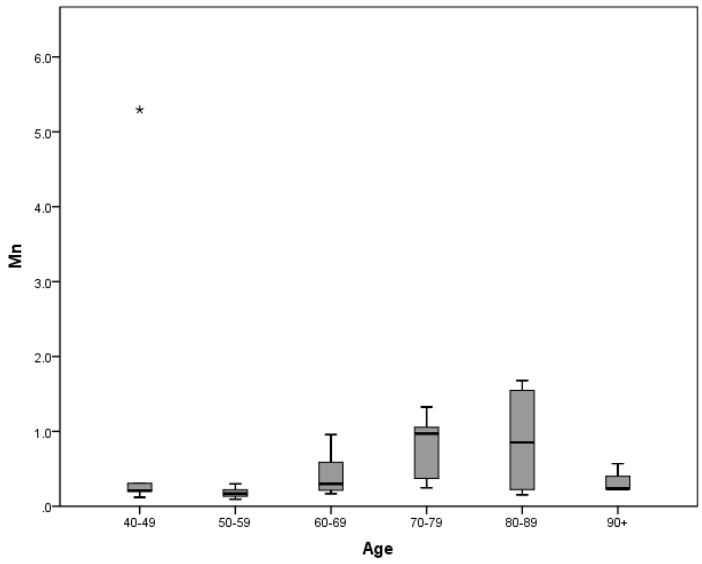
Bone Mn appears, based on data presented in Table 7-10, to vary between age groups, though not in any consistent pattern across all groups. In black males and females, median bone Mn appears to peak between the ages of 80-89, though this is not the case for white males. Statistically however, there is no significant difference in mean bone Mn concentration and age at death. Kruskal-Wallis tests did not yield any differences in age category and bone Mn concentration.

Table 7-10. Bone Mn concentration in $\mu g \cdot g^{-1}$ and age at death by race and sex.

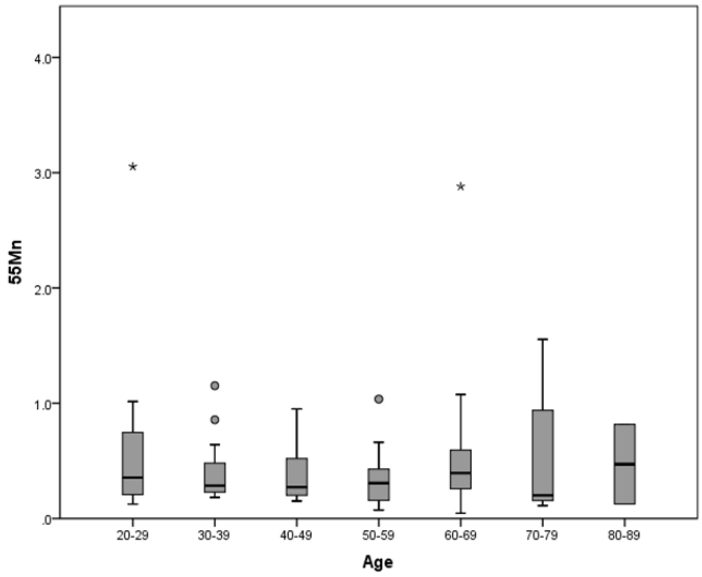
Group	Age Group	N	Mean	SE Mean	Median	SD	Minimum	Maximum
Black Males	20-29	14	0.620	0.201	0.355	0.753	0.125	3.053
	30-39	13	0.412	0.083	0.285	0.298	0.184	1.152
	40-49	26	0.370	0.047	0.272	0.239	0.151	0.951
	50-59	24	0.330	0.044	0.306	0.213	0.072	1.036
	60-69	33	0.632	0.145	0.404	0.834	0.046	4.384
	70-79	16	0.533	0.134	0.201	0.534	0.112	1.554
	80-89	2	0.471	0.345	0.471	0.488	0.126	0.816
White Males	20-29	0	-	-	-	-	-	-
	30-39	0	-	-	-	-	-	-
	40-49	5	0.237	0.041	0.210	0.091	0.121	0.349
	50-59	7	0.182	0.027	0.170	0.072	0.094	0.299
	60-69	10	0.421	0.082	0.299	0.261	0.167	0.958
	70-79	6	0.717	0.182	0.708	0.446	0.245	1.326
	80-89	4	0.884	0.387	0.853	0.774	0.152	1.679
	90-99	3	0.346	0.111	0.235	0.192	0.234	0.568
Black Females	20-29	2	0.231	0.077	0.231	0.109	0.154	0.308
	30-39	14	0.459	0.078	0.451	0.292	0.167	1.241
	40-49	9	0.274	0.058	0.208	0.175	0.105	0.695
	50-59	7	0.397	0.145	0.263	0.384	0.157	1.255
	60-69	10	0.366	0.064	0.351	0.203	0.128	0.738
	70-79	2	0.187	0.002	0.187	0.003	0.185	0.189
	80-89	2	0.793	0.194	0.793	0.274	0.600	0.987

Among all individuals, $H(7) = 12.02$, $p > 0.05$. In black females, $H(6) = 8.601$, $p > 0.05$ indicating no significant difference in bone Mn across age groups. In this group however, the presence of outliers among females between the ages of 30 and 39 and 50 and 59 were suspected of skewing the results. These cases were removed and Kruskal-Wallis test was run again. The results remain insignificant, $H(6)$ 10.344, $p > 0.05$.

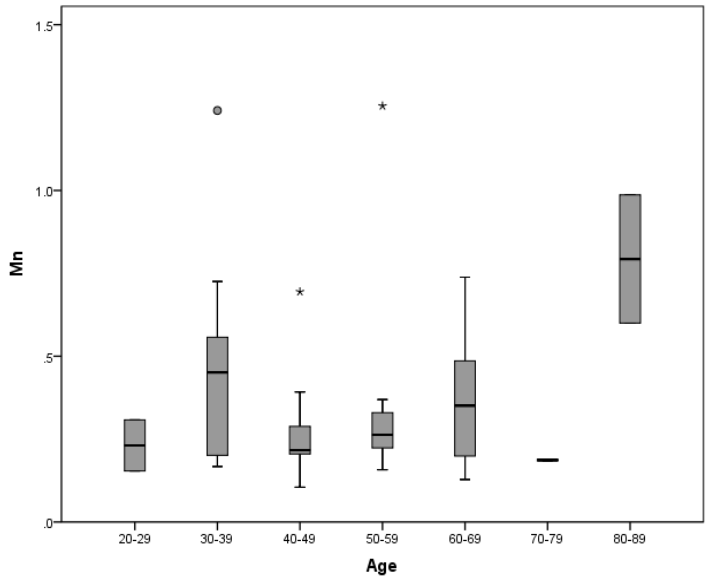
In black males, the result is the same with $H(6) = 4.424$, $p > 0.05$. As is evident in Fig. 7-13, there are two outliers that may potentially affect statistical results, in males aged 20-29 and 60-69. These outliers were removed and the analysis was performed again with little change in results, $H(6) = 3.261$, $p > 0.05$. And in white males, $H(5) = 11.530$, $p > 0.05$, also not a significant relationship between age and bone Mn.



a. White males



b. Black males



c. Black females

Figure 7-13. Age at death and median bone Mn concentration in a) white males, b) black males and c) black females.

7.2.2.2 Temporal trends in manganese concentration

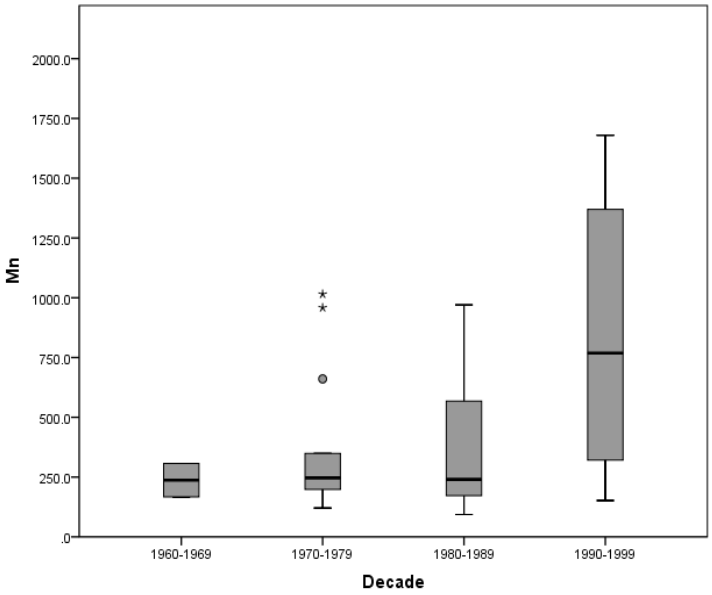
Bone manganese descriptive statistics per demographic group and decade are given in Table 7-11. There is a significant difference in bone Mn across time in all individuals, $H(3) = 9.50$, $p < 0.05$. It is clear from Fig. 7-13, that individuals who died in the 1990s had higher bone Mn concentrations than individuals who died in the previous three decades, particularly those living in the 1960s (Fig. 7-14). Mann Whitney U test was performed with Bonferroni correction (.05/4) to determine if this group differed significantly from other decades. The decade spanning 1990-1999 differs from the 1960s and the 1970s when $p < .016$, but not the 1980s.

Table 7-11. Bone Mn concentration in $\mu g \cdot g^{-1}$ and decade of death by race and sex.

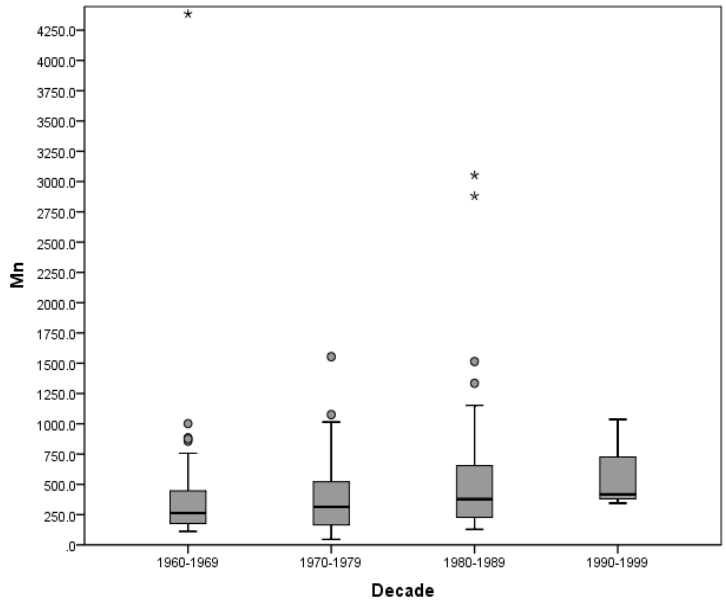
Demographic	Decade	N	Median	SD	Min.	Max.
Black males	1960-1969	41	0.251	0.236	0.112	1.001
	1970-1979	48	0.314	0.316	0.046	1.554
	1980-1989	36	0.377	0.672	0.128	3.053
	1990-1999	3	0.417	0.380	0.344	1.036
White males	1960-1969	2	0.237	0.099	0.167	0.307
	1970-1979	14	0.247	0.291	0.121	1.015
	1980-1989	12	0.240	0.263	0.094	0.970
	1990-1999	7	0.445	0.637	0.152	1.679
Black females	1960-1969	23	0.290	0.154	0.128	0.636
	1970-1979	20	0.416	0.377	0.167	1.487
	1980-1989	5	0.157	0.174	0.105	0.534
	1990-1999	1	-	-	-	-

In black females, there is significant difference in Mn concentration across time, $H(3) = 7.90$, $p < 0.05$, however when this trend is examined graphically (Fig. 7-14), it becomes apparent that women living in the 1970s had the highest Mn concentrations. There is only one female from the 1990s, however this individual has the highest bone Mn concentration (Table 7-11). Mann Whitney test with Bonferroni Correction (.05/4) indicates none of the decades differs significantly in terms of bone Mn in black females.

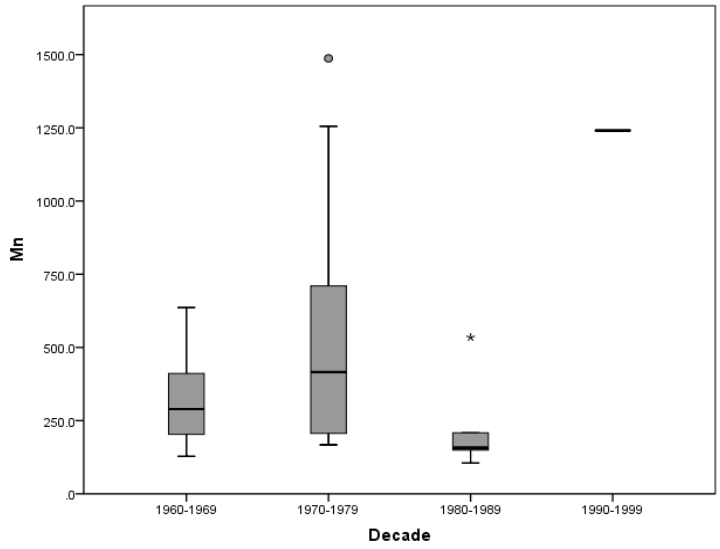
In white males, there appears to be an increase in bone Mn over time (Fig. 7-14), however the difference is not significant, $H(3) = 5.73$, $p < 0.05$. The removal of the outlier (per visual inspection of box plot) does not change the results. In black males the trend is similar, with no significant difference in Mn concentration over time, $H(3)$ 6.10, $p > 0.05$. When apparent outliers are removed, the results remain insignificant.



a. White males



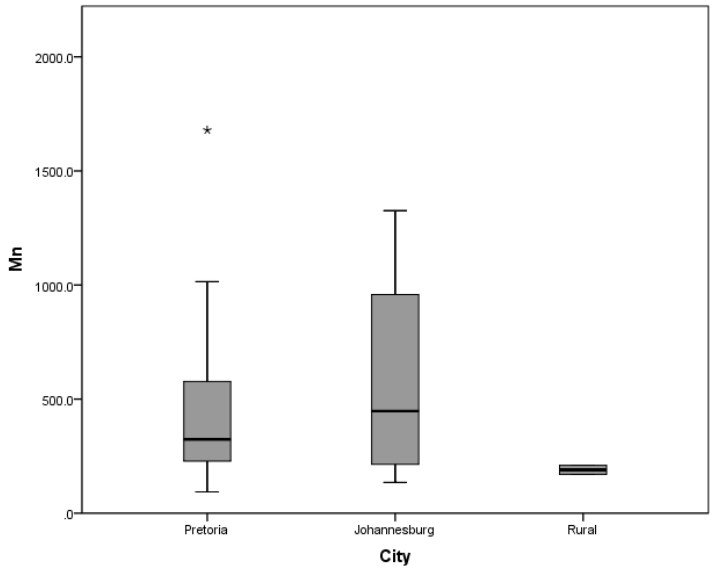
b. Black males



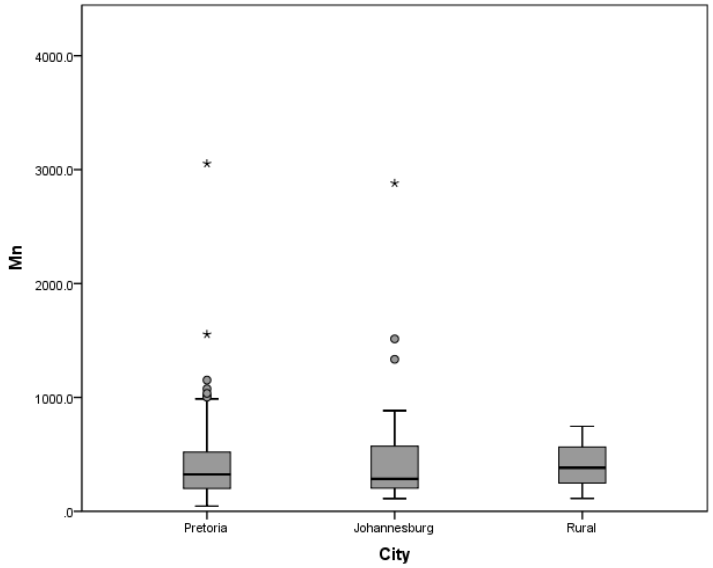
c. Black females

Figure 7-14. Bone Mn (back transformed) by decade of death in a) white males, b) black males and c) black females.

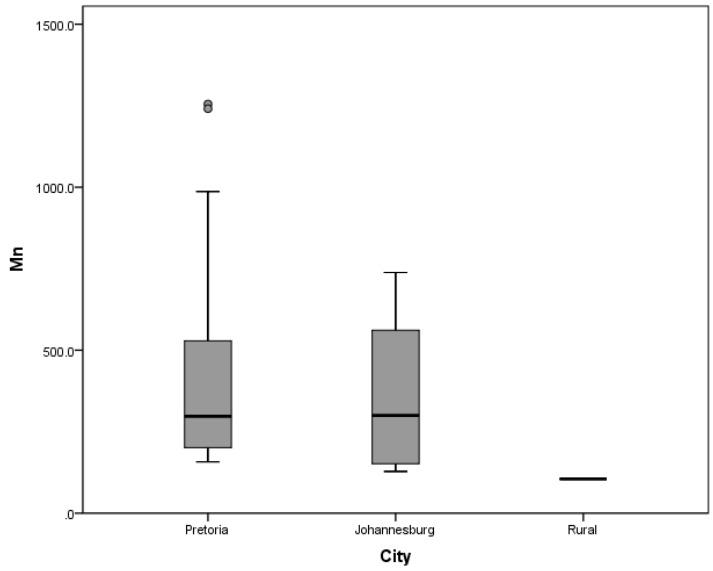
Bone Mn does not vary by city (Fig. 7-15). In all individuals, $H(2) = 2.158$, $p > 0.05$. In black females, $H(2) = 3.197$, $p > 0.05$, indicating that there is no difference in Mn between cities. In black males and white males, $H(2) = 0.10$, $p > 0.05$ and $H(2) = 2.471$, $p > 0.05$, respectively, also demonstrating a lack of difference in bone Mn concentration between individuals in each location.



a. White males



b. Black males



c. Black females

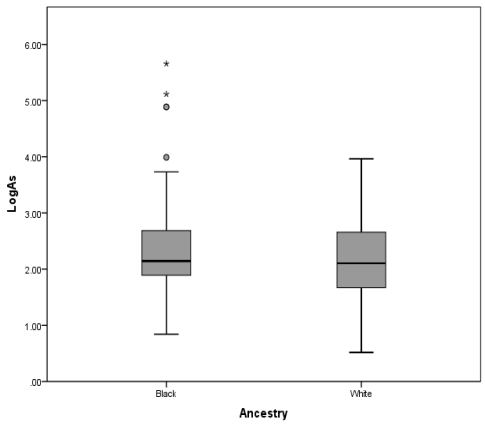
Figure 7-15. Median bone Mn by city in a) white males, b) black males and c) black females.

7.2.3 **Arsenic**

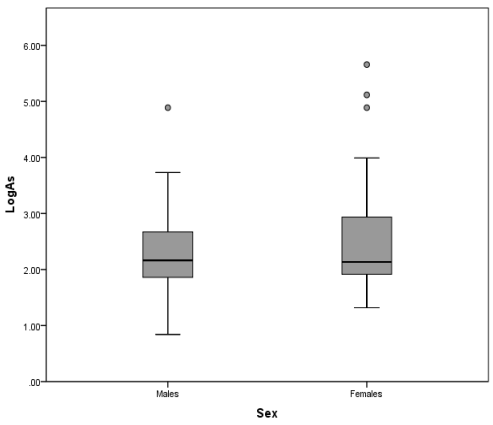
Mean and median As concentration appears to differ by race and sex (Table 7-1). In this analysis, all concentrations reported below detection limits are given as LoD/2.

Unlike lead there is no significant difference between bone As concentration and race among all individuals. Independent samples Mann-Whitney U test confirms that the differences in means are not significant, $U = 2263$, $p > 0.05$. The same is true of the means between white and black males, $U = 2003$, $p > 0.05$. There is no difference in bone As concentration between black males and females, $U = 2717.5$, $p > 0.05$.

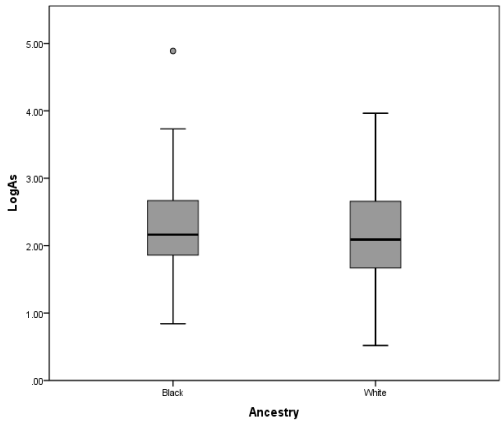
Among black females and white males, there is no significant difference in bone arsenic, $U = 641$, $p < 0.05$. Fig. 7-15 includes box-plots between each group. Log transformed data was used to create histograms as the wide variance of As values in each group makes back transformed data hard to interpret graphically.



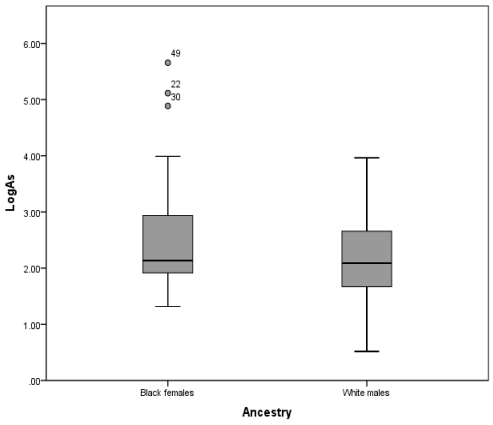
a. All individuals by race



b. Black males and females



c. Black males and white males



d. Black females and white males

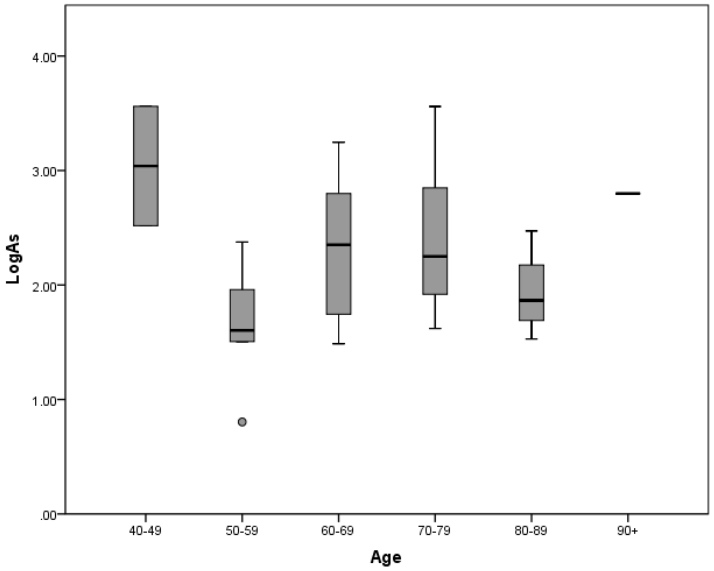
7.2.3.1 Bone arsenic and age

Bone arsenic concentration was explored as a function of age. Using the same categories as with lead, bone As concentration was compared across age categories. Because As is not normally distributed, Kruskal Wallis tests are used as opposed to ANOVA due to non-normal distribution of As. Table 7-12 gives the descriptive statistics for bone As concentration for each age group by race and sex.

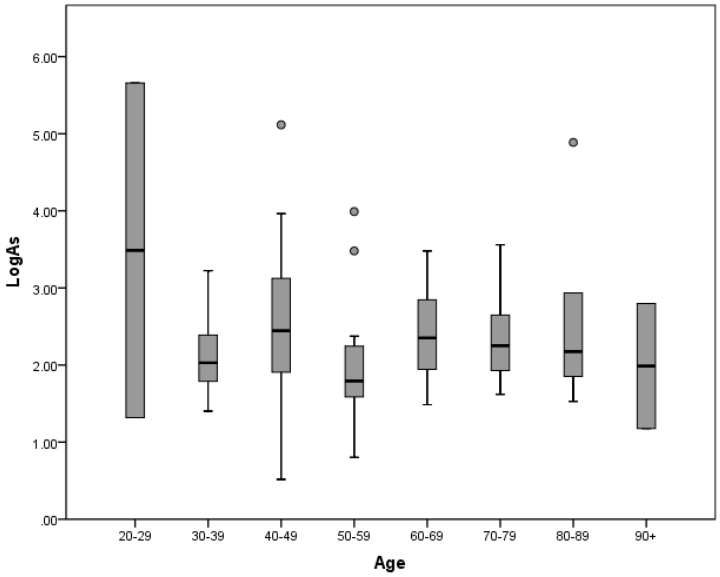
Table 7-12. Bone As in $\mu g \cdot g^{-1}$ by age group by race and sex.

Group	Age Group	N	Mean	Se Mean	Median	SD	Min.	Max.
Black Males	20-29	14	0.879	0.360	0.330	1.347	0.078	4.962
	30-39	13	0.402	0.239	0.079	0.860	0.004	2.609
	40-49	26	3.383	2.956	0.147	15.073	0.004	77.210
	50-59	24	0.590	0.260	0.092	1.275	0.004	5.389
	60-69	33	0.306	0.151	0.111	0.866	0.004	4.985
	70-79	16	0.422	0.217	0.139	0.867	0.004	3.217
	80-89	2	0.108	0.055	0.108	0.078	0.053	0.164
White Males	20-29	-	-	-	-	-	-	-
	30-39	-	-	-	-	-	-	
	40-49	5	0.781	0.716	0.013	1.602	0.004	3.634
	50-59	7	0.065	0.031	0.040	0.082	0.004	0.237
	60-69	10	0.379	0.178	0.174	0.563	0.004	1.766
	70-79	6	1.043	0.617	0.180	1.512	0.042	3.623
	80-89	4	0.119	0.060	0.073	0.120	0.034	0.297
	90-99	3	0.194	0.218	0.015	0.378	0.004	0.629
Black Females	20-29	2	226.81	320.73	226.81	226.80	0.021	453.60
	30-39	14	0.318	0.155	0.104	0.581	0.004	1.674
	40-49	9	14.656	14.493	0.082	43.479	0.004	130.60
	50-59	7	1.889	1.380	0.132	3.651	0.037	9.787
	60-69	10	0.918	0.375	0.303	1.187	0.073	3.016
	70-79	2	0.265	0.180	0.265	0.255	0.085	0.445
	80-89	2	39.036	38.174	39.036	53.986	0.862	77.210

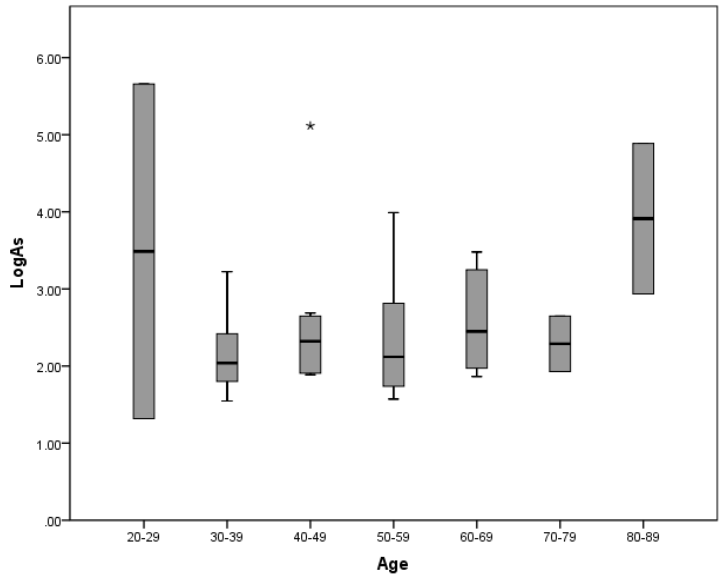
In white males there is no significant difference in bone As concentration between age groups, $H(5) = 4.70$, $p > .05$. Among black females the trend is the same, $H(7) = 6.63$, $p > 0.05$. Among black males, the results are the same, with no significant difference in bone As across age groups, $H(6) = 12.53$, $p > 0.05$. The much higher bone As concentration seen in females aged 20-29 is due to the small number of individuals in this age group (n=2) and the presence of a very high concentration in one of the individuals. Box plots for each age group by race and sex are given in Figure 7-16, below.



a. White males



b. Black males



c. Black females

Figure 7-16. Median bone As by age group in a) white males, b) black males and c) black females. Log transformed data used.

7.2.3.2 Temporal trends in bone As

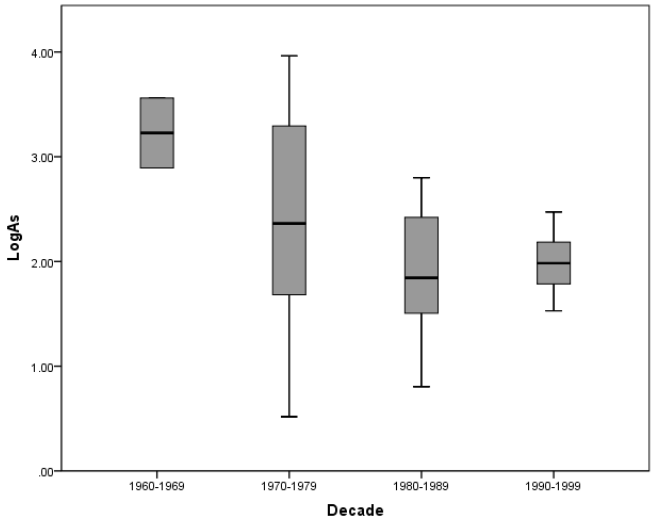
Bone As concentration was examined across time. Across all demographic groups there is significant difference in bone As across time, $H(3) = 22.71$, $p < .001$. Descriptive statistics are given in Table 7-13. Mean values are not given as bone As is not normally distributed.

Table 7-13. Bone As concentration in $\mu g \cdot g^{-1}$ and decade of death by race and sex.

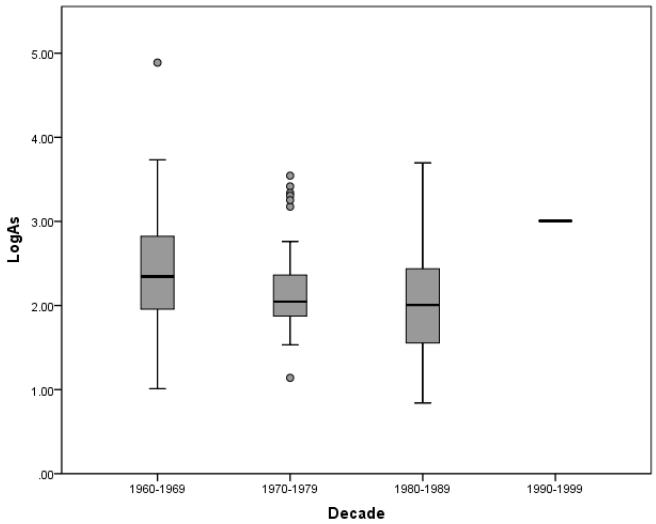
Demographic	Decade	N	Median	SD	Min.	Max
	1960-1969	41	0.202	12.008	0.010	77.210
	1970-1979	48	0.094	0.763	0.004	3.496
	1980-1989	36	0.069	0.843	0.004	4.962
Black males	1990-1999	3	0.000	0.601	0.004	1.015
	1960-1969	2	2.208	2.016	0.782	3.634
	1970-1979	14	0.056	1.110	0.004	3.623
	1980-1989	12	0.047	0.232	0.004	0.629
White males	1990-1999	7	0.075	0.089	0.034	0.297
	1960-1969	23	0.244	97.517	0.004	453.600
	1970-1979	20	0.122	2.216	0.004	9.787
	1980-1989	5	0.079	0.718	0.037	1.674
Black females	1990-1999	1				

In white males, $H(3) = 5.24$, $p > .05$, indicating that bone As does not change over time. The same is true of black females, there is no difference in bone As across time, $H(3) = 2.86$, $p > .05$. In black males, however there is significant difference in bone As over time, $H(3) = 15.50$, $p = .001$. As can be seen in Figure 7-17, there is a reduction in bone As between the 1960s and the 1980s. Mann Whitney U test with Bonferroni Correction (.05/4) confirms that

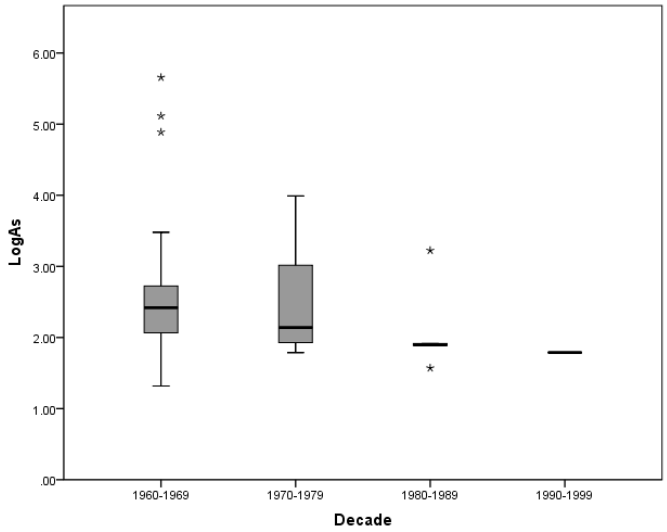
this difference is significant $U = 390.0$, $p < .001$. No other differences were seen between decades.



a. White males



b. Black males

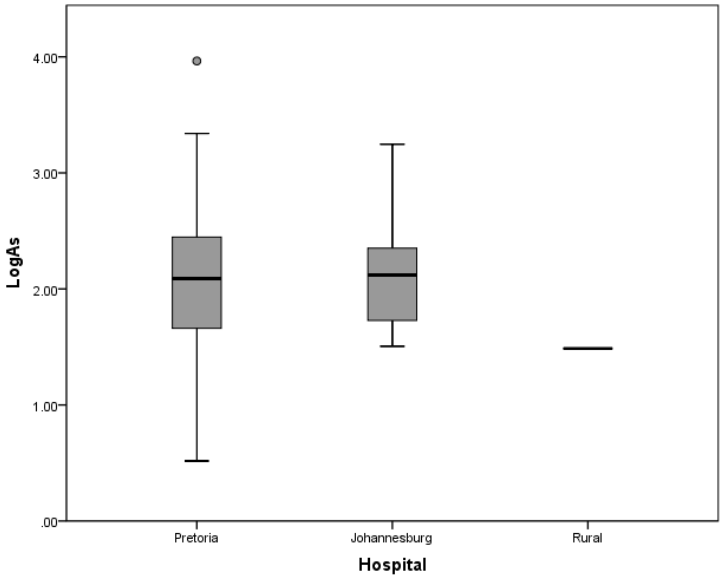


c. Black females

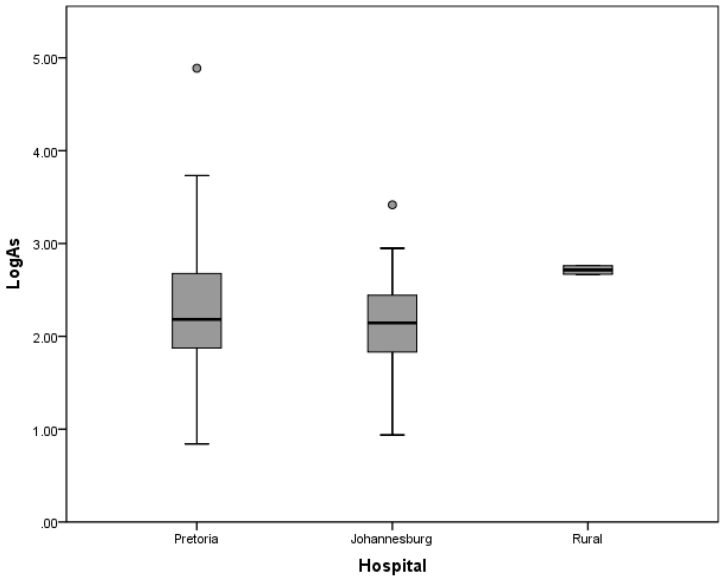
Figure 7-17. Median bone As by decade of death in a) white males, b) black males and c) black females.

7.2.3.3 Geographic trends in bone As

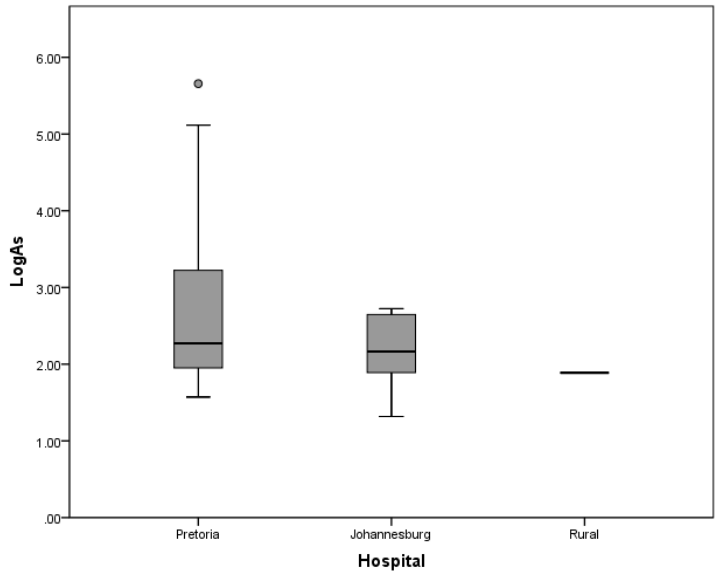
Bone As concentration between Pretoria and Johannesburg was explored. There are no statistically significant differences in bone As concentration by city in any demographic group. In white males, there is no significant difference in bone As between cities, $H(2) = 5.71$, $p > 0.05$. In black males however, there is significant difference between location of residence and bone As, $H(2) = 6.31$, $p < .05$. Mann Whitney U test with Bonferroni correction (.05/4) confirms that the difference in bone As between Johannesburg and Pretoria is significant, $U = 824.0$, $p < .016$. The difference between either city or rural residents is not significant, $p > .05$. There is no difference in bone As in black females across locations, $H(2) = 0.818$, $p > .05$.



a. White males



b. Black males



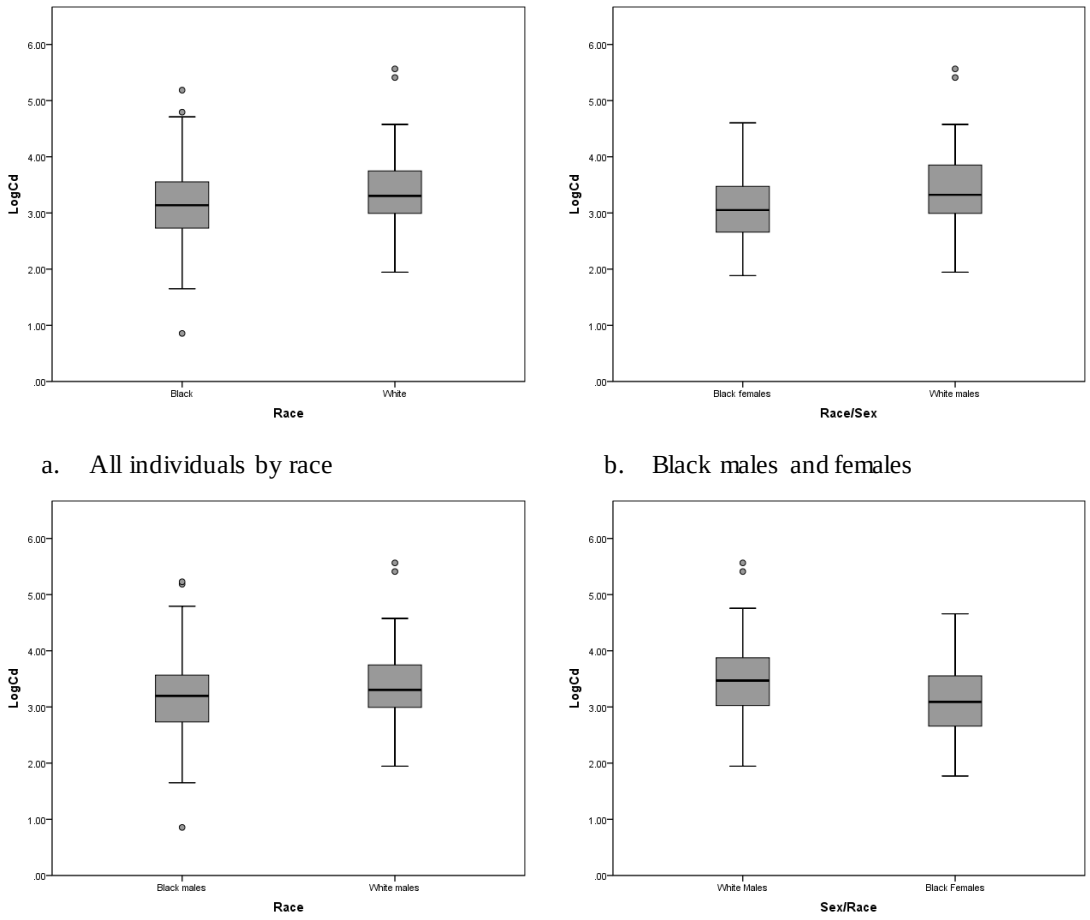
c. Black females

Figure 7-18. Median bone As concentration and city of residence in a) white males, b) black males and c) black females.

7.2.4 **Cadmium**

Log transformed Cd data is normally distributed and so parametric analyses are employed for all tests. Among all individuals, there is significant difference in mean bone Cd between black and white individuals, $t(212) = 2.11$, $p > 0.05$, though the effect size is small, $r = 0.14$.

In black individuals, there is no significant difference in bone Cd concentration between males and females, $t(175) = 0.383$, $p > 0.05$. When only males are compared, there is also no significant difference in bone Cd between black and white males, $t(161) = 1.85$, $p > 0.05$. Among black females and white males, there is significant difference in bone Cd with white males having higher bone Cd concentration than black females, $t(102) = 2.68$, $p < .01$, with an effect size, $r = 0.21$. Box plots of log-transformed bone Cd concentration are given below, in Figure 7-19.



a. All individuals by race

b. Black males and females

c. Black males and white males

d. Black females and white males

Figure 7-19. Median bone Cd concentration in a) all individuals by race, b) black males and females, c) all males and d) black females and white males.

7.2.4.1 Age trends in bone cadmium

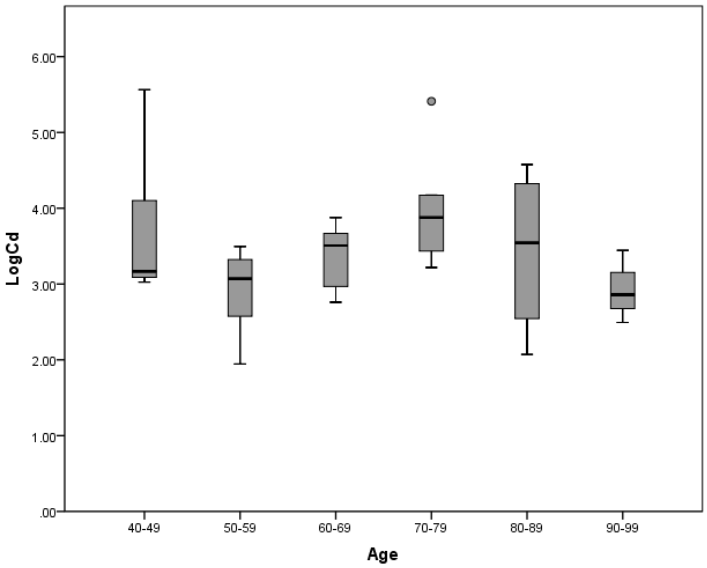
ANOVA was used to explore the relationship between age and bone Cd concentration. Descriptive statistics are given in Table 7-14 and box plots in Figure 7-20. Across all age groups there is no significant difference in bone cadmium concentration, $F(7, 203) = 0.736$, $p > 0.05$. In black individuals, there is also no significant difference in bone Cd in individuals of different ages, $F(7, 179) = 0.457$, $p > 0.05$. Six outliers were removed from analysis of black males and age, the results of ANOVA did not change when these cases were removed.

Among black males only, the results are the same, $F(6,115) = 0.509$, $p > 0.05$, indicating no effect of age on bone Cd concentration. In white males, $F(5, 30) = 1.07$, $p > 0.05$, there is no difference in mean bone Cd across age groups. Three outliers were removed from analysis of white males with no change in results.

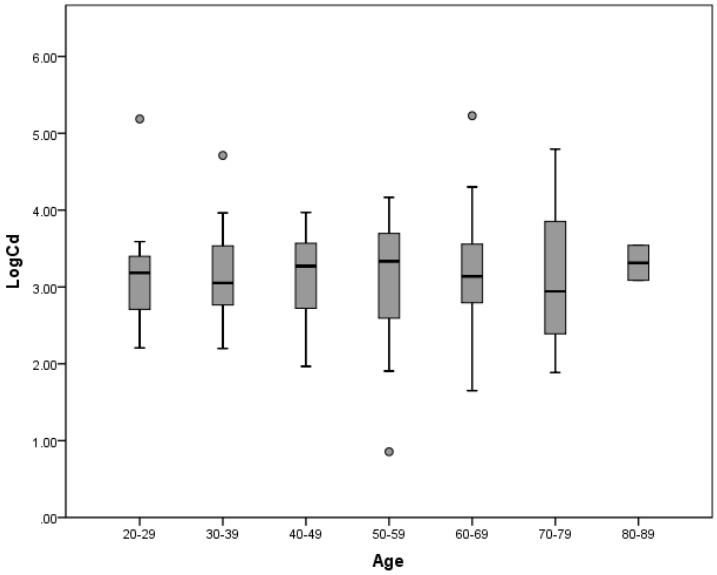
There is no significant difference in mean bone Cd between age groups in black females, $F(5,38) = 1.038$, $p > 0.05$.Three outliers were removed from analysis of black females, with no change in results. Post-hoc procedures (Bonferroni's) were performed on ANOVA for all demographic groups and age, no significant differences were found between any age groups in any of the above analyses.

Table 7-14. Bone Cd in $\mu g \cdot g^{-1}$ and age at death by race and sex.

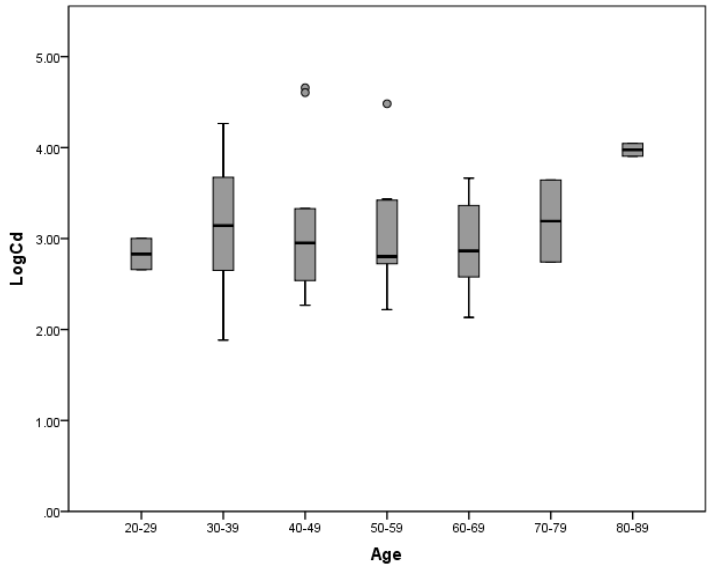
Group	Age Group	N	Mean	SE Mean	Median	SD	Median	Maximum
Black Males	20-29	14	0.033	0.011	0.024	0.043	0.009	0.179
	30-39	13	0.031	0.008	0.021	0.028	0.009	0.111
	40-49	26	0.026	0.003	0.026	0.014	0.007	0.053
	50-59	24	0.029	0.003	0.028	0.017	0.002	0.064
	60-69	33	0.033	0.006	0.023	0.032	0.005	0.187
	70-79	16	0.036	0.009	0.019	0.035	0.007	0.121
	80-89	2	0.028	0.006	0.028	0.009	0.022	0.035
White Males	20-29	0						
	30-39	0						
	40-49	5	0.031	0.007	0.024	0.017	0.021	0.060
	50-59	7	0.021	0.004	0.022	0.010	0.007	0.033
	60-69	10	0.031	0.004	0.033	0.012	0.016	0.048
	70-79	6	0.071	0.031	0.042	0.076	0.025	0.224
	80-89	4	0.046	0.020	0.040	0.040	0.008	0.097
	90-99	3	0.020	0.006	0.017	0.010	0.012	0.031
Black Females	20-29	2	0.017	0.003	0.017	0.004	0.014	0.020
	30-39	14	0.029	0.005	0.023	0.020	0.007	0.071
	40-49	9	0.026	0.009	0.019	0.028	0.010	0.100
	50-59	7	0.029	0.010	0.016	0.027	0.009	0.088
	60-69	10	0.021	0.003	0.018	0.010	0.008	0.039
	70-79	2	0.027	0.011	0.027	0.016	0.016	0.038
	80-89	2	0.053	0.004	0.053	0.005	0.050	0.057



a. White males



b. Black males



c. Black females

Figure 7-20. Median bone Cd and age at death in a) white males, b) black males and c) black females. Log transformed data.

7.2.4.2 Temporal trends in bone cadmium concentration

Bone Cd concentration was analysed across time to identify any significant temporal trends. First, univariate ANOVA was performed between all ages and all individuals, yielding no significant differences in mean bone Cd across time, $F(3,211) = 1.09$, $p > 0.05$. Each demographic group was then analysed independently. Descriptive results are given in Table 7-15 and box plots for each sex/racial group are presented in Figure 7-21.

Table 7-15. Bone Cd in $\mu g \cdot g^{-1}$ by decade of death.

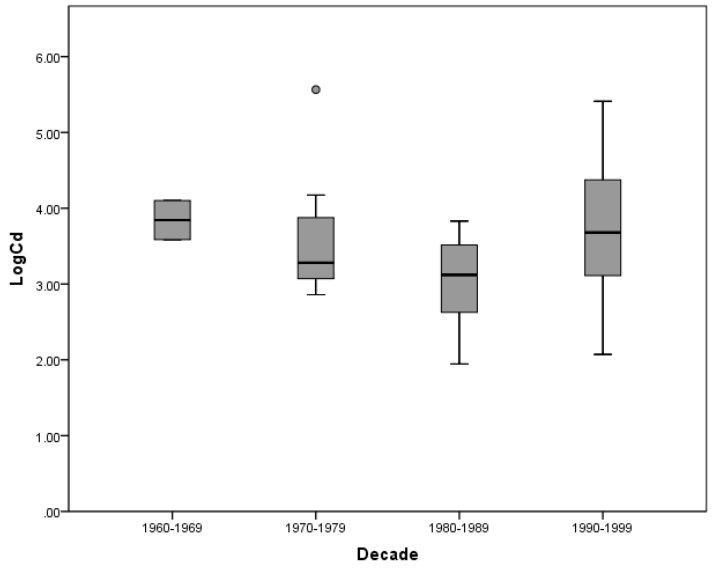
Demographic	Decade	N	Mean	Median	SD	Min.	Max.
	1960-1969	37	0.043	0.026	0.053	0.009	0.261
	1970-1979	41	0.024	0.019	0.021	0.002	0.121
	1980-1989	25	0.036	0.027	0.030	0.007	0.114
Black males	1990-1999	2	0.021	0.024	0.013	0.015	0.033
	1960-1969	2	0.048	0.048	0.017	0.036	0.060
	1970-1979	14	0.048	0.027	0.017	0.017	0.261
	1980-1989	10	0.022	0.023	0.012	0.007	0.046
White males	1990-1999	5	0.035	0.027	0.035	0.008	0.097
	1960-1969	22	0.024	0.020	0.013	0.007	0.050
	1970-1979	15	0.021	0.023	0.013	0.009	0.057
	1980-1989	5	0.020	0.020	0.007	0.013	0.031
Black females	1990-1999	1	-		-	-	-

In white males, $F(3,32) = 1.76$, $p > 0.05$, indicating no significant difference in mean bone Cd across time. Four outliers were removed from this analysis. Removal of outliers resulted in a change from insignificant to significant results. Inclusion of age as a covariate does not change results. Post hoc procedures (Bonferroni's procedure) do not yield any significant

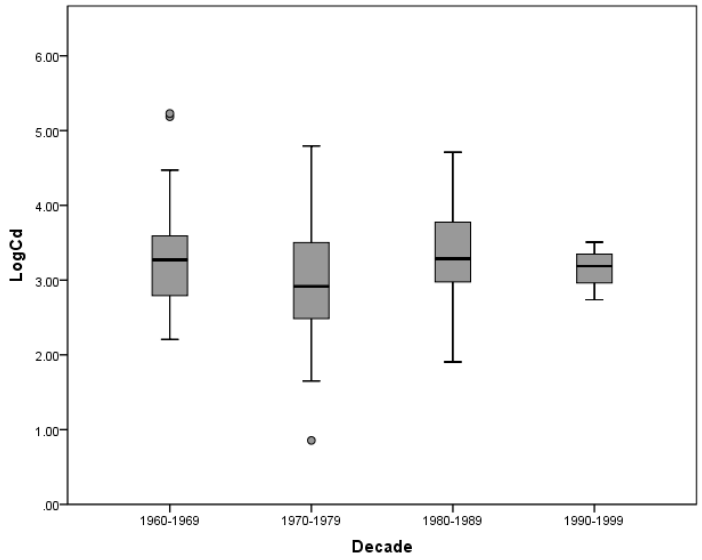
differences between any decade and bone Cd, $p > 0.05$, this is interpreted as a lack of overall difference in bone Cd concentration over time in white males.

In black females, there is no significant difference in bone Cd across time, even when age is taken into account, $F(3,46) = 1.092$, $p > 0.05$. Examination of Cook's distance uncovered one outlier among black females, however this individual died in 1998 – among the most recent date of death among this demographic group and it was determined that the date of death resulted in a large Cook's distance, not a high bone Cd concentration. This outlier remained in the analysis.

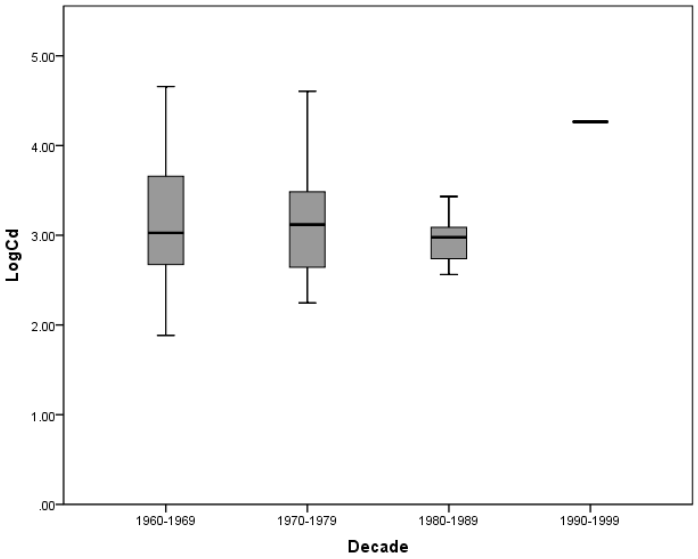
The same true of among black males, $F(3,119) = 1.97$, $p > 0.05$. However, when age is included as a covariate, and ACNOVA is conducted the result is the same. Six outliers were removed from initial ANOVA with no change in significance.



a. White males



b. Black males



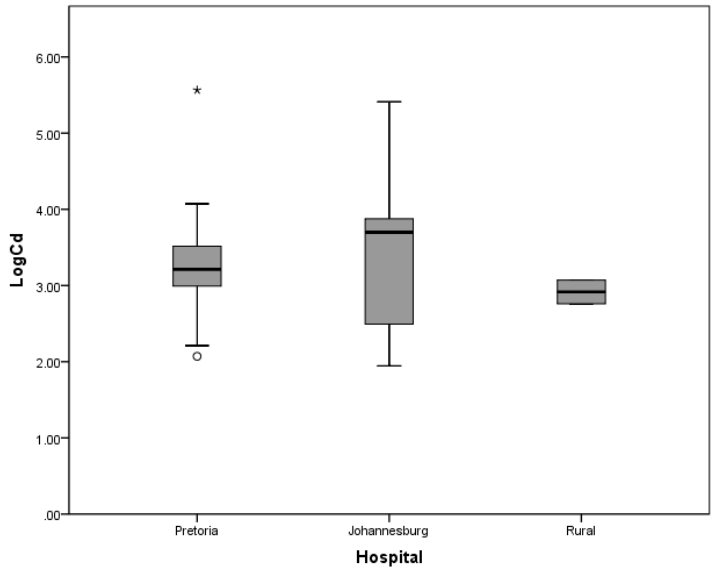
c. Black females.

Figure 7-21. Median bone Cd concentration and decade of death by a) white males, b) black males and c) black females. Log transformed data.

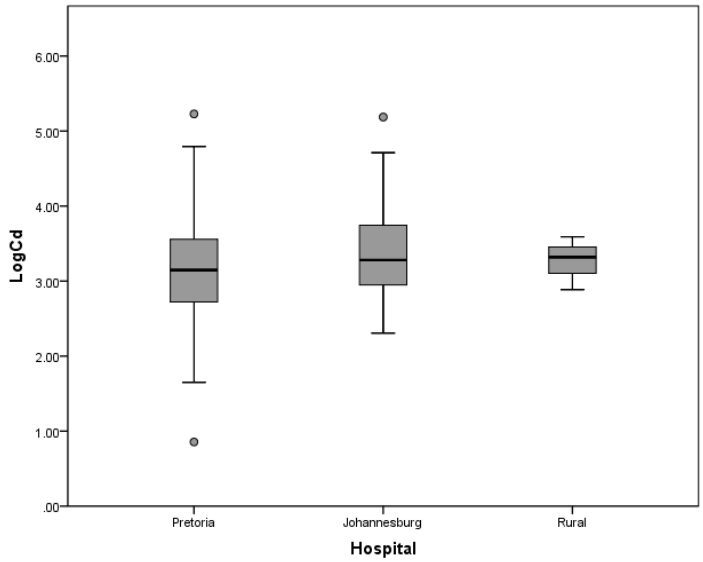
7.2.4.3 Geographical trends in bone Cd concentration

Among all individuals, there is no difference in mean bone Cd concentration between Pretoria and Johannesburg, $F(2,196) = 0.586$, $p > 0.05$. Box plots are given in Figure 7-22.

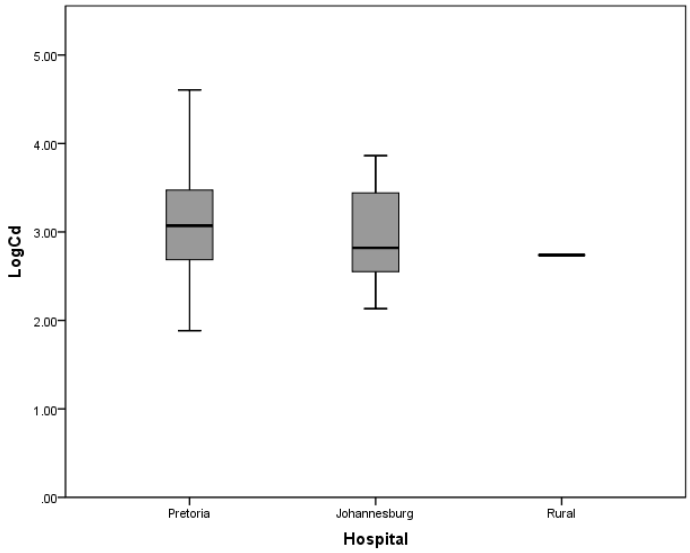
Among white males, there is no difference in mean bone Cd concentration between individuals in Pretoria and Johannesburg, $F(2,22) = 0.352$, $p > 0.05$. In black females, the trend is the same, $F(2,45) = 0.507$, $p > 0.05$. In black males, there is no difference in mean bone Cd concentration between individuals from Pretoria or Johannesburg, and rural residents, $F(2,21) = 0.370$, $p > 0.05$.



a. White males



b. Black males



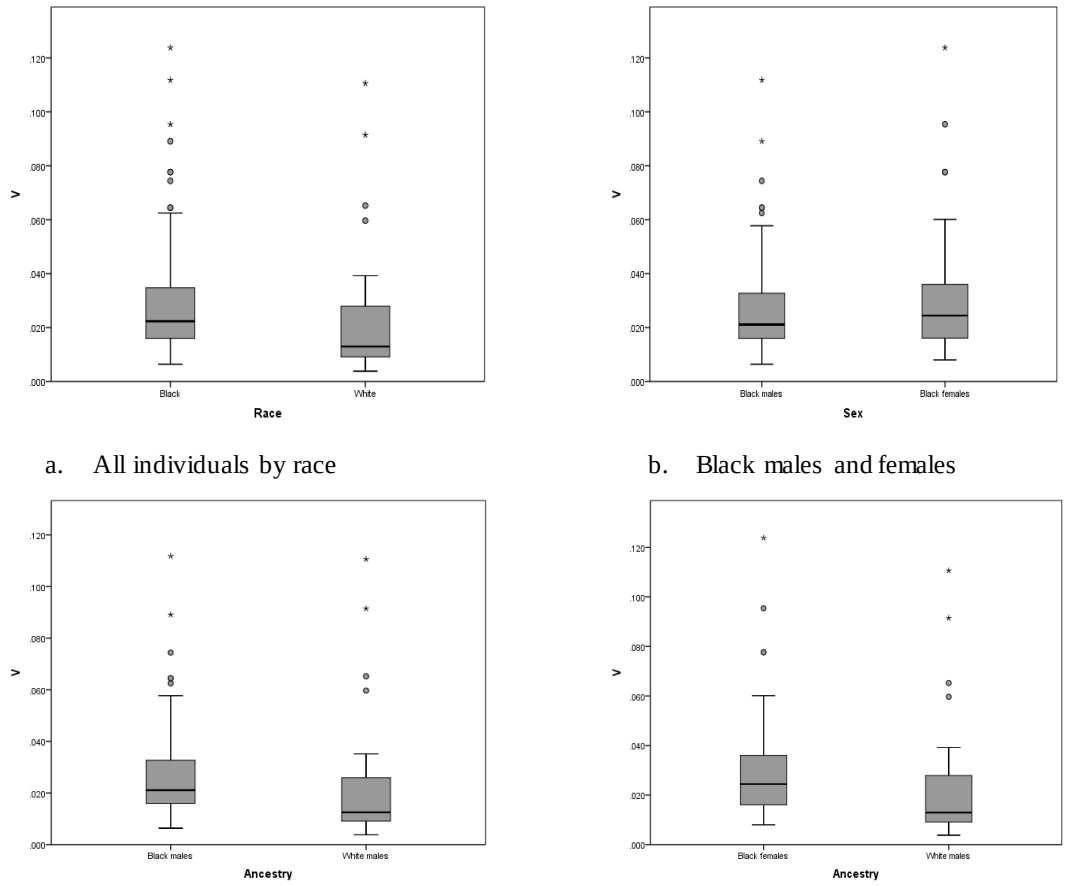
c. Black females

Figure 7-22. Median bone Cd concentration by city of residence (admitting hospital) in a) white males, b) black males and c) black females. Log transformed data.

7.2.5 **Vanadium**

Descriptive statistics for bone V are given in Table 7-1. Vanadium is not normally distributed across any demographic group and was log transformed. Log transformed V concentrations are normally distributed and parametric tests are used.

When all individuals are compared, there is significant difference in bone V concentration between black and white individuals, $t(41.65) = 2.78$, $p < 0.001$, with effect size, $r = 0.40$. Black individuals have significantly higher bone V concentrations than white individuals. Box plots are given in Figure 7-23.



a. All individuals by race

b. Black males and females

c. Black males and white males

d. Black females and white males

Figure 7-23. Median bone V concentration in a) all individuals, b) black males and black females, c) black males and white males and d) black females and white males. Log transformed data.

In males only, the difference in bone V between black and white individuals is also significant, $t(42.09) = 2.66$, $p < 0.001$, with effect size, $r = 0.38$. Among black males and females, there is no significant difference in bone V concentration, $t(136) = 1.35$, $p > 0.05$. When white males and black females are compared, the difference is also significant, $t(61.23) = 3.014$, $p < 0.001$, with effect size, $r = 0.36$. As is evident in Fig. 7-23, in all cases with significant differences, black individuals yield higher bone V concentration than white individuals.

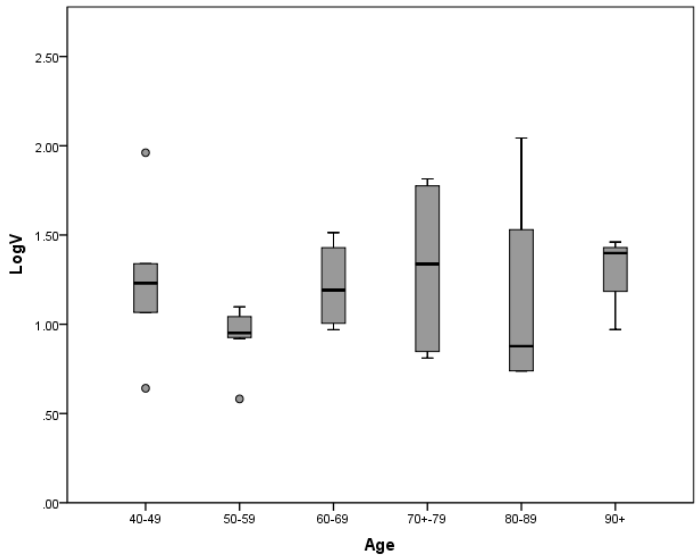
7.2.5.1 Bone V concentration and age at death

Descriptive statistics for bone V concentration across age groups are presented in Table 7-16, below. Bone V concentration was explored by ANOVA to investigate the relationship between V and age at death. In the whole sampled population, there is no significant difference in mean bone V concentration across age groups, Welch's $F(7, 26.74) = 0.967$, $p > 0.05$.

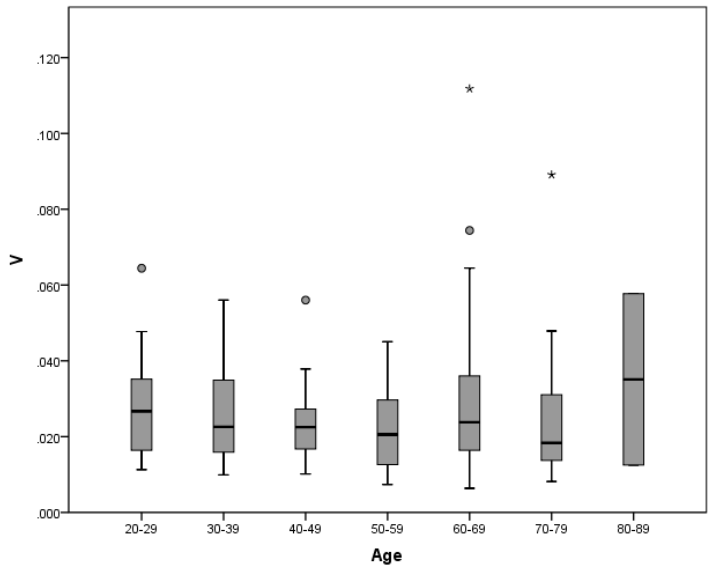
Table 7-16. Bone V concentration in $\mu g \cdot g^{-1}$ by age at death.

Group	Age Group	N	Mean	SE Mean	Median	SD	Min.	Max.
Black Males	20-29	14	0.029	0.004	0.027	0.015	0.011	0.064
	30-39	13	0.028	0.004	0.023	0.015	0.010	0.056
	40-49	26	0.024	0.002	0.023	0.009	0.010	0.056
	50-59	24	0.022	0.002	0.021	0.011	0.007	0.045
	60-69	33	0.030	0.004	0.024	0.022	0.006	0.112
	70-79	16	0.026	0.005	0.018	0.021	0.008	0.089
	80-89	2	0.035	0.023	0.035	0.032	0.013	0.058
White Males	20-29	0	-	-	-	-	-	
	30-39	0	-	-	-	-	-	
	40-49	5	0.014	0.003	0.017	0.007	0.004	0.022
	50-59	7	0.009	0.001	0.009	0.003	0.004	0.013
	60-69	10	0.019	0.003	0.016	0.009	0.009	0.033
	70-79	6	0.031	0.011	0.024	0.026	0.006	0.065
	80-89	4	0.033	0.026	0.008	0.052	0.005	0.111
	90-99	3	0.021	0.006	0.025	0.010	0.009	0.029
Black Females	20-29	2	0.031	0.009	0.031	0.012	0.022	0.039
	30-39	14	0.031	0.006	0.022	0.023	0.010	0.078
	40-49	9	0.023	0.003	0.022	0.008	0.010	0.035
	50-59	7	0.031	0.008	0.034	0.020	0.008	0.060
	60-69	10	0.035	0.010	0.026	0.033	0.012	0.124
	70-79	2	0.019	0.003	0.019	0.004	0.016	0.022
	80-89	2	0.037	0.001	0.037	0.001	0.036	0.038

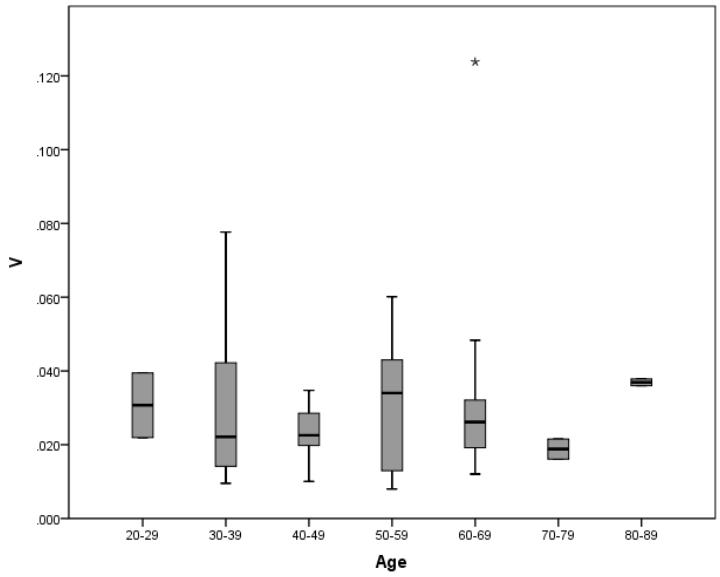
In black women however, there is significant difference in bone V among age groups, Welch's $F(6, 6.68) = 5.085$, $p < 0.05$, with effect size, $\omega^2 = 0.02$. Post hoc procedures (Games-Howell) indicate significant difference between the oldest women, those aged 80+ and women aged 40-49, $p < 0.05$ (Fig 7-24), however among women aged 80+, sample size is only two, so these results are accepted with caution. Neither significance, nor post hoc results change when the outlier is removed from analysis. In black and white males, there is no difference in mean bone V across time, $F(6, 121) = 0.619$, $p > 0.05$, and Welch's $F(5, 9.52) = 2.232$, $p > 0.05$ respectively.



a. White males



b. Black males



c. Black females

Figure 7-24. Median bone V concentration by age at death in a) white males, b) black males and c) black females. Log transformed data.

7.2.5.2 Temporal trends in bone V concentration

In the sample population as a whole, there is no significant difference in mean bone V concentration across time. Descriptive statistics for bone V by decade and demographic group are given in Table 7-17. When each sub-group is explored independently, the trend is the same and no change in bone V concentration is evident over time.

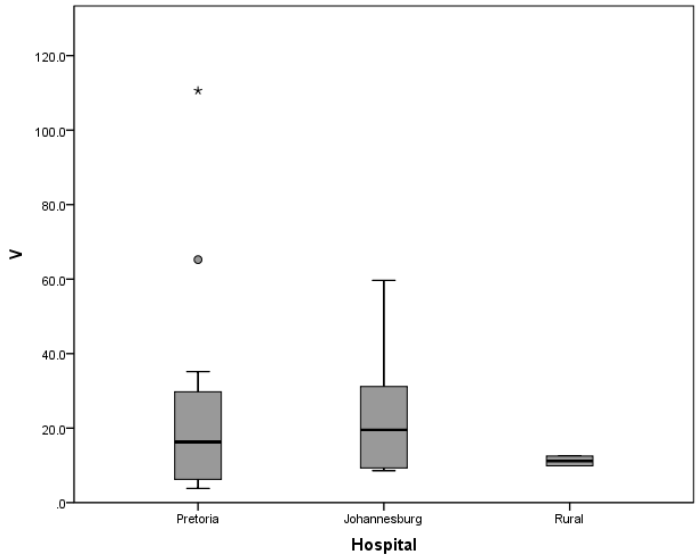
Table 7-17. Bone V concentration in $\mu g \cdot g^{-1}$ by decade of death.

Demographic	Decade	N	Mean	Median	SD	Min.	Max
	1960-1969	41	0.028	0.023	0.015	0.010	0.064
	1970-1979	48	0.024	0.021	0.014	0.006	0.074
	1980-1989	36	0.025	0.020	0.016	0.009	0.089
Black males	1990-1999	3	0.025	0.019	0.018	0.012	0.045
	1960-1969	2	0.019	0.019	0.004	0.016	0.022
	1970-1979	14	0.014	0.019	0.009	0.004	0.035
	1980-1989	12	0.020	0.013	0.017	0.004	0.065
White males	1990-1999	7	0.032	0.010	0.040	0.005	0.111
	1960-1969	23	0.026	0.022	0.010	0.013	0.048
	1970-1979	20	0.040	0.029	0.031	0.008	0.124
	1980-1989	5	0.017	0.012	0.011	0.010	0.036
Black females	1990-1999	1	-	-	-	-	-

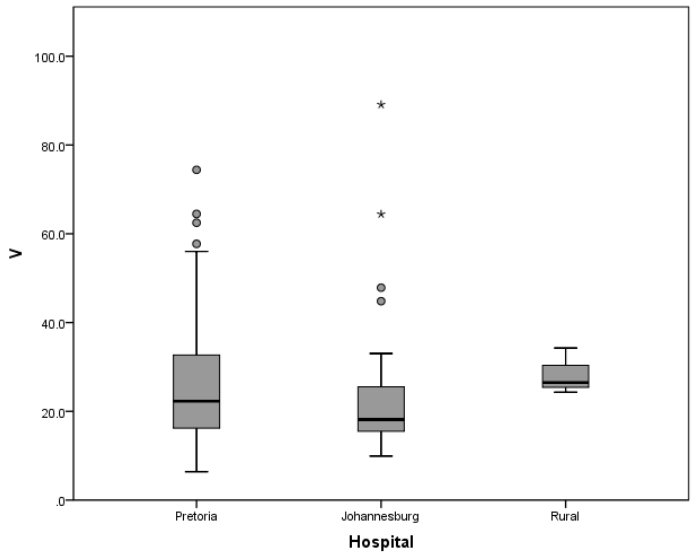
7.2.5.3 Geographic trends in bone V concentration

Across all groups there is no difference in mean bone V between Johannesburg, Pretoria or rural residents, $F(2, 194) = 0.765$, $p > 0.05$. When individual groups are tested, the results

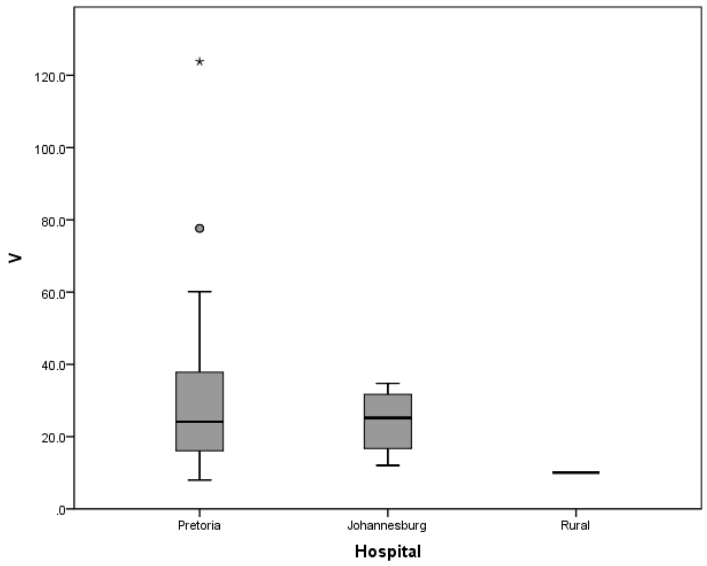
are the same. In black females, $F(2,45) = 1.38$, $P > 0.05$, black males, $F(2, 122) = 0.490$, $p > 0.05$, and in white males, $F(2, 21) = 0.219$, $p > 0.05$.



a. White males



b. Black males



c. Black females.

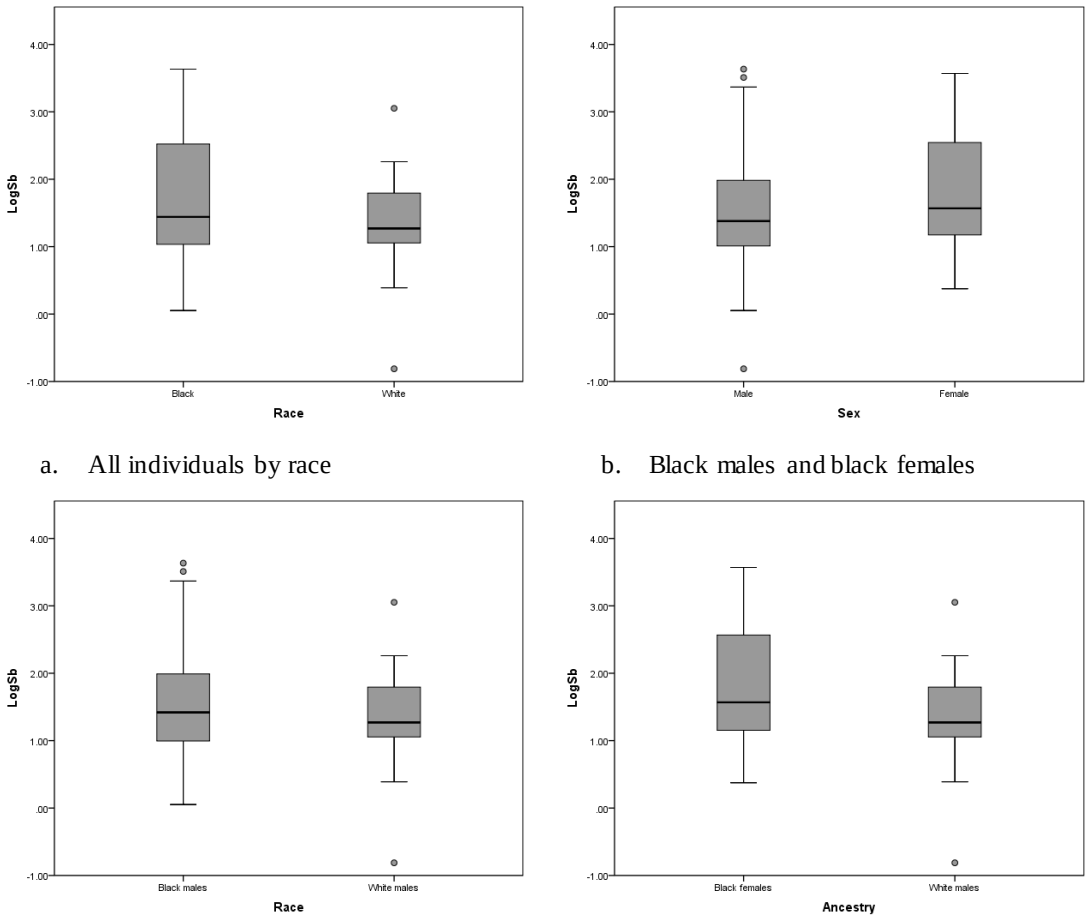
Figure 7-25. Bone V by city in a) white males, b) black males and c) black females.

7.2.6 **Antimony**

There are significant differences between groups with regards to bone Sb concentration. Within the population as a whole, the differences occur between males and females, as opposed to by race. Among white males, and black males and females Mann-Whitney U = 2479, $p > 0.05$. Between females and all males however $U = 3149$, $p < 0.05$.

The primary difference in bone Sb appears to occur between white males and black females. Black females have significantly higher Sb concentrations than white males, $U = 563$, $p < 0.01$, with effect size, $r = 0.31$. Between black males and females, the difference is not significant, $U = 2560.5$, $p > 0.05$.

Within males only, there is no difference in bone Sb between black males and white males, $U = 1914$, $p > 0.05$. Fig. 7-26 includes box plots for each sex/race.



a. All individuals by race

b. Black males and black females

c. Black males and white males

d. Black females and white males

Figure 7-26. Median bone Sb by race and sex in a) all individuals by race, b) black males and females, c) black males and white males and d) black females and white males.

7.2.6.1 Age trends in antimony concentration

There are significant differences in bone antimony concentration among age groups.

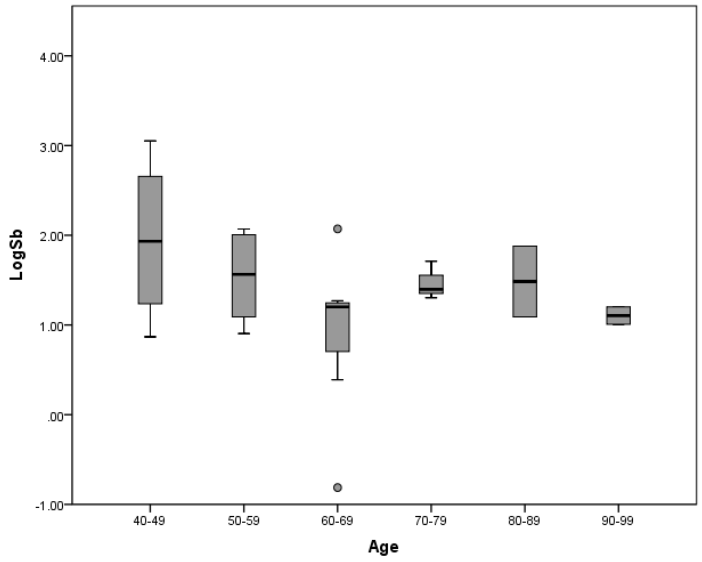
Descriptive statistics are given in Table 7-18.

Table 7-18. Bone Sb in $\mu g \cdot g^{-1}$ per age group by race and sex.

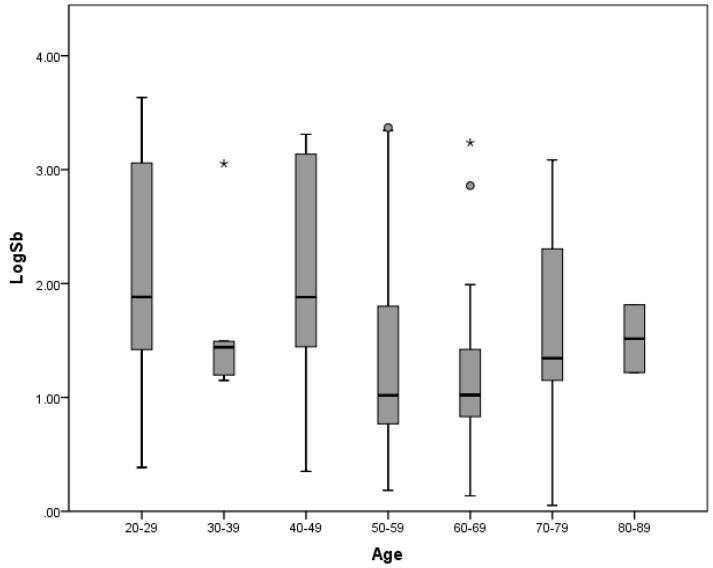
Group	Age Group	N	Mean	SE Mean	Median	SD	Minimum	Maximum
Black Males	20-29	14	0.835468	0.365317	0.063825	1.366891	0.006	4.302
	30-39	13	0.095781	0.086169	0	0.310687	0.006	1.129
	40-49	26	0.485239	0.133241	0.039245	0.6794	0.006	2.045
	50-59	24	0.202007	0.130401	0.005892	0.638833	0.006	2.336
	60-69	33	0.090933	0.057284	0.008342	0.32405	0.006	1.725
	70-79	16	0.152499	0.082533	0.01768	0.33013	0.006	1.215
	80-89	2	0.040815	0.024265	0.040815	0.034316	0.01655	0.06508
White Males	20-29	0						
	30-39	0						
	40-49	5	0.248295	0.220243	0.04025	0.492479	0.006	1.128
	50-59	7	0.039382	0.01874	0.0123	0.049582	0.006	0.1175
	60-69	10	0.018212	0.011331	0.006459	0.035832	0.006	0.1178
	70-79	6	0.01607	0.008396	0.010045	0.020567	0.006	0.05135
	80-89	4	0.021983	0.018119	0.006145	0.036238	0.006	0.07564
	90-99	3	0.008693	0.00465	0.01018	0.008054	0.006	0.0159
Black Females	20-29	2	0.852615	0.842385	0.852615	1.191312	0.01023	1.695
	30-39	14	0.156998	0.072875	0.036275	0.272674	0.006	0.7533
	40-49	9	0.114243	0.062831	0.01568	0.188493	0.006	0.5052
	50-59	7	0.356958	0.245643	0.02357	0.649911	0.005631	1.79
	60-69	10	0.660533	0.387167	0.023515	1.224328	0.006	3.709
	70-79	2	0.06593	0.00591	0.06593	0.008358	0.06002	0.07184
	80-89	2	0.257105	0.230695	0.257105	0.326252	0.02641	0.4878

Kruskall-Wallis test confirms that the difference in Sb between age groups among all individuals is significant, $H(7) = 17.57$, $p < 0.05$. This difference is only significant in black males. In white males and black females, there is no difference in bone Sb across age groups and $H(5) = 2.26$, $p > 0.05$, and $H(6) = 3.01$, $p > 0.05$, respectively.

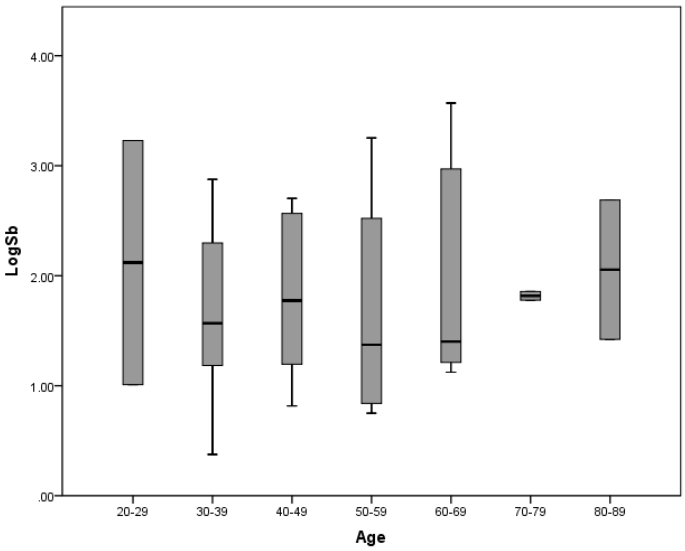
In black males, $H(6) = 21.03$, $p < 0.01$. Mann-Whitney U test with Bonferroni's correction (.5/7) indicates that the difference in bone Sb in black males ages 20-29 and 40-89 are significantly different higher than in other age groups. Bone Sb is lowest in individuals ages 50-59 (Fig 7-27b).



a. White males



b. Black males



c. Black females

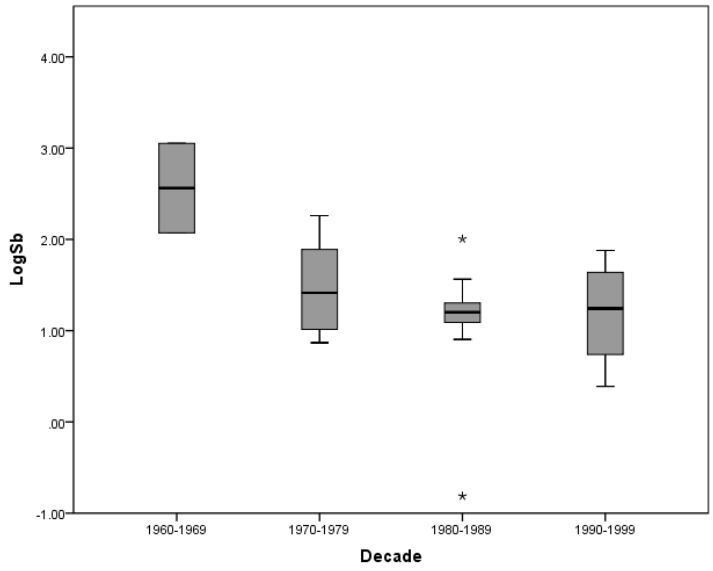
Figure 7-27. Median bone Sb by age in a) white males, b) black males and c) black females.

7.2.6.2 Temporal trends in antimony concentration

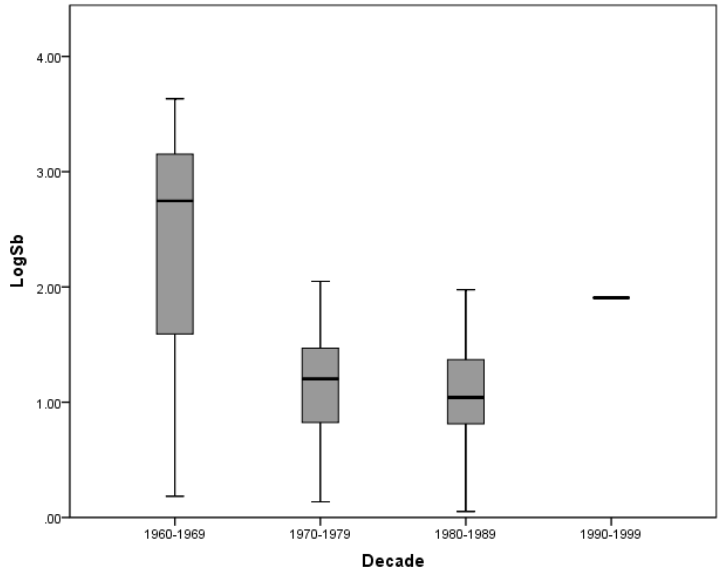
There is significant difference in bone Sb concentration across time and descriptive statistics are given in Table 7-19. Across the population $H(3) = 75.04$, $p < 0.01$. This difference is only significant in black males and females, $H(3) = 48.98$, $p < 0.001$, and $H(3) = 14.96$, $p < 0.01$, respectively. In white males there is no significant change in bone Sb across time, $H(3) = 5.62$, $p > 0.05$, though graphically, bone Sb does appear to be higher in the 1960s. Mann Whitney U test with Bonferroni's correction (.5/4) confirms that bone Sb is significantly higher during the 1960s than in any subsequent decade in black individuals (Fig. 7-28).

Table 7-19. Bone Sb concentration and decade of death in $\mu g \cdot g^{-1}$ by race and sex.

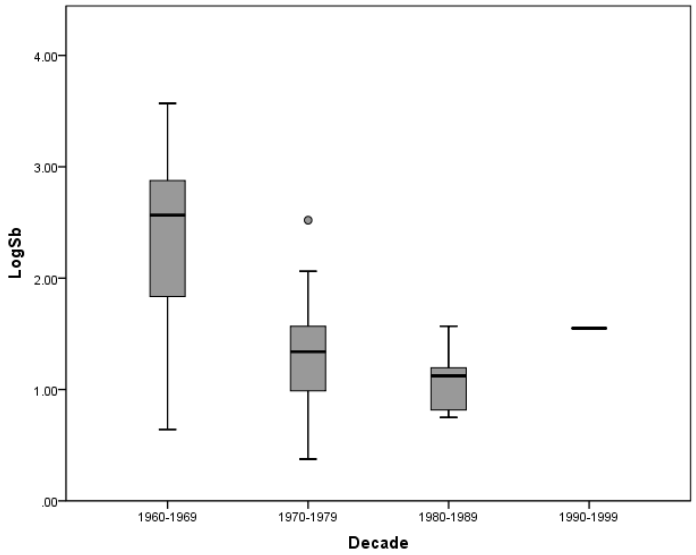
Demographic	Decade	N	Median	SD	Min.	Max
	1960-1969	41	0.507	1.000	0.006	4.302
	1970-1979	48	0.009	0.073	0.006	0.112
	1980-1989	36	0.004	0.069	0.006	0.095
Black males	1990-1999	3	0.000	0.050	0.006	0.081
	1960-1969	2	0.623	0.714	0.118	1.128
	1970-1979	14	0.009	0.035	0.006	0.118
	1980-1989	12	0.014	0.028	0.006	0.101
White males	1990-1999	7	0.002	0.028	0.006	0.076
	1960-1969	23	0.334	0.891	0.006	3.709
	1970-1979	20	0.013	0.075	0.006	0.331
	1980-1989	5	0.013	0.013	0.006	0.037
Black females	1990-1999	1	-	-	-	-



a. White males



b. Black males



c. Black females

Figure 7-28. Median bone antimony by decade in a) white males, b) black males and c) black females.

7.3 Essential element concentrations

Four essential trace elements were explored. Descriptive statistics for each are given in Table 7-20.

Table 7-20. Essential trace element concentrations in $\mu g \cdot g^{-1}$ by race and sex.

Group	Element	N	Mean	SE of Mean	Median	SD	Minimum	Maximum
Black Males	Cu	129	13.083	1.265	8.881	14.312	0.360	78.620
White Males		35	16.203	2.477	10.550	214.758	14.655	1.333
Black Females		44	13.215	2.077	9.586	14.536	1.833	94.720
Black Males	Fe	129	25.460	2.973	13.585	33.633	0.484	234.800
White Males		35	12.246	1.561	9.289	9.237	0.839	42.380
Black Females		44	13.083	1.265	8.881	14.312	0.360	78.620
Black Males	Mg	129	2856.716	59.276	2753.500	670.635	441.800	4805.000
White Males		35	3090.057	126.826	2861.000	750.315	2165.000	4983.000
Black Females		44	2838.306	92.638	2648.000	648.463	2064.000	4805.000
Black Males	Zn	129	87.668	2.570	85.550	29.080	11.320	274.700
White Males		35	102.303	2.450	102.400	14.496	69.840	133.900
Black Females		44	91.011	3.714	88.240	25.998	63.240	232.800

7.3.1 **Zinc**

Descriptive statistics for Zn are presented in Table 7-20. There is significant difference in bone Zn between black and white individuals, $U = 1455.0$, $p < 0.01$, with an effect size $r = 0.354$, with white males having higher bone Zn concentration than either black men or women. Among black individuals, there is no significant difference in bone Zn between men and women, $U = 3013.5$, $p > 0.05$. There is significant difference in bone Zn between black and white males, $U = 1025.0$, $p < 0.01$, with an effect size r= of 0.39.

7.3.1.1 Bone Zn and age

Among all individuals, there is significant difference between bone Zn concentration and age $H(7) = 16.149$, $p < 0.05$. However among individual groups there is no significant difference in bone Zn with age. Among black women, there is no significant difference in median bone Zn concentration across age groups, $H(6) = 1.743$, $p > 0.05$. Among black males, there is no significant difference in median bone Zn across age groups, $H(6) = 6.153$, $p > 0.05$. In white males, the trend is the same, with no significant difference in median bone Zn concentration across age groups, $H(5) = 6.54$, $p > 0.05$.

7.3.2 **Magnesium**

There is no significant difference in bone Mg concentration between black and white individuals as a whole, $U = 2839.5$, $p > 0.05$. Nor is there any significant difference between black males and females, $U = 2935.0$, $p > 0.05$. There is no significant difference in median bone Mg between black and white males, $U = 2044.0$, $p > 0.05$. In black females and white males, there is no significant difference in bone Mg, $U = 665.5$, $p > 0.05$.

7.3.2.1 Bone magnesium and age

Across all age groups there is no significant difference between bone Mg and age group, $H(7) = 7.062$, $p > 0.05$. In black women, the result is the same with no significant difference, $H(6) = 5.691$, $p > 0.05$. Among white males and among black males, there are no significant differences in bone Mg across age groups with $H(5) = 4.762$, $p > 0.05$ and $H(6) = 4.820$, $p > 0.05$ respectively.

7.3.3 **Iron**

There are significant differences in bone Fe between groups. Notably, it is white males, who have the lowest bone Fe concentrations. Among black and white males, white males have significantly lower bone Fe than black males, $U = 1593$, $p < 0.01$, with effect size, r= 0.17. In white males also have significantly lower bone Fe than black females, $U = 580$, $p < 0.016$, with effect size r= 0.12. The in bone Fe between black males and females is not significant, $U = 3096$, $p > 0.05$.

7.3.3.1 Bone iron and age

Bone Fe does not vary with age in any group. In black males, $H(6) = 9.82$, $p > 0.05$. In black females $H(6) = 3.54$, $p > 0.05$, and in white males, $H(5) = 4.48$, $p > 0.05$.

7.3.4 Copper

Bone Cu concentration does not vary by race or sex. In black males and females, $t(178) = 0.831$, $p > 0.05$. In black males and white males, $t(161) = 1.48$, $p > 0.05$. And in black females and white males, $t(82) = 0.773$, $p > 0.05$.

7.3.4.1 Copper and Age

Bone Cu does not vary with age in any group. In black males, $F(6,120) = 0.745$, $p > 0.05$. In black females, $F(6, 39) = 0.432$, $p > 0.05$. The results are the same in white males, where bone Cu does not vary by age, Welch's $F(5, 10.68) = 3.04$, $p > 0.05$.

7.4 Relationships between elements

In the first instance a Spearman's Rank Correlation coefficient matrix was produced to determine which elements were significantly correlated in the bone of the sample population. Matrices for black males, black females and white males were produced. These matrices make up Tables 7-21 to 7-23.

Table 7-21. Spearman's rank correlation matrix for white males. Significant correlations are presented boldface. Bonferroni's corrected critical value for p = 0.0009.

		Ca	Pb	Mn	Cd	Fe	Zn	Mg	Sb	As	Cu	V
Ca	Coef.	1.000										
	Sig.	.										
Pb		-.054	1.000									
		.760	.									
Mn		.356	-.061	1.000								
		.036	.726	.								
Cd		.369	.062	**.630**	1.000							
		.029	.724	**.000**	.							
Fe		.317	.161	**.586**	.276	1.000						
		.063	.356	**.000**	.109	.						
Zn		.507	.168	.004	.228	.065	1.000					
		.002	.334	.983	.188	.710	.					
Mg		**.714**	-.052	.057	.072	.223	**.704**	1.000				
		.000	.767	.746	.683	.197	**.000**	.				
Sb		-.326	.090	.171	.220	.145	-.400	-.411	1.000			
		.056	.606	.326	.204	.407	.017	.014	.			
As		-.092	-.164	.445	**.637**	.215	-.225	-.297	.366	1.000		
		.597	.347	.007	**.000**	.216	.194	.083	.030	.		
Cu		.459	.290	.488	.419	.390	.381	.334	-.061	.002	1.000	
		.006	.091	.003	.012	.021	.024	.050	.729	.990	.	
V		.161	.316	**.583**	.400	**.627**	-.104	.008	.371	.371	.225	1.000
		.356	.065	**.000**	.017	**.000**	.551	.962	.028	.028	.194	.

Table 7-22. Sperma's rank correlation matrix for black males. Significant correlations are presented in boldface. Bonferroni's corrected critical value for p = 0.0009.

		Ca	Pb	Mn	Cd	Fe	Zn	Mg	Sb	As	Cu	V
Ca	Coef.	1.000										
	Sig.	.										
Pb		.284	1.000									
		.001	.									
Mn		.259	.194	1.000								
		.003	.028	.								
Cd		.107	**.549**	**.580**	1.000							
		.230	**.000**	**.000**	.							
Fe		-.098	.100	.148	.118	1.000						
		.272	.259	.096	.183	.						
Zn		**.360**	**.359**	.187	.288	.220	1.000					
		.000	**.000**	.034	.001	.013	.					
Mg		**.602**	.123	.081	.036	.120	**.620**	1.000				
		.000	.165	.363	.686	.178	**.000**	.				
Sb		-.011	.056	.000	.094	**.329**	.028	-.002	1.000			
		.912	.585	.997	.361	**.000**	.789	.987	.			
As		-.009	-.042	**.403**	**.258**	.137	-.096	-.163	.585	1.000		
		.921	.662	**.000**	**.000**	.147	.312	.085	.001	.		
Cu		.149	.091	**.575**	**.308**	.131	.154	.078	-.029	**.353**	1.000	
		.093	.308	**.000**	**.000**	.140	.083	.379	.775	**.000**	.	
V		.180	.183	**.643**	**.443**	.205	.116	.099	**.360**	**.494**	**.501**	1.000
		.042	.039	**.000**	**.000**	.020	.191	.267	**.000**	**.000**	**.000**	.

Table 7-23. Spearman's rank correlation matrix for black females. Significant correlations are presented in boldface. Bonferroni's corrected critical value for p = 0.0009.

		Ca	Pb	Mn	Cd	Fe	Zn	Mg	Sb	As	Cu	V
Ca	Coef.	1.000										
	Sig.	.										
Pb		-.193	1.000									
		.185	.									
Mn		.179	.101	1.000								
		.218	.489	.								
Cd		-.063	.321	**.615**	1.000							
		.669	.024	**.000**	.							
Fe		-.159	.105	.157	.151	1.000						
		.276	.472	.281	.300	.						
Zn		.143	.164	.182	.100	.296	1.000					
		.327	.261	.210	.495	.039	.					
Mg		**.601**	-.407	.155	-.201	.008	.341	1.000				
		.000	.004	.289	.167	.956	.017	.				
Sb		.071	-.046	.160	.057	.138	.389	.104	1.000			
		.654	.772	.313	.722	.383	.011	.513	.			
As		.133	.002	.271	.154	.134	.207	.081	**.653**	1.000		
		.384	.991	.072	.313	.380	.173	.596	**.000**	.		
Cu		.248	-.069	**.549**	.242	.107	.339	.277	.216	.373	1.000	
		.086	.636	**.000**	.093	.464	.017	.054	.170	.012	.	
V		.295	-.256	**.623**	.167	**.329**	.316	.425	**.360**	.083	**.524**	1.000
		.040	.076	**.000**	.252	**.000**	.027	.002	**.000**	.587	**.000**	.

There are several differences and similarities in relationships between elements among white males, black males and black females:

- Sb and Mg are correlated in white males but not in black males or females.
- In black females, Sb and Zn are not correlated and in white males negatively correlated. In black males the two elements are not correlated at all.
- In white males, Fe is strongly correlated with Mn. There is no correlation between these elements in black males or females.
- Fe is also correlated with V in white males but not in black males or females.
- As and Mn are correlated in males but not in females.
- V and Cd are correlated in males but not in females.
- As and Sb are correlated in black males and females but not white males.
- As and Cd are correlated in black males but not in black females or white males
- V and Mn are correlated in all individuals.
- Cd and Mn are correlated in all individuals.
- V and Cu are correlated in black males and females but not in white males.

- Pb and Zn are correlated in black males, but not in white males or black females.
- Pb and Cd are not correlated in white males or black females, but are in black males.

7.4.1.1 Multivariate Analysis – Source apportionment and metabolic processes

Principle Component Analysis with Varimax rotation was conducted on trace elements in bone in the whole population and subsequently in white males and black males independently, to investigate relationships between elements. The purpose of PCA was to generate hypotheses regarding potential sources of toxic element pollution and exposure, and to determine whether groups of elements may be related in meaningful ways.

All elements were included except magnesium which was excluded because the KMO statstic was .291, below the acceptable limit of 0.5. KMO. The overall KMO value was 0.613, indicating sampling adequacy.

Correlations between elements were high enough to allow for PCA, and were measured by the X^2 statistic (Bartlett's test of sphericity) which was $X^2(36) = 306.26$, $p < .001$. In addition the determinant of the correlation matrix is .239, which indicates that multicoliniarity is not affecting the results. The Correlation Matrix is given in Table 7-24. All elements correlate significantly to at least one other element with the exception of iron, which does not correlate to any other element.

Table 7-24. Correlation matrix for all elements include in PCA. Element pairs with significant correlations at either .01 or .05 are in boldface.

	V	Mn	Fe	Ni	Cu	Zn	As	Cd	Pb
V	1								
Mn	**0.540**	1							
Fe	**0.125**	0.07	1						
Ni	0.011	-0.112	0.007	1					
Cu	**0.301**	**0.508**	0.000	0.016	1				
Zn	0.102	0.22	0.027	**0.146**	**0.283**	1			
As	0.058	-0.014	-0.026	-0.043	**0.355**	0.001	1		
Cd	**0.274**	**0.408**	0.030	-0.098	**0.287**	**0.292**	-0.033	1	
Pb	-0.013	0.063	-0.092	0.047	**0.170**	**0.252**	-0.045	**0.249**	1
V									
Mn	.000								
Fe	0.033	0.151							
Ni	0.435	0.05	0.459						
Cu	.000	.000	0.498	0.405					
Zn	0.068	0.001	0.346	0.016	.000				
As	0.198	0.417	0.354	0.263	.000	0.493			
Cd	.000	.000	0.331	0.076	.000	.000	0.314		
Pb	0.425	0.18	0.09	0.245	0.006	.000	0.257	.000	

Initial analysis identified four factors which had eigenvalues above 1 (Kaiser's criterion). The scree plot is given in figure 7-29. Components two, three and four are also included in the analysis based on their eigenvalues.

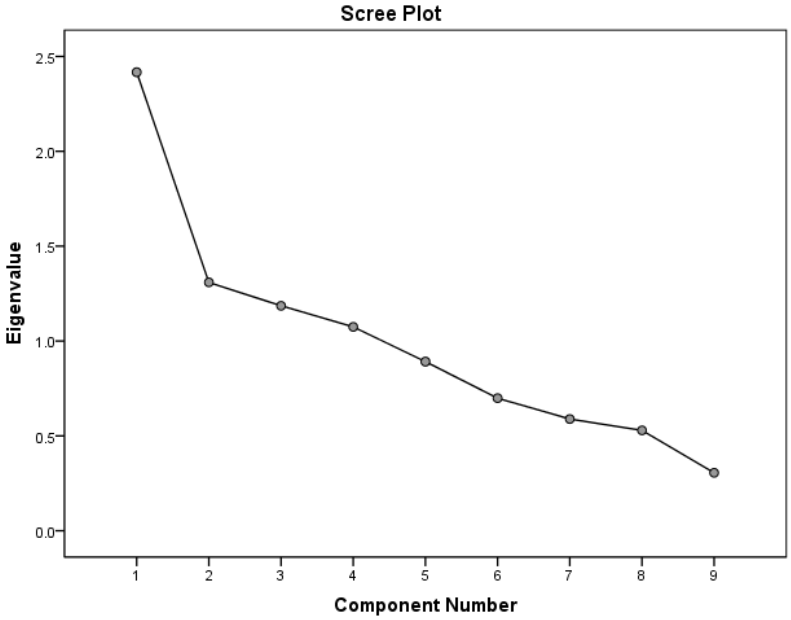


Figure 7-29. Scree plot of all elements (except Mg) showing one primary factor.

Eigenvalues for components 1, 2, 3 and 4 are respectively and account for 66.55% of the total variation.

Initial eigenvalues are given in Table 7-25, below.

Table 7-25. Eigenvalues for each component and the percent variation explained by the component.

Component	Eigenvalue	Percent variance
1	2.417	26.885
2	1.309	14.546
3	1.186	13.175
4	1.075	11.940

Component 1 accounts for 27% of variation and component 2 accounts for 15% of variation, with components 3 and 4 accounting for the remaining 25% of variation.

Rotated factor loadings are given in Table 7-26, below. Factor loadings below 0.4 are omitted.

Table 7-26. Rotated factor loadings for components 1, 2 and 3 in all individuals.

Component	1	2	3
Element			
Mn	0.834		
V	0.773		
Cd	0.559	0.498	
Pb		0.781	
Zn		0.589	
Fe		-0.407	
As			0.900
Cu	0.512		0.628
Sb			

Factor loadings for each component show several trends. Component 1 includes Mn, V and Cd and is likely associated with metal smelting and processing, given the presence of vanadium and manganese. Component 2 includes lead, which initially suggests that this component represents elements associated with vehicle emissions as well as metabolic proesses involving Zn and Cd. Component 3 includes copper and arsenic and is potentially associated with acid mine drainage or gold mining slag. Component 4, including nickel alone is likely to be associated with platinum mining.

7.4.1.2 PCA white males

PCA was conducted on white males and black males independently. In white males, examination of KMO values resulted in the removal of As, Ni, Mg and Pb. Overall KMO value was 0.614 and Bartlett's Test of Sphericity yielded a $X^2(10) = 54.561$, which is above the critical value at $p < .001$. The determinant of the coefficient matrix was .117, indication that there is a lack of multicolinearity between elements.

Table 7-27. Correlation matrix for white males. Element pairs with significant correlations at either .01 or .05 are in boldface. Determinant = .117

	Zn	Cd	Cu	Mn	V
Zn	1				
Cd	**0.355**	1			
Cu	**0.348**	0.271	1		
Mn	0.099	**0.614**	0.244	1	
V	0.003	**0.411**	0.159	**0.769**	1
Zn					
Cd	0.018				
Cu	0.020	0.058			
Mn	0.287	.000	0.078		
V	0.493	0.007	0.181	.000	

Two components had eigenvalues above 1 which accounted for 73.275 of the variance. Initial eigenvalues are given in Table 7-28, and the scree plot is Fig. 7-30, below.

Table 7-28. Eigenvalues for white males and the percent of variation explained by each component.

Component	Eigenvalue	Percent variance
1	2.408	48.167
2	1.255	25.108

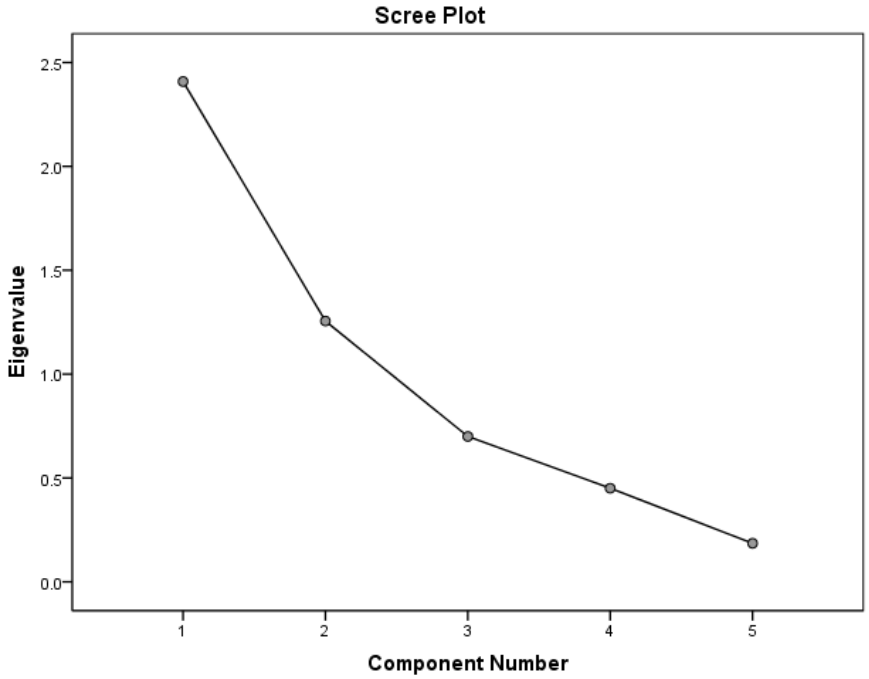


Figure 7-30. Scree plot for components and eigenvalues in white males.

Rotated factor loadings for each component are given in Table 7-29, below.

Table 7-29. Rotated factor loadings for components 1and 2 in white males.

Component	1	2
Element		
Mn	.935	
V	.904	
Cd	.655	.475
Zn		.865
Cu		.723

As in all individuals, Component 1 includes Mn, V and Cd, and is most likely associated with metal processing activities. Component 2 in white males is significantly different than that of the whole population, in that it no longer includes Pb, but instead Zn, Cd and Cu. In

this case, it is hypothesised that this component represents tobacco smoke. It is unclear why Pb is not a factor in any component in white males.

7.4.2 PCA black males

PCA in black males reveals different trends. Ni and Fe were removed from analysis due to KMO values of less than 0.5. When these elements were removed, overall KMO = 0.713 and Bartlett's Test resulted in $X^2(21) = 182.548$, $p < .001$, which is well above the critical value for X^2 with $Df = 21$. The determinant of the correlation matrix is .229, indicating a lack of multicolinearity. The correlation matrix is in Table 7-30, below.

Table 7-30. Correlation matrix for black males. Determinant = .229. Significant relationships between elements are in boldface.

	Zn	Cd	Pb	Cu	Mn	V	As
Zn	1						
Cd	**0.316**	1					
Pb	**0.264**	**0.473**	1				
Cu	**0.3**	**0.34**	**0.283**	1			
Mn	**0.225**	**0.337**	**0.165**	**0.607**	1		
V	0.101	**0.391**	0.127	**0.386**	**0.492**	1	
As	-0.018	0.100	-0.016	-0.006	0.083	0.114	1
Zn							
Cd	.000						
Pb	.001	.000					
Cu	.000	.000	0.001				
Mn	0.005	.000	0.032	.000			
V	0.129	.000	0.077	.000	.000		
As	0.42	0.13	0.427	0.472	0.175	0.099	

Initial analysis reveals three components with eigenvalues above 1, accounting for 54.35% of the variability. Eigenvalues are given in Table 7-31, and the corresponding scree plot in Fig. 7-31, below.

Table 7-31. Components and eigenvalues for black males and the percent variance explained by each component.

Component	Eigenvalue	Percent variance
1	2.651	37.875
2	1.154	16.483

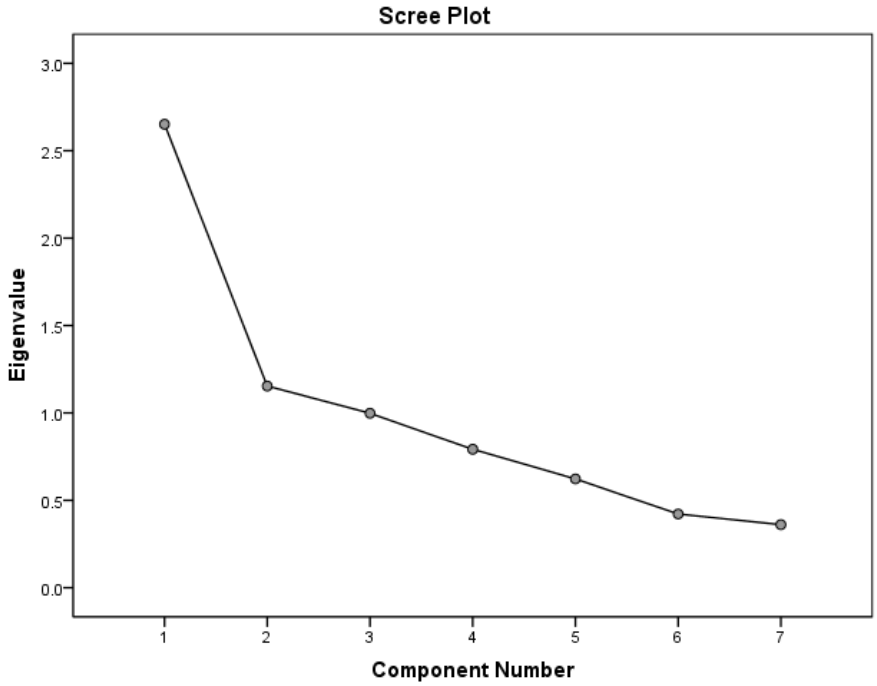


Figure 7-31. Scree plot for components and eigenvalues in black males.

Rotated factor loadings for each component are given in Table 7-32, below.

Table 7-32. Rotated factor loadings for extracted components in black males.

Component	1	2
Element		
Mn	.798	
V	.786	
Cu	.639	.426
As	.412	
Cd	.403	.637
Zn		.676
Pb		.781

Component 1 includes Mn and V, as well as Cu, As and Cd, which are likely due to metalworking processes and mining activities. Component 2 includes Pb, as well as Cd, Zn and Cu, which may represent a combination of metabolic processes and tobacco smoke.

7.4.3 **Relationships between toxic elements**

Relationships between toxic elements are explored. Simple and multiple regression is used to determine whether any of the toxic element factors in each component of PCA form linear relationships. Several significant relationships are evident between toxic elements. The statistical methodology used to compare toxic element concentrations is given in Chapter 6.

In all comparisons, the data reported includes R^2, b, β (R), and the SE of b. Significant β values are noted. All data used is log-transformed.

7.4.3.1 Lead and cadmium

Results of linear regression are given in Table 7-33. Among all individuals, bone Pb is significantly correlated with bone Cd, however this is clearly due to the effect of black males in the sample.

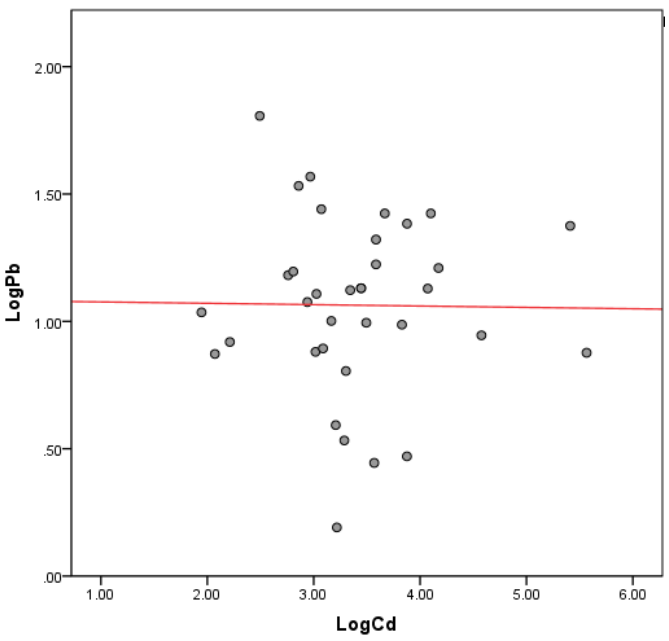
When groups are investigated independently the relationship between bone Pb and Cd differs. There is a weak correlation between bone Pb and Cd in black women and no linear relationship.

Among white males, there is also no significant correlation linear relationship.

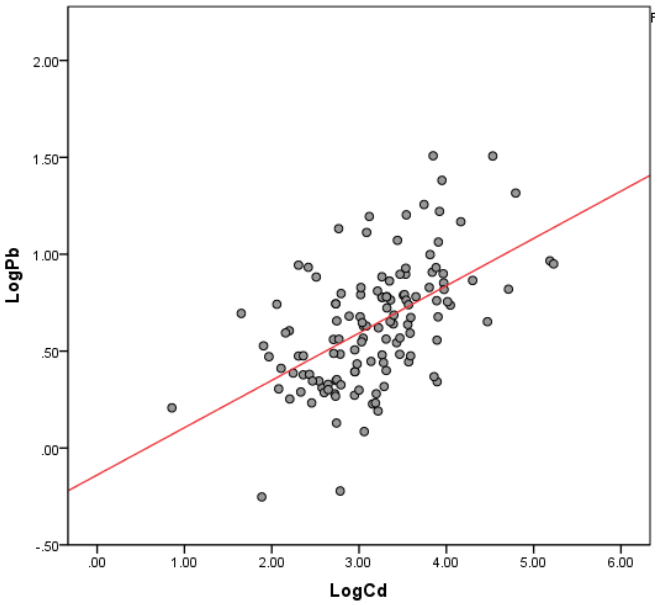
Among black males, there is a not very strong correlation between bone Pb and bone Cd. There is also a clear linear relationship, $R^2 = 0.294$. The results of the simple regression are given below in Table 7-34 and are plotted in Fig. 7-32.

Table 7-33. Regression statistics for Pb and Cd. Dependent variable = Bone Pb. *Sig. at p < 0.001 level. **Not significant at p = 0.05.

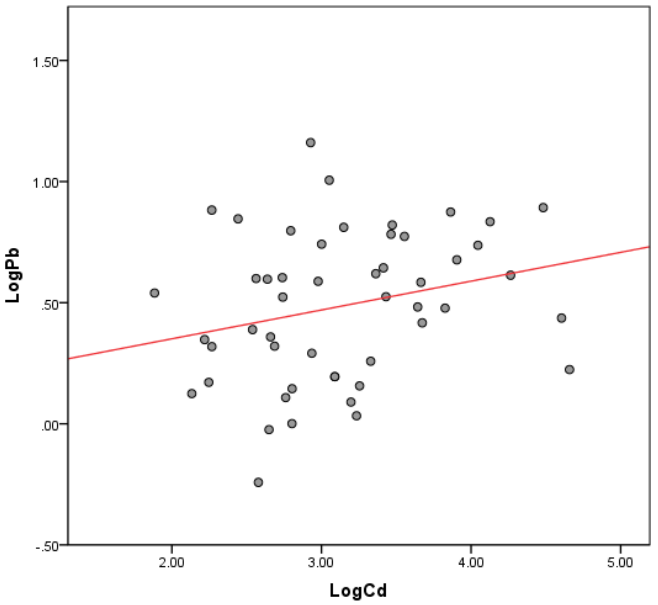
Pb vs. Cd	B	*SE* B	β	R^2	Adjusted R^2
All indiv.	.210	0.026	.471*	.222	.278
Black Fem.	.119	0.065	.256**	.066	.046
White Males	.065	.078	-.144**	.021	-.009
Black Males	.244	0.034	.543*	.294	.289



a. White males



b. Black males



c. Black females

Figure 7-32. Plot of Pb and Cd in a) white males, R^2 = .021, b) black males, R^2 = .294 and c) black females, R^2 = .066

7.4.3.2 Cadmium and Manganese

The relationship between bone Cd and bone Mn concentrations was explored by examining the correlation and potential linear relationships, the details of which are given in Table 7-34 and Figure 7-33.

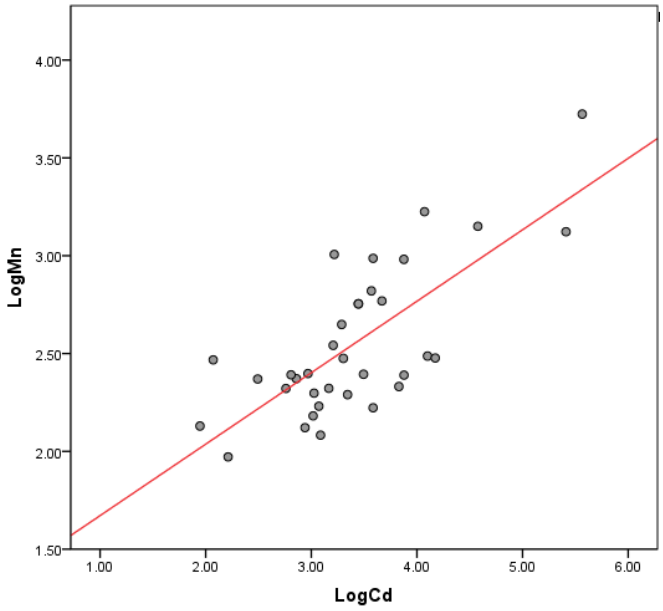
There is a clear linear relationship between bone Mn and bone Cd in all groups. In white males in particular bone Cd shows a moderate linear relationship with bone Mn

concentration, with R^2 = .547. The same is true of bone Cd and Mn in black males, in which R^2 =.368.

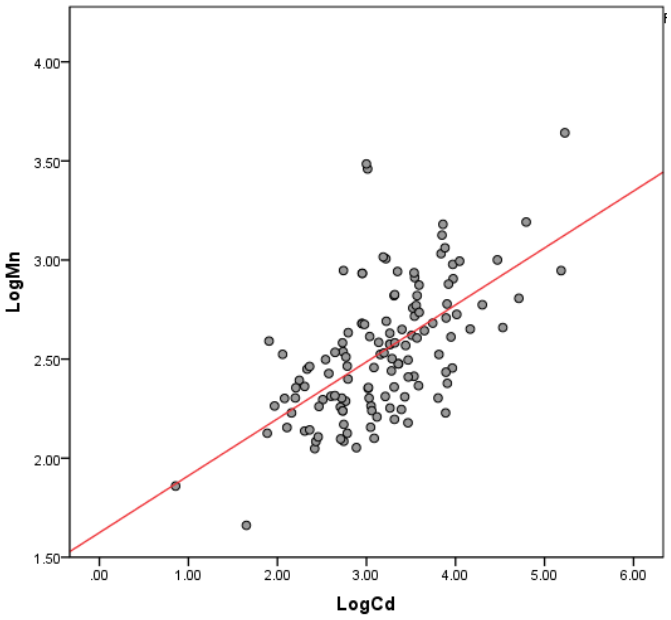
Table 7-34. Linear regression with variables Mn and Cd. *Sig. at 0.05.

Mn vs. Cd	B	*SE* B	β	R^2	Adjusted R^2
All indiv.	.301	.025	.637*	.406	.403
Black Fem.	.248	.048	.595*	.354	.340
White Males	.365	.058	.739*	.547	.533
Black Males	.287	.033	.607*	.368	.363

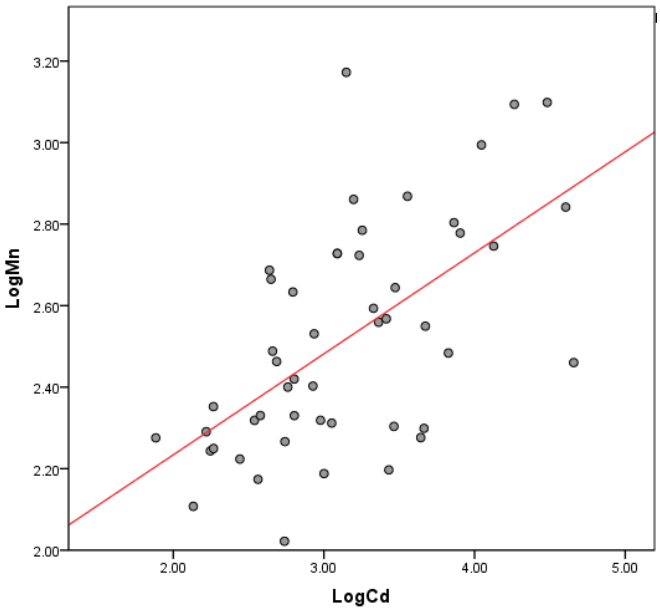
In all regression models of Mn and Cd, residuals were normally distributed and no outliers were identified.



a. White males



b. Black males



c. Black females

Figure 7-33. Linear relationship between Mn and Cd with Mn is dependent variable in a) white males, R^2 = .547, b) black males, R^2 = .368 and c) black females, R^2 = .354.

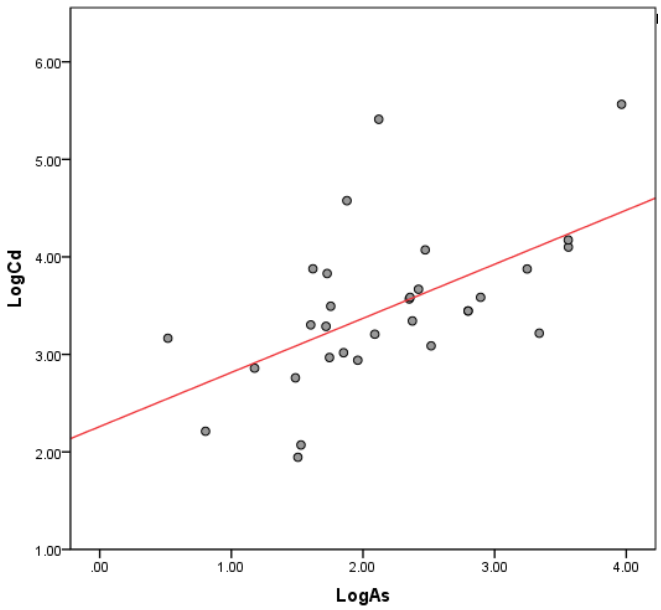
7.4.3.3 Cadmium and Arsenic

Bone As concentration is a weak but significant predictor of bone Cd in some groups and the sampled population as a whole. Statistics are given in Table 7-35 and plots for individual racial groups in Fig. 7-34. In the study population as a whole, as well as with all males and black males, the three residuals chosen (Chapter 6) are not normally distributed despite a weak but significant linear relationship, In white males, there is a significant positive linear relationship between bone As and bone Cd, and no assumptions of the linear regression

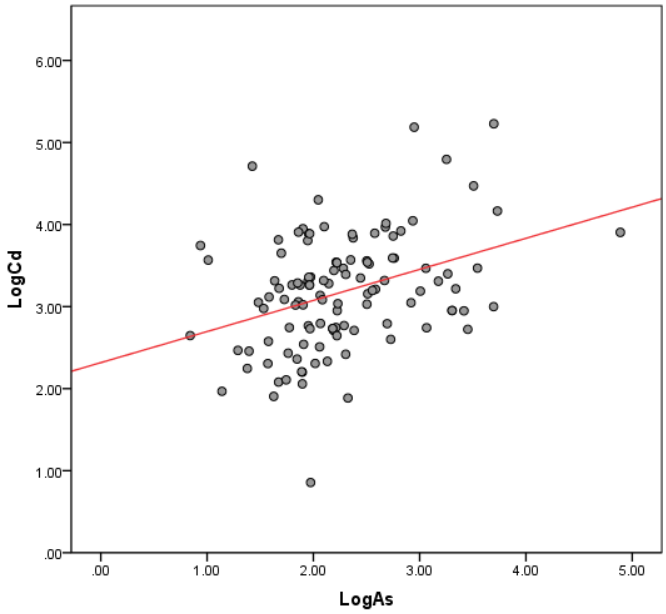
model are violated (residuals normally distributed). There is no linear relationship between bone As and bone Cd in black females.

Table 7-35. Linear regression between Cd and As with Cd as dependent variable. *Sig. at p < 0.01.
**** Not sig.**

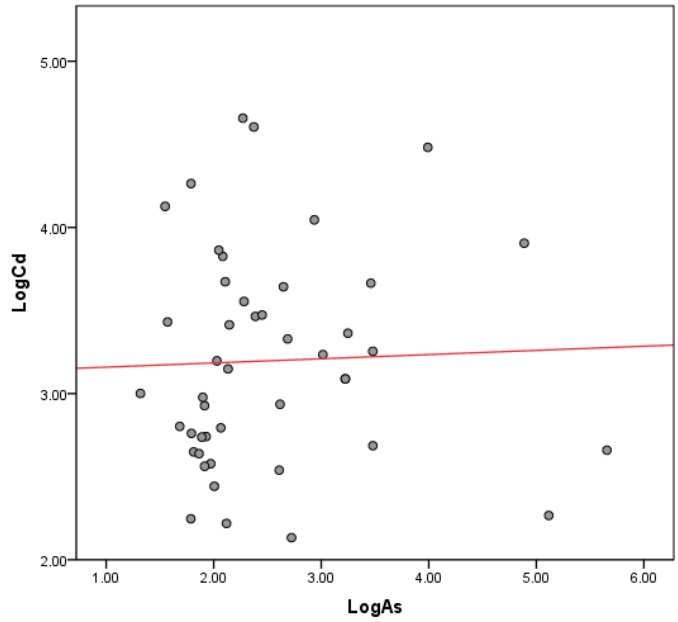
Cd vs. As	B	*SE* B	β	R^2	Adjusted R^2
All indiv.	.322	.074	.298*	.089	.084
Black Fem.	.053	.218	.037**	.001	-.021
White Males	.576	.156	.565*	.319	.296
Black Males	.345	.084	.362*	.131	.123



a. White males



b. Black males



c. Black females

Figure 7-34. Linear relationship between As and Cd where dependent variable is Cd in a) white males, R^2 = .319, b) black males, R^2 = .131, and c) black females, R^2 = .001

7.4.3.4 Manganese and Arsenic

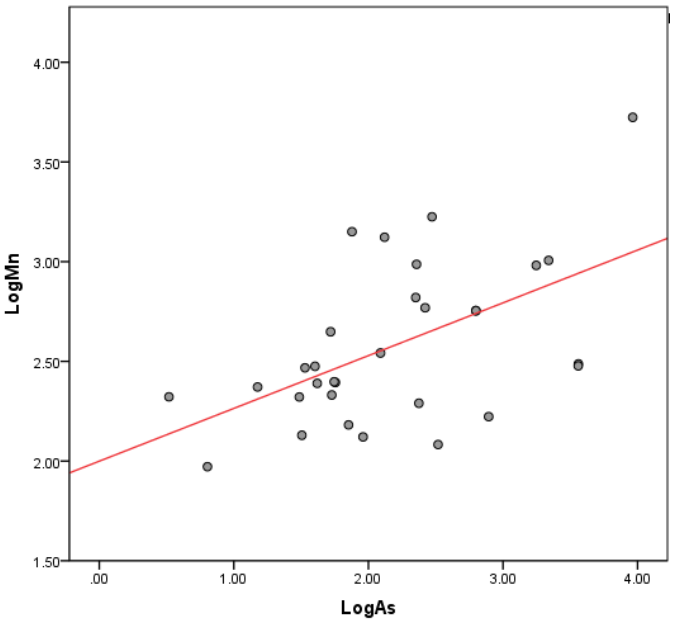
A linear relationship is evident between bone Mn and As in the sample population as a whole. As is a significant but weak predictor of bone Mn. Regression statistics are given in Table 7-36, below.

Table 7-36. Linear regression for Mn and As with dependent variable Mn. *Sig. at p < .001. **Not significant.

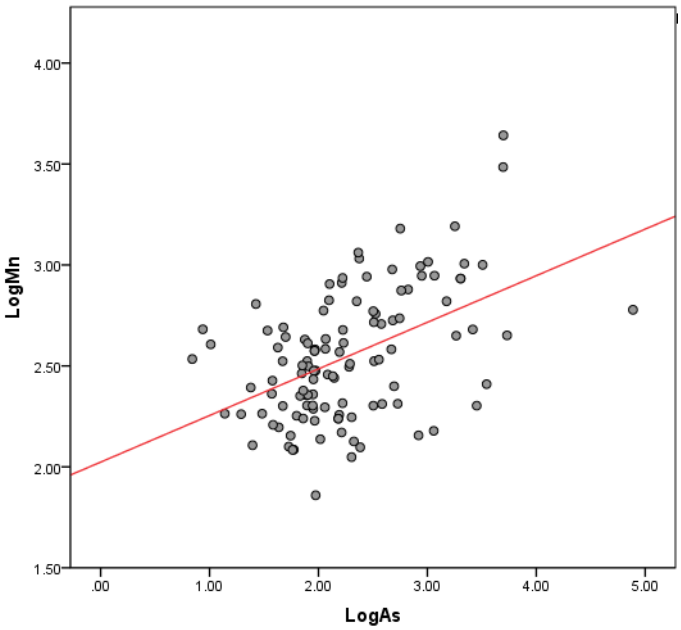
Mn vs. As	B	*SE* B	β	R^2	Adjusted R^2
All indiv.	.166	.028	.395*	.156	.151
B. Fem.	.054	.044	.182**	.033	.011
W. Males	.265	.077	.538*	.289	.265
B. Males	.231	.085	.435*	.235	.228

Linear regression for Mn and As with dependent variable Mn. *Sig. at p < .001. **Not significant.

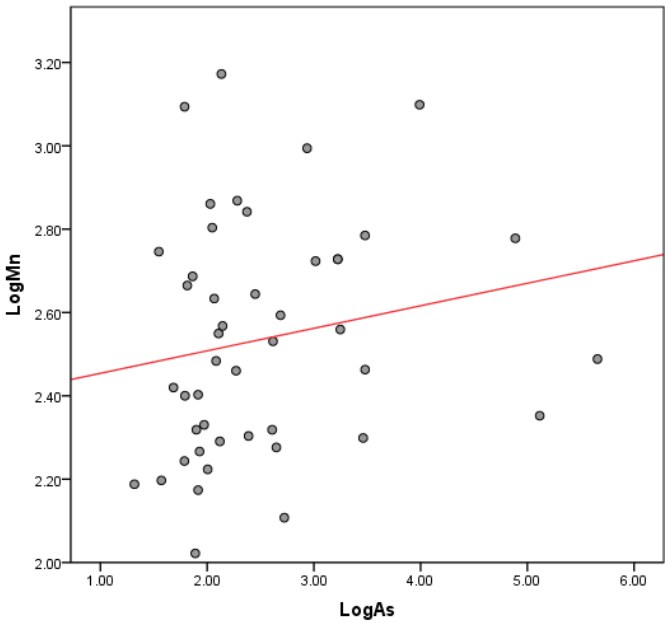
In all males As is a weak but significant predictor of bone Mn. In black females, there is no association between bone As concentration and bone Mn concentration. Regression lines for black females, white males and black males are plotted in Fig. 7-35.



a. White males



b. Black males



c. Black females

Figure 7-35. Linear relationship between Mn and As where dependent variable is Mn in a) white males, R^2 = .289, b) black males, R^2 = .235 and c) black females, R^2 = .033 (not sig.)

7.4.3.5 Lead and vanadium

There is no linear, non-linear or curvilinear relationship between bone Pb and bone V in all individuals. Curve estimates are given in Table 7-37 and plotted in Fig. 7-36.

Table 7-37. Table showing curve estimates for Pb and V. There is no significant relationship, linear or otherwise, when all individuals are included in the model.

Curve	R^2	F	Df 1	Df 2	Sig.
Linear	.003	.591	1	.443	
Logarithmic	.004	.810	1	.369	
Inverse	.005	1.042	1	.308	>.05
Quadratic	.006	.643	2	.526	
Cubic	.006	.464	3	.708	

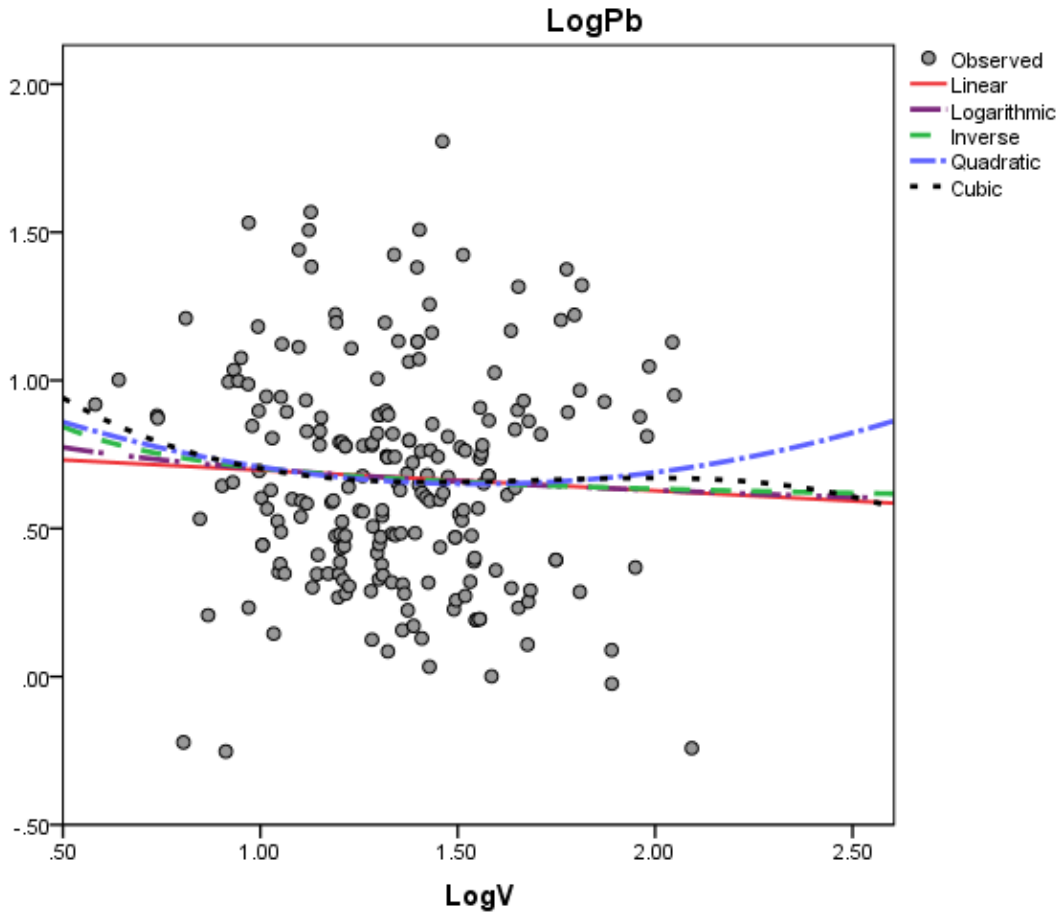
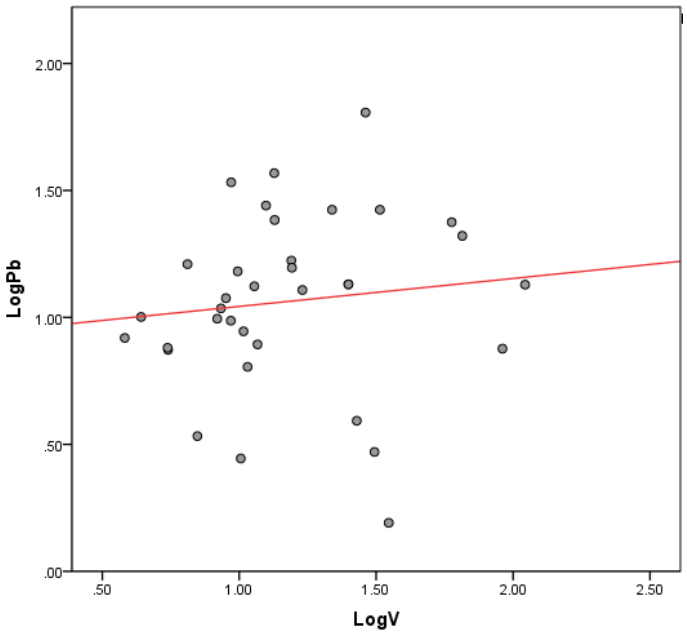


Figure 7-36. Curve estimates for Pb and Vin the total population. There is no relationship between the two varibles.

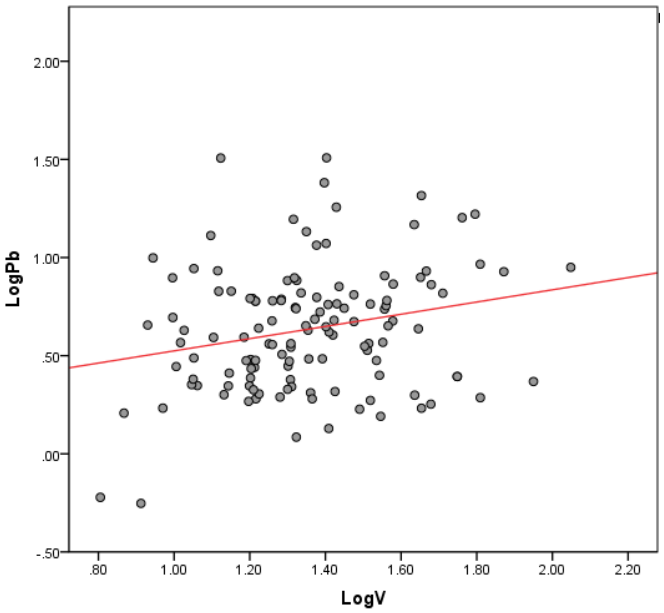
When the groups are examined individually, however, the relationship between Pb and V does become significant in black females and black males, but very weak. In black females, there is a significant negative relationship between bone Pb concentration and bone V concentration, though the residuals of the linear model are not normally distributed according to the K-S statistic (Table 7-38 and Figure 7-37). There is no relationship between the two elements in males.

Table 7-38. Linear regression between Pb and V with dependent variable Pb. *Sig. at p < 0.01, ** Not sig.

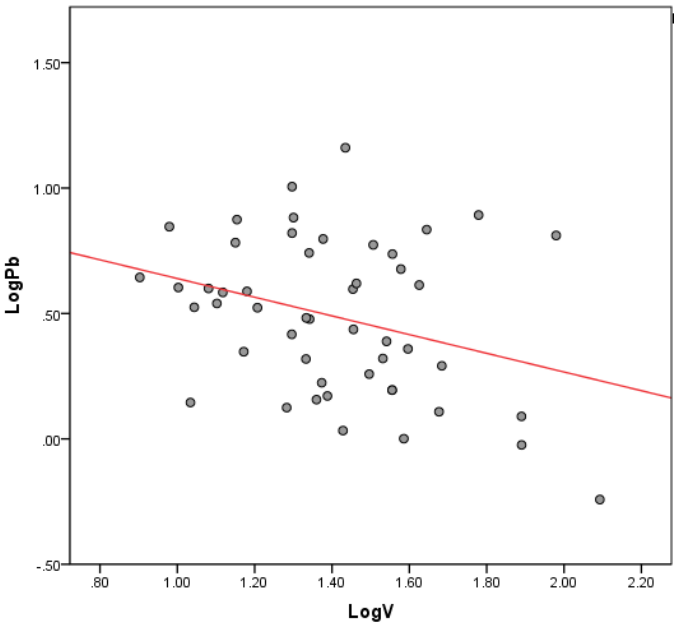
Pb vs. V	B	*SE* B	β	R^2	Adjusted R^2
All indiv.					
B. Fem.	-.373	.160	-.319*	.102	.083
W. Males	.110	.165	.115**	.013	-.007
B. Males	.311	.116	.231*	.054	.046



a. White males



b. Black males



c. Black females

Figure 7-37. Linear relationships between Pb and V with Pb as dependent variable in a) white males, R^2 = .013 b) black males, R^2 = .054 and c) black females, ^{R2} = -.102. In black and white males the relationship is not significant.

7.4.3.6 Manganese and vanadium

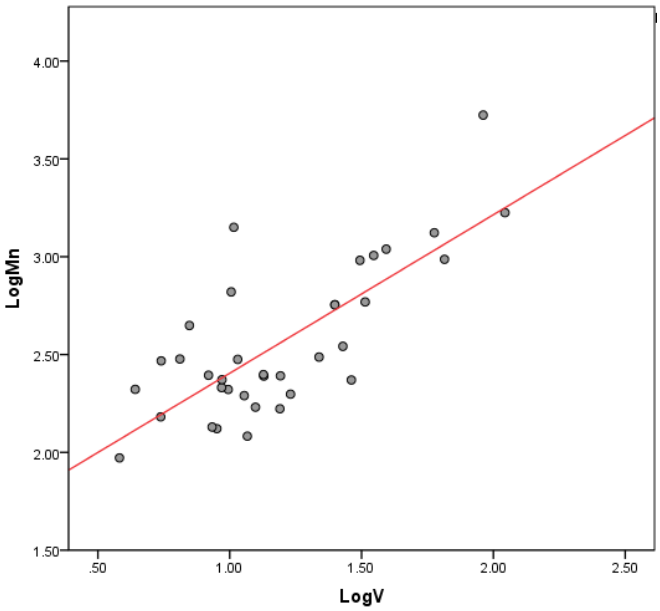
PCA shows that Mn and V are highly loaded factors in Component 1 in both males and across the sample population as a whole. The two elements are highly correlated in all individuals.

Linear regression also yields a significant linear relationship between manganese and vanadium in all individuals (Table 7-39).

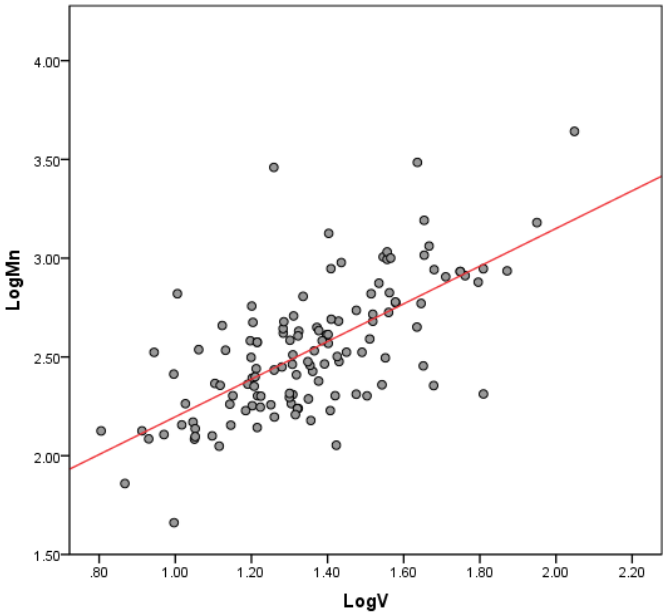
Table 7-39. Linear regression between Mn and V with dependent variable Mn. *Sig. at p < 0.01.

Mn vs. V	B	*SE* B	β	R^2	Adjusted R^2
All indiv.	.780	.063	.646*	.418	.415
B. Fem.	.599	.124	.572*	.327	.313
W. Males	.810	.120	.756*	.571	.558
B. Males	.954	.093	.674*	.454	.450

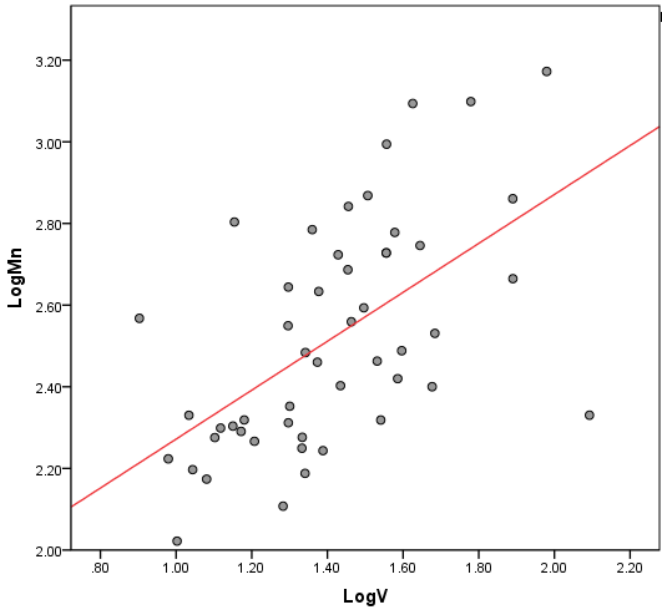
The relationship is strongest in males, but significant in females as well (Fig. 7-38). In white and black males, the correlation between bone Mn and bone V is over .60 (B in table 7-39).



a. White males



b. Black males



c. Black females

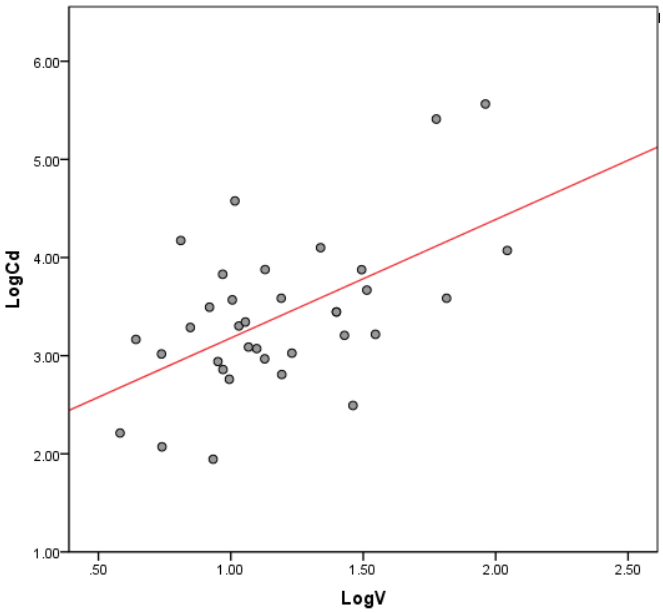
Figure 7-38. Bone Mn and V with dependent variable Mn in a) white males, R^2 = .571, b) black males, R^2 = .454 and c) black females, R^2 = .327

7.4.3.7 Cadmium and vanadium

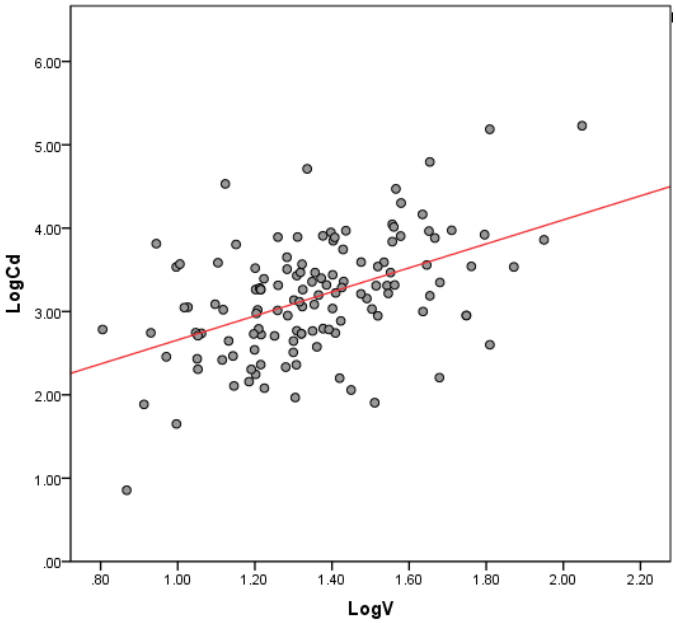
There are weak, but significant linear relationships between bone Cd concentration and bone V concentration in males, but not in black females (Table 7-40). The strongest relationship is seen in white males, followed by black males (Fig. 7-39).

Table 7-40. Linear regression between Cd and V with dependent variable Cd. *Sig. at p < 0.01. **Not sig.

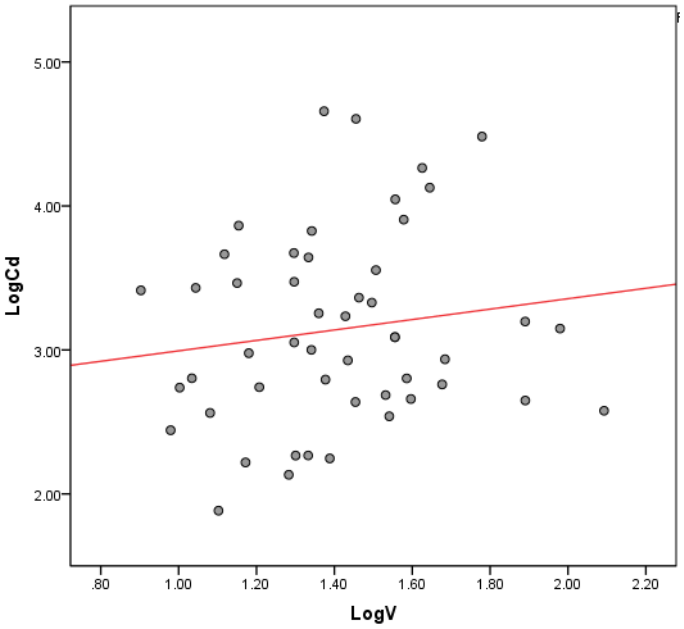
Cd vs. V	B	*SE* B	β	R^2	Adjusted R^2
All indiv.	.968	.162	.378*	.143	.139
B. Fem.	.362	.359	.144**	.021	.000
W. Males	1.207	.312	.558*	.312	.219
B. Males	1.4410	.232	.482*	.233	.227



a. White males



b. Black males



c. Black females

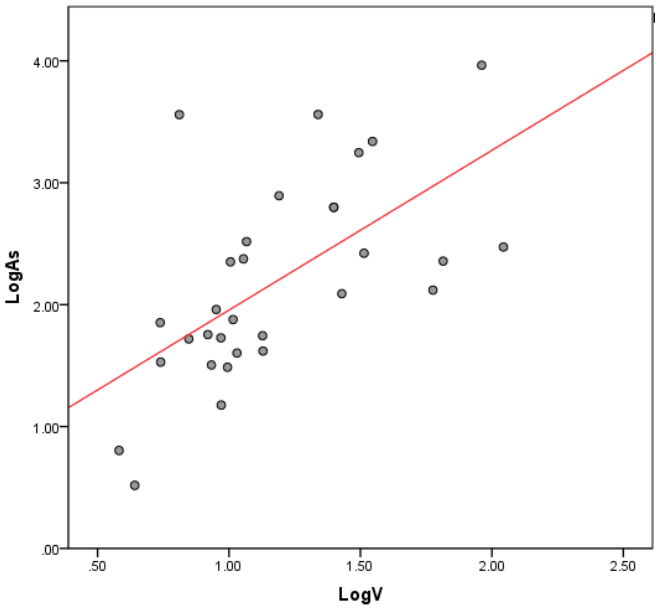
Figure 7-39. Cd and V with dependent variable Cd in a white males, R^2 = .312, b) black males, R^2 = .233 and c) white males, R^2 = .312. black females, R^2 = .021R2 in black females is not significant (p > .05).

7.4.3.8 Arsenic and Vanadium

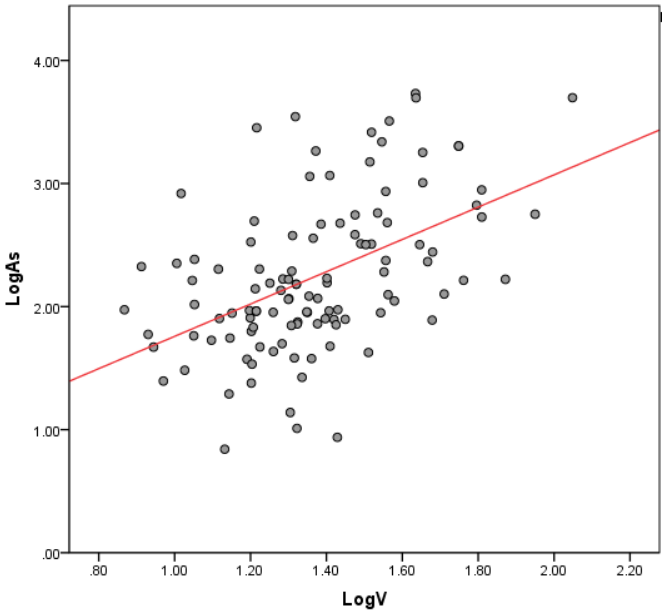
In all individuals, there is a linear relationship between bone As and bone V concentration. The relationship is not evident in black females. In males, a significant linear relationship is present in both black and white males.

Table 7-41. Linear regression between bone As and bone V in with dependent variable As. *Sig at p < 0.001. ** Not sig.

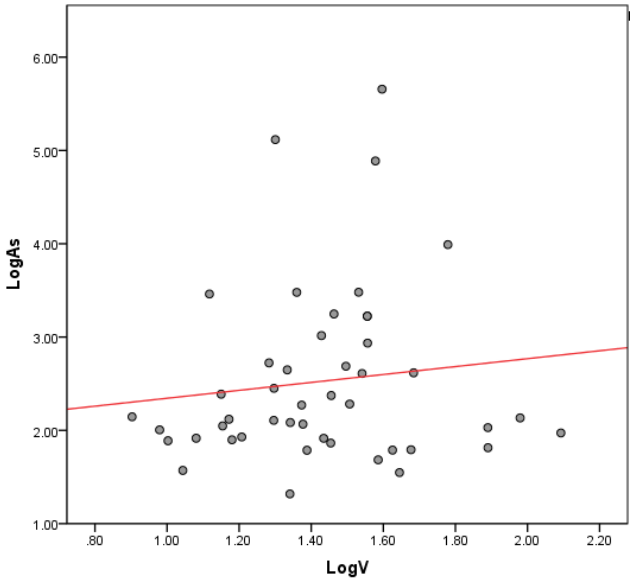
As vs. V	B	*SE* B	β	R^2	Adjusted R^2
All indiv.	1.104	.179	.406*	.165	.160
B. Fem.	.424	.533	.119**	.014	-.008
W. Males	1.311	.313	.613*	.376	.355
B. Males	1.392	.397	.486*	.236	.229



a. White males



b. Black males



c. Black females

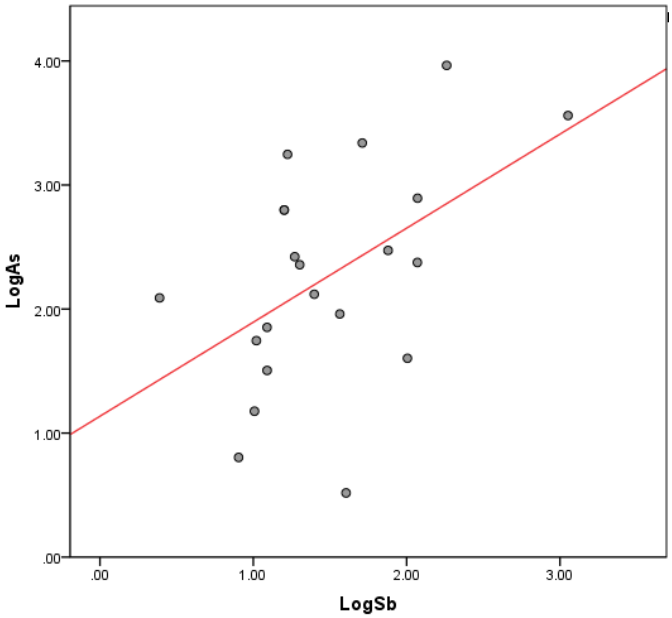
Figure 7-40. Bone As and bone V with dependent variable As in a) white males, R^2 = .376, b) black males, R^2 = .241 and c) black females, R^2 = .014 (not sig.)

7.4.3.9 Arsenic and Antimony

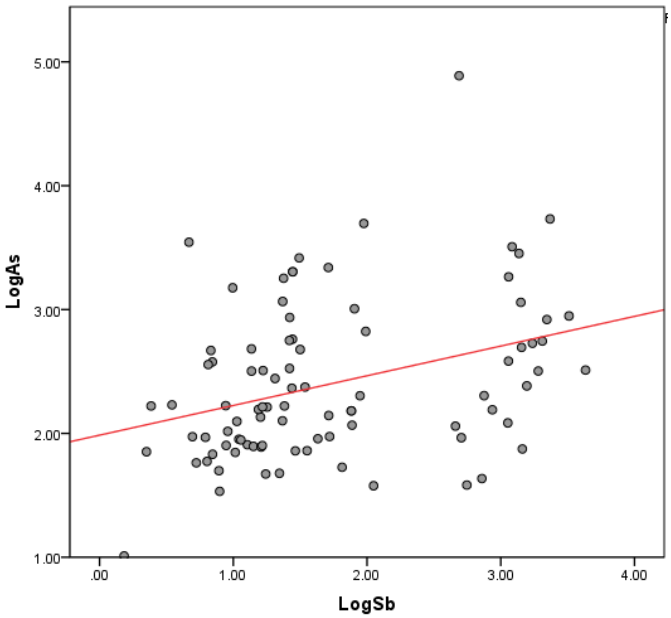
Arsenic and antimony show only a very weak linear relationship, R^2 = 0.029 (Table 7-42). When the relationship is explored by sex and race, significant linear relationships are evident. The relationship is strongest (though still fairly weak) in black females, with R^2 of .292, followed by white males with an R^2 of .251. Scatterplots with R^2 are given in Figure 7-41.

Table 7-42. Linear regression between As and Sb with dependent variable As. *Sig. at p < 0.01.

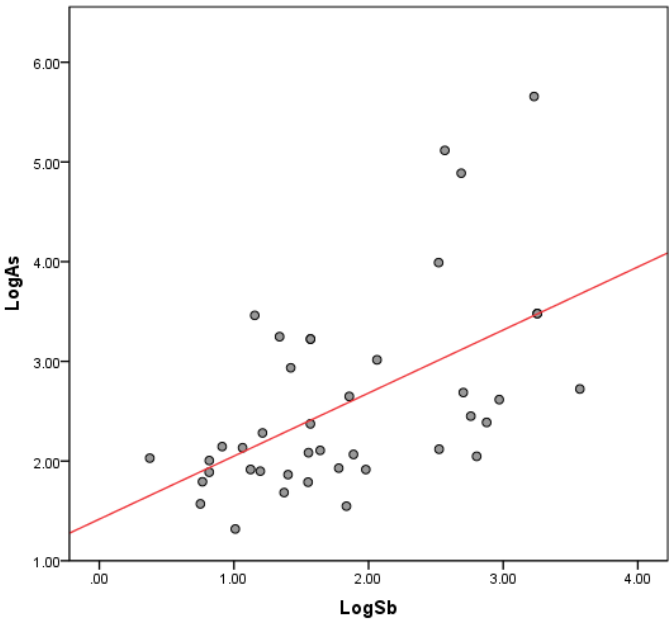
As vs. Sb	B	*SE* B	β	R^2	Adjusted R^2
All indiv.	8.82	3.50	.170*	.029	.024
B. Fem.	.632	.158	.540*	.292	.274
W. Males	.758	.301	.501*	.251	.211
B. Males	.240	.071	.342*	.117	.107



a. White males



b. Black males



c. White females

Figure 7-41. Linear relationship between As and Sb with dependent variable As in a) white males, R^2 = .251, b) black males, R^2 = .117 and c) black females, R^2 = .292.

8 Discussion

This research presents unusual and unexpected findings with regards to toxic trace elements in urban Gauteng. From the 1960s to the 1990s, several demographic trends in toxic element exposure become clear. This chapter will discuss the results presented in the previous chapter and examines the level and degree of exposure for each toxic element measured in the sample population. The potential impact of each element on the health of males and females in the population will also be discussed. The relationships between elements are also discussed, as the presence or absence of these relationships can shed light on potential health effects of exposure as well as the potential source of exposure. Lastly, this chapter includes a discussion of the six research objectives set forth in the introduction and how these objectives were met through the results of the research.

It is all too easy to draw sweeping conclusions from the results of this research and the temptation to fit, refit, and over-fit statistical models to the data is great. As with any biological research, the temptation is also great to infer too much into the results that are obtained and make assumptions about the population at large based on a small segment of that population. This can be particularly true in archaeology and biological anthropology, where despite small sample sizes researchers risk forming assumptions about whole cultures or populations. In public health research where sample sizes are often, but not always, high, and researchers have the benefit of substantially more background information about research subjects, the practice of formulating generalisations about a whole population are more justified.

This research is somewhat unique, in that it straddles the line between bioarchaeology and public health. Certainly these data can be used to infer recent, and to some extent present, public health conditions in a modern urban population and in this way it is firmly rooted in public health. However the use of a skeletal collection, even an identified one, comes with all the drawbacks of conducting research on a burial or archaeological population. Sample sizes are small. Study "participants" cannot be chosen, nor can detailed sociological data be gathered. In this sense, this research is considered bioarchaeological as opposed to public health oriented and every effort is made to avoid making broad generalisations about urban South Africans. Rather, the results discussed here will present trends and relationships in the *sample* population, the 200 individuals on which this research was conducted. It is possible, perhaps even likely, that the results presented here represent population-wide patterns in trace element exposure, but it is inappropriate to assume that this is the case. Any conclusions reached about the population of Gauteng at large are treated as potential, as opposed to definite, realities.

To fully understand the implications of toxic element exposure it is critical to explore these results from a geographical and social context. The distribution of labour, access to health services, as well as demographic trends such as household composition and location of residence were all largely politically determined during apartheid, for both the black and white population. Thus exposure to and interaction with the environment were both predetermined by race and are not equal between groups, which subsequently causes some striking differences in exposure to toxic pollution within the population.

The extreme inequality characterizing apartheid South African cannot be separated from the results presented in this research. However South Africa is not unique in suffering from, or creating, inequality within its population. In almost all countries, particularly less developed countries, exposure to toxic elements and urban pollution disproportionally affects one demographic group or socioeconomic strata more than others. In most cases, such as the United States, the poorest and most disadvantaged groups within the population bear the brunt of toxic element exposure, due to marginal living conditions, proximity to industry and transportation congestion and occupational exposure. This trend has been quantified in populations across the globe. Recent research into lead exposure among children in post-apartheid South Africa has found that children from the lowest socioeconomic strata suffer from greater exposure than their more well-off contemporaries. The results presented here show that this was not necessarily the case for this population during apartheid. For some elements, arsenic, cadmium, antimony, lead and vanadium, there appears to be significant difference in exposure between different demographic groups. In the case of lead, the opposite trend has been identified, in which white males have significantly higher lead exposure than more socioeconomically disadvantaged black individuals. The potential reasons for these trends are discussed in greater detail in subsequent sections of this chapter.

It is suggested from these results that, despite the high level of mining and industrial activities taking place in Gauteng during the three decades in question, the level of exposure to elements such as lead was lower than in many industrial areas in other parts of the world. Lead levels in particular are much lower than expected, given the use of leaded petrol and lead mining and smelting activities in the region. Other element concentrations, such as manganese, cadmium and arsenic are also lower than in many industrial regions. There is no significant difference between toxic elements in individuals living in Pretoria and Johannesburg, indicating that the levels of exposure at this time may have been relatively uniform throughout the region (Chapter 4, Section 4.2.3).

Several essential trace elements have been studied, including iron, magnesium, zinc, and copper. These elements are included and discussed for two reasons. Each of these elements

interacts with specific toxic elements within the human body, and can affect uptake and health consequences of toxic elements. Each of these elements is also a common industrial pollutant in itself, and the relationship between these elements and certain toxic elements can often be due the sharing of common sources within the environment. In this respect, the correlation between these elements and toxic elements can allow for the formulation of basic hypotheses regarding the likely source of toxic elements. The measurement of these elements can also highlight differences in nutrition and overall health that may affect toxic element uptake and toxicity.

It is critical to note at the outset of this chapter, that the interrelationship between toxic element exposure, essential trace elements and the human body is extremely complex. For example, it is known that certain elements share uptake pathways within the human body and subsequently, concentrations in human tissues are likely to be correlated. In other instances, essential trace elements may inhibit the uptake of toxic elements. Calcium, a critical element in bone formation, is known to affect the uptake of cadmium and lead. Lastly, the correlation of elements in the atmosphere due to common sources is also a significant possibility. As such, the nature and cause of the association between given elements in human bone tissue is difficult to determine with certainty. What is most likely is that the interplay between elements in bone tissue is likely the result of environmental, biological, metabolic and kinetic factors in combination. The discussion that follows is not intended to present definitive evidence of any one environmental, behavioural or nutritional causes in regards to relationships between elements or individuals. Rather, this section is an exploration of the data presented in Chapter 7 and a discussion of the statistically-based inferences that may be (cautiously) drawn from them.

Overall, several trends emerge from the results of this analysis in addition to that discussed above. In black women, statistical analysis does not reveal as many significant trends between toxic elements in black or white males. This is evident in PCA, for which women were not included due to a very weak or nonexistent relationships between elements. Within this trend one notable exception occurs, and that is the inverse relationship between magnesium and lead, which suggests that for this sample population, magnesium may play a significant role in lead uptake. Another trend is the clear racial dichotomy between many elements.

In males, both black and white interesting correlations are evident between essential and toxic elements in the bone tissue of the sample population. Conversely, some relationships that would be expected given the level of industrial pollution are not present. More importantly, relationships between toxic and trace elements among males differ between

black and white males, suggesting different routes of exposure and/or differences in nutritional and health status. Given the lack of corresponding environmental data from the study era, it is not possible to determine conclusively the sources of and environmental connections between trace and toxic elements. However the data presented in the preceding chapter does allow for the discussion of reasonable hypotheses, which can provide both an understanding of past health and environmental trends. Perhaps more importantly, the data presented here highlight several gaps in present-day environmental studies, such as an understanding of the way toxic element exposure may have changed across time that may be critical to public health in urban South Africa.

8.1 Lead

8.1.1 Overall trends in bone lead concentration

The overall trend in bone lead concentration in individuals from apartheid-era Gauteng is one of substantially lower lead exposure than would be expected in an industrial environment and one in which leaded petrol was used. In black individuals, in particular, bone lead concentration is lower than that of non-occupationally exposed Europeans during the same time period. Few black individuals have bone lead concentrations that would be expected in occupationally exposed individuals. Black women have significantly lower lead concentration that either black males or white males, which corresponds to trends worldwide (Barbosa et al. 2006; Popovic et al. 2005). These results are in accordance with the lower-than-expected levels of lead found in environmental samples in Gauteng and discussed in Chapter 4. When compared to data from adult men in the United States, the lead levels in black and white males in the study population are significantly lower. Hu et al. (1996b) report an average bone lead concentration of 20.8$\mu g \cdot g^{-1}$ in non-occupationally exposed adult men in Boston. These authors also report that lower socioeconomic background and fewer years spent in school were correlated to higher bone lead. In addition, white individuals had a slightly higher mean bone lead concentration than black individuals at 22.1 and 25.8$\mu g \cdot g^{-1}$ respectively. Lead levels in both black and white adult males in the US were higher than those reported in this research, and in the case of black males, substantially so, a trend also reported by Elmarsafawy et al. (2002) who found higher bone lead levels in occupationally exposed black individuals than in similarly exposed white individuals. In adult women patients in a Boston maternity ward, mean cortical bone lead was 4$\mu g \cdot g^{-1}$, which is quite close to the mean bone lead concentration in women from Gauteng, at 3.92$\mu g \cdot g^{-1}$ (Hu et al. 1996a). Other authors however, studying nurses in Boston, report a higher mean bone lead concentration of 13.3$\mu g \cdot g^{-1}$, significantly higher than that reported here (Korrick et al. 1999).

The lead concentration found in black individuals in the sample population are lower than those reported in occupationally exposed or industrially exposed populations (Table 2-1, Chapter 2) such as those reported by Lindh (1980), Somervaille et al. (1988), Baranowska et al (1995) in continental Europe. The bone lead concentration in white males falls in the lower range of occupationally exposed individuals.

The distribution of lead across the sample population however, is unexpected. Bone lead concentration is highly dichotomous between black and white individuals within the sample population. This finding is counter to trends in developed countries in which disadvantaged populations have higher rates of lead exposure than populations from higher socioeconomic backgrounds (Bellinger et al. 1988; Hicken et al. 2012; Krieger et al. 2003; Tong et al. 2000). This is also counter to recent findings in South Africa, in which lower socioeconomic status is positively correlated with lead exposure (Harper et al. 2003).

There are four primary factors that may contribute to the higher bone lead concentrations in the white males of the sample population: residential patterns, non-petrol related residential lead exposure, age and iron status. It is likely that a combination of one or more of these explains the difference in bone lead concentrations between black and white individuals. Between black males and females, it is hypothesized here that differences in bone lead concentration may be explained first and foremost by differences in bone biology and by age. In addition differences in occupation and time spent within and without the household may contribute to the lower lead levels seen in black women versus those seen in black and white males. Lastly, it is possible that within the sample population men and women had different sources of lead exposure, which may account for some difference in total lead burden within each sex.

8.1.2 **Bone lead trends and potential source and exposure pathways**

The lowest bone lead concentration occurs in black females and is significantly different from that of black males, though the mean and median bone lead concentrations in each group are still low relative to industrially exposed populations elsewhere in the world. Baranowska et al. (1995) in particular measured bone lead in a modern Polish population from the highly industrial and polluted Silesia region and found concentrations as high as 200 $\mu g \cdot g^{-1}$. These authors lowest measured bone lead concentration was approximately 20$\mu g \cdot g^{-1}$. The lowest bone lead concentrations in black males and females – 0.5$\mu g \cdot g^{-1}$ each in South Africa. This is very low for an industrial region, particularly one in which leaded petrol was still in use. In Sweden, 1980, prior to the cessation of leaded petrol, mean bone lead concentration in non-industrially or occupationally exposed individuals was 2.85$\mu g \cdot g^{-1}$. In occupationally exposed individuals this increased to 15$\mu g \cdot g^{-1}$, which is on par with the

highest measured bone lead concentration of 14.48$\mu g \cdot g^{-1}$ among black women in the sampled population. In black males the highest measured bone lead concentration in the sampled population is 32.23$\mu g \cdot g^{-1}$. The results suggest that the majority of black individuals in the sample population were neither occupationally, nor industrially (i.e. living near an area characterized by industrial lead emissions) exposed. Given the level of industrial and occupational activities involving in the Transvaal region during apartheid, this result is unexpected.

The difference in bone lead between males and females has been reported world-wide and in nearly every study of lead exposure in South Africa. Mathee et al (2002), von Schirnding et al (1991) and Naicker (2012) consistently found higher blood lead levels in males. In children this was attributed to boys spending greater time outdoors than girls. Barry (1970) measured bone lead in adults in the north of England in the1960s and reported that males from the UK had bone lead concentrations higher than females at a ratio of 3:2. This is approximately the same ratio observed in the Pretoria sample population, in which male bone lead ratio is higher than that of females at a ratio of 2.9 to 2.

It is notable that the mean bone lead measured by Barry in non-occupationally exposed males and females is 21.03$\mu g \cdot g^{-1}$ and 16.05$\mu g \cdot g^{-1}$, respectively – substantially higher than the mean values reported here. Moreover, in English individuals, there was no dichotomy in the range of bone lead concentration as there is in South African individuals. The range of bone lead concentration in Barry's sample population was the same for both males and females with approximately 0.6 to 49$\mu g \cdot g^{-1}$ for both sexes.

There is a substantial body of research documenting the role that differences in bone biology – specifically different rates of bone turnover – plays in the deposition of lead in bone in men and women (Aufderheide and Wittmers 1992; Theppeang et al. 2008b; Vahter et al. 2007; Vahter et al. 2002). Popovic et al. (2005) note that in women exposed to lead a smelting plant, bone lead concentration was lower than expected, based on comparative studies of men, indicating sex differences in the deposition of lead in bone. Vahter et al. (2002) have also noted sex differences in the deposition of lead in the bones of women. In addition, the higher rate of bone turnover in postmenopausal women may contribute to lower bone lead concentrations but higher blood lead concentrations due to endogenous release of lead into the blood stream and several studies have confirmed this. In pre-menopausal women, however, uptake of lead into bone is expected to be roughly equal to men when exposure is equal (Kosnett Mj 1994).

In the case of the present sample population, the relationship between bone lead and age was examined in both males and females. Bone lead increases between the ages of 20-29 and

peaks in women between the ages of 40 and 49 and declines from the ages of 50-89[2]. In women aged 40-49, bone lead is approximately double that of any other age group at 5.47$\mu g \cdot g^{-1}$. Though these results are not statistically significant, the data is consistent with Walker et al. (1984) in which the average age of menopause in women living in Soweto was 48.9 years. The data presented here would seem to confirm this, as it is well established that bone lead levels in women peak just before and decline after menopause. In white and black males, bone lead does differ significantly between individuals of different ages and peaks substantially later. In black males, bone lead concentration peaks between the ages of 80 and 89 and in white males, in ages 90-99[3]. These data correspond to trends reported by Hu et al. (1996), which report increasing bone lead concentration in adult males from the ages of 47 to 70+. Hu et al. (1996a) also found increasing bone lead concentration with age in adult American women.

In black individuals, the difference in bone lead concentration between men and women may indicate a lower level of exposure among women, and this certainly may be the case, as will be explored in the following section. But it may also indicate that lead is not being deposited in the bones of women at the same rate as males, and is being released from bone in postmenopausal women. This has significant implications for the potential toxicity of lead in women and the subsequent health effects. The results here indicate that 30% of women in the sample population were exposed to a moderate degree of lead exposure. The lower lead concentrations among women aged 50 and older does not indicate cessation or reduction of exposure, but likely indicates that the higher rate of bone turnover post-menopause likely resulted in the release of lead from bone into the blood stream and other tissues where it may have caused a host of health issues. Thus older black women in South Africa may have been at increased risk of lead toxicity than older males.

It has been established by Monna et al.(2006) and Olowoyo et al. (2010) that atmospheric lead in Johannesburg and South Africa is largely concentrated along transportation corridors, showing higher lead concentrations in biomonitors (bark and lichen) located along major roadways than anywhere else, even in close proximity to mining dumps. Monna et al.'s isotopic studies of lead in Johannesburg further demonstrate that the majority of lead in the atmosphere comes from leaded petrol burning, as opposed to mine pollution or domestic coal burning. The results of this research appear to corroborate these results. When the

[2] Bone lead increases slightly in women aged 80-89, however in this group, N=2. The difference in mean bone lead concentration per age is not significant, though this may be likely to small sample sizes in some age groups.

[3] Sample sizes in these groups are small in both black and white males. However, in both groups the minimum and maximum bone lead concentrations in each age group are significantly higher than in lower age groups.

Monna et al. lead concentration map of Johannesburg is juxtaposed with a map of the racial group areas that characterized apartheid-era Johannesburg, the relationship between lead exposure and proximity to transport networks becomes clear (Chapter 3, p. 60 and Chapter 4, p. 83, respectively)

It is clear that the black townships and residential areas are located far from the central business district and urban core, which yield the highest lichen lead concentrations (identified in red, above). The areas with the lowest concentrations are those in the outlying, suburban areas, to which black individuals were relocated following the Group Areas Act. Even biomonitoring sites located near mine dumps adjacent to Soweto (identified in green) show lower lead concentrations than those sites located in the central business district, which is adjacent to white-only residential areas. Most importantly, the one lead monitoring study conducted in Soweto and central Johannesburg during the 1980s confirms that the township experienced lower lead pollution than central Johannesburg throughout the day (Formenti et al. 1998).

This distribution of lead across the landscape has clear implications that are mirrored in the bone lead results from the Pretoria bone collection. Bone lead concentration is highly dichotomous between black and white individuals within the sample population. The median bone lead concentration in white males is significantly higher at 12.82 $\mu g \cdot g^{-1}$ than in either black males or females at 4.34$\mu g \cdot g^{-1}$ and 3.35$\mu g \cdot g^{-1}$. Calcium, a critical element in bone formation, is known to affect the uptake of cadmium and lead. Von Schirnding et al. (1991) found similar correlations between the proximity of schools and major roadways and blood lead concentration in children in Cape Town, though the results do not correspond so strikingly to racial group areas as in Gauteng. This is potentially because in both Pretoria and Johannesburg, the relegation of non-whites to areas outside of the city centre and into suburban areas was more pronounced than in other cities in South Africa (Bickford-Smith 1995). Among the conclusions that can be drawn from the lead levels in this sample population is that residential patterns, in which black individuals outside the urban core may have had lower rates of exposure to emissions from leaded petrol than their white counterparts and thus lower bone lead concentration.

While the bone level concentrations do not correspond with the concentrations expected of occupationally exposed populations, it is possible that the difference in bone lead between males and females is due, in part, to occupation and the daily movements across the urban landscape that result. In urban Gauteng, during apartheid, many males living in the region would have been industrial/mining/construction-related (Pons-Vignon and Anseeuw 2009), whist women were more likely to remain in the townships or work as domestic servants

(Casale 2004). This would likely affect both time spent outdoors and time spent in the urban core or central business district, in short, the time spent exposed to outdoor air pollution. It is interesting to note, however that the main source of employment available to urban black women during apartheid was domestic service, much of which would have taken place in white homes in white residential areas. It is likely that a significant proportion of women in the sample population were domestic workers in either Pretoria or Johannesburg, and it would be expected that women working in white households may have higher bone lead concentrations as a result. Unfortunately, no white women could be included in the sample population, which would have shed light on this matter.

Monna et al.'s lead isotope data concludes that the bulk of atmospheric lead in Johannesburg comes from leaded petrol and that the domestic burning of coal contributes little to overall lead burden in the environment. South African coal is low in lead, but not devoid of it. Moreover, the burning of coal for domestic heating and cooking is common in the townships and black residential areas of both Johannesburg and Pretoria. It is not unreasonable to assume that some lead exposure in black women comes from domestic coal when burned inside enclosed spaces. To date, there have been no isotopic studies of bone or blood lead in either city nor was lead isotope analysis was conducted in women in this sample population. It is possible, however to infer the potential source of lead from its relationship with other toxic elements (Doucet and Carignan 2001).

During apartheid the urban areas experienced high degrees of oscillating migration among the black population, particularly black males. These individuals would have been resident in the cities only as temporary labour, returning to their designated bantustan at the end of seasonal work or the end of a work contract. In this way, these individuals would have been only part-time residents of either Johannesburg or Pretoria and would not have been exposed to the same level of pollution as permanent residents. The permanent white population of the cities and the more permanent black female population would have been exposed to more lead. Though black females have low bone lead concentrations and much lower concentrations than white males, there are other variables that may explain this difference.

The correlations between bone lead and several elements differs between groups. Notably, black females seem to follow the same pattern as white males with regards to correlations between elements, suggesting a potentially similar pattern of exposure. In black males, there is a correlation between bone lead and bone zinc, $r_s = .307$, $p < .001$. There is no significant correlation between lead and zinc in the bones of white males or black females. A correlation between the two is to be expected, given the strong correlation between atmospheric zinc and lead as presented in Chapter 4, based on Monna et al. (2006) and

Olowoyo (2010. To what extent dietary intake of zinc may be influencing these results in unclear. It is known that zinc deficiency is prevalent in black South Africans, and bone zinc is significantly lower in black males and females than in white males in this study population, $U = 1441.5$, $p < .01$, with $r = .354$. There is no difference between bone zinc concentration in black males versus black females.

The discrepancy between black males and the rest of the study population points to either a different source of lead exposure between black males and white males and black females, a significant secondary source of lead exposure in black males, or a different exposure pathway in black males. Environmental data taken from Monna et al. (2006) and Olowoyo (2010) were examined statistically by this author. In high-traffic areas, there is no significant relationship between the two elements. In high traffic areas that form the urban core: taxi ranks and high traffic corridors, there is no correlation between zinc and lead in lichen, $r_s = .608$, $p > .05$. In all areas outside high traffic areas however, there is a strong and significant correlation between zinc and lead, $r_s = .937$, $p < .05$. The relationship between lead and zinc in soil samples taken in Pretoria show the same relationship between lead and zinc with $r = .093$, $p > .05$ in high traffic areas and $r_s = .90$, $p < .05$ in lower traffic areas, including mining and industrial areas.

Results of PCA augment these findings, though unfortunately PCA results in black females are of little use as there are no correlations between elements. In black males, lead, zinc and cadmium form component 2 with cadmium also a factor in Component 1, with manganese, vanadium and copper. In white males, lead is not a factor in either component.

There are several ways to (cautiously) interpret these results. The first relates to potential environmental sources. Many investigations have used PCA to explore toxic element source apportionment in biological tissues (Bechmann et al. 2000; Borgå et al. 2006; Kunito et al. 2002; Samanta et al. 2004). These authors found that it is possible to identify regional and exposure differences in trace element exposure in animals based on relationships between elements in tissues. While it is impossible to determine the exact movements and occupational patterns of individuals within the sample population, it is possible that the black males in the sample population are exposed, at least partially, to a different source of lead than white males (and black females, many of whom would likely be spending time in white neighborhoods near the urban core). This may potentially explain the differences in the relationship between lead and zinc in these groups. It may also be that lead and zinc are metabolically associated.

Worldwide, cadmium is predominantly a soil and water contaminant and it is an element associated with mining in South Africa. Cadmium is mined alongside zinc and lead in

Gauteng and in north western South Africa along the Namibian border. As discussed in Chapter 4, cadmium is found in high concentrations in Gauteng, which previous authors have attributed to vehicle pollution (De Villiers et al. 2010). Yet in high traffic areas, it is not correlated with lead or zinc in lichen, which would be expected if the elements shared a common source. Bivariate correlation performed on environmental trace element data from Monna et al. (2006), Olowoyo et al. (2010) and Naicker et al. (2003) yield correlations between these elements that provide useful comparisons. In mining and smelting areas, lead is not correlated with cadmium, $r_s = .842$, $p > .005$. Cadmium is strongly correlated with zinc in mining and smelting areas, $r_s = .986$, $p < .001$. In Naicker et al.'s (2003) analysis of acid mine drainage from a gold mine near Johannesburg, cadmium was significantly correlated with zinc as well, $r_s = .724$, $p < .001$. As with the relationship between zinc and lead, cadmium and lead are only significantly and positively correlated in black males, $r_s = .304$, $p < .01$. In white males and black females there is no correlation. In black males, zinc and cadmium are also significantly correlated, $r_s = .543$, $p < .001$, with no correlation between zinc and cadmium in black females and white males.

Whilst the correlations between the three elements in bone tissue of black males are not as strong as they are in environmental samples, factors such as bioavailibilty, uptake and element biokinetics are likely affecting the relationships in bone. It is also likely that the source of lead exposure in the three groups is the same – lead from petrol emissions, but the data does suggest a different pathway of exposure in black males, and suggests that the urban core is not the primary area in which black males from this sample population are exposed to lead.

With regards to bone magnesium, the element is not significantly correlated to lead at any site in lichen or soil Luo et al. (2012a; 2012b) have noted that the bioavailability of both lead and zinc in humans is highly dependent on soil pH and the amount of organic material in the soil, with lower pH and soil organic matter associated with greater element uptake in humans. Potentially, black males are more likely to be exposed to ground, dust or soil contaminants from occupations such as mining or industry, than black women or white males, whose exposure to these elements may stem from inhalation of atmospheric pollution[4].

It is not possible to ascertain definitively whether black females and white males share a similar lead exposure source and pathway without isotopic analysis. However the similarities between these two groups in the relationships between lead, zinc and cadmium clearly require further investigation. In addition, apartheid era residential and labour

[4] Mining activities in the Gauteng region create highly acidic soil and water runoff.

patterns, in which whites lived in or near the urban core, and black females worked in white homes, lends credence to the hypothesis that these groups shared similar exposure pathways with regards to lead. The data does not allow for speculation as to the extent to which domestic coal served as a source of lead exposure for black females, however it does not seem likely given the above results. These results suggest that the difference in lead exposure between black women and white males is one of degree as opposed to source or pathway, however clearly more data is needed in order to reach such a conclusion.

When these results are examined for each city independently, some differences arise. In black males who were likely resident in Pretoria, the relationship between bone lead and zinc is slightly stronger, $r = .468$, $p < .001$. However in black males living in Johannesburg, there is no correlation between bone lead and zinc. In Pretoria, bone lead and bone cadmium are correlated, $r_s = .561$, $p < .001$, and not correlated in Johannesburg. In Pretoria, bone cadmium and zinc are correlated in black males, $r_s = .269$, $p < .01$, but not in Johannesburg. In white males and black females, there is no correlation between bone lead and zinc or bone cadmium and zinc. Again, this points to potentially different sources or pathways of exposure in black males between the two cities.

Mathee et al. (2006; 2009b; 2004) have examined lead in paint as a potential source of lead exposure in Johannesburg. Without isotopic analysis of bone lead in this population, it is impossible to say to what extent lead paint is a source of lead for white individuals and, to a lesser extent, black females. Lead paint is not commonly associated with adults, and is generally only a source of lead for children, particularly small children who often exhibit signs of pica and are prone to eating paint flaked from the walls of older or decrepit homes (Gould 2009). Adults can be exposed to lead paint in homes, during periods of renovation or in individuals who work as builders, however this is generally not a long-term source of exposure in adults (Atsdr 2007). In young adults, for whom bone tissue may still include exposure from childhood, exposure to lead paint could, hypothetically, be a source of bone lead and in young females not getting adequate nutrition, osteopenia could release childhood lead sequestered into bone back into the blood stream. None of the individuals in the sample population are below the age of 18 however, and most are too old for lead in household paint to be considered a significant risk factor.

In black males, mining and cottage industries involving automobile repair and lead battery dismantling are potential sources of lead exposure in the sampled population. Cottage industries were and remain common in the townships and squatter settlements in Gauteng. Mining is certainly a potential source of lead, particularly in the case of black males in Gauteng. It is highly likely that some males in the sampled population were involved in

mining, and potentially resided in the mining hostels located just outside each city. During apartheid laws were enacted to strictly regulate the movement of black individuals, specifically black males, from white residential neighborhoods and the central business district. Unless black males worked in these areas they were restricted from spending more than 24 hours within them. Men who were miners would likely rarely enter the central business district. In addition, a large percentage of black miners were technically circular or oscillatory migrants. They would have worked in the mines, lived in a mining hostel on the urban periphery and returned to a bantustan when not working. These males in particular, were unlikely to be exposed to the same lead sources as women and white males.

8.1.3 **Geographic and temporal trends**

There are no temporal trends in bone lead concentration within the sampled population. No differences in lead were uncovered between individuals living in Pretoria versus individuals living in Johannesburg, in any group. This result is unexpected. Environmental monitoring has shown that atmospheric lead is higher in Johannesburg than in Pretoria. Pretoria is a smaller and less industrial city. Furthermore, predominant wind directions for each city result in wind that is not blown from city to city (Fig. 8-1). Wind patterns from Krugersdorp, the major mining area between the two cities would blow some wind north east, towards Pretoria. However environmental monitoring conducted in 1986 included Lanseria, the airport just north east of Krugersdorp (Formenti et al. 1998). This study showed that this site did have low atmospheric lead compared to other sites in Johannesburg. Multiple studies have shown that atmospheric lead emissions from traffic congestion are not limited to the areas adjacent to roadways and that atmospheric lead can travel quite far, and it is likely that overall atmospheric lead in Pretoria was influenced to some extent by lead coming from Johannesburg (Daines et al. 1970; Doucet and Carignan 2001; Komárek et al. 2008). It is not possible to determine the extent to which atmospheric lead moves from one city to the other, however it is clear that within this sample population, the exposure levels of individuals to lead was similar, even if environmental monitoring has not shown the same.

It is possible that individuals living in Pretoria were exposed to lead pollution from the Edendale lead mine, located near Pretoria and active until 1938 some of the adults in the study population may have been exposed to smelting activities that took place at the mine, which could have released metal fumes which included lead, zinc and iron oxides, arsenic and antimony (Glass 2006).

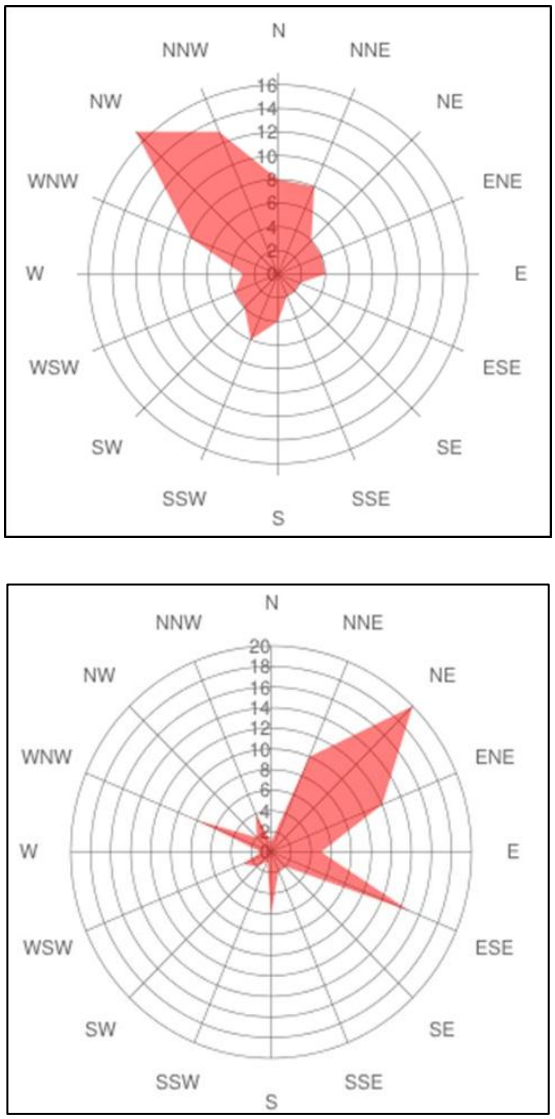


Figure 8-1. Dominant wind direction from Pretoria and Johannesburg. In Pretoria, top, the overall trend is for a north by northeasterly wind. The dominant wind direction in Johannesburg, below, is northwesterly, which would blow wind not into Pretoria but southwest of the city. Source: windfinder.com.

No temporal trends in bone lead concentration were apparent in the sample population. When each demographic group was analysed individually the results were the same with significant change in bone lead concentration over time. During the entire "study period" of this research, from 1960 to 1998, South Africa used leaded petrol. Whist the country began to phase out leaded petrol in 1996, post-phase out studies of environmental lead have shown that lead persists in the atmosphere and in soil and dusts despite cessation of lead use. It was not expected that there would be a decline or increase in bone lead over time.

8.1.4 **Lead and health in the sample population**

8.1.4.1 Lead in males

Lead levels in black males are skewed towards lower concentrations. Approximately 85 percent of black males in the sample population have a bone lead concentration below 10.0µg·g^{-1}. Over 35 percent have bone lead concentrations between 0.5 and 3.0µg·g^{-1}, indicating a lower risk of lead-related health effects than white males in the study. In white males, the results are nearly mirror image, approximately 90% of individuals have bone lead concentrations above 3.0µg·g^{-1}. Nearly 60% of the white males in the study have concentrations above 10.0µg·g^{-1}.

Recent research has demonstrated that even low levels of chronic lead exposure can cause neurological and behavioural consequences, particularly in males. In white males with bone lead concentrations above 10.0µg·g^{-1}, the clinical effects can include movement disorders such as Parkinson's Disease and behavioural disorders such as poorer impulse control and delinquent behavior. Needleman et al. (2002) report in their case-controlled study of adjudicated teenage delinquents that the cases in their study had bone lead levels of 11µg·g^{-1} or greater. At bone lead levels of 25µg·g^{-1} or above, cases were four times more likely than controls to be brought into the criminal justice system. With regards to neurological disorder, it has been demonstrated that individuals with tibia bone lead concentrations of 13µg·g^{-1} or greater are significantly more likely to suffer from Parkinson's Disease than individuals with lower lead exposure (Coon et al. 2006; Gorell et al. 1999a; Weisskopf et al. 2010). The risk of these disorders, given the strong association with cumulative lead exposure, is clearly greater in the white males in the sample population.

Within the male population as a whole, there is potential evidence that a majority of both black and white males have bone levels high enough to cause hypertension which is particularly prevalent in black individuals in South Africa. The degree to which lead exposure contributes to this has not been explored, but given the low levels of chronic lead exposure found in this study, it could be that lead does not play a role. Given the threshold bone lead levels (iliac crest) of 5µg·g^{-1} which Wedeen (1988) associated with hypertension, the majority of males in the sample population could have been at risk - in this sample population approximately 80% of white males and approximately 60% of black males. Bone lead levels of 20µg·g^{-1} and above are associated with hypertension, nephropathy and cognitive decline, which could have impacted approximately 55% of white males in the sample population. Renal disease has been associated with blood lead in South Africa in males working in a battery factory. To put even low bone lead concentrations in perspective with regards to health risk, Schroeder and Tipton's (1968) estimate that a bone lead

concentration of 5μg·g^{-1} equals a body burden of lead of approximately 200mg. And, given that up to 60% of the total lead in blood can originate in bone tissue, even at relatively low bone lead concentrations, endogenous release of lead into the blood stream can be of concern, meaning lead can have severe health consequences years after cessation or reduction in exposure.

Recalling Bandeen-Roche (2009) and Weisskopf et al. (2004b), regarding cognitive decline and bone lead concentration, it is probable that males in the sampled population would have been at risk for mild cognitive impairment due to lead exposure, including those with lower bone lead concentrations. White males with the highest bone lead concentrations may have been at particularly high risk. Anecdotally, the cadaver database lists the white male with the highest bone lead concentration in the sample population, 64μg·g^{-1}, as having died of "senility".

8.1.4.2 Lead in females

The health effects of lead exposure are somewhat different in females than in males, due largely to physiological differences in bone tissue and the effect of reproduction and menopause on bone. Nonetheless, bone lead is a significant predictor of hypertension in females, as it is in males. Another effect of lead exposure in women is the potential for osteoporosis. Women in general are more prone to bone loss than males at all ages, and this has the additional effect of releasing endogenous lead from bone into the blood. In this sense, older women are at greater risk of endogenous lead exposure than older males (Theppeang et al. 2008b). This risk is also heightened during pregnancy, when increased bone turnover releases lead into the bloodstream which may then cause toxicity in both the women and fetus (Gulson et al. 1997).

It is possible that women in the sample population did not suffer greatly from the effects of lead however. In the sampled population, 96% of black females had bone lead concentrations at or below10μg·g^{-1}. Among these 44% of the female population had bone lead at or below 3μg·g^{-1}. There are few health effects associated with bone lead concentrations this low. Given that average bone lead concentration peaks at ages 40-49 and declines thereafter, and that 65% of the sample population is over the age of 40, it is unwise to assume that black females had consistently low lead exposures and were therefore unaffected. However, the low lead concentrations in females relative to males does indicate a lower rate of exposure to lead and potentially lower chances of suffering from toxic health effects. The average bone lead concentration in females just before menopause is approximately 5.5μg·g^{-1}, high enough to cause the same risk of hypertension in males, but below the thresholds for renal effects (Nash et al. 2003; Nash et al. 1999). It would be

unwise as well, to assume that the declining bone lead levels in women over the age of 50 were due to cessation of exposure. Considering the use of leaded petrol in South Africa, this is unlikely. What is more likely is that the decline in bone lead concentration with age is due, in part, to endogenous release of lead in older women.

Bone lead can have negative health consequences in pregnant women and even more serious consequences for children who suffer from pre-natal exposure. Jedrychowski et al. (2009) found that even extremely low pre-natal lead exposure (< 0.5µg/dL) was correlated with cognitive deficits in children up to three years of age, particularly in boys. The levels of lead in women of childbearing age within the sampled population (18-50), coupled with the known increase in bone turnover during pregnancy, would likely release enough lead into the bloodstream to cause negative consequences for children born to them. These consequences could have included loss of IQ. Hernandez-Avila et al. (2002) report that women with tibia bone lead of 16.6µg·g^{-1} or greater had children with significantly reduced length and head circumference than women with lower bone lead concentrations. Reduction in head circumference is known to have negative effects on brain development and is associated with reduced skeletal growth. Notably, only one female in the sample population had a bone lead concentration near this level, and none of the women of childbearing age had bone lead concentrations above 14.5µg·g^{-1}. As will be discussed below, there may be other factors contributing to the low level of and health effects of bone lead in women in the sample population.

8.1.5 Bone lead and essential trace elements

The interaction between lead and essential trace elements is well established and discussed briefly, in Chapter 1. Several trace elements affect the uptake and metabolism of lead, such as iron, magnesium, zinc, and in males in particular, copper. Generally, dietary inadequacies in these elements result in increased uptake and toxicity of lead, and some, like magnesium, are even administered to individuals undergoing chelation therapy for acute lead toxicity. The metabolism of all trace elements, whether toxic or essential is complex and dependent on a number of factors; age, sex, health and lifestyle (smoking, alcohol consumption). As such, it is not possible to know conclusively in this population how or why certain relationships exist in the bone concentration of lead and essential elements, however the data presented here, coupled with known dietary and health trends in South Africa, does allow for the formulation of solid hypotheses.

8.1.5.1 Magnesium and zinc

Among the most interesting relationships to be uncovered among the results of this research is the relationship, or lack thereof in some groups, between bone lead and magnesium. As

discussed in Chapter 2, magnesium has a mitigating effect on lead in the body. It both reduces the amount of lead that is absorbed into bone tissue and reduces the toxicity of lead in the blood stream or other tissues. In South Africa, mild to moderate magnesium deficiency is common, due in part to poor diet and also to the natural lack of the mineral in the water supply (Leary 1986). Magnesium deficiency has its own correlated health consequences, such as cardiovascular disease and heart attack and is common worldwide. Median magnesium concentration in the sample population is $2741 \mu g \cdot g^{-1}$ ±656 and does not vary by race, sex or age. Due to its affinity for bone, like lead, and its ability to reduce bone lead uptake it is expected that there would be a negative relationship between bone magnesium and bone lead.

Bone magnesium and bone lead are not correlated in black males, and negatively correlated, though not significantly correlated in females, $r_s = -.390$, $p < .001$. Unlike zinc, magnesium is not correlated with lead in the environment in any location, which suggests that the difference in lead/magnesium correlation between black males and females is not environmental, but nutritional and/or biological.

The negative correlation between bone magnesium and bone lead in black females is interesting and complex. Magnesium is a bone seeking element and bone magnesium can be seen as a proxy for information regarding dietary intake. Black females with low magnesium levels have higher bone lead concentrations, which is to be expected. Zinc also has a negative relationship with lead in black females. There is evidence demonstrating that zinc has a protective effect on the body in the presence of lead (Hubbs-Tait et al. 2007; Jamieson et al. 2005).

Just why this relationship is not apparent in white or black males is difficult to ascertain. It is possible that the higher levels of lead in males in the sample population obscure any linear trend between lead and magnesium, especially as bone magnesium does not vary between the groups. Among the factors associated with reduced magnesium uptake is alcohol consumption (Elin 1988; R. Rylander 2001). As discussed in Chapter 3, alcohol consumption ranging from moderate to high intake is common in South Africa and substantially more common in men than in women. It is possible that alcohol consumption in the males in the sample population reduced the effects of magnesium on lead. Given the much lower prevalence of alcohol intake in black women than in black or white males in South Africa, it may be that magnesium intake is not impacted and magnesium is providing a mitigating impact on lead uptake in black females. This phenomenon is also a potential factor in the lower bone lead concentration in black females in the sample population.

8.1.5.2 Iron

In the sample population bone iron concentration differs significantly between white males and black and white females. Unexpectedly, it is the white males who have significantly lower bone iron concentration with a median of 9.57$\mu g \cdot g^{-1}$ compared to 13.74 and 14.36 for black males and black females respectively. It is expected that bone iron concentration provides a meaningful estimation of the dietary intake of iron and particularly in an unburied population, can be used as a proxy for iron intake during life, though there is significant disagreement in regards to the use of bone iron concentration as nutritional indicator (Ezzo 1994; Klepinger 1984). Within the black population in present-day South Africa, both men and women tend towards similar levels of iron deficiency, in some areas iron intake is less than half the recommended daily intake (Bourne and Steyn 2000; Macintyre et al. 2002). Studies of cadaver bone from present day populations in Europe report bone iron concentrations of approximately 60$\mu g \cdot g^{-1}$ which is just under three times the median for black males and females and just over six times the median bone iron concentration for white males in the sample population (Helliwell et al. 1996; Wiechuła et al. 2008).

Low iron intake and iron deficiency has been associated with increased uptake of lead and lead toxicity. Few studies have examined the relationship between bone iron and bone lead. Some authors report positive correlations between bone lead and bone iron concentrations which is not expected given the interaction between the two elements (Brodziak-Dopierala et al. 2009; Wiechula et al. 2008). Bone lead and bone iron concentration were not correlated in any of the group in this sample population. Though a significant correlation between the bone elements does not exist, it is notable that white males have both the highest bone lead concentrations and the lowest bone iron concentrations. This may be an indication that there is some influence of lead on iron and vice versa in this group, despite a lack of a statistical relationship in bone tissue.

8.2 Manganese

8.2.1 **Overall trends in bone manganese concentration**

Bone manganese has only recently begun to be considered as a viable biomonitor of manganese exposure in humans, despite research demonstrating that approximately 40% of manganese stored in the body is found in bone. The result is a dearth of data regarding bone manganese concentration and how it relates both to the level of manganese in the environment and how it relates to human health. Nevertheless, the recent research into bone manganese measurement provides useful data by which to compare the sample population in this research. It is possible to consider whether individuals in this population were likely occupationally or environmentally exposed, which is an important factor in establishing the

level of exposure. Subsequently, it may be possible to infer the likely health consequences that would result at the exposure levels experienced by the sample population.

Bone manganese concentrations in non-occupationally exposed populations appear to range from .006 to 0.36$\mu g \cdot g^{-1}$ in developed countries such as Spain, New Zealand, Russia, Taiwan, Korea and Canada (Table 2-1, Chapter 2(Pejović-Milić et al. 2009)). Pejovic-Milic et al. (2009) have examined whether bone manganese can be used as an indicator of occupational exposure in Canada. These authors report that in exposed individuals bone manganese ranged from 3.8 to 9.1$\mu g \cdot g^{-1}$ and in non-exposed individuals the mean was 0.2 to 3.0$\mu g \cdot g^{-1}$. Only one individual in the sample population has a bone manganese concentration above 4$\mu g \cdot g^{-1}$, a black male. Outside of that individual, no other group has any one individual with a bone manganese concentration above 2$\mu g \cdot g^{-1}$. The average bone manganese concentration of 0.453$\mu g \cdot g^{-1}$ lies above the values for these countries and could be considered low to moderate exposure, given that it is above what is expected for non-occupationally exposed individuals and far below what is expected for occupationally exposed individuals. Moreover, the distribution is skewed left, and the median is substantially lower than the mean at 0.302$\mu g \cdot g^{-1}$, below bone magnesium values reported for Russia or New Zealand.

These results are commensurate with the environmental data, which indicates that during the period from 1960 to 1998, manganese, although present in the atmosphere, was low relative to values measured elsewhere, particularly in light of the ferromanganese and alloy activities in the region.

8.2.2 **Manganese demographic trends and potential source and pathway**

There is no significant difference in bone manganese between any of the demographic groups in the sample population. Black females, white males and black males have bone manganese concentrations of 0.41, 0.44 and 0.48$\mu g \cdot g^{-1}$ respectively. The lack of significant difference in mean bone manganese, as well as the low concentration relative to other countries suggests a common environmental source of manganese exposure affecting all individuals. As discussed in chapter 4, Gauteng is home to substantial ferromanganese processing facilities, predominantly iron and manganese smelting and steel and alloy fabrication (Dme 2005; Moja et al. 2013).

PCA analysis and subsequent correlation and linear regression demonstrate significant, positive relationships between manganese and vanadium in the sample population. This is a strong indication that manganese (and vanadium) exposure is related to metal processing, as both elements are used extensively in the manufacturing of steel and other metal alloys. Both elements are, however necessary for bone health and their association may be metabolic as

well as environmental (Smith and Huyck 1999). PCA determined that in the whole sample population, as well as for black and white males, manganese and vanadium were always factors which loaded highly in Component 1 of each analysis.

In the sample population, $r_s = .629$, $p < .001$ between manganese and vanadium. The relationship is stronger in black individuals with $r_s = .635$ and .648, $p < .001$ in females and males respectively, than in white males at $r_s = .583$, $p < .001$. The relationship also follows a positive linear trend. Coal burning is also another potential source of manganese for the sample population. South African coal, whilst low concentrations of most trace metals, has slightly higher manganese and vanadium concentrations than coal from regions such as North America (Wagner and Hlatshwayo 2005).

Despite the scale of manganese processing in South Africa, it is unlikely that any individuals within the sample population were directly involved in manganese smelting or mining. Bone manganese concentrations are well below what would be expected for individuals in these professions (Pejović-Milić et al. 2009). Another potential source of occupational manganese exposure is welding, and it is possible that the individuals with bone manganese concentrations above $2\mu g \cdot g^{-1}$ were involved in some sort of occupation such as welding, where the likelihood of manganese inhalation is high (Pejović-Milić et al. 2009). The low manganese concentrations of the sample population are most likely to result from exposure to low-levels of manganese in the atmosphere resulting from manganese processing and as well as coal burning.

It is difficult to compare manganese concentration in the sample population to present-day studies of manganese distribution in the South African population. The addition of MMT as a lead replacement in South African petrol in 2000 has likely resulted in an increase in manganese exposure in urban areas. This means that present-day tissue concentrations of manganese are likely to be higher than those in the sample population, particularly in high-traffic urban areas.

Bone manganese concentration, unlike lead, does not differ among individuals of different ages. It is unclear what influence age has on bone manganese. Pejovic-Milic et al. (2009) did not find a relationship between age and bone manganese. Early research into bone element concentration did, however find a negative correlation between bone manganese and age, with decreased manganese in older individuals (Tipton et al. 1968). Zaichick et al. (2011; 2009) report decreasing bone manganese with age in women and increasing bone manganese with age in men. In this research, bone manganese does not change significantly with age in either males or females, regardless of race.

Several authors have reported higher levels of bone manganese in women than in men. Zaichick et al. (2011) report mean a bone manganese concentration in women at 0.43μg·g^{-1} compared to 0.27μg·g^{-1} in men. Other authors have reported both sex difference in manganese uptake and postulated that it may be a function of iron uptake, with lower iron stores associated with higher manganese uptake (Finley et al. 1994; Kim and Lee 2011). The relationship between bone iron and manganese will be discussed below.

8.2.3 Geographic and temporal trends in bone manganese

There are no differences in bone manganese concentration between Pretoria, Johannesburg and rural residents. When each demographic group is examined individually, the result is the same with no differences based on city of residence. Similarly, there are no temporal trends in bone manganese. These results indicate that manganese exposure was relatively constant across both the landscape and across time. Unfortunately data regarding manganese concentration between Pretoria and Johannesburg is lacking, and any present-day studies of manganese in the region would be highly influenced by the presence of MMT and would not be a useful tool for comparing past distributions. However, cautious extrapolation is possible. As has been discussed previously, the predominant source of manganese in the Gauteng/Transvaal environment prior to the introduction of MMT was ferromanganese smelting. This activity releases manganese mainly into $PM_{2.5}$ which is known to have a wide dispersal pattern and long airborne "lifetime". Potentially, this may have resulted in a more even dispersal of manganese across the landscape and a more uniform rate of exposure among the population.

There are no significant increases or decreases in bone manganese concentration across time in the sample population. This result was expected, as MMT, the cause of increasing manganese exposure in urban South Africa was not widely introduced until approximately four years after the most recent date of death included in the sample.

8.2.4 Bone manganese and essential trace elements

8.2.4.1 Iron

Iron is known to have a complex relationship with manganese. Previous research has found significant correlations between bone manganese and bone iron in both males and females (Brodziak-Dopierala et al. 2009; Kuo et al. 2000). Recent research suggests that dietary iron can reduce uptake of even inhaled manganese, which is the predominant pathway of exposure in most populations (Davis et al. 1992; Ellingsen et al. 2003; Kim et al. 2012; Kim and Lee 2011; Kim et al. 2005; Thompson et al. 2006). Moreover, manganese is known to inhibit iron absorption (Hansen et al. 2010; Rossander-Hultén et al. 1991).

It is possible that if iron deficiency is causing increased uptake of manganese this process is acting on the whole sample population albeit to different degrees.

With regards to manganese source and pathway, given the predominance of steel production in Gauteng, and the use of both iron and manganese in the South African steel industry, it is unusual that the two element concentrations would not be correlated in the black population. Worldwide, iron and manganese tend to be correlated as they are two of the main constituents of PM_{10} (large particulate matter) and South Africa is no different (Dusseldorp et al. 1995; Karar et al. 2006; Moja et al. 2013).

The difference in bone iron concentration may play an important role in manganese uptake. In white males, there is a significant, positive correlation between bone manganese and iron, $r_s = .615$, $p < .001$, and a moderate linear relationship, $R^2 = .384$, $p < .001$. This relationship is not evident in either black males or females. Several authors have suggested that in states of chronic iron deficiency, manganese uptake into the nervous system is increased. In the case of white males, this is one explanatory factor for why manganese concentration is similar to that of black individuals, yet iron concentration is so low. In these individuals, manganese may potentially be deposited preferentially in the nervous system as opposed to bone (Aschner and Aschner 1990; Erikson et al. 2002; Kim et al. 2012).

8.2.5 **Manganese and health in the sample population**

8.2.5.1 Manganese in males and females

As the use of bone manganese as a biomonitor of human exposure is in its infancy, it is challenging to postulate what effects manganese exposure may have had on the sample population. While there is no benchmark dose in bone to correlate to specific degrees of toxicity, it is certainly possible to make educated generalisations about the potential health consequences associated with the reported bone manganese concentrations. As discussed in Section 8.2.2 above, manganese exposure in males in the sample population can be considered low to moderate, with a few individuals who were more highly exposed.

Low-level manganese exposure is associated with mild cognitive and central nervous system damage. Luccini et al. describe neurological symptoms including tremors, irritability, loss of balance and rigidity (of muscle tissue) in ferroalloy workers exposed to low levels of manganese. The authors however, report environmental manganese concentrations of up to 500 times those reported by Yousefi and Rama (1992) in Johannesburg. It is likely that only the individuals with the higher bone manganese concentrations – those individuals with concentrations above $2\mu g \cdot g^{-1}$,were exposed to this level of airborne manganese. Among these males, the likelihood of mild neurological dysfunction was high.

Nonetheless, like lead, manganese exposure and toxicity is cumulative and exposure across time is associated with increasing toxicity. Mergler et al. (1999) describe a continuum of toxicity in non-occupationally exposed individuals and indicate that very low levels of manganese exposure can cause sub-clinical to mild symptoms that worsen with worsening exposure. Roels et al. (1987) report mild neurotoxic symptoms in a low to moderately exposed population. Exposed subjects showed significant deficiencies in visual reaction time, short term audio-verbal memory along with hand tremor.

Manganese found in finer PM is believed to be more toxic to humans than that found in PM_{10}. The fine particles produced by the manganese smelting activities in Gauteng may have been more hazardous to human health than that from MMT. Research involving rats exposed to inhaled fine-particle manganese demonstrates that smaller particles can remain in lung tissue up to 500 days after exposure. Some of this is taken into bone, where it is safely sequestered, but that which remains in lungs is still available to uptake into the brain and central nervous system (Weiss 2006). In addition very fine manganese particles can be translocated directly to the brain from the olfactory epithelium (Oberdorster et al. 2004; Tjalve and Henriksson 1999). In effect, the lungs become a reservoir for manganese alongside bone (Andersen et al. 1999).

The possible health implications of this in the sample population are not inconsequential. A moderate manganese concentration in the lungs, similar in concentration to that found in bone is a significant amount and can cause more than just mild neurotoxicity. Standridge et al. (2008) found impaired standing balance in adults exposed to low levels of environmental manganese, which would include the majority of the sample population. The study participants who exhibited displays of "postural sway' when standing were thought to be experiencing the effects of subclinical manganese toxicity. Lucchini et al. (2012) found an increase incidence of Parkinson's Disease attributed to manganese exposure in Valcamonica, Italy, adjacent to a ferroalloy processing plant. The authors also report mean airborne and soil manganese concentrations of 49.5 ng/m^3 and 958$\mu g \cdot g^{-1}$ in the same city, significantly lower than the atmospheric manganese concentration reported by Yousefi and Rama in Johannesburg, but higher than the soil manganese concentrations reported by Olowoyo et al. (2009) in Pretoria. Lucchini et al. studied adolescents living in Valcamonica and found significant and marked motor dysfunction and olfactory impairment in teenagers exposed to long-term low level manganese. Hand tremor in the participants was linked to hair manganese concentration. Lastly, the authors note that olfactory impairment is strongly linked to Parkinson's Disease and is an early symptom, with 75% of Parkinson's individuals suffering from olfactory dysfunction (Zoni et al. 2012). Soil manganese testing was not conducted in Johannesburg during apartheid, and it is difficult to compare modern soil

samples from Pretoria with airborne samples from Johannesburg, particularly as limited environmental studies show higher concentrations of all toxic elements in Johannesburg. However the airborne manganese concentrations measured in Johannesburg are much higher than those of Valcamonica, and as PM manganese is likely more toxic, it can be suggested that individuals in Pretoria and Johannesburg were likely affected by manganese in the same way as individuals in Valcamonica. The prevalence and epidemiology of Parkinson's Disease in South Africa is unknown (Carr et al. 2009; Dotchin and Walker 2012).

What is clear is that the sample population were likely exposed to levels of manganese that were high enough to cause subclinical toxicity and mild symptoms, leading to a potentially higher incidence of neurological disease than would otherwise occur. In some individuals, higher bone manganese concentrations would likely have had mild clinical symptoms of manganese neurotoxicity. Bone manganese concentrations suggest chronic long-term exposure, which is known to have a cumulative effect in the body.

8.2.5.2 Manganese in females

There are no health effects of manganese that are specific to women however manganese exposure during pregnancy is particularly dangerous to the developing fetus. To date, few studies have examined the effects of prenatal manganese exposure in children however there is evidence to suggest that low-level environmental manganese exposure in utero is associated with cognitive deficiencies in young children. Takser et al. (2004; 2003) found significant negative correlation between cord blood manganese concentration and attention, non-verbal memory and hand coordination at age three. Henn et al. (2010) have also found that low-level environmental manganese exposure during the early postnatal period is associated with adverse effects on infants neuro-motor development. Other, recent research has suggested that low-level manganese exposure during pregnancy leads to infants with higher body mass (Ponderal Index), which may result in a propensity towards obesity later in life (Eriksson et al. 2003; Yu et al. 2012).

The results of this research show that the sample population was likely exposed to low-to moderate environmental exposure to manganese, enough to cause subclinical to mild clinical neurological affects. Several males, both black and white, showed elevated bone manganese levels indicating that they were potentially involved in smelting activities, or were living near smelting facilities. These males would have shown some cognitive and neurological symptoms of mild manganese toxicity.

8.3 Cadmium

8.3.1 **Overall trends in bone cadmium concentration**

Bone cadmium in the sample population is as low, or lower, than reported bone cadmium concentrations in industrialised countries. Mean bone cadmium concentration for the sample population as a whole is 0.03$\mu g \cdot g^{-1}$. This mean is lower than values reported for Spain, Czech Republic and Russia and substantially lower than reported bone cadmium concentrations from Poland, Korea and Taiwan. The values reported here are commensurate with those reported by Lindh et al. (1980) in non-smoking industrially and non-industrially exposed individuals living in a low-pollution area in Sweden. The bone cadmium concentrations reported by these authors were all below .05$\mu g \cdot g^{-1}$ in cortical bone. The low mean cadmium concentration suggests that few individuals were occupationally exposed to cadmium and that environmental exposure was low. Bone cadmium concentration in non-occupationally exposed individuals has been reported to be as high as 0.2$\mu g \cdot g^{-1}$.

There is little data regarding bone cadmium concentration as it relates to occupational exposure. Less than 10% of white or black males in the sample population have bone cadmium concentrations above 0.15$\mu g \cdot g^{-1}$ indicating potential occupational exposure, though in low concentrations. It is potentially more likely that these males were smokers, which will be discussed below (Morgan et al. 1990). Lastly, the health effects of low-level cadmium exposure, such as that which characterizes the sample population, are just beginning to be recognised.

8.3.2 **Demographic and potential source and pathways**

Mean bone cadmium concentration does not vary significantly between black males and females, nor between black and white males, however the difference between black females and white males is significant, with higher bone cadmium concentrations in white males. Mean bone cadmium for white males, black females and black males is 0.030, 0.036, and 0.027$\mu g \cdot g^{-1}$. However the maximum bone cadmium concentrations in both black and white males are more than double the maximum concentration of black females at 0.187 and 0.261$\mu g \cdot g^{-1}$ respectively. The lack of difference in cadmium concentration in women is not in accordance with the literature, in which the trend is towards higher cadmium uptake in women than in men. Several studies have found higher cadmium uptake in women and higher bone cadmium concentration in women as well (Berglund et al. 2011; Menke et al. 2009; Satarug et al. 2004; Vahter et al. 2002). However, it has been suggested that the difference in cadmium uptake in women may be due to iron status. Olsson et al. (2002) found that despite a lower cadmium intake women had higher cadmium uptake than men, which was attributed to lower iron status in women. In this sample population however, this

phenomenon may be reversed, with white makes having much lower iron status than women, though other researchers have reported that low iron stores are not associated with greater cadmium uptake in males (Satarug and Moore 2004; Satarug et al. 2004). In no group is bone iron concentration correlated with bone cadmium concentration. The individuals in this study with highest cadmium concentrations among both males and females also have lower iron concentrations, though not the lowest, and the relationship is not significant.

The likely sources of cadmium in the sample population are not clear, however in the case of males, PCA does identify a component that suggests tobacco smoke. Dietary ingestion of cadmium is the most common pathway worldwide, and it South Africa unlikely to be different, though studies of dietary cadmium in South Africa are lacking. Cadmium is often present in coal, however levels of cadmium in South African coal are low (Wagner and Hlatshwayo 2005). Street et al. (2008b) found high levels of cadmium in three of the most popular traditional medicinal herbs used by many black South Africans. This indicates the presence of cadmium in soil and reaffirms the possibility of dietary intake of cadmium in the sample population. Potentially, contamination of vegetables and plant foods is responsible for cadmium exposure in the sample population. Were medicinal plants playing a significant role in cadmium exposure, it would be expected that black individuals would have higher bone cadmium concentrations than white individuals, which they do not. As discussed in above, cadmium and lead only appear to be related in black males. PCA analysis of males shows that cadmium is a factor in two components and is only associated with lead in black males. The fact that cadmium is not related to lead at all in white males, who likely had higher exposure to pollution from traffic is curious.

De Villiers et al. (2010) however, found a high correlation between cadmium and lead in soil sample sites near urban Johannesburg, suggesting a common source. This is the opposite trend found in lichen studies conducted in Johannesburg and Pretoria, in which cadmium is not correlated with lead in high traffic areas but highly correlated with lead in mining/smelting areas (Monna et al. 2006; Olowoyo et al. 2011). De Villiers et al. suggest that due to the correlation of lead with cadmium, vehicle emissions is the most likely source of cadmium in Gauteng, however data from lichen studies would seem to contradict this. Naicker et al. (2003) report both cadmium and lead in AMD in Johannesburg, but when bivariate correlation was performed on this data, there was no correlation between cadmium and lead in water samples.

Another potential source of cadmium is gold mining activities, particularly in males. Naicker et al.'s data regarding AMD from gold mine tailings shows a strong correlation between

arsenic and cadmium in mining runoff, r_s= .888, p < .001. In both black males and white males, bone arsenic is significantly correlated to bone cadmium, r_s = .372, p < .001 and r_s = .618, p < .001, respectively. There is no correlation between the two elements in black females. The same relationships are present and significant between cadmium and manganese, in both Naicker's data and the males in this study, but not in black females. PCA identified arsenic, manganese, and vanadium and cadmium in Component 1, in white males and manganese, vanadium and cadmium in Component 1 of black males. Vanadium also correlates strongly to cadmium in both white and black males, but not in black females. As discussed above, manganese and vanadium are both processed in Gauteng, and both are associated with PM in the region. The association with cadmium and these two elements in males indicates that they may be more highly exposed to industrial or mining sources than females.

There is one significant source of cadmium that may be a factor in male exposure: tobacco. Tobacco cigarettes are known to contain cadmium copper and zinc in high concentrations and in heavy smokers can be a significant source of these elements (Chiba and Masironi 1992). Over 50 percent of black males, 40 percent of white males and only 10 percent of black females smoke tobacco in South Africa (Sitas et al. 2004). PCA revealed that cadmium, copper and zinc are factors in Component 2, in both black and white males, however cadmium and zinc are not significantly correlated. Cadmium exposure in black males could potentially be due to inhalation of cadmium (as well as copper) from cigarette smoke, however the same relationship between elements should be found in white males as well. Subsequent bivariate correlation shows that none of these elements are correlated in black females, which would be expected given the low prevalence of smoking among this group. The three elements are significantly correlated in black and only copper and cadmium are correlated in white males.

The possible explanations for higher cadmium in white males versus black females are probably related both to degree and source of exposure. White males in the sample population were more likely to be exposed to traffic pollution, given residential patterns and bone lead concentrations and more likely to be smokers than black females.

8.3.3 **Geographic and temporal trends in cadmium**

There are no differences in bone cadmium between Johannesburg, Pretoria or rural areas. This result was expected. In Chapter 4, bar graphs generated using data from Monna et al. and Olowoyo et al. showed no difference in lichen cadmium between Pretoria and Johannesburg. Though these graphs are based on present day data, it is not expected that environmental cadmium has changed significantly in either city during the last two decades.

There are also no differences in bone cadmium across time in any demographic group within the sample population. Again, this result was expected, as there is no literature suggesting that potential sources of cadmium changed significantly from the 1960s to the 1990s.

8.3.4 Cadmium and health in the sample population

The low bone cadmium concentration across the sample population indicates that the individuals that comprise it were likely not suffering from serious clinical effects of cadmium toxicity. The highest bone cadmium concentrations in the study population are significantly lower than those of individuals suffering from *Itai Itai* disease. Noda et al. (1990) measured bone cadmium concentration in Japanese patients suffering from the disease and controls. Mean bone cadmium concentration in sufferers was 1.9 to 2.7$\mu g \cdot g^{-1}$ and mean cadmium concentration in controls was 0.5. The highest measured bone cadmium concentration in this sample population is 0.2$\mu g \cdot g^{-1}$, lower than Noda et al.'s control group.

Subclinical cadmium toxicity may have affected a small minority of individuals in the sample population. There is a significant and growing body of evidence suggesting that low-level and chronic exposure to cadmium is associated with serious health effects in both males and females. Most prevalent among these effects is damage to bone and renal tissue. Both osteopenia and osteoporosis are found in populations exposed to low level cadmium, particularly in older individuals with long-term exposure (Brzoska and Moniuszko-Jakoniuk 2004). Alfven (2000) report an increased odds ratio of osteoporosis in men and women exposed to cadmium versus controls. The odds ratio was also higher in males than in females at 2.2 and 1.8 respectively, indicating that men are as likely if not more likely to suffer osteoporosis after cadmium exposure than women. Men with low-level cadmium exposure and poor diets (low intake of fruits and vegetables) are particularly at risk for cadmium induced bone fracture (Thomas et al. 2011). Still other studies have found that the bone effects of low cadmium exposure are greater in women than in men (Vahter et al. 2007). This low-level exposure may also cause significant renal damage (including diabetes) as well (Alfvén et al. 2002). In 2004, the Swedish study OSCAR (OSteoporosis – CAdmium as a Risk factor), found that both bone and renal damage caused by cadmium occurs at a concentration far lower than previously believed and that no level of cadmium is safe (Jarup and Alfven 2004).

Interestingly, in the South African population as a whole, the white population has higher rates of both osteoporosis and diabetes. Whilst differences in diet likely play a prominent role in this phenomenon, given the data gathered on the sample population, there may potentially be environmental factors such as exposure to toxic elements that play a role as well.

8.3.5 **Bone cadmium and essential trace elements**

8.3.6 **Iron**

Among the greatest risk factors for increased cadmium uptake and toxicity is low body iron stores (Andersen et al. 2004; Reeves and Chaney 2002). This certainly describes the sample population, particularly white males, who have significantly lower bone iron concentrations than either black males or females. Not surprisingly, it is also white males in the sample population that also have the highest concentrations of bone cadmium. The relationship between iron and cadmium in men however, is unclear, primarily due to the lack of focus on low iron stores in men. Most studies concerning the relationship between iron deficiency and cadmium uptake focus on women, however there is little reason to believe that iron deficient males would not also be affected by increased cadmium uptake. In fact, iron status may be a more significant determinant of cadmium uptake than the amount of cadmium in the environment (Vahter et al. 1996) and iron status is now believed to be the primary reason why, in most studies, women have higher tissue cadmium concentrations than men. This means that the white males in the study population in particular, may have been most susceptible to cadmium uptake. This may, in part, explain the greater bone cadmium concentrations found in white males versus black males and females in the study population.

8.3.7 **Zinc and copper**

Zinc, copper and cadmium have a complex relationship in human tissues. Firstly, the three elements are known constituents of tobacco, and have been associated with smoking (Bernhard et al. 2005). In addition, the presence of cadmium in the body is known to increase zinc and copper uptake in kidney and liver tissues (the relationship in bone has not been assessed) (Satarug et al. 2001). In the sample population this complexity is further emphasized by a lack of consistency between these relationships and previously published studies. In this population, no individuals show positive correlation between cadmium and zinc in bone, and only black males show a correlation between cadmium and copper. As discussed in Section 8.3.2, this could be an indicator of smoking in black males. Bone zinc concentration is significantly lower in black males and females than in white males, adding further complexity to the issue.

The relationship between zinc and cadmium is well researched. Zinc homeostasis within the body is disrupted by cadmium, and zinc deficiency, in turn, may increase uptake of cadmium. The primary mechanism may be competition for metallothionein. However, other elements play a role in zinc uptake, particularly in bone and given the lower concentrations of cadmium in black males, it is unlikely that cadmium alone is affecting zinc uptake.

Vanadium also is known to reduce zinc uptake into bone tissue, and may be causing suppression of zinc uptake in black males in the sample population.

8.4 Antimony, Arsenic and Vanadium

Antimony, arsenic and vanadium are discussed together because little has been reported regarding the concentrations of these two elements in bone tissue. The toxicological effects of each element are just beginning to be understood and vanadium is, like manganese, also an essential trace element. Both are present in PM matter and are industrial pollutants. Antimony has been associated with Heavy Goods Vehicles (HGVs) due to its use in brake pads in HGVs and has been mined in South African provinces adjacent to Gauteng. Vanadium is mined in South Africa and is used in steel and alloy production in Gauteng. The source of arsenic is more ambiguous, but it is present in AMD and is found in lichen in both Pretoria and Johannesburg. Unfortunately these three elements are not generally studied in bone, and it is difficult to determine how the sample population compares to other populations in regards to exposure. However some interesting patterns emerge with regards to these elements, particularly in relation to elements such as lead, cadmium and manganese.

8.4.1 **Overall trends**

8.4.1.1 Antimony

Antimony shows clear differences in distribution within the sample population. Black individuals have significantly higher bone antimony concentrations than white individuals, by an order of magnitude. In black males and females median bone antimony is $0.289 \mu g \cdot g^{-1}$ and $0.280 \mu g \cdot g^{-1}$ respectively, compared to $0.016 \mu g \cdot g^{-1}$ in white males. For black individuals, these concentrations are substantially higher than those reported in bone from Sweden, Czech Republic or Russia. The highest bone antimony concentrations reported in cadaver bone are $0.015 \mu g \cdot g^{-1}$ in Sweden in the 1980s. This is analogous to the concentration found in white males in this sample population. This indicates that black individuals were likely significantly more exposed to antimony and had higher uptake of antimony than white individuals. Despite these few studies examining antimony in bone, there is no literature on the level of antimony at which clinical toxicity emerges, even in tissues other than bone (Filella et al. 2011).

Unlike other elements measured in this study, antimony concentration changes considerably over time. Individuals who died in the 1960s had significantly higher bone antimony concentrations than individuals living in any other decade. This trend is significant in both black and white individuals. This trend was quite unexpected, given the presence of antimony in Pretoria and Johannesburg in the present day. It is unclear why there was a

decline following the 1960s and whether the sample population may have had higher antimony exposure than living populations in South Africa. Also unexpected, given present-day data, is a lack of any difference in antimony concentration between individuals from Johannesburg and Pretoria in the sample population. Data from Monna et al., (2006) and Olowoyo et al. (2010) show that antimony levels are higher in lichen in Johannesburg than in Pretoria, yet that difference is not present in the sample population.

It is unclear as to the source of antimony exposure in the sample population. Present day data shows a clear correlation between high-traffic areas and antimony. In all areas, antimony is highly and significantly correlated with lead, $r_s = .950$, $p < .001$. There is no corresponding correlation between lead and antimony in any group in the sample population. Whether this is due to differences the uptake and kinetics of each element in humans is not clear. It could also be an indication that, at least in the sample population, the primary source of antimony exposure was not traffic-related as it appears to be in the present day. This hypothesis is further supported by the higher concentrations of bone lead found in white males, which are very strongly correlated with traffic. It would be expected that if antimony exposure in the sample population was linked to exposure to automobile emissions, white males would have the highest bone antimony concentrations as opposed to the lowest and that there may be some correlation between the two elements in white males.

The difference in the degree of exposure to antimony between black and white individuals is striking. Were antimony largely atmospheric in origin, it would be expected that whilst differences in exposure may be present, the difference in antimony concentrations between any one group and another would be significantly smaller than the 15-fold difference between that occurs between black and white individuals.

8.4.1.2 Arsenic

Arsenic follows the same trend as antimony, in that it is significantly higher in black individuals than in white males in the sample population. Median bone arsenic concentration is $0.114 \mu g \cdot g^{-1}$ and $0.128 \mu g \cdot g^{-1}$ in black males and females respectively and $0.71 \mu g \cdot g^{-1}$ in white males. These concentrations fall within the values reported for individuals in Poland and significantly below those reported for populations in Taiwan or Korea.

Arsenic concentration in the sample population changes significantly, but not substantially, over time, nor does it differ between individuals between cities. It is not possible to compare arsenic to either present day or historic concentrations. Arsenic is not and has not been widely monitored in South Africa and there is little environmental data with which to compare bone arsenic and environmental arsenic concentrations. It is also difficult to

determine the likely sources of arsenic in Gauteng. Arsenic has been associated with AMD and is present in mine tailings linked to gold mines in Johannesburg. It is unlikely to be associated with coal burning, as South African coal is unusually low in arsenic compared to coals from other regions (Wagner and Hlatshwayo 2005). Kempster (2007) has measured arsenic in ground water in Gauteng and found that arsenic in water in and around Johannesburg and Pretoria are elevated relative to other parts of the province. Other studies have found moderately high arsenic concentrations in groundwater and soil in the region. It is likely then that arsenic is ingested as opposed to inhaled, with water as the most likely source of the element in the sample population. Subsequently, it may be that the location of many of the townships and black residential areas adjacent to mine dumps may partially explain the higher bone arsenic concentrations in black individuals than in white, particularly given the poor quality of water used by residents of townships. In addition, most of the water consumed in Gauteng, particularly in the townships is surface water which is highly susceptible to the uptake of elements such as arsenic from AMD. Similarly to lead, in the case of arsenic, apartheid-era residential policies may have affected the distribution of arsenic exposure across the population.

Bone arsenic concentration in the sample population is not correlated with lead in any group, which would be expected were arsenic exposure linked with pollution from traffic. Arsenic is not correlated with lead in AMD, a potentially significant source of arsenic in the environment and in the sample population. Arsenic is correlated with cadmium in both white and black males as it is in AMD, with $r_s = 0.618$, $p < .001$, $r_s = 0.327$, $p < .001$ and $r_s = 0.888$, $p < .001$ respectively. There is no correlation between bone arsenic and bone cadmium in black females. The correlation in males may be due to the greater exposure to AMD (living in the vicinity of mining operations as mine labour). It could also be due to presence of arsenic in cigarettes. Until the 1980s (and beyond, in many countries) arsenic was a widely-used pesticide in tobacco growing (Lindberg et al. 2010). This leads to elevated levels of arsenic in cigarettes. Smoking may also reduce arsenic methylation, leading to increased arsenic uptake in smokers. The correlation between arsenic and cadmium in males but not females may indicate that in males, smoking may be a source of arsenic. Also notable is the correlation between arsenic and manganese, again in white and black males but not in black females, another indication of different sources in males and females.

Within the sample population, there is significant correlation between antimony and arsenic in all groups. This is expected, as the two elements are similar in chemical properties and are often co-contaminants from mining and industrial activities (Gebel 1998; Gebel et al. 1998). Unfortunately no studies of environmental pollution in South Africa have included both

elements or established a correlation between them in the South African environment. Nonetheless, the association between arsenic and antimony in bone tissue is not surprising, given the presence of mining activities in the region. What is surprising is that whilst antimony concentration in the sample population declines significantly over time, arsenic concentration does not decline nearly as sharply, even when its use in medicine is taken into account (see below). The cause of this remains unknown however, as discussed above, there were alternative sources for arsenic in the environment that are not associated with antimony. The significant drop in antimony concentrations and the more minor drop in arsenic concentration may indicate that one, antimony-arsenic producing exposure source diminished, but that other sources of arsenic persisted.

Among the more interesting findings regarding arsenic in the sample population, is the association between high levels of arsenic ($> 1\mu g \cdot g^{-1}$) and cancer as cause of death. Arsenic is a known carsinogen (Buchet and Lison 1998; Mink et al. 2008; Moore et al. 2002; Ng et al. 2003; Park et al. 2012; Tsai et al. 1999). Arsenic is a well-known component of chemotherapeutic drugs and even in the present day, it is used in chemotherapy as arsenic trioxide for specific types of cancer (Waxman and Anderson 2001). In the 1960s, it was used to treat a broad spectrum of cancers before its toxic side effects caused a more targeted use. Approximately 30% of individuals across the sample population have bone arsenic concentrations well above what would be expected for environmental or even occupational exposure. Closer inspection of cadaver records shows that the cause of death for many of these individuals was cancer, and that a significant majority of individuals with high arsenic concentrations died in the 1960s. In individuals with bone arsenic concentrations above $1\mu g \cdot g^{-1}$, the chi-square distribution shows that the relationship between very high arsenic concentrations and decade of death is significant, $X^2(3) = 8.23$, $p < .05$. This suggests that arsenic compounds may have been used for medicinal purposes in the 1960s, but was not used substantially in the following decades. Despite the carcinogenic properties of arsenic, as well as its use in cancer therapy, high concentrations of arsenic are present in individuals from the sample population whose cause of death was not cancer, causing a lack of significant correlation between cancer and arsenic. Thus the relationship between arsenic and cancer in this population can only be considered anecdotal, at best.

8.4.1.3 Vanadium

Vanadium is present in all individuals in the sample population, though at lower concentrations than suspected, given the use of vanadium in smelting and alloy production. Mean bone vanadium in the sample population is $0.026\mu g \cdot g^{-1}$. Only one published study, from Korea, could be identified in which bone vanadium was included, and the mean bone vanadium concentration reported was $1.3\mu g \cdot g^{-1}$, significantly higher than that reported here.

Navarro et al. (1992) measured similar values in cortical bone but significantly higher bone vanadium concentrations in individuals with chronic renal failure ($2.3\mu g \cdot g^{-1}$). No individual in the study population had a bone vanadium concentration above $0.125\mu g \cdot g^{-1}$.

Like antimony and arsenic, bone vanadium is significantly higher in black males and females than in white males at $0.026\mu g \cdot g^{-1}$, $0.032\mu g \cdot g^{-1}$ and $0.020\mu g \cdot g^{-1}$ respectively. Between black males and females, the difference is significant, with black females having higher bone vanadium concentrations than black males. Also similar to arsenic and antimony, vanadium concentration is highest in black females. It is not clear why this would be so. Little is known about the toxicology of vanadium (or antimony) and there is little knowledge regarding sex differences, if any exist, in uptake and metabolism of either element. Research does suggest that iron is critical to the uptake of vanadium (Sabbioni and Marafante 1981). If the low bone iron concentration in white males is a reflection of in vivo iron status, it is possible that the low iron status of white males is suppressing iron uptake resulting in vanadium. It may also be possible that the higher iron status of black males and females is increasing vanadium uptake from the environment. Unfortunately, despite its affinity for bone, and the recent suggestions by some that bone tissue be explored as a potential biomonitor for vanadium exposure, there are no established reference values for vanadium in bone. There is a strong positive correlation between iron and vanadium in white males, $r_s = .611$, $p < .001$, but not correlated in black males or females. And, despite a lack of statistical significance, it is clear that the low iron stores of white males in the sample population may be affecting bone vanadium concentration.

There are two potential sources for vanadium in the sample population: metal smelting and steel production and fossil fuel burning. As discussed in Chapter 4, vanadium is mined in South Africa and processed in Gauteng, where it is used to make steel and other alloys. It is strongly correlated with manganese – also used in steel processing - in all individuals in the sample population. Vanadium is also a well-known component of PM throughout the world and is associated with fossil fuel (coal, petroleum) burning. Vanadium is significantly correlated with arsenic and cadmium in all males, but not in females which could indicate its presence in tobacco.

8.4.2 **Arsenic, antimony and vanadium and health in the sample population**

The toxicity of vanadium and antimony are not well understood, and levels of these elements in the sample population are likely below any toxicity threshold. Antimony in conjunction with arsenic is known to have carcinogenic properties and is associated with cancer in animals (Gebel 1997). Vanadium, particularly vanadium pentoxide, is associated with increased mortality in urban areas when inhaled as PM (Campen et al. 2001; Dominici et al.

2007; Woodin et al. 2000). To what extent the health of the sample population would be affected by these elements remains unclear.

Low level exposure to arsenic is understudied, but several studies have found that low-level arsenic, particularly in conjunction with elements such as manganese and activities such as smoking, can result in significant health effects (Chen et al. 2009b; Mink et al. 2008; Moon et al. 2012). Low concentrations of arsenic have been associated with the development of Type II diabetes in North America (Steinmaus et al. 2009). Arsenic in low concentrations may play a causative role in QT prolongation (the time between the Q wave and T wave of the heart beat), a risk factor for sudden cardiac arrest. It has been established that high levels of arsenic are associated with long QT intervals, however recent research suggests that even low-level arsenic may affect the QT interval (Mordukhovich et al. 2009). The cardiovascular effects of arsenic may be increased in the presence of manganese and cadmium. In adults, manganese and arsenic may cause hypertension and cardiovascular disease (Mordukhovich et al. 2012). The correlation between arsenic and manganese is significant in all groups in the sample population with r_s = .418 ($p < .001$), .442 ($p < .001$) and .361 ($p < .01$) in black males, white males and black females respectively. In light of these correlations and the exposure of the sample population to low-level arsenic and manganese, there is the potential that some individuals in the sample population suffered from the cardiovascular effects of arsenic exposure.

8.5 Summary and research objectives

This project has set out to address six specific research objectives, each of which is discussed below.

8.5.1 Objective 1: Differences in element exposure between black Africans and white South Africans

This research has aimed to quantify the racial differences in toxic element exposure in the sample population. The bone concentration of six toxic elements was measured using cortical bone from 215 South African adults. Several clear racial trends were apparent when the results of this research were analysed. All toxic elements with the exception of manganese show clear racial disparities between black and white individuals. Most striking among these trends is that of lead exposure. White males show significantly higher bone lead concentration than either black males or females, at all age groups and across time. The potential causes of this are many-fold and include both lifestyle differences and political and social divisions between black and white individuals. In addition, the relationship between lead and other toxic and essential elements differs slightly for white males than for black males (and to a lesser extent black females) indicating that both degree and source of

exposure, as well as diet and health may be affecting the difference in lead concentration between black and white individuals. As discussed in Chapter 8, exposure to traffic is most likely the primary source of lead across the population.

Other elements which are also traffic or vehicle-related do not show similar racial disparity in bone element concentration. Cadmium, often associated with traffic pollution and PM emissions, does not vary between black and white males. It does vary between black females and white males. This is somewhat counter to trends often reported in the literature, in which women tend to have higher tissue cadmium concentrations. In South Africa this reversal of a common trend may be due to the fact that a one potentially significant source of cadmium exposure is tobacco smoke. White males in South Africa are significantly more likely to smoke than black females, which may explain this disparity.

Manganese concentration does not differ significantly between black and white individuals, indicating that between 1960 and 1999, manganese exposure may have been uniformly distributed across the population. The degree to which this may change in the future if South Africa continues to use MMT as a lead replacement in petrol will be discussed in Section 8.5.

There is little literature in South Africa regarding arsenic, antimony and vanadium exposure. To date, this is the first study to quantify these elements in human tissues in South Africa. Clear racial differences in bone antimony concentration are apparent. Unlike lead and cadmium, black individuals, both male and female, have substantially higher bone antimony concentrations than white males. This is surprising given the established association between traffic pollution and antimony, as it is the opposite of what would be expected given the clear trend in lead exposure.

Like antimony, arsenic concentration is also significantly higher in black individuals than white individuals, though the reasons behind this, as with antimony, are not clear. Bone vanadium concentration is also higher in black individuals than in white individuals, particularly among black females. This could be due to differences in iron uptake between black individuals and white individuals as opposed to differences in overall exposure to the element.

Overall, racial differences in toxic element exposure are prevalent in the sampled population. The causes of these differences are complex and vary between individual elements, but it is clear that overall, black individuals may suffer higher exposure to arsenic and antimony, whilst white individuals were more highly exposed to lead and cadmium.

8.5.2 **Objective 2: Toxic element exposure between black males and females**

Sex differences between black males and females were investigated and quantified. This relationship could not be investigated in white individuals due to the lack of white females in the sample population. Several trends are visible in the data. Overall, that data show significant sex differences in all toxic elements except manganese within the black population. For lead and cadmium, males show significantly higher concentrations than females. Bone lead concentration, for example, differs between males and females, with black females showing significantly lower bone lead concentration than black males. This difference concurs with bone lead trends reported in the literature, in which women tend to have lower tissue lead concentrations than males even in instances of similar rates of exposure. The difference in bone lead concentration between black males and females is particularly prevalent at older ages, indicating an expected loss of lead from bone in post-menopausal women, rather than a difference in exposure. At younger ages, particularly from the ages of 20-50, bone lead concentrations between males and females are rather similar and in some cases, are higher in females than in males.

Bone cadmium concentrations in black males are significantly higher than those of black females. As discussed above, this may be due to the presence of cadmium in tobacco, and the low prevalence of smoking among black females in South Africa. Surprisingly, and similarly to lead, cadmium uptake is affected by iron uptake and iron status. For this reason women tend to have higher bone (or other tissue) concentrations of lead and cadmium. In this sample population however, it is white males with the lowest bone iron concentrations, which may have significantly affected cadmium (and lead) uptake in white males.

The results for the elements antimony, arsenic and vanadium show the opposite trend. Black females have significantly higher bone element concentrations for these elements than black males. It remains unclear whether the higher levels of these elements in black women represent differences in exposure or metabolic differences.

8.5.3 **Objective 3: Inferring the health consequences of toxic element exposure in the sample population**

This objective aimed to infer the potential health effects of the recorded bone element concentrations in the population. Analysis of bone element concentration allows for the association between a given concentration and potential health effects that may occur as a result. This particularly true in the case of lead, for which decades of research into bone lead concentration and health has been conducted. In the sample population, it was determined that nearly all white males had bone lead concentrations above the threshold for hypertension and mild cognitive impairment. In black individuals the percentage is

significantly lower, yet over half of individuals still have bone lead concentrations at or below the threshold for hypertension or cognitive effects, indicating that with regards to health, lead exposure may have had a greater impact on the health of white individuals than black.

What is clear from this research is that a significant number of individuals in the population were exposed to toxic elements in high enough concentrations to cause impaired health. In a developing country such as South Africa, these ill-effects may are likely to have contributed to overall poorer health among the most disadvantaged members of South African society. The hypertensive, neurological and renal effects of many of these elements may have contributed significantly to the overall burden of disease for all members of society. In the case of already poorly nourished individuals, dietary inadequacies were likely exacerbated by exposure to toxic metals.

8.5.4 Objective 4: Toxic element exposure rates in relation to the environment in urban Gauteng and world-wide patterns

This objective has entailed the comparison of bone element concentrations in the sample population with cortical bone element concentrations from other industrialised populations worldwide. Overall, the degree of toxic element exposure in the sample population is lower than that of many other industrial regions such as parts of Europe, North America or Asia. Given the highly industrial and mining-based economy, relatively lax environmental regulations and the use of leaded petrol, it was expected that toxic element concentrations in human bone would be higher, particularly in the case of lead. The literature regarding toxic elements in the Transvaal/Gauteng environment is scant, however it indicates that phenomena such as acid mine drainage and PM emissions are widespread. When compared to highly industrialised regions such as Silesia in Poland, the level of human exposure to elements such as lead is far lower than expected. Lead concentrations in particular are lower than expected given the urban environment and the use of leaded petrol until the early 2000s.

Other elements, such as cadmium and manganese are also not as high as expected. The presence of ferromanganese smelting and steelworks in the urban region have not resulted in high levels of environmental manganese, nor have they resulted in high levels of human exposure to manganese. This may change in the future however as South Africa replaces lead with MMT in petrol. It is expected that human exposure to manganese will increase in the future. Initial studies of blood manganese in South African school children would seem to confirm this. Whether manganese exposure follows the same demographic trends as lead, given that it is largely dependent on exposure to vehicle pollution remains to be seen.

Cadmium is not present in the Gauteng environment in large quantities. South African coal, which is burned for heating and cooking in many communities and is often a source of cadmium exposure worldwide, is very low in cadmium. The level of cadmium exposure in the sample population is significantly lower than in parts of Asia, particularly Japan. Tobacco is a major source of cadmium worldwide, and given the trend towards higher cadmium levels in males in the study population and the prevalence of smoking among South African males, it is likely a significant source in South Africa. Whilst there is known cadmium mining in the Gauteng region, it does not appear to significantly contaminate the atmosphere or soil to an extent that it is causing significant human exposure.

Antimony concentrations in bone are significantly higher than in many European countries. Bone antimony concentrations in black individuals in this study are among the highest bone concentrations reported anywhere, and exceed previously reported bone antimony concentrations by an order of magnitude. Antimony is highly correlated with both lead and traffic in environmental studies, indicating that it is an atmospheric pollutant. This further confounds potential reasons behind the significant difference in antimony concentration between black and white individuals in the study who were likely less exposed to traffic pollution. It is unclear then, as to the source of antimony in the environment and human exposure rates are so high in the sample population.

Vanadium is mined and processed extensively in Gauteng, and is present in the atmosphere, though little monitoring has taken place in the urban environment. Despite this bone vanadium concentration is lower in the sample population than in other populations. Bone arsenic is also low in the sample population relative to parts of Asia and is similar to values reported in bone tissue from Poland. Arsenic is present in Gauteng, in higher quantities than other regions of South Africa due primarily to its presence in AMD from gold mining.

Overall, this research demonstrates that with the exception of antimony, toxic element concentrations in bone in the sample population are lower than in other 20th century populations. These results were unexpected given the prevalence of mining, smelting and industrial activities in the region.

8.5.5 Objective 5: Exploring the role of apartheid in explaining demographic differences in toxic element exposure

The potential effect of apartheid on toxic element exposure was explored. Apartheid policy created a unique population distribution in urban South Africa. The strict separation of racial groups and the relegation of black residential areas to the urban periphery adjacent to mining and industrial activities led to the working hypothesis that black individuals would

experience higher rates of exposure to all toxic elements. This has not proven to be the case. However, all elements with the exception of manganese do show striking racial disparities in bone concentration. Such disparities are seen worldwide, and in this sense South Africa is not unique, however it would be naïve to dismiss the impact of apartheid policies, and the corresponding disparities in health and welfare, on toxic element exposure within this population.

In the case of lead, the urban residential pattern created by apartheid policy seems to have highly influenced demographic patterns in lead exposure. The concentration of white residential areas in the urban core with the highest traffic and transportation activities has likely resulted in significantly higher lead exposure in the white population due to greater exposure to leaded petrol. Other studies in South Africa have linked blood lead in children to exposure to traffic, and the very clear residential patterns in both Pretoria and Johannesburg also confirm this. The inclusion of white females in this study may have provided more evidence of this.

In the case of antimony, arsenic and vanadium, the higher exposure of black individuals to these elements may also in some part, be explained by apartheid. As has been discussed in Chapters 4 and 8, arsenic is generally water-borne, and in Gauteng is associated with AMD. It is likely that the formation of townships and the strict residential policies of the Group Areas Act, which relegated black individuals to the urban periphery near mine tailings are partly responsible for the racial difference in arsenic exposure in the population. The extent to which this is true of elements such as antimony and vanadium are unclear, however the very distinct racial disparities in exposure to these elements cannot be ignored. On balance, it is likely that apartheid contributed greatly to racial disparities in toxic element exposure, either by direct exposure differences or differences in health that lead to greater uptake of certain elements. In other regions of the world these differences exist as well, along socioeconomic lines, however in South Africa during apartheid, socioeconomic status and race are largely one and the same.

8.5.6 Objective 6: Comparison of results with present-day studies of toxic element exposure and demographic trends in South Africa

The limited but increasing body of knowledge regarding toxic element exposure in South Africa was compared with the results of this research. No studies of toxic elements in humans were conducted in South Africa prior to the 1980s. Those studies that have been conducted have largely centred on blood lead studies in children. Present day studies indicate that lead exposure is high enough in Gauteng to cause concern, particularly in developing children. Overall, these studies have shown that in children, lead exposure is

higher in children of lower socioeconomic status. The difference in the trend in children versus the trend in adults in the sample population is clear. Lower status adults (i.e. black adults) clearly have lower lead exposure, at least in the sampled population. Studies of children in Cape Town do indicate that children exposed to traffic and main roadways have greater lead exposure than children in more suburban or rural areas. Studies of rural versus urban long-distance runners also show a clear association between traffic and lead. No study has framed this exposure in a racial framework based on apartheid residential policies.

Studies of manganese exposure in South Africa are only just emerging following on the use of MMT. These studies have shown an association with blood manganese and traffic in children. Though this trend is not seen in adults in the sample population, it is likely due to the fact that this population pre-dates the introduction of MMT. It may well be that in the future, white individuals in Gauteng suffer higher rates of manganese exposure and toxicity than black individuals due to residential patterns that persist in post-apartheid South Africa. However, residential patterns may be changing albeit slowly. In Chapter 4, post-apartheid demographic change is discussed. Since the end of apartheid many urban neighborhoods have become increasingly favoured by black individuals moving into the urban core that was once off-limits to them. There is a corresponding movement of white individuals to suburban neighborhoods. The result of this shift in residential patterns may be a shift or reversal of toxic element exposure in the population.

9 Conclusion

The investigation of human exposure to toxic elements is one important facet, among many, in understanding the overall public health status of a given population. As such, the information yielded during such an investigation is of great value. The same can be said of historical investigations such as this project, which provides insight into a substantial public health issue in South Africa, during a period when such issues were largely overlooked. This research has generated quantitative data regarding human exposure to toxic elements in urban South Africa during apartheid. Several population-wide trends have been uncovered, as well as trends among specific race and sex groups. In addition, apartheid-driven racial disparities in toxic element exposure have been uncovered, some of which were unexpected, but which begin to make sense when framed by sociological, political and environmental trends. Toxic element exposure continues to be a public health hazard in South Africa in the present day. It is hoped that by collating quantitative data on toxic element exposure in the recent past, as has been done here, that present day trends may be examined on a more longitudinal scale. This in turn, may allow for a greater understanding of both present-day and future trends in toxic element exposure.

This research began with one overall aim and six specific objectives. The overarching aim of the project was to establish a set of baseline data on toxic element exposure in urban South Africa during apartheid. The dataset generated has met this aim and provides valuable quantitative information regarding human bone element concentration for several toxic elements: lead, manganese, cadmium, arsenic, antimony and vanadium. The second major aim of this research was to investigate and provide quantitative data on the demographic and social trends and health implications of toxic element exposure within the study population, as well as the overall status of toxic element exposure in the study population in comparison with other industrialised nations.

To achieve these aims, this project has quantified toxic element exposure in a small population of urban South Africans who lived and died during apartheid. It has also required the synthesis of biochemical data with detailed sociological, biological and environmental analysis to produce a complete picture of the factors affecting and influencing human toxic element exposure in South Africa during the 20th century.

Toxic element exposure, especially lead, is of growing concern in sub-Saharan Africa. In South Africa in particular, the dominance of mining, metallurgy and heavy industry within the economy makes exposure to inorganic pollution a significant public health hazard. However the traditional reluctance to regulate mining and industry along with the need to

address more pressing public health issues such as AIDS, has meant the relative neglect of this facet of health in the population.

During apartheid, few studies of toxic element exposure were undertaken in South Africa and none concerned adult exposure in Gauteng. This research addresses that critical gap in knowledge and data. In addition this research provides important baseline data by which to contextualize and compare present and future trends in toxic element exposure in South Africa, particularly as the country continues to develop and grow beyond the legacy of apartheid.

From a sociological and anthropological perspective, one question that begs asking is what role lead exposure played and potentially still plays in the violence and social unrest that has plagued South Africa during the latter half of the 20th century. It is tempting to borrow from the philosophy of environmental determinism and infer that some of the social upheaval in the country is the result of less-than-ideal environmental conditions. If one considers that even moderate lead exposure causes a loss of IQ and a propensity towards violence, and many white individuals – males specifically – show lead levels high enough to been affected, it seems logical to conclude that the brutal way in which apartheid was enforced may be at least partly to blame on lead.

Returning to Nevin's (2000) now seminal research on the clear association between crime, violence and lead exposure, it is critical to consider the influence of lead on crime in South Africa. Nevin's data show that the years in which lead concentration in petrol peaked were also the years in which assault, rape and murder peaked across the United States. In 2000, approximately 20 years after lead was removed from the US petrol supply, the rates of these violent crimes have fallen to their lowest point in 40 years. In South Africa cessation of the use of lead in petrol took place less than a decade ago. In South Africa in 1980, the approximate median year of this project, lead concentration in petrol peaked. By the late 1990s, atmospheric lead concentrations were still elevated relative to Europe or North America. By Nevin's calculation, the lag between the drop in crime which corresponds to the reduction or cessation of leaded petrol is 20 years. In 2000, South Africa had among the highest rates of rape and homicide in the world, which corresponds clearly with a peak in lead 20 years prior.

In the urban environment of Pretoria and Johannesburg, the ejection of black individuals from the urban core and the sequestration of white residential areas in the most congested areas of the urban environment resulted in the high exposure of white individuals to lead for over 40 years during apartheid. As discussed in Chapter 1, one of the more bloody periods of apartheid occurred during the late 1980s and early 1990s, when white nationalists struggled,

often violently, to maintain white rule. This rise in apartheid-based violence also corresponds to a steady rise in the concentration of lead in South African petrol, as did the increasing anti-apartheid urban uprisings, which were also often marked by violence. Of course, violence in South Africa is as much a product of income inequity, abuse of human rights and racism as it is a product of environmental conditions, but it is highly likely that exposure to lead played a role. As the new generation of children born into a post-leaded petrol environment reach adulthood it will be most interesting to see if the rate of violence in South Africa declines as a result.

With regards to the association with between lead exposure and IQ, the effects may have been significant on the population during apartheid. Generally speaking a reduction in IQ is more devastating for the most disadvantaged in a given society. In South Africa, although black individuals may have had lower rates of exposure to lead, inadequate education, socioeconomic stress and poor health may have exacerbated even minor reductions in IQ among the exposed population. Children with stable economic situations, educated parents and food security are often better equipped to succeed in school regardless of lead-induced difficulties in concentration and cognition due to greater access to educational resources and support. This is rarely the case with impoverished children who, in urban Transvaal in particular were often attending sub-standard schools, living in chaotic and overcrowded homes and potentially struggling with poor health. The families of these children likely did not have the resources to help lead-affected children compensate for deficiencies. Moreover, the data presented here show that these individuals were more highly exposed to other toxic elements than white individuals, which may have compounded the effects of lead. In this way, the effects of urban lead exposure, while lower in the black population, may have been more pronounced, particularly in children. In turn, the loss of productivity of affected adults may have contributed to reduced economic opportunities within the black population, further exacerbating poverty. The phasing out of leaded petrol can only positively affect this situation.

The question arises however - has South Africa leapt from the frying pan and into the fire? The widespread use of MMT as a lead replacement in South Africa is troubling. The neurotoxic effects of manganese are clear, particularly as they relate to neurological disability. Whereas the population studied in this project had uniform and relatively low (compared to other countries) exposure to manganese, this will most certainly change. Increased manganese exposure is already being reported in urban school children. Will the pattern of manganese exposure mimic that of lead exposure during apartheid? It is not clear. Certainly, the heavy traffic areas of urban Gauteng include many white residential areas, and exposure to manganese in this population will rise. But the end of apartheid has meant the

unrestricted movement of the black African population, who are now free to live in neighborhoods once strictly off limits to them. Yet living conditions among new urban settlers haven't changed significantly and hundreds of thousands of informal urban households have arisen, often near transport networks and potential employment opportunities. These individuals are now likely exposed to similar levels of manganese as their affluent white compatriots. Whilst the effects of manganese exposure are not as dire as those of lead, the potential for disability among the affected may be greater. To date, South Africa has only a fledgling national health service, which is underfunded and overburdened and serves primarily the black population. A rise in the rate of disability such as neurological impairment due to manganese exposure could have serious negative consequences for the already inefficient health service. White individuals, who are overwhelmingly treated in the private medical sector, may not suffer from poor, manganese related health outcomes despite similar exposure.

There are many reasons why leaded petrol was used for so long in South Africa, and why MMT is used as a replacement despite being banned in many developed countries. The strong influence of the mining industry which seeks to protect its interests is one, although this is true of any mining operation in any country. The other is lax government control over the environment. It is easy to point a finger at both the apartheid and the new South African governments and lay the blame for poor environmental conditions at their feet, but to do so would be disingenuous and would ignore the very real and all-consuming tasks these governments have had to deal with in addition to the environment. The apartheid government had its hands full maintaining a brutal, racist regime along with the challenging job of oppressing over three-quarters of its population, a task which did not allow for time spent hand-wringing over the environment.

The new South African government on the other hand, has had the overwhelming task of rebuilding a cohesive, peaceful society out of an angry, resentful and fearful population (both black and white), all whilst attempting to meet incredibly high expectations both internal and external. The construction of sanitary housing, the restructuring of education, the reduction in and treatment of AIDS and the maintenance of peace and order have all taken precedence over the environment and perhaps rightfully so. Yet discussions with South African colleagues whilst conducting this research revealed anecdotal evidence that the state of the environment has become worse since the end of apartheid. Quantitative research by individuals such as Mathee confirms that inorganic pollution is still a very prevalent problem in urban South Africa. Herein lay the problem. Without a clean environment, the priorities of the new South Africa would appear to be just out of reach. Healthy and equitable housing development requires water that is not contaminated by

AMD. Equal educational opportunities for all is not possible when some children are faced with neurological and developmental impairment due to pollution. An efficient health service run on limited resources cannot survive the effects of widespread toxic pollution. The removal of lead from petrol was one important step in the right direction. If, in just over 10 years' time, South Africa experiences a drop in violent crime on par with that experienced in the United States the lives of everyone in the population will benefit.

As with most research, as many questions are raised as have been answered by the results of this research. There is substantial work to be done in South Africa on both historical toxic element exposure and present-day exposure. First and foremost issue that needs to be addressed is that of the status of white women with regards to lead exposure during apartheid. Unfortunately, no white females could be included in this study. However the Pretoria Collection includes the remains of many white females, and the sampling of some of these females would shed light on the overall rate of exposure among white adults from 1960 to 1999. It is hypothesized that white females would follow an exposure pattern which would yield higher bone lead concentration than black individuals, but slightly lower concentration than white males.

Additionally, skeletal material from other South African collections, such as that which exists at the University of Cape Town would also be valuable in determining whether the element exposure patterns uncovered in Transvaal/Gauteng were present in other cities in South Africa. Cape Town was never as severely segregated as Pretoria or Johannesburg and it would be quite informative to see if toxic element exposure was as racially dichotomous in this city. In addition, data from more regions within South Africa would allow for greater context for present-day studies and the tracking of toxic element exposure over time.

With regards to lead, lead concentrations in skeletal material from collections such as Pretoria, Witwatersrand and Cape Town can be measured non-destructively by X-ray fluorescence (XRF). This method would allow for the "sampling" of an entire collection, yielding information on thousands of individuals. Such a study could generate vast amounts of data which could be used to uncover population-wide trends in lead exposure.

It is unclear whether all of the lead bioavailable to humans comes from petrol. Certainly Monna et al. show that atmospheric lead is dominated by lead from petrol, but that study did not take into account the potential role of indoor air pollution from coal burning in human exposure. Analysis of lead isotopes in a cross section of the sampled population may shed light on this. Data regarding the isotopic signature of both leaded petrol and South African coal is available and can be compared with the isotopic signature of lead in humans to investigate the source of exposure in different groups. A selection of 25 males among the

skeletal remains was sampled for lead isotope analysis. This analysis was conducted in the Summer of 2012. Black and white males with low, median and high bone concentrations were included for analysis to investigate whether lead source was the same in at all groups and at all exposure levels. The analysis of results was not yet underway at the time this document was submitted.

With regards to present-day research into toxic element exposure in South Africa, there are numerous questions to be answered. Firstly, there are no studies of toxic elements other than lead and manganese in the present. Whilst cadmium, arsenic and antimony appear in low bone concentrations in the sampled population, other biomarkers exist that may be more accurate. These elements are not currently monitored in the South African population, making comparisons with historical data difficult. Has exposure to these elements changed significantly since the 1960s?

In addition, there is little to no monitoring of adult element exposure, particularly to that of lead or manganese. The monitoring of these elements can be safely accomplished without the need for blood (a concern in a highly AIDS-affected country) by XRF. Such data would not only dovetail nicely with the historical data presented here, but would also allow for the monitoring of those groups most at risk for lead- and manganese-related health effects. It may also allow for targeted occupational and environmental intervention in high-risk groups.

Lastly, there is no research to date regarding the effect of toxic element exposure on HIV/AIDS infection and vice versa. As both public health issues are prevalent in South Africa, data regarding the influences of one on the other may be quite valuable. Do individuals with HIV/AIDS have higher body burdens of some elements? If so, is this a cause or a consequence of infection?

These are only a few of the potential research directions that could follow on the research presented here. However each question serves to highlight the need for further investigation into both present-day and historical toxic element exposure in South Africa.

Despite the number of subsequent questions raised, the information yielded as a result of this project is both important and significant. Prior to this project, there was no published research on adult toxic element exposure in urban Transvaal/Gauteng either during apartheid or in the present day. As such, this project has generated quantitative data that addresses this gap. The analysis presented here has shown that exposure to most toxic elements in urban South Africa was determined by one's racial group as classified by apartheid law, and that in most instances the difference in exposure between racial groups would have resulted in different degrees of impaired health. In addition, the research has generated a database of

bone element concentration in 215 urban South Africans during the latter half of the 20th century that can be used to compare present-day toxic element studies. Such comparison allows for the identification of temporal and geographic trends in toxic element exposure in urban South Africa, which was heretofore impossible.

The ongoing value in data such as those presented here is the way in which they can be used and built upon in the future. Studies such as Nevin's are difficult to replicate in South Africa due to a lack of lead exposure monitoring. By generating a small data set and research which can hypothetically be expanded to include more data, it is possible to track human lead exposure from its peak in the 1980s to its decline in the future, and to juxtapose urban crime and social trends onto the data set across time. The data can also be used to track changes in the rate of toxic element exposure in at-risk populations.

The racial divide in exposure for all but one toxic element investigated shows, quite succinctly, the way in which apartheid policy had a negative impact on all parts of the population. It highlights the extent of the racial division within South Africa during apartheid – to the point at which even the air that black and white individuals breathed was distinct. Above all else, this research has demonstrated that apartheid had hidden and unexpected impacts on South African society, many of which have yet to be uncovered.

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Appendix A. Intra-individual comparisons of long bone element concentration

It was hypothesized that different long bones within the same individual would yield no difference in mean element concentration for each element. This was hypothesis was explored statistically for two reasons. Firstly, if false, and not taken into consideration, it would greatly increase the odds of both Type I and Type II errors in any statistical analysis including all bones, due to either false degrees of variation or lack thereof. Secondly, the investigation of bone element concentrations between bones of the same individual may yield significant correlations that enable both the present author and subsequent researchers to compare bone element concentrations of different individuals with meaningful results.

Not all bones were present in all individuals, however two individuals were complete (six bones). Mean element concentration for each bone in a compared pair varies depending on the individual.

Differences in means: dependent t-tests vs. Repeated Measures ANOVA

The use of a General Linear Model (GLM) was rejected in this analysis. This is due to the small number of complete individuals and the substantial number of missing values that result from incomplete individuals. RM ANOVA was explored, however, the missing data resulted in a substantially high α-level, that effectively rendered the results meaningless. Subsequently, it was decided that dependent, pairwise, two-tailed *t*-tests between each bone for mean each element concentration (using the log transformed concentration) would be a more appropriate method. For each element, 15 pairwise comparisons were analysed, and for all comparisons, a 95% confidence interval was calculated. Effect sizes (r) for dependent t-tests were calculated from the following equation:

$$r = \sqrt{\frac{t^2}{t^2 + df}}$$

Bonferroni's correction

With any tests involving small sample sizes, the potential for Type I error, and the data analysed here is no different. To correct for this, and to ensure that H_0 is not rejected in error, Bonferroni's Correction was applied to all t-tests on each bone type. For all tests, H_0 is rejected if $p < 0.003$ (0.05/15), where the number of comparisons is 15.

Correlation and Regression

Correlation and regression were conducted on all bone pairs where the difference in mean element concentrations was significant *and* significant correlation was found to exist between bone element concentration. The correlation coefficient was compared with the critical value for Pearson's correlation coefficient for a two-tailed test with sig. 0.05, (*df* = N-2) to determine whether Pearson's *r* was legitimately significant or could have occurred by chance given the small sample size. These tests are included to show the potential relationships between long bone element concentrations. Due to small sample sizes, even significant *r* values below the critical value for *r* should be viewed with caution. Instead the results suggest that a significant linear relationship *may* exist between bones for certain elements, and a larger sample size is needed.

Lead

No significant differences in bone Pb concentration was apparent between any bone type.

Table A-1. Intra-individual bone Pb concentration.

Element/Bone	N	*t*	Df	Sig.
PbFemur - PbFibula	10	-2.068	9	.069
PbFemur - PbTibia	8	-1.283	7	.240
PbFemur - PbRadius	12	-1.399	11	.189
PbFemur - PbUlna	8	-1.807	7	.114
PbFemur - PbHumerus	8	-.764	7	.470
PbFibula - PbTibia	3	3.554	2	.071
PbFibula - PbRadius	6	-.227	5	.829
PbFibula - PbUlna	4	-.186	3	.864
PbFibula - PbHumerus	6	1.796	5	.132
PbTibia - PbRadius	3	-.361	2	.753
PbTibia - PbUlna	3	-2.801	2	.107
PbTibia - PbHumerus	4	-1.626	3	.202
PbRadius - PbUlna	6	-2.631	5	.046
PbRadius - PbHumerus	6	.502	5	.637
PbUlna - PbHumerus	3	1.222	2	.346

Manganese

No significant difference in bone Mn was apparent in any bone type.

Table A-2. Intra-individual bone Mn concentration.

Element/Bone	N	t	Df	Sig.
MnFemur - MnFibula	10	*-2.505*	*9*	*.034*
MnFemur - MnTibia	8	.287	7	.783
MnFemur - MnRadius	11	-.772	10	.458
MnFemur - MnUlna	8	-1.428	7	.196
MnFemur - MnHumerus	8	-1.027	7	.339
MnFibula - MnTibia	3	.059	2	.959
MnFibula - MnRadius	5	-.031	4	.977
MnFibula - MnUlna	4	-1.477	3	.236
MnFibula - MnHumerus	6	1.121	5	.313
MnTibia - MnRadius	3	-.160	2	.888
MnTibia - MnUlna	3	-.721	2	.546
MnTibia - MnHumerus	4	-.268	3	.806
MnRadius - MnUlna	6	*-4.856*	*5*	*.005*
MnRadius - MnHumerus	5	2.030	4	.112
MnUlna - MnHumerus	3	1.792	2	.215

Cadmium

No significant difference in bone Mn was apparent in any bone type.

Table A-3. Intra-individual bone Cd concentration..

Element/Bone	N	*t*	Df	Sig.
CdFemur - CdFibula	10	-3.451	9	.007
CdFemur - CdRadius	11	-1.465	10	.174
CdFemur - CdHumerus	8	-.441	7	.673
CdFibula - CdRadius	5	.152	4	.887
CdFibula - CdUlna	4	-.108	3	.921
CdFibula - CdHumerus	6	4.337	5	.007
CdRadius - CdUlna	6	-1.336	5	.239
CdRadius - CdHumerus	5	2.300	4	.083
CdUlna - CdHumerus	3	1.286	2	.327

Zinc

No significant difference in bone Zn was apparent in any bone type.

Table A-4. Intra-individual bone Zn concentration.

Element/Bone	N	t	Df	Sig.
ZnFemur - ZnFibula	10	-1.573	9	.150
ZnFemur - ZnTibia	8	1.281	7	.241
ZnFemur - ZnRadius	11	1.999	10	.073
ZnFemur - ZnUlna	8	-.058	7	.955
ZnFemur - ZnHumerus	8	1.056	7	.326
ZnFibula - ZnTibia	3	1.416	2	.293
ZnFibula - ZnRadius	5	1.055	4	.351
ZnFibula - ZnUlna	4	.211	3	.846
ZnFibula - ZnHumerus	6	1.930	5	.111
ZnTibia - ZnRadius	3	-.083	2	.942
ZnTibia - ZnUlna	3	-.426	2	.712
ZnTibia - ZnHumerus	4	.228	3	.835
ZnRadius - ZnUlna	*6*	*-3.709*	*5*	*.014*
ZnRadius - ZnHumerus	5	1.962	4	.121
ZnUlna - ZnHumerus	3	1.645	2	.242

Antimony

No significant difference in bone Sb was apparent in any bone type.

Table A-5. Intra-individual bone Sb concentration.

Element/Bone	N	t	Df	Sig.
SbFemur - SbFibula	*5*	*-2.820*	*4*	*.048*
SbFemur - SbTibia	7	-1.337	6	.230
SbFemur - SbRadius	8	-1.204	7	.268
SbFemur - SbUlna	7	.417	6	.691
SbFemur - SbHumerus	5	-1.332	4	.254
SbFibula - SbUlna	2	-.437	1	.737
SbFibula - SbHumerus	2	2.388	1	.252
SbTibia - SbRadius	2	-.084	1	.947
SbTibia - SbUlna	2	.622	1	.646
SbTibia - SbHumerus	2	-1.542	1	.366
SbRadius - SbUlna	*4*	*3.373*	*3*	*.043*
SbRadius - SbHumerus	3	.345	2	.763
SbUlna - SbHumerus	2	-.985	1	.505

Arsenic

No significant differences in As concentration were found between bones of the same individuals.

Table A-6. Intra-individual bone As concentration.

Element/Bone	N	t	Df	Sig.
AsFemur & AsFibula	10	-1.794	9	.106
AsFemur & AsTibia	8	1.539	7	.168
AsFemur & AsRadius	10	-1.367	9	.205
AsFemur & AsUlna	8	.093	7	.929
AsFemur & AsHumerus	7	-.405	6	.700
AsFibula & AsTibia	3	2.022	2	.181
AsFibula & AsRadius	5	.508	4	.638
AsFibula & AsUlna	4	.774	3	.495
AsFibula & AsHumerus	6	1.780	5	.135
AsTibia & AsRadius	3	-1.346	2	.311
AsTibia & AsUlna	3	-.340	2	.766
AsTibia & AsHumerus	4	-.029	3	.979
AsRadius & AsUlna	6	.402	5	.704
AsRadius & AsHumerus	4	1.237	3	.304
AsUlna & AsHumerus	3	-.258	2	.820

Iron

No significant differences in Fe concentration were found between bones of the same individuals.

Table A-7. Intra-individual bone Fe concentration.

Element/Bone	N	t	Df	Sig.
FeFemur - FeFibula	10	-.884	9	.400
FeFemur - FeTibia	8	.463	7	.657
FeFemur - FeRadius	11	-.585	10	.571
FeFemur - FeUlna	8	.776	7	.463
FeFemur - FeHumerus	8	1.459	7	.188
FeFibula - FeTibia	3	-.005	2	.997
FeFibula - FeRadius	5	.674	4	.537
FeFibula - FeUlna	4	.531	3	.632
FeFibula - FeHumerus	6	1.524	5	.188
FeTibia - FeRadius	3	2.033	2	.179
FeTibia - FeUlna	3	1.352	2	.309
FeTibia - FeHumerus	4	.966	3	.405
FeRadius - FeUlna	6	.877	5	.421
FeRadius - FeHumerus	5	.624	4	.567
FeUlna - FeHumerus	3	3.570	2	.070

Magnesium

No significant differences in Mg concentration were found between bones of the same individuals.

Table A-8. Intra-individual bone Mg concentration.

Element/Bone	N	t	Df	Sig.
MgFemur & MgFibula	10	1.475	9	.174
MgFemur & MgTibia	8	-.878	7	.409
MgFemur & MgRadius	11	.522	10	.613
MgFemur & MgUlna	8	-.375	7	.719
MgFemur & MgHumerus	8	.793	7	.454
MgFibula & MgTibia	3	-1.084	2	.392
MgFibula & MgRadius	5	-.391	4	.716
MgFibula & MgUlna	4	-2.139	3	.122
MgFibula & MgHumerus	6	-.699	5	.515
MgTibia & MgRadius	3	.224	2	.843
MgTibia & MgUlna	3	-.777	2	.518
MgTibia & MgHumerus	4	-1.168	3	.327
MgRadius & MgUlna	6	-1.582	5	.174
MgRadius & MgHumerus	5	-.912	4	.413
MgUlna & MgHumerus	3	-.297	2	.794

Copper

There are significant differences in mean bone Cu concentration between bones. For femora and fibulae, $t(14) = 5.659$, $p < 0.00$. In fibulae and humeri, $t(6) = 8.306$, $p < 0.001$. However, Cu is not among the primary elements of interest in this research.

Table A-9. Intra-individual bone Cu concentration.

Element/Bone	N	t	Df	Sig.
CuFemur - CuFibula	15	-5.659	14	.000
CuFemur - CuTibia	11	-.207	10	.840
CuFemur - CuRadius	16	-.543	15	.595
CuFemur - CuUlna	11	-2.480	10	.033
CuFemur - CuHumerus	10	.601	9	.563
CuFibula - CuTibia	5	1.844	4	.139
CuFibula - CuRadius	9	.391	8	.706
CuFibula - CuUlna	6	2.170	5	.082
CuFibula - CuHumerus	7	8.306	6	.000
CuTibia - CuRadius	4	-.013	3	.991
CuTibia - CuUlna	4	.195	3	.858
CuTibia - CuHumerus	5	1.952	4	.123
CuRadius - CuUlna	7	-.357	6	.733
CuRadius - CuHumerus	6	1.087	5	.327
CuUlna - CuHumerus	4	1.029	3	.379

Calcium

There are no differences in Ca concentration between bones.

Table A-10. Intra-individual bone Ca concentration.

Element/Bone	N	*t*	Df	Sig.
CaFemur - CaFibula	15	-1.342	14	.201
CaFemur - CaTibia	11	.155	10	.880
CaFemur - CaRadius	16	.637	15	.534
CaFemur - CaUlna	11	-4.003	10	.003
CaFemur - CaHumerus	10	-1.826	9	.101
CaFibula - CaTibia	5	1.071	4	.344
CaFibula - CaRadius	9	-.795	8	.450
CaFibula - CaUlna	6	-1.585	5	.174
CaFibula - CaHumerus	7	-.787	6	.461
CaTibia - CaRadius	4	-4.364	3	.022
CaTibia - CaUlna	4	-4.134	3	.026
CaTibia - CaHumerus	5	-3.743	4	.020
CaRadius - CaUlna	7	-1.159	6	.291
CaRadius - CaHumerus	6	.289	5	.784
CaUlna - CaHumerus	4	-.852	3	.457

Discussion

These results demonstrate no significant differences in the mean bone element concentration between pairs of bones from the same individuals.

Differences in means

The presence of differences in mean bone element concentration among cortical bones of the same individual has immediate consequences for this and similar research. Firstly, it indicates that all long bones can be compared in the wider study. Secondly, the small sample sizes available in this study require a bit of caution. H_0 was rejected in these analyses largely due to small sample sizes. Were a larger study to be conducted, it may be that there would be significant differences in mean bone element concentration between bones.

To date, no studies have focused solely on calculating differences in bone element concentration between long bones of the same individual. Wittmers et al. (1988) analysed differences in relationships between several long bones and trabecular bone, in an attempt to model the relationship between the two bone types and to establish a model that allows for the prediction of whole body lead burden from the concentration of one or more bones. However researchers have long assumed that bone element concentration would be similar

among long bones of the same individual, and the results in this chapter do not challenge this assumption. There is evidence that bone element concentration can differ significantly *within* the same bone, for example bone samples removed along the diaphysis of the same bone can yield different concentrations (Aufderheide and Wittmers 1992). It is possible that the results above are influenced by this phenomenon, however every effort was made to sample each bone in precisely the same location along the diaphysis. In addition, Aufderheide and Wittmers (1992) point out that few studies of bone lead distribution across the skeleton have taken into account age and sex. This is true, and the above small study is no different. Sample sizes were simply too small to investigate whether age, sex or health influence differences in bone element concentration within the same individual (Rabinowitz 1991).

Among the potentially interesting questions that arise from these results is how variation in element concentration may be affected by age, sex and race. Wittmers et al. (1988) found that the variation in lead concentration between bones decreased with increasing age. These authors included both trabecular and cortical bone, unlike the present study which is concerned only with cortical bone. To date, there is scant research investigating the intra-individual variability in trace element concentration among long bones. The variation in means found here, particularly between femora and fibulae and radii and ulnae suggest that even among bones with similar rates of turnover, trace element uptake and release may differ significantly.

Aufderheide, A. C., and Wittmers, L. E., 1992. Selected aspects of the spatial-distribution of lead in bone. *Neurotoxicology*, 13 (4), 809-819.

Rabinowitz, M. B., 1991. Toxicokinetics of bone lead. *Environmental Health Perspectives*, 91, 33-37.

Todd, A. C., Parsons, P. J., Tang, S. D., and Moshier, E. L., 2001. Individual variability in human tibia lead concentration. *Environmental Health Perspectives*, 109 (11), 1139-1143.

Wittmers, L. E., Wallgren, J., Alich, A., Aufderheide, A. C., and Rapp, G., 1988. Lead in bone .4. Distribution of lead in the human skeleton. *Archives of Environmental Health*, 43 (6), 381-391.

Appendix B: Method

Analytical Method: Theoretical considerations

Pre-analytical Method – Theoretical considerations

Trace element analysis of bone tissue consists of two distinct phases: the pre-treatment, or digestion phase, and the analysis phase. Digestion involves the dissolution of bone by acid, dilution of digested materials and preparation for analysis by ICP-MS or other method. As the science behind chemical analysis of bone is not new, there are myriad methods to choose from when conducting this type of analysis. The following sections discuss the most widely used methods for pre-analysis and the rationale behind the pre-analysis method used in this research.

Sample pre-treatment

Sample pre-treatment involves the decomposition of a sample of mixed organic and inorganic phases such as human bone. In the first instance, sample particles must be reduced in size in order to facilitate nebulisation by a mass spectrometer or any other analytical device. Mixed samples must also be both mineralised and dissolved prior to analysis. Mineralisation is the removal of the organic phase of the sample, leaving only the inorganic phase and analytes of interest. Dissolution is the process by which the remaining inorganic phase is dissolved into solution in preparation for analysis (Hoenig 2001). In bone samples this means the elimination of bone collagen, lipid material and any residual organic material that may have adhered to the bone tissue itself.

Inorganic sample preparation consists of breaking down the substance of interest into inorganic components that can be easily dissolved into liquid form and analysed by the spectrometer. Bone is a highly complex matrix consisting of both inorganic (primarily calcium and phosphorus forming hydroxyapatite) and organic (collagen) components. In order for bone tissue to be analysed, it must first be mineralized and dissolved. Mineralisation consists of "digesting" the organic component of the bone. The remaining inorganic component is dissolved, dried, and reconstituted within an aqueous matrix suitable for ICP-MS analysis; generally 3 to 5% HNO_3.

There are several potential sample digestion methods that are widely used in trace elenment studies of human and animal bone tissue. Among these are dry ashing, and wet "ashing" or wet digestion. There are benefits and pitfalls to each method, and each is accepted in the literature as adequate for trace element analysis of bone. To date, there is no concensus regarding which method is best for trace element analysis of mixed samples (Hoenig 2001).

Table B-1, below lists several studies of trace elements in bone comparable in methods and objectives to the present study and the acid combinations used.

Table B-1. Comparative digestion methods. Studies similar to the present study in which trace element concentration in bone was measured with ICP-AES, ICP-MS or AAS and the digestion acids used.

Author, Year	Acid Combination
(Baranowska et al. 1995)	HNO_3
Degryse et al. 2004	HNO_3 (dry ashed)
Drasch 1982	HNO_3 (room temperature 10 days)
Gonzalez-Reimers et al. 2005	$HNO_3 + H_2O_2$
(Grotti et al. 2005)	HNO_3
Jaworowski et al. 1985	$HNO_3 + HClO_4$
Klepinger et al. 1986	HFl + HCl
Kosugi et al. 1986	HNO_3
Kuo et al. 2000	HNO_3
Martinez-Garcia et al. 2005	$HNO_3 + HClO_4$ (dry ashed)
Özdemir et al. 2010	HNO_3 (dry ashed)
Reinhard & Ghazi 1992	HNO_3 (dry ashed)
Roberts et al. 1996	HNO_3 + HCl
Shafer et al. 2008	HNO_3 (microwave)
(Todd et al. 2001)	HNO_3

The technology used in digestion procedures also varies. Dry ashing, the superheating of a sample (above 300º C) takes place with only small amounts of dilute acid and involves the use of a muffle furnace. Microwave digestion in either closed or open vessels uses a pre-set heating protocol on a microwave oven (laboratory quality) and high pressure in conjunction with acid to complete digestion. The advantages of microwave and dry ashing procedures are that they take a relatively short amount of time (hours as opposed to days) and can reduce contamination as samples are generally covered (Hoenig 2001)

The primary benefit of dry ashing, particularly in regards to bone, is that after ashing, the ashed weight of the sample can be determined. This allows for the final element

concentration to be expressed in concentration per ashed weight, which is increasingly standard practice in anthropological and archaeological literature. Drawbacks to dry ashing are potentially slower processing times – particularly problematic when large numbers of samples are being analysed, and potential loss of some analytes due to high temperatures. Generally, however, heavy metals such as lead, cadmium and manganese are not lost.

The alternative method is wet "ashing", generally referred to as wet digestion. Wet digestion involves the dissolution of the bone sample in a highly concentrated acid or combination of acids with oxidative properties.Wet digestion aims to achieve the complete oxidation and elimination of the organic phase of the sample and the simultaneous dissolution of the inorganic phase (Hoenig 2001).

Wet digestion is generally facilitated by heat, either on a conventional hotplate or heating block or by microwave digestion. Samples are dissolved in small quantities (< 5mL) of concentrated acid. Samples can then be evaporated to dryness and reconstituted with deionised water or dilute acid, or the acid containing the dissolved sample be diluted to a specific volume with deionised water. In some cases, subsequent digestions can be carried out if the sample is not digested completely, however this increases the potential for contamination. Insoluble materials that remain in the solution can also be filtered. Potential complications arising from wet digestion methods relate primarily to the potential for incomplete removal of organic material and contamination from impure reagents. Open tube digestion methods, while useful in regards to facilitating evaporation, leave samples vulnerable to lab contamination. Other pitfalls include the possibility of co-precipitation of an analyte of interest with the precipitate formed from a matrix element with the acid reagent.

As is demonstrated in Table B-1, there are a range of potential acids that can be used in wet digestion. The choice of one particular acid over another is determined largely by the composition of the sample and the analytical objectives. The primary acids used in wet digestion are nitric, hydrochloric, sulphuric, perchloric, and hydrofluoric acids as well as hydrogen peroxide and aqua regia (hydrochloric and nitric acids). HFl is a highly dangerous, primarily used in geological sciences to dissolve silicate-based materials. It cannot be used with borosilicate glassware and requires specialized PTFE digestion containers. Another acid commonly used in digestion procedures is sulphuric acid. Sulphuric acid, when combined with a sample high in calcium, like bone, can result in the formation of calcium sulphate which can cause certain elements, namely lead, to precipitate and results in low analyte recovery (Hoenig 2001).

ICP-MS Analytical Method: Theoretical Considerations

ICP-MS differs from axially viewed ICP-AES in that it measures the molecular weight of an element or compound. Ions of each element of interest are separated by the mass/charge ratio *(m/z)*, which represents the atomic mass of a given element divided by its charge. Because the atoms are ionised by the ICP, i.e. an electron is removed from each neutral atom, the charge of each atom is generally positive. In this case, z=1.

ICP-MS also allows for the quantification of different isotopes of a given element. This is referred to as resolution (R), and refers to the ability of the ICP-MS to detect two adjacent *mz* ratios.

Sample introduction and quadrapole mass spectrometer

Bone samples in solution of dilute (<5%) nitric acid are introduced into the ICP-MS via the nebuliser, which sprays the liquid sample into an argon jet. Droplets are directed into the argon ICP, where temperatures of approximately 8000ºC dry, decompose and dissociate the sample into individual atoms. These atoms are then ionised and introduced into the mass analyser (MS) via a multi-chambered vacuum system. Once in the MS, the ions are scanned across a *m/z* range. Each mass is passed into an electron multiplier (EM) where mass counts are measured. This information is then processed by the attached data processor (PC) and converted into analyte concentrations.

Quadropole MS

The standard mass analyser, and the one used in this research is the quadrapole mass spectrometer (QMS). The advantages of a QMS system is a relatively fast scan speed and a broad atomic mass unit (amu) range. This means that nearly all the natural and radiogenic isotopes can be detected. In addition, the high resolution of the QMS means that analytes separated by as little as 1 amu can be detected.

Method validation and determination of analytical robustness

Method validation is the process by which the method is deemed fit for purpose. The primary factors used in determining whether the methods used in this project are fit for purpose are: precision (including repeatability and robustness), bias, LoD and uncertainty. The following sections describe the theoretical aspects of analytical method and the theoretical considerations that must be made when choosing an analytical method.

Potential interferences

Interferences in ICP-MS analysis are generally more readily overcome than those of ICP-AES, but can still prove problematic. The primary types of interferences are spectral and non-spectral (Dean 2005).

Spectral interferences

Spectral interferences consist of molecular and isobaric interferences. Molecular interferences are further divided into polyatomic and doubly charged polyatomic interferences.

Isobaric interferences occur when two different element isotopes have the same mass, for example, ^{40}Ca and ^{40}Ar and in fact, is among the most common potential isobaric interferences. Polyatomic interferences are those which occur as a result of the interaction between a given analyte, the sample matrix, aqueous solution into which the sample has been dissolved and the plasma (Dean 2005). Simply, the QMS cannot distinguish between polyatomic ions and atomic ions with the same *m/z*. As with isobaric interferences, these interferences can largely be avoided by the addition of a collision cell to the QMS or by selection of a different acid matrix (Dean, 2005). In the collision cell, the ion beam is passed through an additional cell filled with a given gas. Which gas is used is dependent on the analyte(s) of interest and the type(s) of interference needing correction. The selected gas interacts with the ions and removes the interfering species, allowing the analytes of interest to pass into the mass analyser. This project will use dilute nitric acid as a sample matrix. The choice to use nitric acid for sample dissolution was determined largely by its superior oxidative qualities and efficiency at digesting bone tissue. In addition, the analytical matrix will consist of dilute nitric acid (3%). There is some consensus regarding the preferential use of nitric acid as an analytical matrix, as many polyatomic interferences can be avoided (Mccurdy and Woods 2004). The only potential interferences with nitric acid come primarily from Ar, O, H (from water) and C (from the CO_2 produced from any remaining organic residue).

Non-spectral interferences

Non-spectral interferences include those that affect the total mass range of analytes under analysis and generally include signal suppression or enhancement and drift. Signal suppression or enhancement occurs when there are changes in the number of ions reaching the detector (Linge and Jarvis 2009). Samples which area high in total dissolved solids

(TDS) can cause signal suppression and often this is caused by the sample matrix. Matrix effects, in which the sample matrix is so abundant as to interfere with ion detection.

Signal drift is also caused primarily by high TDS in samples. When drift occurs it is generally due to deposition of particulate matter on the sampler, skimmer cone or ion lenses. These affect the ion extraction process and can result in signal suppression over time. As with signal suppression or enhancement, reduction of TDS is recommended to reduce drift. Normally, it is advisable to ensure that TDS in the sample solution are not higher than 200-300 $\mu g/mL^{-1}$ (Linge and Jarvis 2009).

Monitoring and correcting non-spectral interferences and matrix effects

The primary means by which to monitor and quantify non-spectral interferences is through the addition of internal or external standards. In both cases it is critical for standards to be matrix-matched as closely as possible to the matrix of the analytical sample (Vanhaecke et al. 1992). This is to allow for detection of matrix effects. Because non-spectral interferences depend on many factors, are unpredictable, and can vary from run-to-run and on given days, the use of standards becomes critical.

Internal standards

Internal standards involve the addition to each sample, blank and external standard, an analyte that is not present in the sample materials. Vanhaecke et al. (1992) noted that for certain acid matrices, such as sulphuric acid, signal suppression depended heavily on the mass number of the element or nuclide monitored and signal suppression dependent on the atomic mass of the target analyte, and where the lighter the nuclide the greater degree of signal suppression. Conversely, when the same analysis was conducted in a matrix of hydrochloric acid, mass dependent suppression was also observed, but with signal suppression of the heavier nuclides (Vanhaecke et al. 1992). In short, the choice of internal standards is critical to the accurate measurement of signal strength. Internal standards that closely match the target analyte behaviour will also undergo the same signal enhancement or suppression as the analyte (Agatemor and Beauchemin 2011). Because signal accuracy varies depending on element mass, elements across a range of masses (predominantly encompassing the total mass range of the target analytes) must be included in the internal standard.

It should be noted that in many cases, specifically in any analysis that includes a wide range of elements, it is not always possible to include individual standards that closely match the mass and behaviour of the individual target analytes In this instance, multiple standards may

be used to compensate for matrix effects altering the signal for one specific element. Agatemore and Beauchemin (2011) offer a clear example of this: in ICP-MS analysis of samples containing platinum, it was discovered that using both ^{197}Au and ^{191}Ir as internal standards provided better detection of matrix effects than either element alone.

An alternative method involves common analyte standardisation, in which only one analyte is added, but one that is highly susceptible to signal drift, suppression or enhancement. This addition need not be mass-matched or FIP-matched (first ionisation potential). A drift correction equation must then be calculated. This method however, is unproven in complex matrices (Al-Ammar 2003).

Matrix-matched external standardisation

The matrix includes both dissolved solids in the sample as well as the water and acid or solvent solution, can be minimised by matching calibration solutions to samples and by dilution of the sample solution, both methods are employed here. In addition, matrix effects can be caused by the sample consistency and viscosity and equipment settings, including sample introduction systems, sample introduction flow-rate, size of droplets formed in the nebuliser, ion generation, ion movement into the sampler and ion transport into the mass detector (Agatemor and Beauchemin 2011). These effects can also vary from sample to sample, or across an entire sample run.

Reducing matrix effects

Ideally, matrix effects should be minimised as best as possible before ICP-MS analysis. There countless ways that this can be achieved and methods for matrix effect reduction are specific to the matrix itself, target analytes, and equipment set-up and calibration.

Sample dilution

When the sample matrix is of a high concentration, as is the case with human bone samples, among the first steps to reduce matrix effects is through sample dilution (Agatemor and Beauchemin 2011). When LoD are low enough to allow for sample dilution, it is possible to reduce the concentration of the matrix to levels low enough to reduce or eliminate matrix effects. This has the secondary effect of reducing the TDS in the matrix. Beyond this, a low sample uptake rate and low nebuliser gas flow rate can act as a secondary dilution by reducing the amount of sample that reaches the plasma.

Determining analytical fitness for purpose

Precision

Precision in this context is measured as standard deviation of the mean or standard error of the mean where *n* is the number of replicates and *SE* is the standard error:

$$SE \div \sqrt{n}$$

In this case the mean is the mean percent recovery of the analytes of interest for each sample. A low standard of error relative to the sample mean indicates that the sample means are close to the population mean. Population mean in this case is 100%, or the ideal percent recovery of each element from the standard reference material.

Precision is determined by repeatability, which is the process of repeating the same independent experiment with the same method under the same experimental conditions (in this case the experimental conditions are the method, the laboratory, the equipment and the researcher and all samples are treated independently of each other. The experiment is repeated *n* number of times and the results obtained for each repeat are used to determine the standard of error. The number of times the method will be repeated is determined by the confidence interval between the population (sample) and standard deviations of the sample. In this project it is assumed that the data are distributed normally and the confidence interval should be 95% so that *z* = 1.96. So where $\bar{x}$ = the population mean and *SE* = the standard error:

$$\textit{Lower confidence interval} = \bar{x} - (1.96 * SE)$$

$$\textit{Upper confidence interval} = \bar{x} + (1.96 * SE)$$

The probability of a normal distribution of means is assumed when number of samples (i.e. independent tests) is large because the larger the sample size, the more likely it is that the sample mean represents the population mean. However the number of repeat independent tests in this project is limited by time and the cost of the SRM. Fortunately, Cullen and Barwick (2004) have demonstrated that beyond 15, the number of replicate tests has little effect on confidence interval and that below six the interval is too high.

Trueness

Trueness is the percentage variation between the reported value true value (i.e. actual analyte concentration) (Linge and Jarvis 2009). It is a measure of systemic error. Trueness cannot be calculated for unknown samples, but is measured by analysis of certified reference material

Bias

Bias refers to the measurement of two types of statistical error: systematic and random. Bias can be referred to as the degree of closeness between the average value of a set of results and the accepted reference value (Cullen and Barwick, 2004).

Bias is measured through the use of NIST-SRM 1486 Bone Meal, which will be used to obtain the accepted reference value. During repeat testing, the average recovery of analyte is measured against the reference value. Accepted bias in this project is 15%. That is, a result within 15% of the accepted reference value will be accepted, as this is what is generally deemed acceptable within the literature.

Limits of Detection and Limits of Quantification

LoD and LoQ were calculated using the raw counts per second (cps) data. The values given in Table 6-2 are for the analytical equipment.

The following procedure is used to calculate LoD for each element:

1. Calculate mean cps for blanks
2. Calculate SD for cps for blanks
3. Calculate LoD as mean blank cps +3SD
4. Calculate LoQ as mean blank cps +10SD

Sensitivity (blank corrected lowest standard cps) was measured using the following procedure:

1. Subtract blank cps from lowest standard cps.

LoD and LoQ are transformed to ppb:

1. LoD and LoQ values are divided by sensitivity and then by 1000 to give LoD and LoQ in $\mu g \cdot g^{-1}$

*Detection limits for the samples are calculated by multiplying the LoD and LoQ by the average dilution factor for the analytical samples which is 69.48.

LoD and LoQ are determined by running approximately ten blanks through the method from start to finish. In addition, internal standards are added at both the LoD and LoQ for each sample run to monitor these values. Blank solution is made up of 3% HNO_3 in distilled water.

Detection range and linearity

The detection range is essentially the range of values across which the analyte can be detected and the method used. The working range must be calculated to ensure that it encompasses the entire range of values yielded by the analysis. In general, the lower limits of the working range are determined by the LoD and LoQ. The upper limits are determined by the concentration of a given analyte, at which detection sensitivity is compromised. The range of detection for a given analyte must fall between these two limits.

The linear range falls within the working range. The linear range is that in which the response of the instrument to the analyte is directly proportional to its concentration. Linearity is established by the use of internal standards and is determined by conducting a least squares regression and correlation coefficient to the results for these standards.

Role of CRM in method validation

Certified reference material (CRM) is used to ensure quality control and fitness for purpose of the chosen analytical method. The CRM used must conform to ISO Guidelines in that any uncertainty in reference values must be small in comparison to the uncertainty in routine analytical data. CRM can also be used for equipment calibration, as the concentration of analytes in the material is known, however this was not the case in this project (Kane 2001). CRM should match as closely as possible the matrix of the analytical samples and contain each of the analytes of interest. The primary role of CRM in this analysis is not for calibration but for method validation.

Method for trace element analysis of human cortical bone.

1. Samples were removed from bags and placed into clean, acid washed 13mL polyethelene tubes and weighed to 0.0001 accuracy.
2. Samples are then rinsed 3 times in MilliQ Millipore water by filling each tube with water and agitating.
3. Aspirate 1mL pipette 3 times in 6M HCl and rinse w/ MilliQ. 1mL concentrated (15.2M, or approx. 69%), sub-boiled HNO_3 is added to each tube and tube is capped. 15 reagent blanks and CRM (NIST 1486 Bone Meal) prepared alongside analytical samples.
4. Samples left to digest at room temperature (20°C) for 72 hours.
5. 9mL MilliQ added to each tube.
6. Samples placed in ultrasonic bath for 5 min.
7. Each sample is diluted to 100ppm Ca by calculating dilution factor for each sample based on sample weight and estimated % Ca in bone material.
8. Aliquot based on DF is transferred from tube to acid washed 20mL bottle by (cleaned and aspirated 3 times 6M HCl and MilliQ) pipette. Pipette tip is changed and cleaned in between each sample. Bottle is weighed, tared, and aliquot is added. 3% HNO_3 is added to bottle until total weight is 10g.
9. 100µL internal standard solution is added to each sample. Solution: 0.5 ppm In, 0.5 ppm Re, and 2 ppm Be in 3% HNO_3.

Matrix-matched standards

Standards were calculated by determining the likely analyte concentrations in bone. 5 working standards were prepared with the following element concentrations:

Secondary standards

A set of 7 secondary standards were made adding aliquots of individual element solutions (Inorganic Ventures) to 3% HNO_3. After each aliquot was added, HNO3 was added to each acid washed bottle until total solution weight was 20mL. Secondary standards were formulated as follows:

1. 5 ppm Sr and Zn
2. 1000 ppm Fe and Mg
3. 5 ppm Fe and Mg
4. 5 ppm Cu, Se, V, Sn, Cd, Sb
5. 5 ppm Ni, Pb, Mn, As
6. 200 ppb Ni, Pb, Mn, As
7. 200 ppb, Cu, Se, V Sn Cd, Sb

Working standards

Five working standards were created. Aliquots of each secondary standard were added to acid washed bottles with (aspirated) pipette tips. 200µL spike solution added to each bottle and 3% HNO_3 added until total solution weight was 20g. The five working calibration standards were as follows:

Std.	Elements	Target Conc.
Std 5	Fe Mg	10 ppm
	Zn Sr	150 ppb
	Ni Pb Mn As	10 ppb
Std 4	Fe Mg	4 ppm
	Zn Sr	60ppb
	Ni Pb Mn As	4ppb
	Se V Sn Cd Sb Cu	4ppb
Std 3	Fe Mg	1 ppm
	Zn Sr	15 ppb
	Ni Pb Mn As	1 ppb
	Se V Sn Cd Sb Cu	1 ppb
Std 2	Fe Mg	100 ppb
	Zn Sr	1.5 ppb
	Ni Pb Mn As	100 ppt
	Se V Sn Cd Sb Cu	100 ppt
Std 1	Fe Mg	5 ppb
	Zn Sr	75 ppt
	Ni Pb Mn As	5 ppt
	Se V Sn Cd Sb Cu	5 ppt

Calcium standards

Calcium standards were mixed separately. Five calcium standards were made using 1000 ppm Ca solution. Ca solution was added to clean bottles with (aspirated) pipettes. 300µL spike solution was added to each bottle. 3% HNO_3 was added until total solution weight was 30g. Calcium standard concentrations were as follows:

Standard 1: 70 ppm

Standard 2: 80 ppm

Standard 3: 90 ppm

Standard 4: 100 ppm

Standard 5: 110 ppm

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Appendix C: Certified Reference Materials

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material 1486

Bone Meal

This Standard Reference Material (SRM) is intended primarily for use in evaluating analytical methods used for the determination of selected major, minor, and trace elements in bone and in material of a similar matrix. It consists of steamed bone meal that was sieved and blended to a high degree of homogeneity.

The base material for this SRM was obtained from a commercial supplier. The entire material lot was sieved through a nominal 355 μm sieve (45 mesh), blended in the NIST cone blender, radiation sterilized and bottled into units of 50 g.

Certified and Non-certified Concentrations of Constituent Elements: The certified concentrations of the constituent elements are shown in Table 1. These concentrations are based on the results of a definitive analytical method or the agreement of results by at least two independent methods. Non-certified concentrations, for information only, are provided in Table 2.

NOTICE AND WARNINGS TO USERS:

Expiration of Certification: This certification is valid for five years from the date of shipment. Should any of the certified values significantly change before then, purchasers will be notified by NIST. Please return the attached registration form to facilitate notification.

Storage: The material should be kept tightly closed in its original bottle away from sunlight or ultraviolet radiation.

Use: The bottle should be mixed well by rotating the bottle before each use. Samples of this SRM should be dried under vacuum for 24 h or for 2 h at 105 °C in a conventional drying oven. A minimum sample of 150 mg of the dried material should be used to relate analytical determinations to the certified values in this certificate.

Dissolution Procedure: Samples may be dissolved by heating with hydrofluoric and nitric acids, followed by heating to dryness with perchloric acid, cooling, and adding dilute nitric acid.

Coordination of the analyses was performed by W.F. Koch of the NIST Inorganic Analytical Research Division.

Statistical analysis of the experimental data was performed by S.B. Schiller and L.M. Oakley of the NIST Statistical Engineering Division.

The technical and support aspects involved in the certification and issuance of this SRM were coordinated through the Standard Reference Materials Program by R. Alvarez and T.E. Gills.

Gaithersburg, MD 20899
December 18, 1992

William P. Reed, Chief
Standard Reference Materials Program

(over)

Material Source: The material for this SRM was obtained from the Espoma Company, Millville, NJ.

Homogeneity Assessment: Samples from randomly selected bottles of SRM 1486 were tested for homogeneity using x-ray fluorescence spectrometry. No evidence of material heterogeneity was observed in any of the elements measured which included strontium, zinc, copper, iron, phosphorus, calcium, and potassium.

Certified Concentrations and Uncertainties: The certified value is the weighted mean of method results from a definitive analytical method or the weighted mean of results from at least two independent analytical methods or laboratories. The uncertainty is the half-width of a 95% confidence interval for the mean, with an allowance for systematic differences between methods.

Table 1. Certified Concentrations of Constituent Elements

Element	Concentration, wt. percent	Element	Concentration µg/g
Calcium	26.58 ± 0.24	Iron	99 ± 8
Magnesium	0.466 ± 0.017	Lead	1.335 ± 0.014
Phosphorus	12.30 ± 0.19	Potassium	412 ± 4
		Strontium	264 ± 7
		Zinc	147 ± 16

Non-certified Concentrations: Elements other than those certified are present in this material. Those that were determined but not certified are provided as additional information on the composition.

Table 2. Non-certified Concentrations of Constituent Elements

Element	Concentration, wt. percent	Element	Concentration µg/g
Silicon	(<0.02)		
Sodium	(0.5)	Aluminum	(<1)
Carbon (Total)	(18.6)	Arsenic	(0.006)
Moisture		Cadmium	(0.003)
2 h @ 105 °C	(2.4)	Copper	(0.8)
		Fluorine	(800)
----------		Manganese	(1)
Loss in Ignition		Selenium	(0.13)
@ 1000 °C	(31.5)		

Table 3. Methods and Analysts for Certified Elemental Determinations

Element	Method Code	Element	Method Code
Calcium	GRAV INAA TITR	Potassium	FAES IDTIMS
Iron	ICP IDTIMS	Strontium	FAES IDTIMS
Magnesium	INAA IDICPMS	Phosphorus	GRAV ICP
		Lead	IDTIMS
		Zinc	ICP IDTIMS

Methods Used for Analysis of SRM 1486:

FAAS = Flame Atomic Absorption Spectrometry
FAES = Flame Atomic Emission Spectrometry
ICP = Inductively-Coupled Plasma Emission Spectrometry
ID ICPMS = Isotope Dilution, Inductively Coupled Plasma Mass Spectrometry
ID TIMS = Isotope Dilution, Thermal Ionization Mass Spectrometry
INAA = Instrumental Neutron Activation Analysis
RNAA = Radiochemical Neutron Activation Analysis
TITR = Titrimetry
XRF = X-ray Fluorescence Spectrometry
GRAV = Gravimetry

Analysts, National Institute of Standards and Technology

D.S. Braverman
R. Demiralp (Guest Researcher)
J.D. Fassett
K.M. Garrity
R.R. Greenberg
J.R. Moody
P.J. Paulsen
P.A. Pella
T.A. Rush
J.M. Smeller
S.F. Stone
T.W. Vetter
R.D. Vocke
L.J. Wood

Cooperating Analysts

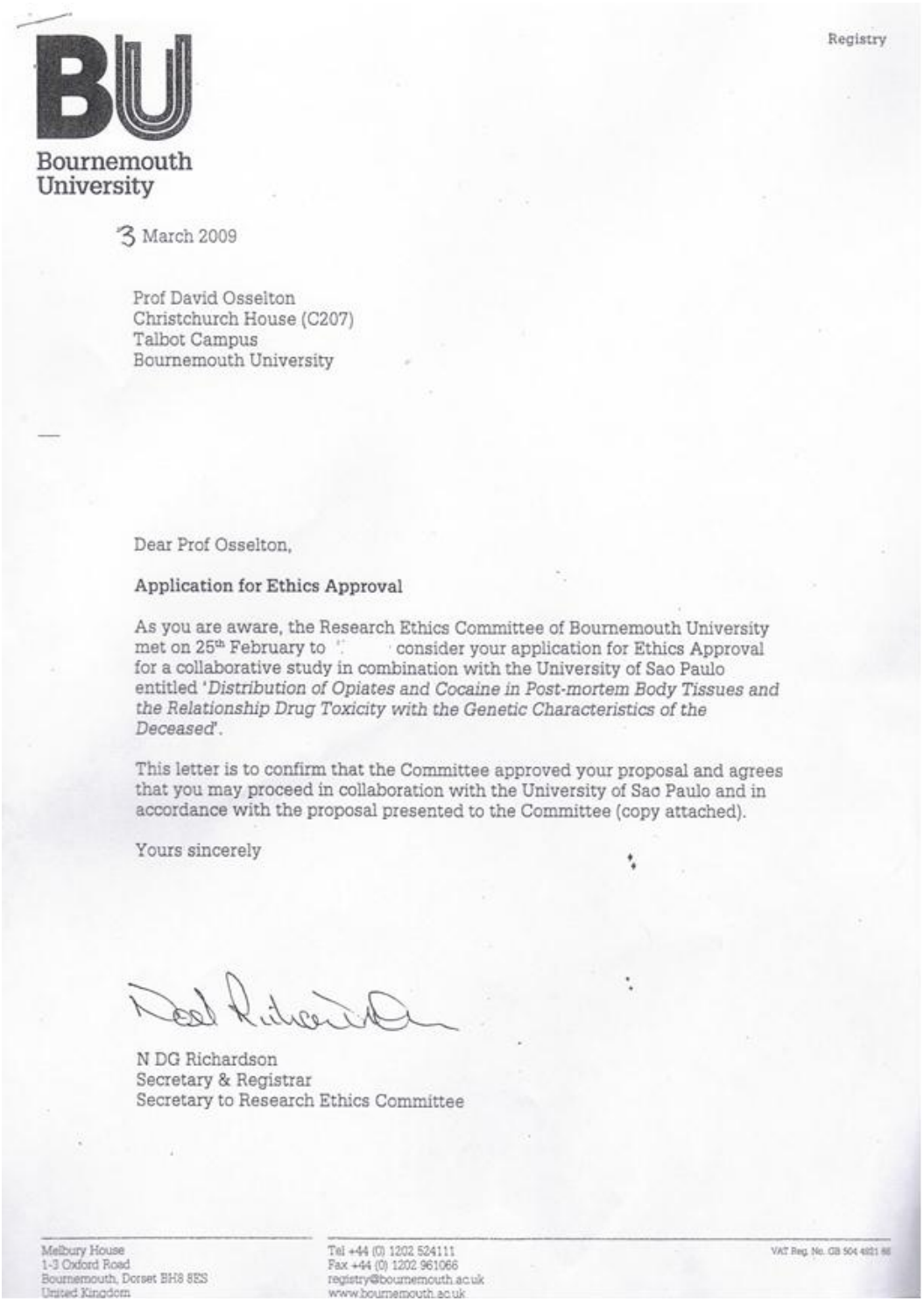
A.R. Byrne, Jozef Stefan Institute, Ljubljana, Slovenia, Yugoslavia.

N. Miller-Ihli, Nutrient Composition Laboratory, U.S. Department of Agriculture, Beltsville, MD.

J.B. Bodkin, College of Earth and Mineral Sciences, Mineral Characterization Laboratory, The Pennsylvania State University, University Park, PA.

Appendix D: Ethics Review

This project was granted Ethics Approval by the Bournemouth University Research Ethics Committee on 27 April, 2010.



Registry

Bournemouth University

3 March 2009

Prof David Osselton
Christchurch House (C207)
Talbot Campus
Bournemouth University

Dear Prof Osselton,

Application for Ethics Approval

As you are aware, the Research Ethics Committee of Bournemouth University met on 25th February to consider your application for Ethics Approval for a collaborative study in combination with the University of Sao Paulo entitled '*Distribution of Opiates and Cocaine in Post-mortem Body Tissues and the Relationship Drug Toxicity with the Genetic Characteristics of the Deceased*'.

This letter is to confirm that the Committee approved your proposal and agrees that you may proceed in collaboration with the University of Sao Paulo and in accordance with the proposal presented to the Committee (copy attached).

Yours sincerely

N DG Richardson
Secretary & Registrar
Secretary to Research Ethics Committee

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VAT Reg. No. GB 504 4821 88

From: David Osselton
Sent: 27 April 2010 13:40
To: Catherine Hess
Cc: Martin J. Smith
Subject: FW: Ethics enquiry

-----Original Message-----
From: Geoffrey Rayment
Sent: 27 April 2010 12:26
To: David Osselton
Cc: Noel Richardson
Subject: RE: Ethics enquiry

Dear David,

Further to Noel's e-mail (below), I have consulted Dr Chapman, Chair of the University Research Ethics Committee, and he has agreed that this does not constitute a significant change to the original application and the previous ethical approval still stands. You do not, therefore, need to submit a new request for approval.

Regards

Geoff Rayment,

Committee Clerk,

Student & Academic Services Directorate.

Tel. 01202 961073

E-mail. grayment@bournemouth.ac.uk

From: Noel Richardson
Sent: 27 March 2010 13:58
To: David Osselton
Cc: Geoffrey Rayment
Subject: RE: Ethics enquiry

Dear David,

In my view the prior approval should be sufficient if the work is of the same nature as that proposed with Sao Paulo. It would, however, be prudent to inform the Ethics Committee that this extension of the scope of the work is proposed, so that you can't be accused of trying to evade due process!

However, I'm not now in charge of these matters, so I've copied this to Geoff Rayment so that he can seek the opinion of the new Chair of Ethics Committee.

Best Regards

Noel

Noel DG Richardson

Clerk to The University Board

Bournemouth University

From: David Osselton
Sent: 26 March 2010 11:59
To: Noel Richardson
Cc: Catherine Hess (i7801430)
Subject: Ethics enquiry

Dear Noel

You may recall that the ethics committee gave approval for us to work on tissues with the University of Sao Paulo. One of my PhD students has been offered an opportunity to undertake analytical work on a bone collection in South Africa where subjects have donated their bodies for medical science. Consent to undertake work has been given by the South African authorities. In view of the fact that this is closely allied to the project which has already been granted approval, do I have to submit another request for consideration or can I take it that the prior approval can be applied?

Best wishes

David

Appendix E: Pretoria Agreement

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

16 September 2011

To whom it may concern

Bone samples for scientific study: Me C Hess

These samples for scientific analysis were taken from skeletal material in the Pretoria Bone Collection. The skeletal material originates from two sources, namely donations and unclaimed bodies. Under the Human Tissues Act of South Africa, law no. 65 of 1983, anyone may donate their body for tissue transplants, medical training and research. The act also provides for destitute individuals who die in a public hospital to have the body donated. If an individual dies in a public institution, the body can be handed over to an approved institution, like the University, for the purpose of research.

Upon arrival, an accession number is given to each body. This is linked to the individual's personal details. After registration, the body is embalmed and usually stored for another year before being made available for use in dissection or other research. Skeletal remains retain the accession number for identification purposes. If, at any stage, family members want to reclaim the body, they are free to do so. To date, no such claims were made with regard to any of the skeletons in question. Skeletons used in this study were accessioned from approximately 1950 to 1980, which makes any such claims at this stage extremely unlikely.

All skeletons in the Pretoria Bone Collection are thus from donations or unclaimed bodies; and curated as stipulated by the Human Tissues Act of 1983. The remains are only made available to bona fide researchers who supply an approved protocol. A small subsection of the entire collection, mostly bone material destined for burial or destruction, is available for destructive sampling.

The Department of Anatomy has

1. Accepted the protocol supplied by Ms Catherine Hess.
2. Accepted ethical clearance as acquired by Ms Hess from Bournemouth University.
3. Approved sampling of the collection.
4. Approved export of the samples for scientific analysis at an appropriate laboratory in the United Kingdom.

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Appendix F: Wits Agreement

From: Desire Brits [Desire.Brits@wits.ac.za]
Sent: Friday, July 29, 2011 5:06 PM
To: Catherine Hess
Cc: Brendon Billings
Subject: RE: Dart Collection Inquiry

Dear Catherine,

The School of Anatomical Sciences has decided to allow you access to sample femora from the Teaching bone collection, however; as mentioned before the teaching collection has limited demographic information and therefore it was suggest that you spend a day in the collection to determine how much information you can obtain, if any. Unfortunately we currently have no electronic database with demographic information for these specimens and you will have to manually document cadaver numbers and follow this up with the technical staff that has a book with all the necessary information.

Best wishes,

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Appendix G: Data sets

Datasets are provided in Microsoft Excel (2010) format on the CD attached to this document. The disc contains three data files:

Main Dataset: This spread sheet includes the sample numbers, cadaver numbers, variable coding, sample weights, dilution factors and element concentrations for every sample, including duplicate bones from single individuals.

Lichen: This spread sheet includes the data taken from Monna et al, 2006 and Olowoyo et al. 2010, which was used to calculate element correlations in the urban environment in present-day Gauteng.

Precision: This dataset includes precision (%RSD) data for each element/sample repeat calculated by ICP-MS.

Appendix H: Lead exposure in adult males in urban Transvaal Province, South Africa during the apartheid era

Hess CA, Cooper, MJ, Smith, MJ, Trueman CJ, Schutkowski H. *In press*. Lead exposure in adult males in urban Transvaal Province, South Africa during the apartheid era. PLoS One.

Abstract

Human exposure to lead is a substantial public health hazard worldwide and is particularly problematic in the Republic of South Africa given the country's late cessation of leaded petrol. Lead exposure is associated with a number of serious health issues and diseases including developmental and cognitive deficiency, hypertension and heart disease. Understanding the distribution of lifetime lead burden within a given population is critical for reducing exposure rates. Femoral bone from 101 deceased adult males living in urban Transvaal Province (now Gauteng Province), South Africa between 1960 and 1998 were analyzed for lead concentration by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Of the 72 black and 29 white individuals sampled, chronic lead exposure was apparent in nearly all individuals. White males showed significantly higher median bone lead concentration (ME = 10.04$\mu g \cdot g^{-1}$), than black males (ME = 3.80$\mu g \cdot g^{-1}$) despite higher socioeconomic status. Bone lead concentration covaries significantly, though weakly, with individual age. There was no significant temporal trend in bone lead concentration. These results indicate that long-term low to moderate lead exposure is the historical norm among South African males. Unexpectedly, this research indicates that white males in the sample population were more highly exposed to lead.

Introduction

Population-wide exposure to lead pollution is a problem that has, for the most part, been addressed in Europe and North America. However inorganic environmental pollution, specifically that of lead and other toxic heavy metals is a major public health concern in sub-Saharan Africa [1-5]. Lead in particular, is of growing concern because of its known toxicity at low levels. The problem of environmental lead pollution was largely overlooked in South Africa in the 20th century, despite its substantial mining and industrial activities, and perhaps more surprisingly, despite the country's persistent use of leaded petrol [1,6]. Few studies of human lead exposure in Transvaal were published prior to the formation of the New South African Republic in 1994, which has left a prominent lack of baseline data with which to compare the growing body of public health research into the issue. Among the aims of this research is to address this gap in data through analysis of the skeletal remains of South African individuals who died before 1998. The Pretoria Bone collection, from which the

study population derives, contains fully identified individuals and its use in this research comprises an unparalleled opportunity to study recent historical trends in human lead exposure. In addition, bone tissue is an endogenous repository for lead. Due to its low turnover rate compared to other human tissues – approximately 10 years for compact bone – bone lead concentration is an excellent indicator of chronic lead exposure [7]. Within Africa, the highly industrial Gauteng Province is among the more polluted regions. The region forms the backbone of the industrial and mining economy of South Africa and is home to the country's most industrial city, Johannesburg and its capital, Pretoria. Urban pollution is a significant public health concern as is human exposure to lead [4,5]. The results of this research may provide valuable background information to more recent studies involving human blood lead concentration in the region.

South Africa began monitoring lead exposure in children in the 1980s [8-11]. Studies conducted by von Schirnding et al. found that as many as 13 percent of children living in Cape Town had blood lead levels greater than or equal to 25μg/dL – more than twice the threshold considered dangerous by the US Centers for Disease Control - and noted that proximity to traffic was a significant risk factor for elevated blood lead, as was lower socioeconomic status, overcrowding and homes in disrepair [10-12]. Deveaux et al. also conducted blood lead monitoring in young children in Cape Town and found that children whose blood lead was greater than or equal to 29μg/dL were also living in homes with leaded paint [9].

Analysis of teeth from individuals buried in Cape Town before the introduction of leaded petrol show higher than expected lead concentrations which were also significantly higher than those measured in the mid-1980s and before the reduction in the lead concentration of petrol [13]. It was determined that the prevalent use of lead pipes in residential plumbing was responsible. To date only one study of bone lead has been conducted in South Africa. Todd et al. measured tibia lead in employees of a lead-acid battery factory [14]. They report a mean bone lead concentration of 53.4$\mu g \cdot g^{-1}$.

Despite these early studies, as late as 2005 the country had no national lead monitoring program [15]. In addition, we could find no studies of lead exposure conducted in Transvaal during the apartheid era, leaving a gap in the understanding of the historical and demographic patterns associated with lead exposure. In addition, because of the cumulative nature of bone lead, this measure is widely considered to be a valuable indicator of chronic, as opposed to acute, lead exposure, and from an epidemiological standpoint, may be a more reliable indicator of demographic and long-term exposure patterns than blood lead [7,16-18]. In light of these observations, this study aims to quantify lead exposure among urban South

African males during apartheid by measuring bone lead concentration in an identified skeletal collection.

The authors wish to note that the racial terms "black" and "white" are used in this study to denote ethnic ancestry. This is wholly due to the fact that that the sampled population is classified in this way in associated cadaver records and because the population would have been segregated purely by racial classification during the time period being studied. These terms have social, demographic and political connotations the implications of which appear to have influenced patterns of lead exposure within the study population.

Materials

Skeletal material was sampled from the Pretoria Identified Bone Collection at the University of Pretoria, South Africa and the Dart Student Bone Collection at Witwatersrand University, Johannesburg. The Pretoria Bone Collection is an identified reference collection held at the University of Pretoria, School of Medicine. The skeletal remains are those of individuals who died in the Pretoria area between 1943 and 2012 and whose bodies were either unclaimed or donated. In the former case, unclaimed bodies become the property of the University of Pretoria to be used for teaching and research, subject to the South Africa Human Tissues Act of 1983 [19]. The collection consists of individuals who range in age from neonates to 95 years of age. The predominant demographic within the collection is black males. This is largely to do with both overall demographic patterns within South Africa and to economic conditions during Apartheid, in which circulating migration brought black males to urban areas from Bantustans for work [19-21]. No information regarding the occupation of any of the individuals in either collection was available. The Raymond Dart Collection is housed at the University of Witwatersrand, School of Medicine and is similar in demographic composition to the Pretoria Collection. Skeletal remains in the Dart collection date to 1928 [22]. Only 12 of the femora included in this study are from the Dart collection. For both collections, ancestry was determined by the admitting hospital and based on the racial classification set forth in the 1950 Population Registration Act, which categorized individuals as black, white or colored based on physical appearance, parentage (an individual with one white and one black or colored parent could not be classified as white) and socio-cultural considerations. For the purpose of this paper, these classifications were not re-examined, as this research is primarily concerned with the way this racial division would have contributed to different lead exposure rates. In addition, because of the unique lack of fluidity between racial groups imposed by Apartheid, and because these groups largely defined socio-economic status at the time, the two factors are considered one and the same in this instance.

Ethics Statement

This research was approved by the Bournemouth University Ethical Review Committee and the University of Pretoria, Department of Anatomy. In addition, the project met the requirements set by the UK Human Tissues Act (1994) and bone samples were imported into the UK and analyzed in accordance with the Act.

Methods

Analytical methods

Cortical bone samples of approximately 0.250g were removed from the right or left femora of 101 individuals who lived in Gauteng Province at the time of their death between 1961 and 1998. Bone samples were removed from femora with a 10mm diamond-tipped core drill attached to a drill press. Cores were taken from the posterior-distal surface of the right or left femur, just above the intercondylar fossa and placed into sealed plastic bags until analysis. Due to the demographic composition of the collections which are biased heavily towards black males, the remains sampled were primarily black males. Analysis was conducted at the University of Southampton Geochemistry Class 100 Clean laboratory at the National Oceanography Centre Southampton. All reagents used were Fisher Trace Element grade and further sub-boiled in Teflon® stills to ensure ultra-purity. Water used was MilliQ® Millipore ultra-pure water (18.2 MΩ).

Sample preparation

Samples were weighed, washed three times with MilliQ® water to remove any surface contaminants and placed into acid-washed 13mL polyethylene tubes. 1mL of concentrated, sub-boiled HNO_3 (69%) was added to each tube and left at room temperature for 72 hours. After initial digestion, 9mL MilliQ® was added to each tube and samples were left to digest at room temperature (approx. 20° C) for a further 72 hours. To facilitate ICP-MS analysis, all samples were diluted to approximately 100$\mu g \cdot g^{-1}$ calcium concentration with 3% sub-boiled HNO_3.

Sample analysis

Samples were analyzed by ICP-MS (Thermo Scientific XSeries 2) calibrated with synthetic mixed element standards made from single element ICP-MS standards (Inorganic Ventures). All samples and standards contained 20$ng \cdot g^{-1}$ Be and 5$ng \cdot g^{-1}$ In and Re as internal standards. The elements were analyzed in one of two instrument modes depending on signal size and susceptibility to interferences. These were standard mode and CCT mode with 2mL/min. of

a mixed He/H_2 gas added to reduce interferences. Ten reagent blanks of 3% HNO_3 were analyzed and Pb concentration in all blanks was below the limit of detection. Detection limit for Pb is 0.0004 $\mu g/L^{-1}$. Method validation was established by the inclusion of ten, 0.1g samples of NIST SRM 1486 Bone Meal and Pb concentration is reported in Table 1. Mean Pb recovery rate in CRM was 90%. Sample duplicate precision was measured at 0.82 (*SD* = 0.32).

Statistical methods

Kolmogorov-Smirnov tests confirmed that bone Pb concentration was not normally distributed for either black males, $D(74) = 0.255$, $p < 0.001$ or white males $D(29) = 0.277$, $p = 0.001$. Pb concentration data was log transformed and was found to be normally distributed with $D(74) = 0.084$, $p > 0.05$ and $D(29) = 0.133$, $p > 0.05$, in black and white males respectively. Independent t-tests, ANCOVA and multiple regression were performed on log-transformed data. All Pb concentrations reported are back-transformed values.

Results

Median bone Pb by race and age group are presented in Table 2. There was a high degree of variability within the subject population as a whole. Of 72 black males, the median Pb concentration is 3.80 $\mu g \cdot g^{-1}$. For the 29 white males median Pb concentration is 10.04 $\mu g \cdot g^{-1}$. Results of Pb concentration for black males are presented in Table 3 and white males in Table 4. In some cases, samples from both right and left femora were taken from the same individual, in these cases Pb concentration in both femora within a single individual was averaged and is indicated by an asterisk.

White males show significantly higher bone Pb concentration than black males (Fig. 1). An independent t-test confirmed that the difference in means is significant, $t(100) = 5.5$, $p < 0.001$. Among all samples, the highest individual concentrations occur in white males – samples 82 (64.09 $\mu g \cdot g^{-1}$) and 60 (24.8 $\mu g \cdot g^{-1}$). Among black males, the highest concentrations occur in samples 11 (32.23 $\mu g \cdot g^{-1}$) and 10 (18.05 $\mu g \cdot g^{-1}$).

There were significant difference in bone Pb concentrations between the 12 black males from the Dart Collection, and black males in the Pretoria Collection, $t(71) = 2.23$, $p < 0.05$. Median bone Pb from males in the Dart collection is 6.14 $\mu g \cdot g^{-1}$ and 3.36$\mu g \cdot g^{-1}$ in males from the Pretoria Collection. However this result must be accepted with caution, as there are only 12 samples from the Dart collection and there is considerable overlap in the admitting hospitals between the two collections, indicating that the individual remains in the two collections did not come from two distinct populations.

It is possible that some of the apparent differences in bone lead concentrations between black and white origin males could be attributed to age, as the white individuals are on average older than the black individuals (Table 2). Analysis of Covariance (ANCOVA) demonstrates that the covariate, age affects bone Pb concentration, $F(1, 99) = 6.66$, $p < 0.05$, though the effect size is small, $r = 0.06$. After controlling for the effect of age, the effect of ancestry on bone Pb concentration remains significant, $F(1, 99) = 19.20$, $p < 0.001$, though the effect size is relatively small, $r = 0.145$.

When multiple regression was used to test the relationship between age and ancestry, a significant linear trend between age, ancestry and bone Pb concentration is apparent. Both age and ancestry explain 28.4% of the variance in bone Pb concentration ($R^2 = .284$, $F(1, 102) = 19.82$, $p < .001$). Both age and ancestry significantly predicts bone Pb ($\beta = .235$, $p < .001$ and $\beta = .395$, $p > .001$).

Discussion

Demographic trends and lead exposure

The results of this study are particularly informative, in that they do not correspond to world-wide trends in human lead exposure. In general, and especially in developing countries, the poorest and most disadvantaged sectors within the population tend to encounter greater exposure to lead [23-25]. These groups also tend to yield the highest body concentrations of lead (bone or blood) [26,27]. This is the case in present day South Africa. von Schirnding et al. [10] have reported blood lead levels among children residing in Cape Town, with children from lower income households having the highest levels. Mathee et al.

[28] reported that high blood lead levels were associated with lower socioeconomic status in a study of children in Johannesburg. Other researchers have reported similar findings [2,3].

The higher bone lead levels of white individuals reported in this study are interesting and may be the result of a variety of factors. The finding is counter to results of lead studies conducted in the United States. Research from the National Health and Nutrition Examination Surveys (NHANES) have consistently reported higher blood and bone lead concentrations in African American individuals regardless of age or sex [29,30]. Data from the US Veterans' Association Normative Aging Survey have also shown that white males tend to have lower bone and blood lead than African American males[31]. Similar results were found in the Baltimore Memory Study, in which authors reported significantly higher cortical bone lead in African American versus white males [32]. These patterns have persisted in the United States, even as overall lead exposure rates have fallen [33]. Most notably, Hu et al. [31] report a median bone lead concentration of $20 \mu g \cdot g^{-1}$ in community exposed males living in Boston. This is higher than the median concentrations values found in South African males in this study. Hu et al. also report higher bone lead concentration in black males, and a significant increase in bone lead in males who did not complete high school versus those who completed graduate or professional school. The latter indicates a strong socioeconomic relationship with lead exposure.

With regards to our study, apartheid-mandated urban residential patterns, with white residents living closer to urban core and major roadways (with subsequent exposure to lead from petrol), may be the significant factors. Recent studies have reported greater atmospheric lead concentration in central business areas in Pretoria and Johannesburg, which during Apartheid were primarily white areas [34-37]. von Schirnding et al. found that atmospheric lead levels in the Cape Town city center were 2.5 times greater than in suburban areas [38]. The presence of lead paint in residential buildings and the possibility that homes built in the early 20th century and before may be plumbed with lead pipes is another, though the latter appears to be rare [39-42]. Investigation into the source of lead in bone by analysis of lead isotopic ratios is currently underway, which may shed light on this phenomenon.

Bone lead and age

This research confirms, though weakly, the previously reported association between age and bone lead concentration [43-45]. It has been estimated that 90% of the lead that is stored in the human body is stored in bone tissue [7,46,47]. This has the effect of sequestering lead from other tissues and organs where it may cause toxicity. However as individuals age and bone is resorbed, lead is released from bone tissue. The correlation between age and bone

lead is well established and given the likelihood that bone acts as an endogenous source of lead within the body, releasing lead into the bloodstream as bone is resorbed and remodeled, it is clear that high lead levels in old age may have a significant impact on individual health[17].

Bone lead and public health

With regards to toxicity, it has been previously reported that bone lead levels as low as 5$\mu g \cdot g^{-1}$ have been associated with clinical symptoms of toxicity such as hypertension [48-52]. In this study, 38% of black individuals and 86% of white individuals had bone lead levels above this threshold. Overall, however, the bone lead concentration in males in this study population is relatively moderate. Baranowska et al. [53] reported bone lead levels between 100 and 200 $\mu g \cdot g^{-1}$ in an industrial district in Poland. Nevertheless, in the past decade it has become increasingly clear that chronic low-level exposure to lead is a substantial threat to individual and public health [54].

Reported health effects of chronic lead exposure include renal disease, diminished IQ and developmental delay (in children), and impaired cognitive function in adults [55-64]. Most recently, the drop in violent crime rates in urban areas in the United States has been attributed to the fall in lead pollution following the banning of lead in petrol [56,65,66]. Many of these pathologies are evident even at the subclinical level and at relatively low levels of exposure. Norman et al. [51] report that in South Africa in 2000, nearly 1,500 deaths could be attributed directly to lead exposure. Other studies have found that low-level lead exposure in men leads to diminished cognitive function on the order of five years accelerated mental aging [67]. From the results of this study, it is likely the negative effects of lead on public health have been acting on the population for some time. In addition, the data above suggest that persistent lower-level exposure to lead may be the norm in South Africa (even after the cessation of the use of lead in petrol). This low but chronic level of exposure may be particularly pernicious, as subclinical or sub-acute symptoms are often overlooked in marginalised populations due, in part, to differential access to medical care and lifestyle [68-70]. Potentially then, despite lower lead exposure overall, black males may be more susceptible to unfavourable health effects.

It is critical to acknowledge that, though black individuals may show lower bone lead concentrations, the burden of disease resulting from lead may be higher in this demographic group. Numerous studies have demonstrated that individuals who may be physically or nutritionally stressed are also likely to suffer from the effects of lead toxicity at lower exposure levels than healthier individuals [71-73]. Within these populations lead exposure

may also be associated with other illnesses such as asthma and iron deficiency anemia, both of which are prevalent in low income households in South Africa [74-78].

In summary, bone lead analysis of apartheid-era skeletal remains has yielded unexpected results. White males show significantly higher bone lead concentration than black males. This difference could be attributed to use of exposure to leaded petrol and exacerbated by residential patterns in urban areas in which white individuals resided closer to the congested urban core.

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Figure 1. Boxplot of median bone Pb concentrations (in $\mu g \cdot g^{-1}$) in black and white males. White males show significantly higher bone Pb concentration than black males. Horizontal line = median, boxes = 2nd and 3rd quartiles, error bars = range.

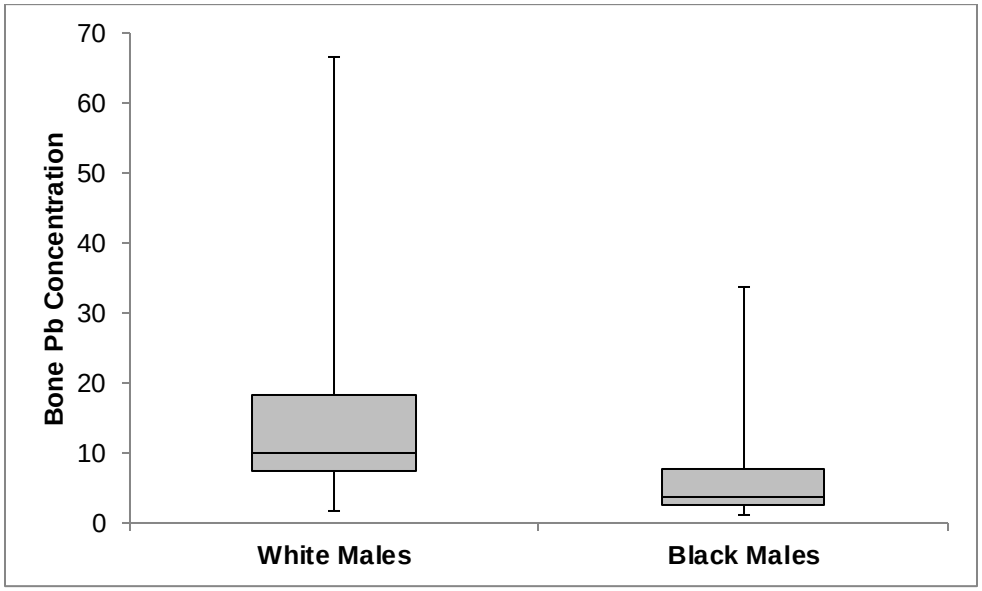

Table 1. Pb concentration and recovery rate for NIST 1486 Bone Meal.

Sample	Pb $\mu g \cdot g^{-1}$	% Recovery
NIST 1486	1.33±.014	-
CRM 1	1.166	87.2
CRM2	1.034	78.0
CRM3	1.167	87.7
CRM4	1.217	92.0
CRM5	1.344	101.0
CRM7	1.229	92.4
CRM 8	1.248	75.1
CRM 10	1.259	95.0
Avg.		**88.5**

Table 2. Median bone Pb ($\mu g \cdot g^{-1}$) in black and white urban South African males in relation to age.

Race		N	Pb	SD	IRQ	Min.	Max.
	Black	72	3.92	5.69	4.12	1.22	32.23
	White	29	10.04	13.61	9.58	1.55	64.09
	Black						
Age	20-29	9	2.22	2.7		1.7	9.25
	30-39	12	4.14	3.47		1.87	13.56
	40-49	18	3.3	2.48		1.22	11.56
	50-59	12	3.67	5.1		1.61	18.1
	60-69	11	4.53	1.72		1.9	6.73
	70-79	10	7.2	11.66		2.02	32.23
	80-89	1	12.95				
Median	49					18	80
	White						
	20-29	0					
	30-39	0					
	40-49	5	10.04	7.9		7.53	26.54
	50-59	7	10.85	7.02		6.38	27.6
	60-69	9	12.7	10.7		2.78	37
	70-79	3	3.41	12.56		1.55	24.18
	80-89	3	7.59	0.22		7.45	7.88
	90-99	2	49.07	21.25		34.04	64.1
Median	62					42	95

Table 3. Total bone lead concentration in $\mu g \cdot g^{-1}$ dry weight in femora of black males.
*Denotes averaged Pb concentration between right and left femora.

Specimen	Age (years)	Death (year)	Pb $\mu g \cdot g^{-1}$	Specimen	Age (years)	Death (year)	Pb $\mu g \cdot g^{-1}$
2	30	1987	4.26	134	20	1964	1.80
3	38	1988	6.60	137	50	1965	14.71
4	36	1988	7.89	141	40	1961	2.19
5	67	1991	2.23	143	49	1966	4.49
6	40	1988	3.92	144	70	1969	7.63
7	30	1987	7.93	147	42	1979	2.72*
8	65	1985	4.76	148	48	1975	1.95
9	50	1988	3.45	150	61	1983	4.17
10	59	1987	18.05	151	65	1972	6.19
11	75	1988	32.23	152	50	1979	3.60
12	51	1991	6.14	156	55	1972	1.61
20	35	1988	2.51	158	50	1972	8.79
23	50	1983	2.95*	159	60	1982	4.53
29	30	1984	2.44	168	70	1979	6.74
48	30	1970	13.56	169	69	1983	1.85
51	58	1975	3.65	174	65	1979	6.73
61	56	1983	3.37	192	35	1967	1.87
63	48	1967	7.65	198	27	1964	6.00
64	40	1967	2.98	199	35	1966	2.48
79	45	1979	5.52	300	44	1979	3.53
83	40	1972	2.96	301	65	1965	2.72
86	44	1979	2.05	306	49	1976	3.05
88	24	1967	9.25	312	25	1966	2.13
89	80	1970	12.95	313	66	1967	6.02
90	70	1966	8.56	314	70	1966	15.66
92	56	1979	5.81	315	26	1972	3.08
95	47	1963	1.91	317	60	1983	4.95
99	40	1965	4.37	319	49	1967	11.56
101	48	1969	1.22	320	37	1966	4.36*
104	26	1966	1.69	321	43	1967	5.76
113	20	1979	2.18*	325	72	1979	2.99
115	70	1979	2.02	326	73	1980	32.13
121	40	1965	3.83	329	70	1983	3.93
123	59	1964	3.69	333	28	1965	6.46
125	70	1973	2.25	334	60	1982	2.38*
131	34	1970	4.03	335	18	1982	2.22

Table 4. Total bone lead concentration in $\mu g \cdot g^{-1}$ dry weight in femora of white males. *Denotes averaged Pb concentration between right and left femora.

Specimen	Age (years)	Death (year)	Pb $\mu g \cdot g^{-1}$	Specimen	Age (years)	Death (year)	Pb $\mu g \cdot g^{-1}$
17	67	1983	15.18	124	83	1993	7.59
39	62	1980	9.70	126	56	1982	8.30
59	84	1998	7.45	178	47	1977	7.53
60	71	1977	24.18	183	62	1983	12.70*
74	72	1998	3.40	185	68	1975	2.95
78	52	1983	6.38	190	66	1997	3.91
82	95	1982	64.09	191	74	1972	1.55
84	82	1997	7.88*	195	60	1973	2.78*
85	50	1976	13.26	295	44	1977	12.30
93	56	1979	9.34*	298	67	1976	37.00
94	42	1977	10.04	305	68	1964	18.44*
105	43	1964	26.54	322	69	1984	15.69
116	59	1982	10.85	324	57	1976	27.58
119	56	1982	12.15*	332	48	1973	7.82
120	91	1979	34.04				